SENSORY, RHEOLOGICAL AND MICROSTRUCTURAL CHARACTERISTICS OF MODEL EMULSIFIED DAIRY SYSTEMS

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ROGERIO B. PEREIRA

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ABSTRACT

Texture is an important sensory property of foods. It is sensed mostly in the mouth during the process of mastication and is an indicator of food quality. To enable faster and cheaper prediction of textural characteristics, extensive research has been done to establish instrumental techniques to produce data that correlates well with the sensory appraisal of texture. In this study, model emulsified dairy systems, represented by processed cheese analogues, were characterised using sensory, microstructural and rheological techniques. Correlation between instrumental and sensory textural data was investigated.

A range of experimental cheeses with differing textural attributes was produced by modifications to the moisture content of the products and the mixing speed during manufacture. Twelve experimental cheeses were used in a partial response surface design in four experimental blocks. These cheeses were subjected to compositional analysis. Two weeks after manufacture, the cheeses were texturally evaluated using a trained sensory panel and quantitative descriptive analysis (sensory), confocal laser scanning microscopy and image analysis (microstructure) and fundamental physical tests including frequency sweep, creep compliance and compression to 70% (rheology). Sensory-instrumental correlations included the chemical data and were performed using pairwise correlation, stepwise regression, principal component analysis and canonical correlation analysis.

Significant differences in moisture, total protein, fat content and pH were found between the experimental cheeses, as expected by the formulation changes. The confocal micrographs showed that fat globule size decreased with decreasing moisture content, but little effect was found for mixing speed. Reduction of the globule size resulted in cheeses that were firmer, better emulsified and stickier.

Sensory evaluation of the cheeses in the mouth was not used in this study because of oral fatigue. Instead, seven hand evaluated attributes were selected from a sensory profiling session. Fracturability, rubberiness and

greasiness proved not to be good sensory attributes to discriminate between these cheeses. Significant differences between the cheeses were found for firmness, curdiness and stickiness. Cheeses with lower moisture content were, in general, firmer, more curdy and less sticky than cheeses with higher moisture content.

Frequency sweep, creep compliance and compression to fracture were all useful tests for providing rheological discrimination between the experimental cheeses. Cheeses with lower moisture content showed higher values of storage and loss moduli, Young's modulus, peak stress and work in compression as well as lower values for compliance. These results provide an indication that these cheeses are firmer, better emulsified and more stable products than those with higher moisture content.

Pairwise correlation was used to correlate the microstructural results to the sensory, chemical and rheological data. It was shown that the area occupied by the protein matrix in the micrographs correlates significantly with most chemical and rheological parameters as well as those sensory attributes that adequately discriminated between the experimental cheeses. Microstructural information was insufficient for use in regression analysis.

Stepwise regression analysis was a useful technique for generating simple models to fit the sensory scores with rheological and chemical data. The regression equations for firmness, stickiness and curdiness produced R-square values above 85%, indicating good predictive ability. Principal component analysis was used to tackle the problem of multicollinearity of the predictive parameters. However, combining those instrumental parameters that were not independent from each other did not improve the quality of the correlation coefficients obtained. Firmness in compression and curdiness were the only two sensory attributes satisfactorily modelled using the first rheological principal component, with R-squares of 88.4% and 90.0%, respectively.

Canonical correlation analysis proved to be a useful statistical tool for maximising the correlation between individual sensory textural attributes and instrumental data. Similarly to the stepwise regression analysis,

fracturability, rubberiness and greasiness could not be satisfactorily modelled. In general, firmness (compression and cutting), stickiness and curdiness were very satisfactorily modelled using only the results from the frequency sweep and creep compliance tests. Compression test data appeared not to lead to any improvement in the correlation coefficients.

Overall, the present study showed that sensory, microstructural and rheological characteristics of the processed cheese analogues investigated do correlate. It is possible to generate predictive models for some individual hand evaluated sensory attributes using chemical and instrumental (rheological) parameters. Prediction using microstructural information has yet to be verified.

"The public sees only the accomplished trick;
they have no conception of the tortuous, demanding
preliminary self-training that was
necessary to conquer that fear."

"The secrets of Houdini" (J.C.Cannell)

To J.

For the love and support,

for the beauty and joy brought into my life,

for the infinite patience

and for making a better me.

Thank you.

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1.0. INTRODUCTION

Sensory evaluation of foods was once considered a purely subjective technique. Scientific progress over the last century has demonstrated that it can become an objective technique, by the use of trained panels and standardised methodology (Lawless & Heymann, 1998). Human beings, who are usually quite subjective in their appraisal of characteristics in food materials, can be thoroughly trained in descriptive sensory evaluation to behave as instruments and rate or rank different products based on specific, well defined attributes within a frame of reference provided to them (Fox *et al.*, 2000; Roberts & Vickers, 1994).

In addition to objective descriptive analysis, subjective evaluation of food materials using consumer panels remains an important feature of the technique. However, regardless of the approach taken during evaluation, the process of characterising a food product using sensory evaluation is time consuming and expensive, with limitations on its practical usefulness to the industry. Numbers generated by sensory techniques are often not completely trusted with regards to their objectivity, which is a result of the inherent variability present among panellists.

In view of that, cheaper and faster alternatives to be used in the food industry for product development and quality control have always been pursued. Instruments have been developed and tested over the years to try and mimic the ways consumers perceive the sensory attributes of foods, with varying degrees of success (Fox *et al.*, 2000; Lawless & Heymann, 1998). In addition, there is much interest in correlating sensory perception of texture with instrumental measurements of textural parameters in foodstuffs.

It is important to consider, however, that instruments will only provide meaningful results, though objective and reproducible, if calibrated against the human senses (Bourne, 1992). Good, precise physical calibration and operation of instruments is not, per se, sufficient for prediction of sensory perception. When measuring physical properties in food materials, it does not

seem reasonable to measure properties that are not perceived by the human senses or simply judged unimportant by consumers. In view of that, development of an instrumental methodology that will allow sensory perception of texture to be estimated or even predicted has been the topic of extensive research in recent years (Drake *et al.*, 1999b; Lawless & Heymann, 1998; Zoon, 1991).

Empirical methodologies used for correlation with sensory response are sometimes criticised for their lack of reproducibility between laboratories and for not really measuring the fundamental properties of the food materials under investigation. At present, a trend exists regarding the use of structural information from image analysis techniques and fundamental rheological tests that measure well defined physical and mechanical properties of foods for correlation with textural attributes as assessed by sensory panels (Aguilera & Stanley, 1999; van Vliet, 1999).

The present research project used model emulsified dairy systems, represented by a range of processed cheese analogues with differing textures, to investigate the possible correlation between sensory evaluation of texture and instrumental measurements using microscopy/image analysis and some fundamental rheological tests. Moisture content of the formulations and mixing speed during manufacture were the only parameters used to create the range of textures for study.

2.0. LITERATURE REVIEW

The aim of this research work was to establish correlation between sensory, microstructural and rheological measurements of textural properties in a model emulsified dairy system. The dairy systems developed for the study were equivalent to a processed cheese analogue, which means that the application of the principles in processed cheese manufacture was required for product development. Hence, a limited review of the relevant literature concerning those principles is presented, together with the perspectives for analogue products in the current market. In addition, a review is presented with background information on the use of the different evaluation techniques of interest. Some relevant published material regarding the application of sensory, microstructural and rheological evaluation of food materials, in particular natural and processed cheeses, is also reported.

2.1. PROCESSED CHEESE

2.1.1. Introduction

Processed cheese is a relatively young food product compared to natural cheese, being invented in 1911, in Switzerland. It was possibly an idea originating from the national dish, fondue, in which natural cheeses of different types are heat treated in the presence of wine, a source of tartaric acid. It was not until 1917, however, that Kraft started the independent development of processed cheese in the United States.

At present, processed cheese is a food product of growing interest in the international dairy products trade. A recent report from the International Dairy Federation (Hetzner & Richarts, 1997) shows that the production of processed cheese increased at a relatively constant rate (2.4%, in average) in the period from 1985 to 1994, especially in countries of the European Union, the United States and Japan. It is pointed out in this report that New Zealand, still with a minor role in the international market for such product, is rapidly

expanding production and exports (doubled production to 12000 tonnes between 1990-1994).

Consumption, unlike production, seems to have stagnated in traditional dairy countries for the past few years. In countries of growing economy and also Japan, however, consumption appears to continue rising. In order to maintain markets and to take advantage of the existing growth potentials, where consumer incomes are still relatively low despite the growth in purchasing power, competitive pricing is decisive (Hetzner & Richarts, 1997).

2.1.2. Processed cheese products

One of the key factors that contribute to the success of processed cheese products is their versatility. Processed cheese is easily adaptable to different market needs and can reach the consumer in a variety of flavours, consistencies and functional properties, showing better keeping quality in comparison to natural cheeses. Furthermore, such products allow for the economical use of defective cheese (to some extent) as well as the addition of value or nutritional benefits to the natural cheese used as raw material (Caric *et al.*, 1985; Caric & Kalab, 1993; Guinee, 1987; Thomas *et al.*, 1980).

Processed cheese consists of a mixture of natural cheeses of different types and degrees of maturity, which are shredded, blended and melted to a homogeneous mass under mechanical agitation (shear), vacuum, heating and the incorporation of emulsifying salts.

According to the ingredients, compositional specifications and consistency, processed cheese products can be classified into processed cheese blocks, processed cheese foods, processed cheese spreads and processed cheese analogues (Caric & Kalab, 1993). Depending on the product type, optional dairy and non-dairy ingredients can be added, including spices and vegetables, meats, stabilisers, preservatives, colours, flavours, whey and whey proteins, skim milk, among others. Each of these added ingredients is discussed in detail in Berger *et al.* (1989). Table 1 summarises some characteristics of the different types of processed cheese products.

Table 1. Ingredients, chemical parameters and common processing temperatures of different processed cheese types

Type of product	*Ingredients*	*Cooking temp (°C)*	*Composition*	*pH*
Processed cheese block	Natural cheese, emulsifiers, NaCl, colouring	71-80 80-85 74-85	Moisture and fat content correspond to the legal limit for natural cheese	5.6-5.8 5.4-5.6 5.4-5.7
Processed cheese food	Same as above, plus optional ingredients such as milk, skim-milk, whey, cream, albumin, skim-milk cheese, organic acids	79-85	≤ 44% moisture < 23% fat	5.2-5.6
Processed cheese spread	Same as processed cheese food plus gums for water retention	88-91 85-98 90-95	≥ 44% and ≤ 60% moisture ≤ 55% moisture	5.2 5.7-5.9 5.8-6.0
Processed cheese analogue	Sodium caseinate, calcium caseinate, suitable vegetable fats (soybean, coconut) emulsifying agent, salt, artificial flavour	As for processed cheese food	As for processed cheese food	5.8-5.9

Source: Caric & Kalab, 1993

Analogue production is based on the use of vegetable oil/milk fat – caseinate/rennet casein blends and, although similar to processed cheese products in terms of manufacture procedures and product characteristics, are not processed cheese products per se (Fox *et al.*, 1996). They usually do not incorporate natural cheese as an ingredient.

2.1.3. Manufacturing protocol

Selection of natural cheese is the very first step in processed cheese production and plays an important role in the successful manufacture of such products. This is because the characteristics of natural cheese are passed

onto the finished product, and the desirable sensory attributes are going to dictate the sort of materials to be used.

Proper selection of good quality natural cheeses, however, is not a guarantee of good quality processed cheese. Aspects such as heating time and temperature, shear rate applied, type and amount of emulsifying salts are just as important for the manufacture of products with the desired flavour and textural attributes (Caric & Kalab, 1993).

Following the selection of the raw materials, the batch/blend is prepared and the cooking, which is a crucial step in the process, follows. The molten product is then transported, filled/packaged and cooled, before being sent to storage and later quality assessment (Fox *et al.*, 1996; Guinee, 1987; Sorley, 1997).

In its definition, processed cheese is said to include natural cheeses of different types and degrees of maturation. It is the unique combination brought about during formulation that will guarantee a product with the intended flavour and functional characteristics. Mature cheeses and those mould-ripened are used in a blend to impart flavour. Body, firmness, elasticity and viscosity of the product will result from the appropriate combination of young and medium cheeses and their levels of intact, non-hydrolysed casein (Sorley, 1997).

2.1.4. Cooking

Cooking is one of the crucial steps of processed cheese manufacturing with regards to the quality of the final product.

Natural cheese, physically considered, is essentially a three-dimensional protein network in which fat globules and moisture are entrapped (Brooker, 1979; Kalab, 1979; Guinee, 1987; Fox *et al.*, 1996). This network, as pointed out by these authors, consists of non-linear strands of *para*-caseinate micelles, intra- and interlinked by means of calcium cross-linking, hydrophobic bonding, electrostatic bonding and van der Waals forces.

If subjected to mechanical agitation (shear) and heating at temperatures above 70°C, as in processing, a heterogeneous mix with a tendency to oiling-off and moisture separation results. That is because the cheese matrix integrity is partially destroyed and its porosity increased. If, however, stabilisers are added, reemulsification of the free fat and rehydration of the protein become feasible and a stable, smooth, homogeneous product can be obtained. This role is played, in processed cheese manufacture, by the emulsifying salts (Caric & Kalab, 1993; Fox *et al.*, 1996; Shimp, 1985).

The shear speed, temperature reached during heating and mixing time during processing are important parameters to be considered in achieving a successful product. Agitation will assist in the breakdown of the protein structure and bring protein and emulsifying salts together. As a rule of thumb, the faster the agitation, the firmer the resulting product (Berger *et al.*, 1989; Sorley, 1997).

As far as temperature is concerned, a minimum of 74°C is considered necessary in order to pasteurise the product and prevent fermentation on storage. Optimum temperatures are in the range of 80-86°C, according to the literature (Berger *et al.*, 1989). The higher the temperature, the more protein hydration and emulsification, and the firmer the product. Emulsification, however, progresses even after heating stopped, not ceasing until the product is brought to a temperature below 30°C. Temperatures of up to 145°C can be utilised for sterilised processed cheese products, especially spreads (Berger *et al.*, 1989).

Cooking time should be as short as possible, since longer times tend to increase browning reaction and increase the viscosity of the mass and hardness of the final product.

2.1.4.1. Processing equipment

Processed cheese products can be manufactured either in continuous cookers or in batch cookers, the latter being the predominant one in the industry (Kosikowski & Mistry, 1997, Zehren & Nusbaum, 1992).

Similar design features in construction and fittings are shared among the different types of batch processing cookers. The melting pans, either single or double walled, can be removable (smaller pans) or fixed (larger pans), mounted in such a way that they are capable of being tipped. Those that can not be tipped display a discharge valve at the bottom (Berger *et al.*, 1989; Kosikowski & Mistry, 1997). Jacketed pans, usually stainless steel or aluminium, are equipped for indirect heating or cooling, important for keeping the temperature of the molten cheese mass after heating, if further mixing is required, or for cooling the mass quickly when the possibility of burning the product arises. In addition to that, melting pans have fittings for steam, vacuum, water injection and monitoring of the process (Berger *et al.*, 1989; Zehren & Nusbaum, 1992).

Examples of batch processors and processing speeds range are the Voegele (60-180 rpm), the Kustner (60-120 rpm), the Blentech (90-180 rpm) and the Stephan (1500-3000 rpm) kettles (Berger *et al.*, 1989). When in process, the temperature-time combination used varies, depending on the blend composition, the desired texture for the finished product and shelf life. As mentioned above, at a given temperature, the processing time generally decreases with agitation rate (Guinee, 1987). The Stephan cooker is an example of a cooker-cutter that has been in use for over 20 years. These machines, fitted with a scraper to ensure thorough mixing in addition to the cutting blades, are particularly suited for the production of processed cheese spreads. Those with lower agitation rates, such as the Blentech, are usually used for blocks and slices (Berger *et al.*, 1989; Sorley, 1997).

In continuous cookers, the blend is usually heated by pumping through tubular heat exchangers to temperatures around 130-145°C for a few seconds and then quickly cooled to 80-90°C (release of pressure under vacuum), where it is held for 2 to 5 minutes for the "after creaming" process. This is a relatively common practice, since UHT temperatures produces identifiable changes in the consistency of the processed cheese. The "after creaming" consists of further stirring the product in a vessel before pumping it to the packing machines (Berger *et al.*, 1989). The higher temperatures achieved in continuous cooking increase the shelf life of the products by destruction of

spore forming bacteria (Guinee, 1987; Berger *et al.*, 1989; Kosikowski & Mistry, 1997). Damrow, Schnell, Choc-Krieg, Kombinator and Votator models are examples of continuous systems.

2.1.4.2. Emulsifying salts

The term emulsifying salt is widely used in the processed cheese literature despite the fact that these salts do not possess emulsifying properties per se (Fox *et al.*, 1996). They are not surface-active substances, but have the power to trigger a number of physico-chemical processes that will ultimately enhance the emulsifying properties of the cheese proteins. Because of this, the name melting salts is preferred at times.

Among the processes triggered, ion exchange, pH displacement and stabilisation, protein peptisation/swelling/hydration and emulsification of the fat are important events that ultimately lead to a new matrix /structure formation during the subsequent stage of cooling (Berger *et al.*, 1989; Caric *et al.*, 1985; Caric & Kalab, 1993; Fox *et al.*, 1996; Shimp, 1985).

2.1.4.2.1. Ion exchange

The calcium *para*-caseinate network that characterises natural cheese is, in general, very slightly soluble. The divalent cation Ca^{2+}, found attached to casein via carboxyl and phosphoseryl residues, is greatly responsible for this lack of solubility of the proteins in water, which in turn reduces the emulsifying properties of these same proteins. By exchange/replacement of the calcium for the monovalent sodium (Na^{+}) of the emulsifying salts, the intra- and interstrand links keeping the protein network integrity are destroyed, the matrix continuity is reduced and a new sodium and/or sodium phosphate *para*-caseinate is formed (Figure 1). The latter show enhanced hydrophilic properties (Caric & Kalab, 1993; Shimp, 1985).

2.1.4.2.2. pH shift and stabilisation

The range of pH values within which processed cheese products fall is narrow, between 5.3 and 5.9, and slightly above the values commonly found

for natural cheeses. It is the correct blend of salts that will shift the natural cheese pH to a value in this range and maintain it by virtue of its buffering capacity (Gupta *et al.*, 1984; Guinee, 1987; Rayan *et al.*, 1980).

The upward pH shift promotes a more reactive caseinate with better hydration and emulsification properties. This is due to the fact that the new pH promotes an increased negative charge in the *para*-caseinate and enhances the calcium binding ability of the salt per se (Fox *et al.*, 1996; Irani & Callis, 1962; Shimp, 1985). It is, therefore, an important factor in determining the characteristics of the finished product.

2.1.4.2.3. Protein dispersion and hydration / fat emulsification

Protein dispersion or protein peptisation, as the process is also referred to in the literature, relates to the disaggregation of the network strands in natural cheese and conversion of the calcium *para*-caseinate into a hydrated/swollen sodium and/or sodium phosphate *para*-caseinate (Fox *et al.*, 1996). The sodium caseinate is very water soluble and able to absorb water to a level that will depend on the balance of factors. These relate to the mechanical (mixing time and speed), thermal (processing temperature) and compositional (intact protein in raw material, quantity and type of emulsifying salts, rework level) inputs of processing.

The calcium cross-linkages, as seen in Figure 1, tend to lock the hydrophobic regions of the protein to the emulsification reaction. As the network is destroyed during processing, exposure of these highly hydrophobic regions occurs and, concomitantly to protein hydration, emulsification of free fat droplets takes place. As shown by Guinee (1987), the hydrophobic regions of the casein strands form a film that coats and also penetrates the oil droplet, while the hydrophobic regions protrude from the droplet surface into the surrounding aqueous phase in a hairy-like conformation (Figure 2).

Stabilisation of the emulsion is achieved by means of the immobilisation of the free water, as it is absorbed by the sodium *para*-caseinate, and increase of the viscosity of the system, which reduces the frequency of collision of the fat droplets.

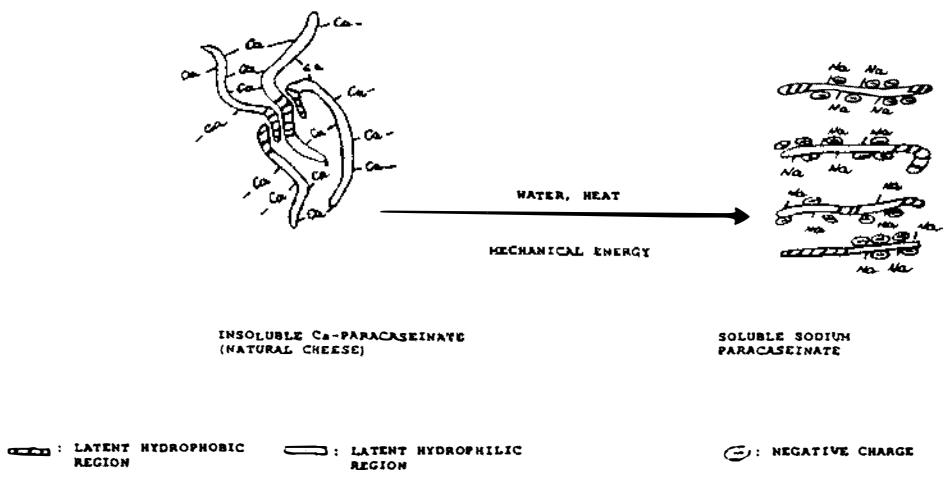


Figure 1. Schematic representation of ion exchange and protein peptisation (Guinee, 1987)

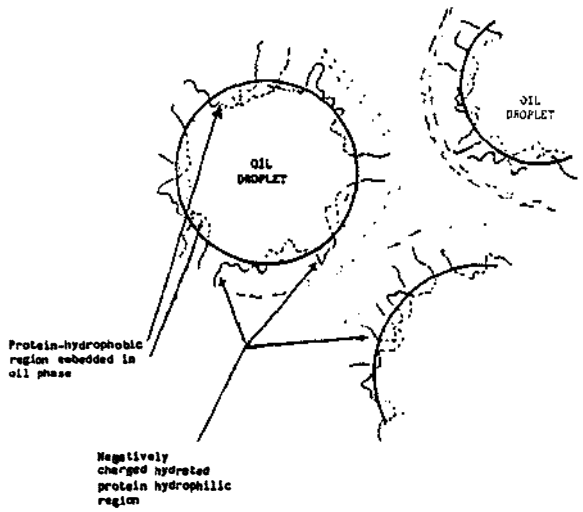


Figure 2. Schematic representation of emulsification in processed cheese (Guinee, 1987)

The process of protein hydration and fat emulsification, leading to the thickening of the mass and increase in its viscosity, is called creaming (Caric & Kalab, 1993; Sorley, 1997). There are, in the literature, many unclear, vague definitions for creaming. Fox *et al.* (1996) defined it as the development of body, creaminess and sheen during processing, especially when spreadable processed cheese is produced. Berger *et al.* (1989) referred to creaming as the production of a melt having a short structure. It is agreed, however, that processed cheese should "cream" only to a limited and controlled extent. Excessive processing, which leads to excessive hydration and emulsification, can break the stability of the system and cause overcreaming. Beyond the point of critical emulsification, the surface area of the fat globules becomes too large, with not enough available protein to cover it completely. The emulsion loses its stability and phase separation tends to follow (Berger *et al.*, 1989; Shimp, 1985). An overcreamed product will appear pale, whitish in colour (Sorley, 1997).

2.1.4.2.4. New structure formation

Having undergone all these physico-chemical changes during processing, the resultant homogeneous mass of molten cheese has to be cooled down and it is during the cooling that the product sets, giving rise to a body with the desired characteristics for each particular type of processed cheese product. This depends not only on the processing conditions, but also on blend formulation and cooling rate (Fox *et al.*, 1996; Guinee, 1987).

As the cheese mass cools, the viscosity decreases, the fat crystallises and interactions occur between the protein strands that lead to the build up of a new matrix. As pointed out by Guinee (1987), the new matrix strands are thinner than those of natural cheeses and the structure resembles that of a cheese gel prior to syneresis and contraction. The protein phase shows varying degrees of continuity, with evenly distributed fat globules. These range in diameter between 0.3 and 5.0 μm, depending on the degree of emulsification (product type). In the newly formed product, fat and moisture do contribute to structural integrity, unlike in natural cheese, where they are mechanically held.

In general, the network of a hard processed cheese consists of interconnecting strands of casein particles that resembles a pearl necklace structure. Soft processed cheeses, on the other hand, show a lesser degree of interparticle connection. The protein-protein interactions in the latter are avoided by means of a fast cooling rate, which brings the product to a temperature below 30°C in a short period of time (Fox *et al.*, 1996).

Not much can be found in the literature about the mechanisms involved in the matrix formation. It is speculated, however, that hydrophobic interactions as well as electrostatic and van der Waals attractions are reactivated during cooling, and that the hairy-like surface of the emulsified fat globules, as seen in Figure 2, might act as loci for the connection of protein strands (Guinee, 1987). Further research to elucidate the physico-chemistry of these mechanisms is necessary.

2.1.4.3. Types of emulsifying salts

Emulsifying or melting salts are of vital importance in processed cheese manufacture. There are several different chemical compounds that could be used for processed cheese manufacture; salts consisting of a monovalent cation and a polyvalent anion possess, however, the best emulsifying properties (Caric *et al.*, 1985). It is worth emphasising that these compounds are not emulsifiers per se, but chemical substances that ultimately enhance the emulsifying properties of caseins during processing. Some surface-active compounds could, however, be added to commercial salt mixtures.

In practice, the sodium salts of phosphoric acid and citric acid are well known in the manufacture of processed cheese products. Sodium aluminium phosphate is also used at times, although only on a limited scale (Ellinger, 1972; Fox *et al.*, 1996). Salts of tartaric and lactic acid are seldom used, due to the impossibility of achieving a product of acceptable quality. The same can be said for potassium salts, due to the bitter taste they can impart to the product. Gupta *et al.* (1984), however, observed that potassium could be used as an alternative for sodium in reduced-Na diets, since cheeses made with potassium salts were similar to those made with their sodium counterparts. Some studies regarding these less commonly used emulsifying salts can be

found in the literature (Gupta *et al.*, 1984; Mann, 1990, 1999; Templeton & Sommer, 1936).

Citrates

Among the several possible salts of citric acid, the only one significantly used in commercial processed cheese manufacture is trisodium citrate ($Na_3C_6H_5O_7$), which is added either alone or combined with phosphates (Berger *et al.*, 1989; Caric & Kalab, 1993; Fox *et al.*, 1996). Mono- and disodium citrates are too acidic for use in processing and tend to produce a product that is unstable, mealy, crumbly and prone to water separation and oiling-off (poor emulsification).

As far as the different processes triggered by emulsifying salts during cooking are concerned, trisodium citrate is known for having low calcium sequestration and protein hydration/swelling ability. Its bacteriostatic potential is likewise low or nil, but its high buffering capacity is comparable to that of orthophosphates (Caric & Kalab, 1993; Fox *et al.*, 1996; Kosikowski & Mistry, 1997; Rayan *et al.*, 1980; Templeton & Sommer, 1936). Berger *et al.* (1989) stated that processed cheese made with citrates possess a long, elastic structure, which to some extent rules out its exclusive use in the production of spreadable processed cheese products.

In terms of their flavour effects, it is generally accepted that sodium citrates impart a clean flavour to the finished product (Fox *et al.*, 1996), while occasional bitterness can be detected when potassium citrates were used in processing (Templeton & Sommer, 1936).

Phosphates

This group of emulsifying salts is subdivided into monophosphates (also called orthophosphates) and condensed polyphosphates (including poly-, pyro- and metaphosphates), each one imparting specific characteristics to the finished product.

Among the orthophosphates, mono-, di- and trisodium phosphates are used in processed cheese manufacture, although the mono- and trisodium forms are seldom used alone. This is because they result in a product that is

either too acid or not acid enough, respectively (Karahadian, 1984, cited by Caric *et al.*, 1985; Templeton & Sommer, 1936). Disodium phosphate is therefore the most popular among the orthophosphates, with properties that are very similar to those shown by trisodium citrate, as previously mentioned. Orthophosphates, however, appear to have intermediate bacteriostatic/bacteriocidal properties between citrates and polyphosphates (Caric & Kalab, 1993; Fox *et al.*, 1996; Kosikowski & Mistry, 1997; Rayan *et al.*, 1980; Templeton & Sommer, 1936).

The subgroup of condensed phosphates includes several different salts, such as disodium, trisodium and tetrasodium pyrophosphates ($Na_2H_2P_2O_7$, $Na_3HP_2O_7.9H_2O$ and $Na_4P_2O_7.10H_2O$, respectively), pentasodium tripolyphosphate ($Na_5P_3O_{10}$), sodium tetrapolyphosphate ($Na_6P_4O_{13}$) and sodium hexametaphosphate ($(NaPO_3)_n$). In general, these salts are superior to orthophosphates and citrates in terms of their ability to promote calcium sequestration, pH shift, protein peptisation and hydration and fat emulsification. The buffering capacity of these condensed polyphosphates tend to decrease as the chain length increases (Berger *et al.*, 1989; Fox *et al.*, 1996). The bacteriostatic/bacteriocidal effect of the components of this subgroup is greatest, possibly due to their interactions with bacterial proteins and chelation of calcium, a cofactor for some microbial enzymes (van Wazer, 1971, cited by Fox *et al.*, 1996).

One important aspect regarding the use of phosphates in processed cheese manufacture is the occurrence of hydrolytic degradation of long chain salts to orthophosphates. This is a well documented process (Berger *et al.*, 1989; Caric & Kalab, 1993; Fox *et al.*, 1996; Kosikowski & Mistry, 1997) and takes place not only during processing itself (ranging from 7 to 50% of total hydrolysis), but also in the course of cooling and storage. Complete hydrolysis usually takes longer in block type cheeses rather than spreads, due to pH and moisture content differences between these products. Significant implications of this fact are the possible legal difficulties in declaring the emulsifier used in the ingredient list, possible variations in the functionality of the salts and, consequently, in product characteristics, and the increased risk of salt crystal formation and precipitation throughout storage (Fox *et al.*, 1996).

Salt mixtures

It is relatively unusual, in commercial manufacture of processed cheese products, to find one particular type of citrate or phosphate used exclusively. On the contrary, salt combinations are common practice and a useful tool for combining the best effects of their individual components (Caric *et al.*, 1985; Caric & Kalab, 1993). These same authors reviewed several studies involving the use of salt combinations as well as combinations of phosphates and monoglycerides, which seem to be satisfactory in the production of processed cheese with improved rheological properties and storage stability. Mono- and diglycerides have been used on experimental scale as an alternative to reduce the introduction of sodium and phosphorus into processed cheese and the diet.

Other studies on mixtures of salts commercially exploited are described by Becker & Ney (1965), Berger *et al.* (1989), Gupta *et al.* (1984), Kosikowski & Mistry (1997), Shimp (1985), Sood & Kosikowski (1979) and Thomas *et al.* (1980).

Gupta *et al.* (1984) evaluated 17 different emulsifying salts, used in 3 different concentrations, and their effects on processed cheese textural and flavour attributes, as assessed by sensory and instrumental analyses. Results from their research showed that acidic emulsifying salts, which cause the pH of the product to be less than 5.2, resulted in dry, crumbly products exhibiting no sliceability or meltability. Condensed phosphates generally imparted non-melting properties to the cheese, while sodium potassium tartrate resulted in grittiness in the final product. Salts normally used in processed cheese manufacture, such as disodium phosphate and trisodium citrate, produced good and desirable characteristics in the experimental cheeses; higher meltability was found for the products prepared with citrate. The influence of salt concentration on processed cheese hardness was extremely variable, increasing or decreasing depending on the salt used. In general, data from sensory analysis supported the instrumental data, although statistical correlation was not attempted. Both assessments, however, indicate the possibility of preparation of salt combinations to achieve a wide range of properties in reduced-sodium processed cheeses.

A study performed in Brazil on the formulation of emulsifying salts for the manufacture of requeijao and other processed cheeses was reviewed by Mann (1987). On the basis of the literature review and Brazilian legislation, six mixtures of emulsifying salts, based on tetrasodium pyrophosphate, sodium tripolyphosphate, acid sodium pyrophosphate, sodium hexametaphosphate, sodium citrate and disodium monophosphate, were used. Rennet curd was used for manufacture of requeijao, and optimal scores for flavour and texture were obtained for products made from a mixture of 60% tetrasodium pyrophosphate, 25% sodium tripolyphosphate and 15% acid sodium pyrophosphate.

Sorley (1997) reported that in a mixture of citrates and orthophosphates, the usual combination is 80% trisodium citrate (TSC) and 20% disodium phosphate (DSP). Use of higher amount of DSP is usually not an option, since the product ends up with a strong and undesirable soapy flavour. When polyphosphates are being used in the combination, however, the mixture can be at any desired proportion.

2.1.4.4. Rework

The term "rework" is used, in processed cheese manufacture, to refer to cheese that has been processed and later added back to new batches. For reasons that include damaged packs, overcreamed batches, leftovers in the cooking kettle, trims from the slice production line, higher or lower viscosity if compared to product specifications, this material is not used for sale (Fox *et al.*, 1996; Shimp, 1985). Instead, it is used to seed and speed up the emulsification reaction in new batches, acting as a catalyst. The physico-chemistry in the mechanisms that lead to this enhanced emulsification with rework is not yet known.

In commercial formulations, rework is always accounted for, usually at levels that range from 5% to a maximum of 20% (w/w). Special attention is required, however, to guarantee that the rework used does not introduce unwanted salts into the new product. In other words, one formulation should

always be put back to the same formulation, or at least one that uses the same salts.

Another important aspect to consider, as far as rework is concerned, is the transportation of the molten cheese to the filling area. This is because, depending on the pipework (too long or with excessive amount and degree of bends), a film of cheese starts to be formed along the system and to act as rework for subsequent batches through the same pipeline (Sorley, 1997). Rework, if not carefully controlled, can easily lead to overcreaming/overemulsification. Small diameter pipes, as well as high speed/high shear, non-continuous pumps, will add to the effect of rework by increasing the turbulent flow of the molten mass and, consequently, creaming (Berger *et al.*, 1989; Sorley, 1997).

2.1.5. Imitation/analogue cheese

Kosikowski & Mistry (1997) and McCarthy (1990) report that great interest arose in commercially producing vegetable fat-casein blends resembling processed cheese after the liberalisation of the definition of imitation foods by the US Food and Drug Administration. Analogue cheeses are essentially oil-in-water emulsions, where the fat or oil phase is dispersed as droplets in the continuous protein/aqueous phase (Ennis & Mulvihill, 1997).

A detailed document from the International Dairy Federation (IDF), published in 1989, provides an insight on the present and future importance of imitation (or analogue, as they are sometimes called) dairy products. According to this document, two major factors influence the production and consumption of imitation dairy products. First, the legislation, which sometimes applies strict prohibitions on the marketing of these products. Second, the preference pattern of consumers, as a result of price and organoleptic differences between natural and imitation products.

As far as the first factor is concerned, wide differences can be found among countries. New Zealand joins countries such as Germany, Italy and

Canada as countries with very strict prohibitory legislation, apparently as a way to protect their dairy industry and its products. Australia, the US, Spain, Norway and Sweden, on the other side of the scale, are highly liberal with regard to legislation on imitation products. In the latter group, marketing is allowed but legislation exists to provide certain protection for dairy products by means of regulations governing packaging and labelling (IDF, 1989). The legal status of imitation dairy products in the United States and some changes in legislation in the European market are also discussed by Kautter *et al.* (1981) and McCarthy (1990).

With regard to patterns of consumer preference, also according to the report from IDF (1989), the main sales argument in countries where marketing is allowed is the low price of such products. This is mainly because vegetable oils normally used are cheaper than milk fat, making especially interesting the possibility of replacing the latter in products with high fat content, such as butter and cheese. Health aspects, particularly related to cardiovascular disease and lactose intolerance, are sometimes also persuasive to consumers, despite the unequivocal adverse effect of these products' flavour on their sales (Lindsay *et al.*, 1980). Analogue cheeses should be nutritionally at least equal to the natural product, if not superior, since the formulation may be changed to suit special dietary needs and consumer preferences (Ennis & Mulvihill, 1997).

2.1.5.1. Imitation/analogue processed cheese

Among imitation cheeses, imitation Mozzarella, Cheddar and Gouda processed cheeses are the majority, with increasing application in the pizza sector (60%), school lunches, catering firms or composite foods (Ennis & Mulvihill, 1997). These authors pointed out a number of advantages of imitation/analogue processed cheeses over their natural counterparts. Worth noting are the lower production costs due to cheaper ingredients, product performance tailored to suit envisaged applications as a result of selection of appropriate ingredients, better quality control of the product, longer storage time of individual ingredients and final products made as consumer demand dictates. Analogues also eliminate the need for starter cultures and the generation of whey and associated whey processing capacity needs.

The principles involved in processed cheese technology are widely applied to the manufacture of analogues (Ennis & Mulvihill, 1997). Shredded natural cheeses used as raw materials for processed cheese are usually replaced with casein or caseinates, advantageous because of lower cost relative to cheese protein and consistent level of intact protein (Fox *et al.*, 1996). Likewise, individual or combinations of vegetable oils are used instead of milk fat. Some limitations for highly saturated oils apply.

Abou El-Nour *et al.* (1996) and Kosikowski & Mistry (1997) reported on the use of vegetable protein isolates, especially soy, peanut and wheat (gluten), to partly replace the milk protein products. In general, however, casein (especially rennet) and caseinates are highly favoured by manufacturers, possibly due to the better perception of flavour. Water, emulsifying salts and sodium chloride are used in the blend as in normal processed cheese manufacture, but nutritional adjustments and additives (flavourings, preservatives, colorants) are often required in the analogues to ensure the marketability of such products. The principles for manufacture of imitation or analogue processed cheeses are presented, in detail, by Kosikowski & Mistry (1997).

2.1.5.1.1. Vegetable oils

An argument often put forward in favour of the use of vegetable oils is their cholesterol content (which is nil) and higher levels of polyunsaturates. This, however, raises some concern about the susceptibility of such ingredients to oxidative processes and the possibility of imparting rancid flavour to the finished product. According to Berger *et al.* (1989), this danger virtually only exists in the event of any fat separation (oiling off). Ennis & Mulvihill (1997) stated that the oil or fat used in analogues manufacture should be liquid or liquefy in the temperature range 32°C to 45°C, in order to give appropriate mouthfeel to the final products.

Soya oil is, by far, the most used among vegetable oils in analogue processed cheese, due to its availability and price. The effects of this oil, used in fat blends for cheese analogues, on the textural characteristics of the

finished products were studied by Lobato-Calleros *et al.* (1997). Occasionally, other oils might be favoured on the basis of a desired nutritional/physiological improvement. Hydrogenated vegetable oils, due to lower degree of unsaturation, are sometimes preferred to other sources.

Berger *et al.* (1989) pointed out that, despite having no adverse effect on flavour (as long as not rancid), no positive contribution on flavour, such as the one from butter, takes place with the use of vegetable oils.

2.1.5.1.2. Vegetable protein

Great resistance to the utilisation of vegetable protein is found among manufacturers, not only due to their different chemical composition and structure, but also because good isolates are hardly any cheaper than the various casein derivatives. However, due to the growing market for analogue cheeses, evaluation of the potential of proteins other than caseins and caseinates is likely to proceed.

Vegetable protein isolates, as reported by Berger *et al.* (1989), tend to swell excessively in the presence of emulsifying salts, producing a consistency that is much thicker than that of a well creamed processed cheese. Some studies reported by these authors showed that isolates can only be used at a maximum of 30% of the protein content of the blend, still resulting in a consistency that is, at the most, acceptable. Examples of the use of vegetable protein can be found in Chen *et al.* (1979b), Abou El-Ella (1980), Kim-Lee *et al.* (1992) and Lee and Marshall (1981).

2.1.5.1.3. Casein and caseinates

Among the different types of casein and caseinates industrially manufactured, calcium caseinate and rennet casein are usually utilised for imitation or analogue processed cheeses (Fox *et al.*, 1996; Gouda *et al.*, 1985; Hokes *et al.*, 1989; McCarthy, 1990).

Acid casein and sodium caseinate, as described by Berger *et al.* (1989), contain very little calcium and are hardly capable of forming a framework

upon which to build and contribute little to a firm structure. Because of that, these forms are usually added in quantities between 3 and 5% as "functional additives" in normal (non processed) imitation cheeses, contributing in structural terms, for example, when low levels of intact protein are used in the blend. Occasionally, they can be used in analogue processed cheese spreads. Added in excessive amount (higher than 3.5%), however, acid casein can have an adverse effect on the flavour of the product (Ennis & Mulvihill, 1997).

Sodium caseinate was used by Marshall (1990) in the manufacture of a model system for study of the relationship between composition and texture. Sodium caseinate is readily soluble in water and forms suitably viscous solutions at relatively low concentrations, but analogues prepared with it tend to show tendency to burn, puff and blister upon heating. This effect is not so apparent when calcium caseinate is used. Sodium and calcium caseinates can be used together to combine the best features of the proteins, but the functionality of the final product will depend on the ratio of sodium to calcium (Ennis & Mulvihill, 1997).

Unlike acid casein and the usual forms of caseinates, rennet casein is hydrophobic in the same way as normal cheese. Actually, rennet casein is, in principle, a very young cheese, in which the original calcium content and the ability to form structure (intact protein) are maintained in full (Berger *et al.*, 1989). Because of these characteristics, adding rennet casein in a formulation is particularly well suited to the manufacture of blocks and slices.

The long structure and neutral, bland flavour of rennet casein make it a great choice for manufacturers of products used for toasting, such as in the pizza sector (Aimutis, 1995; Ennis & Mulvihill, 1997; McCarthy, 1990). On the other hand, it can cause sealing problems due to tailing, if used in spread preparations with salts of low creaming action. Suitable emulsifying salts to be used with rennet casein are those combinations in which long chain polyphosphates predominate. With rennet casein, the degree of casein aggregate cross-linking can be controlled by the calcium-binding strength of the emulsifying salt during processing, so as to achieve the desired degree of hydration and emulsification, and consequently the desired degree of

meltability and stretchability on cooking. Caseinates appear to overhydrate in this application (Fox *et al.*, 1996).

Rennet casein hydrates more slowly than other caseins during the manufacturing process. In the event of a very rapid, poor hydration, the formation of agglomerates of wet casein particles that are difficult to dissolve is observed. These hard glassy lumps of protein are usually referred to as "fish eyes" (Aimutis, 1995; Ennis *et al.*, 1998). It is also a prevalent problem with the readily soluble sodium caseinate (Ennis & Mulvihill, 1997; Gatland, 1987).

The supposedly good re-melting properties and tailing of the cheeses made with rennet casein, always desirable for application as pizza cheese, can sometimes be counterbalanced by these undissolved casein particles left behind in the finished processed cheese. These tend to make the melt a lot thicker and difficult to flow. As described by Ennis & Mulvihill (1997) and Gatland (1987), rennet caseins from different manufacturers or even different batches of rennet casein from the same manufacturer can exhibit widely different hydration properties and behaviour in processing of analogue cheeses.

In an earlier study, Ennis & Mulvihill (1996) observed that different batches of nominally identical rennet caseins exhibited considerable variations in hydration times and solution viscosities when hydrated in a solution of emulsifying salt. These observations were researched further, using a model system, by Ennis *et al.* (1998). Considerable variations in the stability of the hydrated protein dispersions and in the times taken for different rennet caseins to swell, clump and reach maximum viscosity index were found. These findings have important and serious implications for manufacturers of analogue cheeses and end users alike. The variations reported introduce undesirable variability in the rheological properties of the analogue cheeses, thus compromising the ability of manufacturers to reliably reproduce the desired functional properties of the final products.

The amount of rennet casein used in a formulation can go up to 20% of the raw materials, as long as allowed by legislation and the casein is of good

quality, without causing undesirable effect on the flavour of the finished product. This is particularly important in the case of an imitation processed cheese where rennet casein is used as the sole protein source (Berger *et al.*, 1989; Ennis *et al.*, 1998).

2.1.6. Perspectives for imitation cheeses

Interest in the study of the relationship between composition of processed cheese and its textural properties, both instrumentally and sensory assessed, is great. The use of real processed cheese for such studies can present difficulties because, despite the homogeneity of processed cheese in relation to natural cheeses, the former also displays, or can display, variability in composition.

Variations in the cheese milk, the starter culture used and changes during maturation promote considerable variability in the natural cheeses used as raw material for processed cheese manufacture, and ultimately affect the finished processed product. (Marshall, 1990; Sorley, 1997). That is why the selection of natural cheese to be used in a specific formulation is such a major step in the manufacture of quality processed cheese.

One advantage of the use of analogue or imitation products for textural studies is that uniformity in composition and consistency of textural attributes are more easily achieved. This is possible by means of precise knowledge of the composition of the raw materials.

It has been reported that some extent of proteolytic activity can occur even in imitation processed cheeses made solely with rennet casein as protein source (Mulvihill & McCarthy, 1993, 1994). This could be primarily due to residual proteolytic activity of plasmin in the casein. Marshall (1990), however, found that the model cheese analogues used in his research were texturally stable for at least 10 days. The use of cheese analogues, according to this author, facilitated the making of a model of virtually the same composition on different occasions and the attainment of similar results each time. Guinee (1987) calls attention, however, to the fact that discrepancies

exist between results obtained with model systems on casein hydration as influenced by emulsifying salts. Likewise, higher casein concentrations, lower pH values and different ionic environments limit the extent to which results from model systems can be extrapolated to cheese processing.

Situations in which the same formulation results in analogue cheeses of reasonably differing performances due to variability in ingredient characteristics are not satisfactory or acceptable. Gatland (1987) had pointed out the lack of extensive information on the mechanisms taking place during imitation/analogue cheese manufacture. Hence, more detailed understanding of the processes involved in producing ingredients, of the function of the individual ingredients in the final analogue cheese product and of the impact of analogue manufacturing processes on the functional properties of the final products will continue to drive the research in this field of study.

The potential for the pizza and convenience food industry to keep on growing is still vast. There are markets in expansion and demand for cheese analogues is likely to at least be maintained. Delivery of consistent quality will be one factor, if not the major one, in the success of such expansion and imitation or analogue cheeses and their processed counterparts are likely to play an important role in this process. Direct sales to the consumer, however, are expected to remain limited, unless extensive work on flavour improvement is done.

2.2. MICROSTRUCTURAL EVALUATION

2.2.1. Introduction

The texture of a food product has been defined as all the rheological and structural (geometric and surface) attributes of a product perceptible by means of mechanical, tactile and, where appropriate, visual and auditory receptors (ISO, 1981). As emphasised in the very definition of the term texture, knowledge of the structural organisation or spatial arrangement of individual components and their interactions in food products is vital for a

proper understanding of the behaviour of any food material. Although quality factors such as appearance, colour and flavour are all affected by such organisation of the structural elements, no attribute appears to be more strongly influenced than texture (Heertje, 1993; Langton *et al.*, 1996; Stanley *et al.*, 1996; Vodovotz *et al.*, 1996; Aguilera & Stanley, 1999).

2.2.2. Structure and texture

A thorough knowledge of component organisation at ultrastructural and microstructural levels and of structure response to applied forces is necessary to better understand the texture of food products, from a sensory or instrumental perspective. Hence, the ultimate goal of structural examination studies is to determine to which microstructure or microstructures the instrumental probe or human tooth responds (Green *et al.*, 1981; Holcomb *et al.*, 1992; Stanley *et al.*, 1996; Aguilera & Stanley, 1999).

Examination of the microstructure of food materials was limited while direct magnification using glass lens (conventional light microscope) was the only available tool. Basic research on electron beams, however, led to a revolution in microstructure analysis. In more recent years, the advent of electron microscopy (transmission and scanning modes) and the developments and sophistication achieved in light microscopy were responsible for profound changes in food research. One of them was the shift from the merely analytical approach, such as the detection of adulteration (Stasny *et al.*, 1981), to a more scientific, microstructure-function perspective (Lewis, 1981; Aguilera & Stanley, 1999).

Among the many advanced techniques available to date, the method of choice in research relies on factors such as the nature of the food, the microscopic information of interest and the level of resolution required (Vodovotz *et al.*, 1996). It is important to note, however, that enhanced resolution or higher magnifications alone are no guarantee of enhanced image detail or increased structural information. Aguilera & Stanley (1999) stressed the fact that a serious risk of misinformation results from artefacts of

magnification or sample preparation, as well as those due to psychological errors of mental interpretation.

2.2.3. Transmission electron microscopy

The transmission electron microscope (TEM), available initially in 1940, is an important and powerful tool in the study of biological structures. It is based on the principle that a tungsten filament, when heated, works as an electron gun by emitting a narrow beam of electrons travelling at high speed. This electron beam acts as the source of illumination.

A magnetic field (magnetic lens) is used in TEM to focus the electron beam by deflecting it, in an analogous way to the converging glass lenses of light microscopes. The electrons that have passed through the specimen can either be focused on a fluorescent screen for viewing of the final image or onto a photographic plate. It is essential, for the transmission microscopy to work, that a high vacuum environment be created (Fleger *et al.*, 1993; Kalab, 1983; Aguilera & Stanley, 1999). The possible magnifications achieved in these microscopes go up to 300000 X, with a resolution of 0.2 to 1 nm (Stanley & Tung, 1976). A detailed discussion of the principles behind the technique can be found in Reimer (1989).

Despite the evident advantages that arise from such a powerful technique, TEM has severe limitations with regard to the types of specimens that can be examined. In general, such specimens must be completely dehydrated and very thin (≈ 100 nm) to allow transmission of the electrons, but still strong enough to resist beam damage. The increase in the thinness of sections is, however, accompanied by a decrease in contrast (Aguilera & Stanley, 1999). In addition, TEM provides static 2-dimensional images that, despite the advances in image analysis software, are difficult to reconstruct 3-dimensionally from serial sections (Wright *et al.*, 1993).

In general, preparation of biological material for TEM is much more complicated and difficult than for other types of microscopy (Fleger *et al.*, 1993). As pointed out by Aguilera & Stanley (1999), sample preparation

procedures invariably cause structural artefacts, mainly resulting from the drying steps. Aspects relating to the damaging effects of electron beams to biological specimens are discussed in Talmon (1987) and Reimer (1989).

2.2.4. Scanning electron microscopy

While TEM contributed positive and significantly to the study of the ultrastructure of biological materials, including food, it appears that this technique can be counterproductive with regards to viewing a wider spectrum of structural organisation. In this context, scanning electron microscopy (SEM) was developed to combine the best features of light microscopy and TEM.

In many aspects, as pointed out by Pomeranz (1976), Cohen (1984) and Aguilera & Stanley (1999), SEM is more powerful than light microscopy (magnifications up to 100000 X and depth of field 500 X that of light microscopes at equivalent magnifications) and less complicated than TEM. Sample preparation is easier than TEM and introduces fewer artefacts, but that does not imply that sample preparation can be done without care. The final results (SEM image) will mirror the quality of the preparative technique (Aguilera & Stanley, 1999). Furthermore, both surface and internal features can be studied depending upon the preparative technique used.

The final data resulting from SEM consist of electronic signals rather than just a visible image. This enables the images to be computer stored and processed with appropriate image analysis software. The principle of the SEM technique, available commercially since 1965, is very similar to that of TEM, with an electron beam that is passed through a magnetic field under vacuum and focused onto the specimen (Aguilera & Stanley, 1999).

When the electron beam strikes the specimen surface, electrons not only get transmitted, they also generate secondary electrons near the sample surface that can escape and be later collected to form an image of the sample topography. Hence, in SEM, the electron beam is focused obliquely on the specimen surface and then made to scan this surface repeatedly in a

rectangular raster pattern. Secondary electrons generated are subsequently collected, amplified and reproduced onto the screen of a cathode ray tube. The depth of penetration of the primary beam and the intensity of the secondary emission are functions of the accelerating voltage and the density of the specimen (Pomeranz, 1976; Aguilera & Stanley, 1999).

The fact that the sample is still exposed to very high vacuum (implying the necessity of total dehydration) and bombarded by a potentially damaging electron beam (for live tissues samples) constitutes a drawback of the technique (Talmon, 1987). Another one, relatively important but easy to overcome, is the need for some arrangement for conducting the absorbed electrons from the sample (biological materials are usually poor conductors) to the ground. This is usually achieved with the coating of the specimen with a thin film of evaporated metal such as gold or palladium, which ultimately increases the cost of running SEM examinations.

With regards to the necessity of dehydration, Aguilera & Stanley (1999) emphasised that the major problem of electron microscopy usage for food studies is that the materials are composed mainly of water, present not only in bulk form (free water) but also interacting with and dictating specific structures of macromolecules (bound water). If not properly considered, a sample containing residual moisture will lead to structural distortion and even loss of vacuum in the system (microscope). Other volatile components such as fats and oils also need to be removed when food samples are under electron microscopy investigation.

Preparative procedures are discussed in further detail by Carroll & Jones (1979), Chabot (1979), Davis & Gordon (1980), Glauert (1967), Goodhew (1972), Lewis (1981), Read *et al.* (1983) and Steinbrecht & Zierold (1987).

2.2.5. Confocal laser scanning microscopy

Light microscopy is a well developed and widely used technique for the study of microstructure and composition of biological materials, including food systems. The range of magnification achieved with the technique is much

inferior to that of electron microscopy (with resolution ranging from 200 to 500 nm), yet it is an indispensable tool for the food scientist for its versatility, its ease of use and relative ease of sample preparation. In light microscopy, samples are investigated at atmospheric pressure and do not necessarily require dehydration procedures (Blonk & van Aalst, 1993; Aguilera & Stanley, 1999).

One of the major problems found in conventional light microscopy, however, is the fact that all the light that is reflected by the specimen under investigation is collected to produce an image. This includes light from above and below the focal plane, resulting in poor resolution and an image that is often blurred or diffused (Corle & Kino, 1996; Vodovotz *et al.*, 1996).

Due to the limited depth of field of the light microscopy technique, thick specimens are not appropriate for microstructural investigation. On the contrary, good quality and high resolution images are only possible from smears, squashes or very thin sections of the sample, obtained by microtomy. Such preparative procedures can, in some cases, lead to distortion or destruction of the structural elements in fragile specimens. Likewise, they can introduce artefacts through the chemical processing steps for sectioning, which can make image interpretation difficult (Brooker, 1995).

Unlike conventional light microscopy, confocal microscopy, initially developed by Minsky and Petran & Hadravsky in 1961 (Boyde, 1990; Blonk & van Aalst, 1993), allows the investigation of bulk samples with minimal preparation and without prior requirement for embedding, sectioning and fixing (Brooker, 1993; Vodovotz *et al.*, 1996). Confocal microscopy, usually using a laser beam as the source of illumination, has enormous potential in microstructural examinations (Heertje *et al.*, 1987b; Brooker, 1991; Blonk & van Aalst, 1993). This is firstly due to its ability to penetrate deeply yet non-invasively into the specimen (Inoue, 1990; Wright *et al.*, 1993). It can also generate large numbers of sequential thin optical sections that can be computer processed to produce 3-dimensional images (Heertje *et al.*, 1987b; Smart *et al.*, 1995; Vodovotz *et al.*, 1996). In addition to that, the technique enables identification and localisation of more than one chemical component

at a time (depending on the number of laser lines available) by specific labelling technique (Inoue, 1990; Herbert *et al.*, 1999).

In confocal microscopy, sectioning of specimens is optical rather than physical, reducing considerably the occurrence of image artefacts. The technique is also valuable in the study of dynamic processes and transport phenomena (Heertje *et al.*, 1987b, Heertje *et al.*, 1990; Blonk & van Aalst, 1993; Wright *et al.*, 1993; Vodovotz *et al.*, 1996; Ramkumar, 1997).

2.2.5.1. Principles of confocal microscopy

The principles and theory behind confocal microscopy have been thoroughly discussed in the literature (Boyde, 1990; Brooker, 1995; Corle & Kino, 1996; Draaijer & Houpt, 1993; Wilson, 1993; Wright *et al.*, 1993).

The instrument (microscope) uses a focused light source (laser) to provide optimal illumination to a single point at a sub-surface layer of the specimen (Figure 3). The light is then moved across the specimen, point by point, by means of an x,y scanning mirror (beam scanning), although stage scanning, i.e., moving the specimen under stationary laser beam, is also possible (Vodovotz *et al.*, 1996).

Illumination in confocal microscopy is thus sequential rather than simultaneous (conventional light microscopy). This sequential illumination is done in such a way that only the light from the defined focal plane is able to pass back through a confocal aperture, reach the detector (photomultiplier) and produce an image (Blonk & van Aalst, 1993; Brooker, 1993; Aguilera & Stanley, 1999).

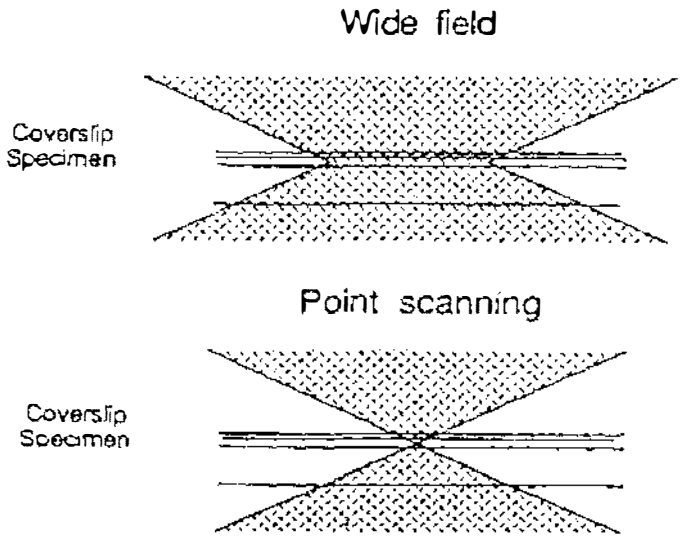
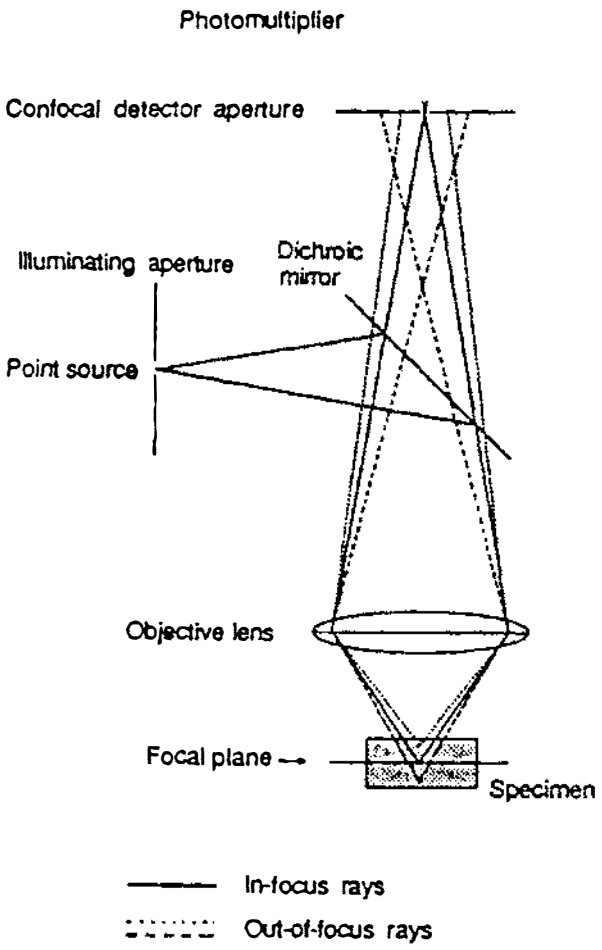


Figure 3. Comparative diagram of illumination in conventional light microscopy (wide field) and confocal laser microscopy (point scanning) (Wright *et al.*, 1993)

As a result of the placement of the confocal pinhole in front of the detector, light reflected from other levels in the specimen, both above and below the focal plane, is screened out. This, in turn, results in an optical slice through the specimen without out-of-focus blur, since the out-of-focus planes appear as black background (Brooker, 1995). Contrast, clarity and detection sensitivity are consequently increased. A diagrammatic representation of the light path from the laser source to the detector can be seen in Figure 4.

The lens in a confocal microscope acts as a condenser as well as a collector. Illumination, specimen and detector all have the same focus, i.e., they are confocal. According to Wright *et al.* (1993), the illuminated spot size may be as small as 0.25 μm in diameter and 0.5 μm deep, depending upon the specific microscope design, the wavelength of light, objective lens and microscope settings.



The dichroic mirror is actually a beam splitter, used to separate the illuminating and imaging rays. It reflects the excitation light on to the objective lens and also directs the fluorescent emission through to the imaging aperture and detector.

Figure 4. Schematic diagram demonstrating the confocal principle (elimination of out-of-focus rays) (Wright *et al.*, 1993)

The laser used is able to penetrate beneath the specimen surface to a depth that depends not only on the optical density of the material being investigated but also on the power output of the laser. A power balance must be sought to guarantee good penetration without local heating of the specimen during examination (Brooker, 1993). Such lasers will display greater versatility for multicomponent analysis with increasing number of lines or wavelengths produced and with larger differences between these wavelengths (Vodovotz *et al.*, 1996). Commonly used laser sources are the argon ion laser,

which produces two lines (488 and 514 nm), and the argon/krypton laser, more versatile, which produces three lines (488, 568 and 647 nm).

2.2.5.2. Confocal fluorescent microscopy

Confocal microscopy can be operated with reflected light (useful for the study of surfaces), transmitted light and, more commonly used in biomedical and food microscopy, with fluorescent incident/emitted light (Blonk & van Aalst, 1993; Brooker, 1995). This is due to the fact that several structural elements in biological and food materials do not differ largely in reflective power.

In order to produce contrast and to image specific structures, chemically specific fluorescent dyes (fluorophores) are often introduced or incorporated and allowed to diffuse into the specimen (Blonk & van Aalst, 1993; Vodovotz *et al.*, 1996). This is done by direct application of the powdered dye to the surface of the specimen or by molecular contact of the specimen with a solution of the dye. Some time is required before examination to allow for the diffusion of the fluorophore, when working with solid materials. For liquid materials, however, the dye can be dissolved by stirring and examination can proceed without delay (Brooker, 1995).

Individual components such as proteins, carbohydrates, lipids and ions can be identified and localised after excitation of the dye with the laser light. Since most confocal microscopes, as pointed out by Blonk & van Aalst (1993) and Herbert *et al.* (1999), are equipped with more than one detector, simultaneous imaging of several components can be achieved, as long as the fluorescent dyes used excite and emit at different wavelengths. Multiple images can then be superimposed to show relative distributions. Another possibility for localisation of chemical components is the use of antibodies, enzymes and ligands labelled with appropriate fluorescent dyes (Brooker, 1995; Ramkumar, 1997).

2.2.5.2.1. Fluorescent probes

According to Vodovotz *et al.* (1996), selection of the appropriate fluorescent dye will depend on the level of specificity for the component of interest, the nature of the fluorescence that can be excited by the narrow band of laser light and the presence of background fluorescence. For unfamiliar materials, experimentation will help define the best dye to be used.

Information on a number of fluorophores normally used for biomedical and food applications can be found in Tsien & Waggoner (1990) and Haugland (1992). These probes are characterised by their absorption and fluorescence wavelengths. By selection of a dye that absorbs and emits at fairly different wavelengths, background signals are reduced and sensitivity improved. The sensitivity of the fluorescence detection, which is maximum for a full spectrum of wavelengths, needs to be balanced with the desired/required resolution, which will be maximum with a narrow detection bandwidth (Vodovotz *et al.*, 1996).

Blonk & van Aalst (1993), Brooker (1995) and Vodovotz *et al.* (1996) discussed some potential problems that arise from working in the fluorescence mode in confocal microscopy. These include possible damage to live specimens caused by the absorption of radiation to excite the fluorescent probe or possible damage to the integrity or structure of the specimen when labelled by soaking in a solution of the dye. In addition, long exposure to the laser light may result in photobleaching of the fluorescent dye, thus potentially affecting subsequent quantitative measurements (Aguilera & Stanley, 1999). It is important to note, however, that such problems are relatively easily controlled and do not compromise the advantages of the confocal microscopy technique. Moreover, photobleaching recovery as a result of diffusion or flow can be used advantageously in the study of physical processes such as the rates of diffusion of molecules (flavour compounds, colouring agents or functional ingredients) through food matrices in relation to food structure (Blonk *et al.*, 1993).

Environmental variables such as solvent polarity, proximity and concentration of quenching species and pH of the aqueous medium can affect

the fluorescence characteristics of a probe. Consequently, probes that work in one application may not necessarily work well in others.

The literature describes a number of commonly used fluorophores used in food systems to stain and label proteins, lipids and carbohydrates (Heertje *et al.*, 1987a; Blonk & van Aalst, 1993; Brooker, 1993; Vodovotz *et al.*, 1996; Herbert *et al.*, 1999). Carbohydrates, for example, are often localised with the aid of carbohydrate-binding proteins, lectins, conjugated with specific dyes (Fulcher & Wood, 1983; Liener *et al.*, 1986; Yiu, 1993).

Lipids are traditionally labelled with Nile Red, considered the best fluorophore for oils due to its resistance to photobleaching and the intense fluorescence in hydrophobic environments (Greenspan *et al.*, 1985; Blonk & van Aalst, 1993; Brooker, 1993). These authors draw attention to the fact that, when dealing with continuous water systems, the best way of applying Nile Red is in the form of Nile Blue, which contains trace amounts of the former. Nile Blue was used by Heertje *et al.* (1987a) in their experiments. While the dye suitably stained the lipid phase in fat spreads, the fluorescence of the hydrophilic components of Nile Blue dominated that of the lipophilic components in the examination of mayonnaise (oil-in-water emulsion). Thus, the continuous water phase became more fluorescent than the discontinuous, dispersed fat phase. In this case, the fat appeared as black holes, as if only the protein had been stained. Any air bubble present in the product would not be distinguished from the fat in such condition.

When working with materials in which the lipid fraction appears as fat crystals, staining is problematic; hence, crystals are usually seen by negative contrast, so long as there is a strongly fluorescing and dominant oil phase in the background (Brooker, 1995).

Several different dyes are used for protein labelling and protein distribution examination. Fluorescein isothiocyanate (FITC) and acridine orange are probes that excite at about 500 nm, while rhodamine, Texas Red and Fast Green FCF excite at about 560 nm (Blonk & van Aalst, 1993; Brooker, 1995; Faraay, 1995; Ramkumar, 1997).

2.2.5.2.2. *Multiple labelling*

Multiple labelling can be easily achieved for investigation of different chemical components in confocal microscopy (Brooker, 1995; Herbert *et al.*, 1999; Mossberg & Ericsson, 1990; Paddock, 1991; Wright *et al.*, 1993;). Even three or more different fluorescent probes can be imaged simultaneously so long as each dye presents strict specificity for one component. Paddock (1991) and Vodovotz *et al.* (1996), however, emphasise the fact that interference may result from multiple labelled specimens bleeding through from one fluorescence channel to another; hence, the importance of working with dyes that have narrow absorbance and of using more restrictive excitation wavelengths and filters. Likewise, it is important that dyes being used in multiple labelling present emission fluorescence spectra with a minimum of overlapping, so that emissions can be completely separated and mixed imaging avoided (Herbert *et al.*, 1999).

Double staining of protein and fat in the study of emulsions and other food materials has been described (Blonk & van Aalst, 1993; Everett *et al.*, 1995; Faraay, 1995; Ramkumar, 1997).

Blonk & van Aalst (1993) discuss the use of FITC and Nile Red for the double staining of protein and fat, respectively, in food systems. As pointed out by Herbert *et al.* (1999), this combination is not an ideal one for clear imaging of these two components as the fluorescent emissions of FITC (about 500 nm) and Nile Red (about 488 nm) can not be completely separated. Some fluorescence of the protein is then visible in the lipid image. In view of that, Herbert *et al.* (1999) tried several different dyes to simultaneously label proteins, lipids and whey in a model dairy gel.

Another good example of double labelling is reported by Brooker (1991), concerning the study of competition between molecules (mixture of proteins) at the air/water interface of foams. An aqueous system containing two egg proteins, fluorescein-labelled ovalbumin and rhodamine-labelled avidin, is described.

The application of multiple labelling techniques in food systems is still quite incipient, despite the enormous potential they bring to the investigation of distributions of specific chemical components. Developments in laser technology as well as in computer hardware/software and new fluorescent materials that can be used to avoid overlapping of images (proper and efficient separation of emissions) will greatly extend multiple labelling applications.

2.2.5.3. Imaging and quantitative analysis

Confocal laser scanning microscopy (CLSM), in its current technological status, is a powerful tool for the generation of high resolution images of biological specimens and food systems from which qualitative information can be derived (Brooker, 1993). In addition to that, computer control and advanced data acquisition features create excellent possibilities for two and three-dimensional image analysis and derivation of quantitative information (Heertje *et al.*, 1987b; Brooker, 1995; Aguilera & Stanley, 1999).

Most commercial instruments to date display some sort of image analysis device interfacing with them. Because the technique generates signals from the detector that are already digitised, transference of images from the microscope to the image analysis system is highly facilitated (Carlsson & Aschund, 1987; Robert-Nicoud, 1989; Brooker, 1995).

Computer storage of signals (images) allows contrast and brightness improving enhancements, such as the electronic reduction of the range of grey levels in the digital images which, in turn, reduce the amount of processing normally necessary before quantitative analysis can begin (Brooker, 1993). Computer mediated image analysis can help quantify features such as sizes and shapes of cellular components and oil droplets (Jokela *et al.*, 1990). It also enables analysis of the thickness of cell walls or particle networks in gels, pore sizes and size distributions in gels, as well as the relative proportions of various phases (Aguilera & Stanley, 1999). Other geometrical features such as length, area, diameter and shape of biological and food structures can be measured (van der Voort & Smeudlers, 1993).

Additional advantages of CLSM imaging include the filtering out of unwanted noise, the possibility of reconstruction of 3-dimensional views obtained in deep scanning and the compilation of digital movies to show time sequences (Aguilera & Stanley, 1999).

Theoretical description and discussion of confocal imaging and different image processing techniques can be found in Boyde (1990), Majlof & Forsgren (1993), van der Voort & Smeudlers (1993), Wilson (1993), Wright *et al.* (1993), Corle & Kino (1996) and Aguilera & Stanley (1999).

2.2.6. Microstructural studies of milk and dairy products

Confocal laser scanning microscopy is still a relatively new technique among food scientists and technologists. Its potential in the study of food microstructure has been demonstrated in a number of publications and reviews (Blonk & van Aalst, 1993; Brooker, 1995; Vodovotz *et al.*, 1996; Ramkumar, 1997; Guinee *et al.*, 1999; Gunasekaran & Ding, 1999; Herbert *et al.*, 1999). The range of applications in food research is, however, yet to be realised.

As pointed out by Smart *et al.* (1995), electron microscopy in both its transmission and scanning modes has been the technique of choice for the vast majority of studies, especially structural ones, in the published literature. According to these authors, confocal microscopy of foods is not as straightforward as that of biological specimens, mostly because of the higher limitation to the depth one can see into thick samples when food materials are concerned. Yet, confocal microscopy has important advantages over electron microscopy, as discussed in section 2.2.5, and is likely to expand as a routine method to probe the structure of foods (Brooker, 1995).

2.2.6.1. Scanning electron microscopy

Scanning electron microscopy has been successfully used by several researchers over the years to examine microstructures of a variety of milk products. These include milk gels (Kalab & Harwalkar, 1973, 1974; Harwalkar

& Kalab, 1981; van Vliet & Dentener-Kikkert, 1982; Langton *et al.*, 1996), soy protein curds (Lee & Rha, 1978; Lee & Marshall, 1981), yoghurt (Kalab & Emmons, 1975), ice cream/whipped cream (Schmidt & van Hooydonk, 1980; Brooker *et al.*, 1986; Stanley *et al.*, 1996), butter (Heertje *et al.*, 1987a; Shukla & Rizvi, 1996) and natural and processed cheeses (Kimber *et al.*, 1974; Kalab, 1977; Glaser *et al.*, 1980; Kalab *et al.*, 1987; Lee & Marshall, 1981; Yang & Taranto, 1982; Anderson & Mistry, 1994; Marchesseau *et al.*, 1997). Textural characteristics of these foods, evaluated instrumentally or through sensory techniques, correlated well, in general, with their microstructure.

Kalab *et al.* (1981) used SEM to comparatively study the microstructure and sensory properties of cream cheeses. Cheeses in which fat globules were small and their membrane intact rated high in firmness, while those in which manufacturing processes led to membrane rupture and coalescence of fat globules were superior in spreadability. Analogue Mozzarella cheeses were also comparatively studied (Taranto & Yang, 1981; Yang & Taranto, 1982). In this case, SEM micrographs showed that gums with lower viscosity used as ingredients formed a uniform and delicate gel network, while those with higher viscosity tended to form lumps in the gel network, adversely affecting the stretching properties of the analogue.

Schmidt (1982) reviewed the many problems and possibilities associated with the use of electron microscopy in studies with milk and milk products. Despite the higher resolution achieved, which provide good insight into the microstructure of foods, formation of artefacts was frequently observed and specimen preparation procedures considerably influenced the final result.

Cheddar cheese microstructure was observed by Anderson & Mistry (1994) to be smooth, reflecting a rubbery body to the product. Condensing of the cheese milk prior to cheese manufacture affected the microstructure of the final product, which became crumbly and mealy. This was due to an uneven protein matrix with poor curd particle fusion.

2.2.6.2. Combined electron microscopy techniques

Different microscopy techniques are often combined for the study of food materials, in an attempt to collect as much and as detailed information as possible on the micro and ultrastructure of the sample. Examples are the published researches of Taranto *et al.* (1979), Rayan *et al.* (1980), Heertje *et al.* (1981), Caric *et al.* (1985), Tamime *et al.* (1990), Patil *et al.* (1992), Langton *et al.* (1996) and Tamime *et al.* (1999). Most of these authors applied the microscopy techniques on natural and processed cheeses.

Taranto et al (1979) comparatively studied the microstructure of Cheddar and Mozzarella and tried to use these data to explain rheological differences between these cheeses. Artefacts could be detected for both cheeses when comparing SEM micrographs without fixation and fixed in glutaraldehyde. Chemical composition differences were reflected in the micrographs obtained from SEM and TEM. Mozzarella appeared to have a compact protein matrix with little fat globule aggregation. This reflected in higher cohesiveness, adhesiveness and springiness of this cheese over Cheddar. Lower firmness of Mozzarella was attributed to higher moisture content. Cheddar cheese, on the other hand, exhibited an open, fibrous protein matrix with tendency to fat globule aggregation.

Green *et al.* (1981), however, pointed out that Cheddar cheese microstructure is highly dependent on the concentration factor (CF) of the cheese milk. In their study, coarseness of the protein matrix and abnormality of the cheese texture increased as the CF of the milk increased, as observed by SEM, TEM and conventional light microscopy.

Processed cheese has been a product of interest for the study of the relationship between microstructure and texture. Rayan *et al.* (1980) used different emulsifying salts to produce 4 types of processed cheese from the same Cheddar cheese. It was observed that, regardless of the individual salt effect on the physical properties of the product, a general decrease in the dimension of the fat globules occurred during processing. Likewise, an increase in the degree of emulsification was seen, which translated texturally into firmer, more elastic and less meltable processed cheeses.

Changes over time (during processing) were also detected by Heertje *et al.* (1981). These authors found, using TEM, an increased incidence of string-like forming elements of the protein matrix, in addition to the expected granular structure of casein micelles. Taneya *et al.* (1980) had reported the occurrence of a network structure with long protein strands in hard processed cheeses, but failed to detect these strands in soft cheeses. The ability of hard processed cheeses to retain their shape upon heating was attributed to these protein strands.

The effects of emulsifying salts on the microstructure of processed cheeses were reviewed by Caric *et al.* (1985). They reported that, in addition to the dispersion of fat, electron microscopy is useful in documenting the presence of crystalline inclusions in processed cheese, the result of the poor solubilisation of the emulsifying salt or the formation of calcium phosphate/lactate/citrate. These crystalline inclusions had been reported by Rayan *et al.* (1980) in their work, mostly due to incomplete solubilisation of the salts used in processing. Caric *et al.* (1985) also pinpointed the need for detailed studies on the relationship between microstructure of processed cheeses and their composition and physical properties. SEM and fluorescence microscopy are promising techniques for that purpose mentioned in the review.

Tamime *et al.* (1990) worked with 10 different types of processed cheese, made from both Cheddar cheese and a cheese base from reconstituted skim milk powder. Their goal was to investigate the effect of the cheese bases, one without and another with added proteolytic enzyme to induce proteolysis, on the microstructure of block type processed cheese. Electron microscopy revealed that marked differences in microstructure existed for the raw materials, but not so much for the final products. The cheese base without added enzyme had a compact structure, while the one in which proteolysis was induced was porous and open. Both cheese bases, when compared to full fat Cheddar, consisted of a protein matrix in which no fat was noticeable, in agreement to their composition. Regardless of the differences between the raw materials, processing resulted in the development of structures that were, in general, similar. Slight differences observed through TEM in the shape of the

fat particles in processed cheese stored at 30°C were found to be statistically not significant. Occurrence of salt crystals in the protein matrix was reported.

More recently, Tamime *et al.* (1999) investigated the effects of fat substitutes in processed cheese analogues on the microstructure, rheology and sensory perception of texture of those products. SEM and TEM were used and revealed differences in microstructure. Products made with anhydrous milk fat contained a higher concentration of fat globules compared with analogues made with fat substitutes. In addition, analogues made with high protein skim milk powder displayed a much denser protein matrix. Micrographs showed electron dense particles (possibly undissolved protein aggregates or fat substitutes) in all products, but no correlation was found between such images and the rheological properties of the analogues.

2.2.6.3. Fluorescence and confocal laser scanning microscopy

Yiu (1985) used fluorescent microscopy and cryomicrotomy (cryosectioning) on different types of natural cheeses and commercial processed cheese. Emphasis was put into the fact that this technique employs rapid and simple procedures compared to electron microscopy and achieves considerably higher resolution than bright-field microscopy. Staining of the protein matrices with Acridine Orange illustrated the denseness of the matrix at the curd junction of cheeses like Cheddar. These fusion areas could not be detected in processed cheese, expectedly smoother and non-granular in texture. The technique also proved useful for monitoring of quality in cheeses like Brie and Camembert. The protein matrices in the ripe and less ripe zones of Camembert cheese were not structurally the same. Nile Blue, used to dye the fat, was used to show the absence of fat globules from the ripe zone immediately below the surface moulds in Camembert.

Fat distribution and globule size in cheeses had previously been investigated by Shimmin (1982), using fluorescent microscopy, and more recently by Everett *et al.* (1995), Sutheerawattananonda *et al.* (1997) and Gunasekaran & Ding (1999). It has been reported from these studies that the specific dye for fat, Nile Red, undergoes severe photobleaching, causing rapid fading (1 to 2 s) of images during observation. Alternatively, fat globule

investigation was carried out by staining the protein and analysing the black holes (fat) by contrast. One important aspect to be considered in data interpretation, however, is that any air pockets in the cheese structure also appears as black holes and can not be distinguished from fat particles (Everett *et al.*, 1995; Sutheerawattananonda *et al.*, 1997).

Everett *et al.* (1995) investigated the size and shape of fat globules in reduced fat Cheddar cheese. Native milk protein and lactalbumin were used as coating for the globules. It was found, through confocal microscopy and 3-dimensional image analysis, that lactalbumin whey proteins appear to cause destabilisation of the fat globules. Large pools of fat, 20 to 50 μm in diameter, can be identified within the casein network and are attributed to the poor emulsification ability of lactalbumin.

Poor emulsification of fat was also observed by Sutheerawattananonda *et al.* (1997) in processed cheese manufactured without an emulsifying salt. Microstructurally, this was shown by lower circularity of fat globules and unchanging fat globule size during cooking and mixing. Normal processed cheese manufacturing, in which emulsifying salts are used, leads to a decrease in fat globule size during cooking/mixing, as shown in previous studies (Rayan *et al.*, 1980).

Blonk & van Aalst (1993) reported on the micrographs obtained with full fat and low fat Gouda cheese and related microstructure to the perceived texture by consumers. Double staining of protein and fat (rhodamine 123 and Nile Red), used in combination with the Ar/Kr mixed gas laser, showed an open protein matrix with agglomerated fat droplets in the full fat Gouda. This structure is quite favoured by consumers, since it promotes fast disintegration of the cheese during chewing and rapid release of taste from lipid-soluble flavours. Unlike the full fat counterpart, low fat Gouda has a massive, compact protein matrix with small and dispersed fat droplets, responsible for a rubbery texture and mouthfeel.

The fluorescent dyes reported by Blonk & van Aalst (1993) were confirmed by Brooker (1995) as appropriate for the examination of protein and fat in dairy products. Nile Blue, for fat, and FITC, Acridine Orange and Fast

Green FCF, for protein, were mentioned as useful dyes. A recent investigation on multiple fluorescent labelling of proteins, lipids and whey in dairy products is presented by Herbert *et al.* (1999).

Faraay (1995) used confocal microscopy to study differences between two types of commercial processed cheese slices. It was shown that the fat globules were more uniformly distributed and smaller in individually wrapped slices (IWS) than in slice-on-slice (SOS) processed cheeses. Also reported was the occurrence of fat crystals in IWS cheeses, with fewer crystals being detected in SOS. The differences were attributed to processing conditions (temperature x time and cooling regime). An attempt was made to relate the microstructure of the cheeses to fundamental rheological properties of the slices, but no statistical processing of the data was used.

Ramkumar (1997) investigated the effect of pH shift on the microstructure of a cheese curd during maturation. Using confocal microscopy, the microstructure was found to be affected by the pH at setting, with the fat globules appearing globular in cheese curds of higher pH values. Lower pH values resulted in elongated and less evenly dispersed fat globules. Possible reasons for those findings were the differences observed in the quantities of mineral retained and differences in the distribution of casein resulting from differences in pH. A methodology was developed to allow investigation of the water phase distribution in cheese during maturation, usually impossible with electron microscopy due to the required dehydration steps. This involved binding of the water soluble β-lactoglobulin to the dye tetramethylrhodamine-5-(and-6)-maleimide and adding the fluorescent dye-protein conjugate to the cheese milk.

Three of the most commonly used cheeses in cooking applications were used by Guinee *et al.* (1999) in their research. The microstructures of Mozzarella, Cheddar and analogue pizza (processed) cheese were examined and a relation with heat induced changes in viscoelasticity sought. While the analogue cheese displayed a uniformly distributed protein matrix with discrete and round fat droplets of varying size, natural cheeses showed orientation in their casein matrix. The protein in Mozzarella cheese appears as longitudinally aligned fibres with entrapped fat columns (coalesced fat

globules) of similar orientation. Less orientation of the casein and fat was observed for Cheddar. The microstructural information appears to be in accordance with the rheological differences measured during heating of the cheeses (shear modulus, G', and phase angle, δ).

The problem of orientation of the structures, fat globules in particular, during microscopic examination was also discussed by Gunasekaran & Ding (1999). These authors performed both 2-dimensional and 3-dimensional image analysis of fat globules number, size and shape. Their findings show that 2-dimensional views are affected by the viewing direction and location of the sample. Three-dimensional analysis is, therefore, a more accurate approach to characterising fat globules properties in cheese. It is pointed out, however, that the limitation of observation depth can be a potential problem, especially in full fat cheeses, since many of the large fat globules are cropped at image boundaries.

Problems associated with image analysis and direct statistical comparisons between different cheese samples had been previously discussed (Sutheerawattananonda *et al.*, 1997). The difficulty lies on the fact that cheese microstructure is heterogeneous and, thus, data are not usually normally distributed or have homogeneous variances. Despite the possible difficulties, however, Gunasekaran & Ding (1999) reported that quantification of 3-dimensional image features could serve as an objective criterion for evaluating quality or effect of a number of variables of interest in cheese making.

2.2.7. Final remarks

Electron microscopy, briefly discussed in this review, offers superb resolution and ultrastructural detail, making it widely used in the study of structure and composition of food systems. However, the disadvantages and limitations of its application as well as the increasing interest in the use of microstructural data for prediction of functional properties of foodstuffs have forced the development of new and more user friendly imaging techniques. Among these, confocal laser scanning microscopy has been gaining popularity

among researchers in biological and non-biological areas for its versatility, ease of use and reduced generation of artefacts.

2.3. SENSORY EVALUATION

2.3.1. Introduction

Sensory analysis of food, as a science, has shown an impressive development during the second half of last century, with the expansion of the processed foods industry. Many researchers attribute its formal beginning to the development of the triangle test in Scandinavia in 1940 and also to wartime efforts to providing more acceptable food for the American military personnel (Cardello & Maller, 1987; Jellinek, 1985; Meilgaard *et al.*, 1991; Stone & Sidel, 1993). It is agreed, however, that acceptability and preference of food are just as old as humans themselves, and many of our present day food taboos and habits have developed throughout the evolution process.

In the past, the trade of goods for use or consumption required that the buyer evaluated the goodness or badness of the different products. The practice of grading developed, and in the particular case of food products, organoleptic testing materials were used to denote supposedly objective measurement of sensory attributes. Because those measurements were, in reality, often subjective, with few and biased tasters and poor control of variables, opening the interpretations to biases and prejudice, they were and are far apart from the more formalised, structured and codified methodology that characterise sensory science as seen at present (Amerine *et al.*, 1965; Jellinek, 1985; Meilgaard *et al.*, 1991).

As a result of continuous changes in the marketplace, more and more a consumer-oriented environment, where different brands, flavours, colours and prices compete intensely with each other and where new products are developed and released at high pace, opportunities for the ongoing development of sensory evaluation are not limited. Development of new methods and refinement of existing ones, as well as identification of new

applications for available methods, can be seen in the great number of books, journals and articles published on the field of sensory science (Sidel & Stone, 1993). Eating will always be, as pointed out by Pangborn (1984), a very emotional and personal experience and whether a product is recognised as food and consumed with satisfaction and pleasure depends extensively on its several sensory attributes.

With the successes, however, come also the pitfalls. It can be quite disappointing, in contrast to the advances already achieved, to occasionally observe the lack of proper definition of the test objectives, adherence to a test method by researchers regardless of application, misuse of results and improper panellists use and selection procedures. All of these, among other factors, serve to reinforce the need for continuous improvement of sensory evaluation as a scientific speciality (Pangborn, 1984).

2.3.2. Definition

One widely used definition for sensory evaluation states that it is a scientific discipline used to evoke, measure, analyse and interpret the responses/reactions to those characteristics of foods and materials as they are perceived by the senses of sight, smell, taste, touch and hearing. It has been used since the 1975 and is accepted by sensory evaluation committees within several professional organisations, such as the Institute of Food Technologists or the American Society for Testing and Materials (Lawless & Heymann, 1998; Stone & Sidel, 1993).

By using the terms "food and materials", the definition tries to be as inclusive as possible, extending its arms to food products (either processed or not) and ingredients, as well as non-food products (cosmetics, environmental odours, pure chemicals and pharmaceuticals, to name but a few). It also highlights the fact that all five senses are involved, instead of just taste, as it is incorrectly assumed most of the time. Some of the confusion regarding this "exclusion" of other senses seem to be aggravated by the fact that terms such as tasting sessions (as to refer to sensory evaluation sessions) and tasters (as to refer to panellists) are commonly used, also erroneously.

Meiselman (1993) points out that, being an analytical test procedure, sensory evaluation requires precision, accuracy and sensitivity. In sensory evaluation, human subjects are used as instruments and, as such, are variable over time, variable among themselves and very prone to bias. Therefore, reliable sensory results depend heavily on the precise definition of the problem to be investigated, a very efficient test design (taking into account sources of bias, allowing for no subjectivity and minimising the amount of testing), proper selection and training of the test subjects (refinement of sensory instrumentation, although variability of responses will never be totally eradicated) and proper interpretation of results by using the most adequate statistical procedure and drawing only conclusions which are warranted by the results (Lawless & Heymann, 1998; Meilgaard *et al.*, 1991).

Sensory evaluation should not be mistaken for market research. In the latter, labels, products image and nutritional claims, among other factors, are all used to try and make the product more appealing to its consumers. It is widely reported in the literature the complex ways in which product information interacts with consumer attitudes and expectations (Cardello & Sawyer, 1992; Deliza & MacFie, 1996; Gacula *et al.*, 1986).

Sensory evaluation, on the other hand, is performed on products whose identities are totally hidden from those evaluating them. Such procedure tends to isolate opinions based on sensory properties only, thus providing important and useful information to product developers, food scientists and managers about the sensory characteristics of their products (Lawless & Heymann, 1998; Moskowitz, 1985). The sample populations on which each of these tests is performed are also likely to be different, thus allowing for possible contradictory results obtained for the same product investigated under both tests. Likewise, quality grading, which is essentially a qualitative procedure usually relying on one single individual, is not to be confounded with sensory evaluation, the latter being both a qualitative and quantitative science (Bodyfelt, 1981; Lawless & Heymann, 1998; Sidel *et al.*, 1981; Stone & Sidel, 1993).

2.3.3. Importance of sensory evaluation of foods

Sensory evaluation is a powerful analytical technique, if properly applied. It provides unique and useful information about sensory attributes of different products which, ultimately, serve as a base for further research in quality control and assurance, as well as product development, improvement and acceptability. The current role of sensory evaluation in the food industry is thoroughly discussed by Eggert (1989), Lawless & Klein (1989) and Sidel & Stone (1993), while its applications for personal care products or within the home care business are covered by Aust & Oddo (1989) and Dethmers & Boomsma (1989).

At the (food) industry level, changes in ingredients, processing conditions and/or packaging materials and procedures are relatively common practice. Such changes provide means of improving product quality (sensory, nutritional, microbiological stability) and manufacturing productivity, reducing production costs or simply adjusting to changes in the suppliers of raw materials (Labuza & Schmidl, 1988; Lawless & Heymann, 1998; Nakayama & Wessman, 1979; Reece, 1979). Sensory data, in the context of a well-defined and efficient business strategy, provide management with product information not readily available from other sources. The ultimate goals are, most of the times, product stability on storage and consumer acceptance of and preference for the product, and only human sensory data provide the best model for how consumers are likely to perceive and react to food products in real life.

As pointed out in the literature (Amerine *et al.*, 1965; Lawless & Heymann, 1998; Meilgaard *et al.*, 1991; Stone & Sidel, 1993), human perceptions of foods and other consumer products are the results of complex sensory and interpretation processes. There is a chain of perception involved, rather than a one step process of stimulus and response. The human brain plays an important intermediate function of interpreting the sensory experience, giving it meaning within a frame of reference and evaluating it relative to expectations. In other words, the brain receives the stimulus (sensation) and organises and integrates it into perceptions, based on which a response is formulated (Amerine *et al.*, 1965; Meilgaard *et al.*, 1991). These

processes are impossible to mimic or predict from instrumental measures, since instrumental assessments give values that miss an important perceptual process, i.e., the interpretation of sensory experience by the human brain prior to responding (Jellinek, 1985; Lawless & Heymann, 1998).

In synthesis, sensory evaluation is primarily a source for product information. However, it also plays a role in research activity related to the development and refinement of methods and procedures (Lawless & Klein, 1989). Sidel & Stone (1993) called attention to the fact that, because each company has unique products and problems, standard practices quite often require modifications or adjustments, sometimes even completely new approaches. Qualified staff, available time and funding will provide the minimum conditions for sensory evaluation to demonstrate its value.

2.3.4. Factors influencing sensory response

Due to the nature of the instrumentation used in sensory evaluation (human subjects), it is important to recognise and understand the basic physiological and psychological factors that may influence sensory perception and cause biases in sensory judgement. These contextual factors are discussed by Amerine *et al.* (1965), Frank *et al.* (1993), Larmond (1977), Lawless & Heymann (1998), Meilgaard *et al.* (1991), Mellers & Birnbaum (1982); Rankin & Marks (1991) and Stone & Sidel (1993), among others. Since they are likely to be present in every situation a product or its characteristics are to be assessed, they need to be minimised to an extent that guarantees the validity of results (scores) and the reliability of the conclusions drawn regarding differences among products (true sensory-based differences). By proper test design and, most important of all, careful and efficient selection and training of panel members, the panellists are put in a frame of mind, i.e., build a desirable frame of reference, that enables them to understand the exact characteristics or attributes that are to be measured.

The physical conditions of the panellists should be regularly monitored and panellists excused from sessions if, by any means, their judgements in a session can be influenced by external factors such as a cold, emotional upset,

heavy pressure at work, to name but a few. Smoking or coffee prior to a session can alter the senses accuracy and should be avoided during the hours that precede an evaluation session. Some aspects related to health and nutrition influencing sensory perception are described by Mattes (1986).

2.3.4.1. Physiological factors

Adaptation can be defined as a decrease or change in responsiveness to a stimulus under conditions of continuous exposure (constant stimulation). It is a common occurrence that can be particularly problematic in the variability of responses in threshold or intensity ratings. This aspect is discussed by O'Mahony (1986), who stresses out that not always adaptation is a disadvantage, but, on the contrary, can also be used to advantage in sensory testing procedures.

Likewise, enhancement and suppression are quite common effects observed during sensory sessions (Lawless, 1986). The former is defined by an increase in the perceived intensity of a substance after exposition to a previous one, whereas the latter represents a decrease in the perception of the second substance or mixture of substances. Synergy represents a special form of enhancement, in which the perceived intensity of a mixture of substances is greater than the sum of intensities of the individual components (Meilgaard *et al.*, 1991). In cases like these, the best approach to tackle the problem is an appropriate experimental design.

2.3.4.2. Psychological factors

Unlike the previous group of factors, psychological errors are not so much related to the specific product being tested, but with panellists being naïve or unfamiliar with the test method and/or product or product category (Stone & Sidel, 1993). Efficient panellists training is key in this particular case.

2.3.4.2.1. Expectation error

This kind of error appears when information about the product or product differences are provided to panellists, who then evaluate the samples with preconceived ideas (Cardello & Sawyer, 1992). Even well-trained panellists, aware of the need to put any previous knowledge aside, might have difficulty adjusting his/her verdict on the product due to subconscious autosuggestion. Because errors such as this are likely to destroy the validity of a test, secrecy about the sample source and characteristics, as well as a test design that accounts for proper sample coding and randomised order of presentation are strongly recommended (Amerine *et al.*, 1965; Meilgaard *et al.*, 1991).

2.3.4.2.2. Habituation error

Because human beings are creatures of habit, it is not uncommon to observe panellists incurring the error of repeating scores when a series of systematically changing stimuli are presented. If not accounted for and minimised, it can lead to erroneous conclusions about the products under evaluation (Larmond, 1977; Meilgaard *et al.*, 1991; Stone and Sidel, 1993).

2.3.4.2.3. Logical error

When samples are not uniformly presented or their differences properly masked, logical errors can occur. These refer to a condition in which two or more characteristics of the samples are associated in the minds of the assessors, who would not hesitate to modify their verdict and disregard their own perception in favour of what seems logical to them. The self-determination of panellists need to be controlled by means of proper training in the specific task they are expected to perform (Amerine *et al.*, 1965; Meilgaard *et al.*, 1991).

2.3.4.2.4. Stimulus error

Another classical problem in sensory evaluation, the stimulus error occurs when the assessors know or presume to know the identity of the

stimulus and draw conclusions thereupon. One example of stimulus error involves the assessment of wines from screw-capped and cork-closure bottles. Aware that the former tends to be cheaper, panellists would rate those wines lower than the more expensive cork-closure bottled wines, even when the better quality of the latter does not exist. The sensory analyst must, in order to avoid problems such as this, avoid the access of cues or any irrelevant information to panellists (blind testing) and randomise order and manner of presentation of samples (Amerine *et al.*, 1965; Lawless & Heymann, 1998; Stone & Sidel, 1993).

2.3.4.2.5. Halo effect

This is an important effect long noted by experimental psychologists, such as Thorndike (1920), in which a very positive attribute influences judgement on others, no matter how unrelated the attributes might be. Amerine *et al.* (1965) and Clark & Lawless (1994) also described it as a carryover from one positive product to another or positive correlation of unrelated attributes, but negative effects can occur as well (Lawless & Heymann, 1998; Meilgaard *et al.*, 1991). Like all other types of error, minimising the halo effect is important for the validity and reliability of the results. Therefore, when more than one attribute is of interest, presentation of separate sets of samples for evaluation of each characteristic is recommended.

2.3.4.2.6. Time-order error

The order in which samples are presented to panellists can result in different types of bias if not carefully controlled, and can create difficulties in the interpretation of the results (MacFie *et al.*, 1989). Presentation of a sample of good quality after one of poor quality, for example, tends to result in the second being rated higher than if it had been evaluated as a single sample. The same applies if the order is inverted, except that, in this latter situation, the rating for the second sample tends to be lower. This type of effect is known as contrast effect. If, however, a good quality sample is presented with a whole group of poor quality samples, the ratings for that good one are likely to be lower than they would be, had the sample been evaluated unimodally (Lawless & Heymann, 1998; Stone & Sidel, 1993). Any pattern in the order of

presentation of samples can be detected by panellists as a cue and used to their advantage, as getting the answers right has a strong and positive psychological effect. This serves to reinforce the need for proper experimental design, with balanced and randomised order of presentation of samples, if results of a sensory session are to be relied upon. MacFie *et al.* (1989) present a discussion on possible designs to balance the effect of order of presentation.

With regards to time in sensory evaluation, it was observed that anticipation and hunger can produce bias for the first sample, while fatigue or loss of interest can do so for the last one. Short term tests and long term tests will tend to produce similar sort of biases, respectively, for the first and last samples (Meilgaard *et al.*, 1991).

2.3.4.2.7. Central tendency error

As discussed in most sensory books (Amerine *et al.*, 1965; Larmond, 1977; Lawless & Heymann, 1998; Meilgaard *et al.*, 1991; Stone & Sidel, 1993), the error of central tendency is a common occurrence, in two different ways. Firstly, it is noted that samples placed in the centre of a set tend to be preferred over those placed at the ends. Balance and randomisation are procedures recommended to minimise the problem. Secondly, it was also noted that panellists, when using a scale for their ratings, tend to use the central part and not use the ends of the scale, in the expectation that an extreme sample, either side, is yet to come among the set. This can be a problem because it tends to bring the differences between samples to a minimum, even when they are not so subtle. For this particular type of problem, training is the key. The panellists should be provided with an adequate and comprehensive frame of reference to use during evaluation, with the ends of scales clearly anchored and understood for each particular attribute panellists are expected to evaluate.

2.3.4.2.8. Suggestion and lack of motivation

The effect of suggestion on sensory results appears when booths are not used in the sensory laboratory and panellists let themselves be influenced by other panellists reactions, either verbal or facial. As far as motivation of

panellists is concerned, it seems unnecessary to point out that interested panellists are always the most efficient ones. The degree of effort made to detect a subtle difference, to search for a proper term for a given impression or to be consistent in assigning scores is of decisive importance for the sensory results. It is the job of the sensory analyst to create a good atmosphere for it to happen. Feedback is vital, just like praising the panellists whenever suitable. The positive psychological effect of knowing how well the panellists are performing should not be underestimated. Panellists should not be seen as subordinates, but rather as co-workers.

As pointed out by Lawless & Heymann (1998), unless the sensory professional is aware of these sources of biases and guards against them, time, effort and money put into a sensory evaluation process can be wasted in conclusions that are inappropriate and unreliable.

2.3.5. Types of sensory tests

The different tests used in sensory evaluation of food and consumer products can be classified into 3 categories, namely affective testing, discrimination testing and descriptive analysis.

2.3.5.1. Affective testing

Affective testing, also referred to in the literature as hedonic testing, has the primary purpose of assessing the preference and/or acceptance of a product or specific product characteristics by actual and potential customers. It is a valuable and necessary component of every sensory evaluation program, if management is to have a clear picture of how a product will be accepted and preferred to others when released to the market (Meilgaard *et al.*, 1991; Stone & Sidel, 1993).

Untrained, consumer panels comprising a minimum of 50 assessors are normally used to represent different groups of the consuming population, which, as noted by Meilgaard *et al.* (1991), would exclude the use of employees or residents local to the company offices, technical centre and

plant. This is due to the high risk of biased results taken with such a test group. Individuals qualified for the other types of tests (discrimination and descriptive) should not be used in affective tests, and vice-versa, because the approach for assessors in discrimination and descriptive testing tends to be more analytical, instead of the more integrative fashion that marks the action of consumer panellists.

In a context of multiple objectives of sensory evaluation, regarding a food or consumer product, a sequence of different tests is required, in which case affective testing will come last, after the analytical approach has been pursued. Further details on affective testing can be found in Amerine *et al.* (1965), Lawless & Heymann (1998), Meilgaard *et al.* (1991), Meiselman (1988), Shepherd *et al.* (1988) and Stone & Sidel (1993).

2.3.5.2. Discrimination testing

Amerine *et al.* (1965) and Peryam (1958) noted that discrimination testing, or difference testing, as it can also be referred to, is the most fundamental approach to sensory analysis of foods. It is also emphasised that it is one of the most useful tool available to the sensory professional, since it is on the basis of a perceived difference between products that a justification is found to proceeding to descriptive analysis (Pangborn, 1984; Meilgaard *et al.*, 1991; Stone & Sidel, 1993). The former is not actually concerned with the identification of the basis for a detected difference, but with whether a sensory difference exists between samples (overall difference tests) or, with a certain extent of training, how a specific attribute differ between samples (attribute difference tests).

When the differences between samples are large, the application of a discrimination test is wasted, and should therefore be avoided. These tests only make sense when the differences are subtle and not always easily picked up by those testing the samples.

Regardless of the purpose underlying the application of discrimination testing, it is fundamental to understand the behavioural aspects of the discriminative process and combine that information with knowledge about

the various test methods, so that objective, unambiguous, reliable outcomes are guaranteed for the decision-making process (Stone & Sidel, 1993). Some theoretical aspects of sensory discrimination are discussed by Ennis (1993) and Ennis & Mullen (1986).

Frijters (1988) attributed the popularity of these methods to the simplicity of the experimental procedure involved. According to this author, the tests can be carried out quickly and be easily performed by panellists. Such opinion is not shared by Stone & Sidel (1993), who observed a tendency toward oversimplification in a complex field, a lack of appreciation of the true difficulties involved. Facing the apparent simplicity, it was noted that many test developers devise a multitude of variations that, for the most part, are intended to increase the amount of information output. In doing so, the sensory professional incurs in ill-conceived practices and misinterpretation of results that seriously put at risk the validity and reliability of the data, and the credibility of the sensory evaluation program.

Several text books and journal papers discuss in detail the advantages and disadvantages of each method in discrimination testing, as well as guidelines on test selection and delivery and panellists screening and selection (Amerine *et al.*, 1965; Berglund *et al.*, 1993; Ennis, 1993; Francois & Sauvageot, 1988; Frijters, 1988; Lau *et al.*, 1995; Lawless & Heymann, 1998; Meilgaard *et al.*, 1991; Pangborn, 1984; Stillman & Irwin, 1995a,b; Stone & Sidel, 1993). They are, therefore, not covered in this review.

2.3.5.3. Descriptive analysis

Descriptive sensory analyses are the most sophisticated tools in the arsenal of the sensory scientist (Lawless & Heymann, 1998). There are different methods and techniques to choose from, all of which involve the detection (discrimination) and detailed description of both the qualitative and quantitative sensory aspects of a product or a group of products (Gillette, 1984; Meilgaard *et al.*, 1991). Descriptive analyses take into account all sensations that are perceived as the product is evaluated, be it appearance, aroma, flavour, texture or sound properties. These characteristics are to be detected, described and their intensity quantified or rated, i.e., panellists

must define to what degree each characteristic or qualitative note is present in a sample.

The results of a descriptive analysis provide a basis for determining which sensory characteristics are important to the acceptance of a product, as well as a means of identification of underlying ingredients or processing variables. In other words, they provide a means of assessing the effect of specific changes in formulation or processing conditions on the sensory characteristics (Stone & Sidel, 1993).

The techniques can be applied to shelf-life studies, product improvement or quality control and assurance, as described by Gillette (1984), Lawless & Heymann (1998) and Meilgaard *et al.* (1991). In time-intensity studies, descriptive analysis is used to measure short-term changes in the intensity of specific attributes over time. Because of its highly analytical approach, descriptive analysis can also be used to pursue or define sensory-chemical-instrumental relationships (Azanza *et al.*, 1994; Edmister & Vickers, 1985; Noble & Shannon, 1987; Szczesniak, 1987; Vickers, 1987). It is, however, a group of techniques quite expensive for day-to-day quality control situations.

Lawless & Heymann (1998) note that any competent sensory professional should be able to perform a descriptive analysis study by following three basic steps, namely training of assessors, determination of assessors reproducibility/consistency and final evaluation of samples. This apparent simplicity, however, can easily be overrated. Training of panellists, for example, is a very time consuming and expensive process and, unless efficiently done and panellists assessed for their detection ability and scoring reproducibility, can put at risk the validity and reliability of the whole sensory program. Performance of a descriptive panel, however, appears not to be significantly affected by the extent of testing experience (Chambers IV & Smith, 1993) or training procedure (Wolters & Allchurch, 1994).

Meilgaard *et al.* (1991) called attention to four components of descriptive analysis that apply not only to the training of panellists but also to the actual evaluation session and establishment of reliable conclusions. These authors refer to the components as the qualitative aspect (characteristics), the

quantitative aspect (intensity), the time aspect (order of appearance of characteristics) and the integrated aspect (overall impression).

At first, the characteristics or descriptors that are going to be used for the perceived sensory parameters must be identified and defined, and this relates to teaching the panel or having the panel create their own scientific language (jargon) for the product or product category of interest. Scientific language differs from colloquial and lexical in the sense that the terms are precisely defined and specifically created for scientific purposes (Lawless & Heymann, 1998; Stone & Sidel, 1993). It is a goal that all panellists use the same concepts and be able to communicate precisely with one another. The formation of the concepts does not represent much, in itself, if they are not labelled in order to facilitate communication. Civille & Lawless (1986) discussed the several desirable characteristics of descriptors that must be kept in mind when establishing descriptive jargon.

The second aspect, or the intensity in descriptive analysis, is concerned with expressing the degree to which each of the descriptors is present in the sample and is usually done by assignment of a value along a measurement scale (category, line or magnitude estimation scales). Training panellists on doing so depends on providing them with a frame of reference for each particular descriptor or, alternatively, on asking them to generate reference standards needed to describe differences among the products. This is usually done by coming to some consensus among the panellists themselves (Lawless & Heymann, 1998; Meilgaard *et al.*, 1991; Stone & Sidel, 1993).

Having evaluated a broad array of products that define the product category, panellists are able to generate a list of best descriptors, which is comprehensive yet not overlapping. Most important is that the terminology be developed and derived by a panel that has been exposed to the underlying technical principles of each modality (flavour, aroma, texture, sound) to be described. Panellists must undergo thorough training on the use of scales in a similar way across samples and time, as well as on the use of the frame of reference for intensity of different descriptors to ensure consistent use of scales across panellists and repeated evaluations (replicates).

The validity and reliability of the quantitative assessment is also dependent upon the selection of a scaling technique that is broad enough to encompass the full range of descriptor intensities. In addition, the scale must have enough discrete points to pick up all the small differences in intensities between samples (Meilgaard *et al.*, 1991).

Occasionally, differences among products can be detected in the order some parameters manifest themselves. This constitutes what Meilgaard *et al.* (1991) called the time aspect of descriptive analysis. By controlling the manipulation of the product (sample) during testing, the panellists induce the manifestation of only a limited number of attributes at a time. Aftertaste and afterfeel are important characteristics that are taken into consideration during evaluation of the order of appearance of attributes. Like all other characteristics studied for their order of appearance during sensory assessment, these can be as indicative of the product profile as the individual aroma and flavour notes or textural characteristics and their respective intensities.

Last but not least, management quite often is also interested in the overall impression of the sensory attributes of the product. This integrated aspect of descriptive analysis can be achieved by measurement of the overall impact of all aroma or flavour components of the product, by assessment of the degree to which various characteristics fit together in the product (balance/blend) or by adding important information about the size and source of attribute differences in an overall difference testing (Meilgaard *et al.*, 1991).

The use of a descriptive panel for hedonic ratings, once description has been completed, is something to be avoided at all times. This is because descriptive panels must undergo a very thorough training process to be able to perform such tests and, through the very same training process, have been removed from the world of consumers. These people that constitute a descriptive panel are not representative of any section of the general public; instead, they are more aware of the various attributes of a product and tend to weigh attributes differently from the way a regular consumer would. Accepting hedonic ratings from trained panels for the decision-making

process is, therefore, quite risky. Conversely, using consumers for a descriptive analysis would be just as inappropriate.

The language used by consumers to describe sensory characteristics is almost too imprecise and non-specific to allow the sensory professional to measure and understand the underlying concepts in a way that will provide meaningful data (Lawless & Heymann, 1998; Meilgaard *et al.*, 1991; Shepherd *et al.*, 1988). Some controversial aspects concerning this topic can also be found in Dungle (1997), Moskowitz (1996) and Moskowitz (1997).

Details of each of the methods in descriptive analysis, except for those usually applied on textural assessment, are not presented here. The most commonly used techniques are the flavour profile analysis (FPA), the texture profile analysis (TPA), the quantitative descriptive analysis (QDA), the time-intensity methods and the free choice profiling (FCP). Reviews on these several different methods/techniques can be found in Amerine *et al.* (1965), Einstein (1991), Lawless & Heymann (1998), Meilgaard *et al.* (1991), Neilson *et al.* (1988), Pangborn (1984), Powers (1988), Skinner (1988), Stone & Sidel (1993) and Zook & Pearce (1988).

2.3.5.3.1. Texture profile analysis (TPA)

This method was developed and first reported in 1963 (Brandt *et al.*, 1963; Szczesniak *et al.*, 1963) with the goal of devising a sensory technique that would allow the assessment of all the texture characteristics of a product (mechanical, geometrical, fat and moisture), from first bite through to complete mastication, using engineering principles. This meant it would be built on a well-defined and rational foundation. The theoretical basis upon which the texture profile is grounded was reviewed by Moskowitz & Kapsalis (1975).

The method has been modified throughout the years (Civille & Dus, 1990; Civille & Liska, 1975; Civille & Szczesniak, 1973; Munoz, 1986; Schwartz, 1975; Szczesniak, 1963a; Szczesniak, 1966; Szczesniak, 1975a; Szczesniak *et al.*, 1963; Szczesniak *et al.*, 1975). Modifications included changing of some food products used as anchors for intensity scales (Munoz,

1986), adding the evaluation of the surface properties of the products to the initial phase of evaluation and adding standard scales to evaluate liquids and semisolids. Reviews of the terminology used (Civille & Szczesniak, 1973; Szczesniak, 1975a; Szczesniak & Bourne, 1969) were also required over the years.

By definition, the texture profile analysis is a time-dependent method, consisting of an initial phase (first bite), a masticatory phase (chewing) and a residual phase (post-swallowing)(Lawless & Heymann, 1998; Skinner, 1988). One very important aspect of this method is the selection and training of panellists. This is covered in detail by Bourne (1982), Civille & Szczesniak (1973) and Skinner (1988). During the training, panellists are exposed not only to the basic concepts associated to texture, but also to flavour. The reason for this, as explained by Stone & Sidel (1993), is the considerable integration of different stimuli in the brains of the assessors as they evaluate a sample. They point out the fact that requesting panellists to ignore flavour and evaluate exclusively the texture of a product may have disastrous effects, because the several sensory properties of a food product interrelate. If unaware of this fact, the panellists take the risk of overlooking subtle but important differences.

An example of the basic TPA score sheet is illustrated in Bourne (1982) showing that different scale sizes are sometimes used for different descriptors. Data from the texture profile are usually displayed in a tabular or graphical form, without much (or any) mathematical/statistical manipulation.

2.3.5.3.2. Free Choice Profiling (FCP)

This method appeared in 1984 and brings about two unique features, despite sharing many aspects with the other techniques in descriptive analysis. Firstly, the way the descriptive vocabulary is generated and the evaluation protocol is structured. Panellists are not expected to come to a consensus as to which descriptive terms and definitions should be used, but are free to rate their sensations on a scale using individually generated terms and to evaluate the product in different ways. The terms must, however, be

applied consistently by each panellist during evaluation (Lawless & Heymann, 1998; Marshall & Kirby, 1988).

The second feature is the statistical treatment of the scores from the panellists, which is done through a procedure known as Generalised Procrustes Analysis. It provides a consensus picture of the data from each individual panellist in a two or three-dimensional space (Arnold & Williams, 1986; Gower, 1975; Langron, 1983; Oreskovich *et al.*, 1991). In other words, it adjusts for the use of different parts of the scale by different panellists and then manipulates the data to combine terms that appear to measure the same characteristic. These combined terms provide then a single product profile (Meilgaard *et al.*, 1991).

Having pointed that out, a distinct advantage of the method comes to the foreground: the avoidance of panel training. This means that experiments may be completed faster and at less expense. Besides, panellists can still be regarded as representing naïve consumers (Meilgaard *et al.*, 1991).

Several studies using FCP are listed in the literature (Beal & Mottram, 1993; Beilken *et al.*, 1991; Guy *et al.*, 1989; Jack *et al.*, 1993a; Marshall & Kirby, 1988; McEwan *et al.*, 1989; Piggott & Watson, 1992; Rubico & McDaniel, 1992; Williams & Arnold, 1985; Williams & Langron, 1984). However, the method has been under scrutiny from sensory professionals who are somewhat sceptical that the results from this technique are not subject to the desired interpretation of the researcher (Lawless & Heymann, 1998; Meilgaard *et al.*, 1991).

2.3.5.3.3. Time-intensity methods

The existence of different phases of food breakdown had been recognised earlier, long before the time-intensity methodology became more widely used (Brandt *et al.*, 1963; Holway & Hurvich, 1937; Meiselman, 1968; McNulty & Moskowitz, 1974; Neilson, 1957). Brandt *et al.* (1963) separated textural characteristics of foods into first bite, mastication and residual phases over time. Practical situations, such as the lasting flavour of a chewing gum or the variation in flavour that a wine displays from the moment the bottle is open

and the wine starts to "breathe" in the glass, help illustrate the dynamic nature of the perception of aroma, taste, flavour and texture of foods, described by Dijksterhuis (1996), cited by Lawless & Heymann (1998). Processes such as chewing, breathing, salivation, tongue movement and swallowing all influence this dynamic phenomenon.

Time-intensity methodology is an attempt to escape the limitation of uni-point methods (time-averaged response) and provide the sensory professional and users of the data with important temporal information about perceived sensations. Not always are the single intensity or the peak intensity values sufficient to provide a flavour or texture profile able to show differences between two different products. For situations in which only the continuous perceptual changes in a specified attribute or multiple attributes over time allows for discrimination between products, time-intensity analysis is the appropriate evaluation technique (Lawless & Heymann, 1998; Lee & Pangborn, 1986; Lee III *et al.*, 1992; Lundahl, 1992; Stampanoni & Noble, 1991a).

Uni-point techniques provide a time-averaged response or the peak intensity value from the panellists. Time-intensity methods, unlike the former, provide information on the maximum intensity perceived, the time to maximum intensity, the rate and shape of the increase in intensity to a maximum point, the rate and shape of the decrease in intensity to half maximal intensity and to extinction points, and the total duration of the sensation (Lawless & Heymann, 1998; Lee, 1989). Not always, however, is all this information required for assessment of differences, in which case the single uni-point responses would suffice.

The best approach to obtain a detailed profile for a product over time is a continuous tracking of flavour or texture, since the use of discrete points in time may miss the quite rapid onset of tastes and odours, as well as textural changes. In terms of texture and phase changing, time-intensity methodology is a powerful tool for the study of products that undergo melting in the mouth, such as ice cream, puddings and chocolate (Lawless *et al.*, 1996; Moore & Shoemaker, 1981; Pangborn & Koyasako, 1981). Time-intensity has

also been used for taste and odour adaptation studies, flavour release and temporal aspects of hedonics, as reviewed by Lawless & Heymann (1998).

Despite the popularity of this technique over the last decade, some problems can not be overlooked. Cost efficiency (expensive and time consuming data collection versus usefulness of the information derived), redundancy of the information derived and high tendency to biases (factors affecting response behaviour almost unknown), to name but a few, are some of the aspects which should be given consideration when opting for time-intensity studies (Lawless & Clark, 1992).

2.3.5.3.4. Quantitative Descriptive Analysis (QDA)

Quantitative descriptive analysis was developed as an alternative to overcome the weaknesses or flaws identified in previously developed methods, mainly the flavour and texture profile techniques (Stone *et al.*, 1974). The latter, according to Stone & Sidel (1993), fail to take into account behavioural issues, use data that are generated by consensus discussion (allowing for the influence of panellists with stronger personality), use product characteristics sometimes established by the experimenter, and lack appropriate statistical treatment of the data. In contrast, QDA was created to have a primarily behavioural approach, with considerable emphasis on the use of replication and statistics as a basis for assessing the quality of the sensory results (Lawless & Heymann, 1998; Meilgaard *et al.*, 1991; Stone & Sidel, 1993).

Important features of the QDA, as it was conceived, involve responsiveness to all sensory characteristics, a multi-product test approach, usage of limited number of subjects that are all qualified (screened/selected) before participation, employment of a language development process free from leader influence, quantitative approach, and existence of a useful data analysis system and of data processing capability. Stone & Sidel (1993) discuss each of these topics in detail.

Similarly to the flavour and texture profile techniques, the training of panellists for QDA expose assessors (previously screened and selected based on their discrimination ability, reproducibility for scoring and commitment to

the sensory program) to many possible variations of the product or product category of interest. This is done in order to facilitate concept formation. Panellists generate terms to be used as descriptors, terms to describe the differences between products, reference standards and verbal definitions, as well as the sequence for evaluating each attribute. The panel leader or sensory professional is not an active participant on this process, but a facilitator to direct discussion and supply materials required by the panel (Lawless & Heymann, 1998; Powers, 1988; Stone & Sidel, 1993; Stone *et al.*, 1980).

Evaluations are performed in individual booths, not through consensus discussions, usually employing unstructured line scales anchored with words generated by the panel to describe the intensity of rated attributes. Products are sensory described from initial visual assessment to aftertaste/afterfeel, although sometimes restrictions are applied in order to focus in particular attributes of interest (Lawless & Heymann, 1998). The resulting data, including replications, can be analysed through analysis on variance (ANOVA) and multivariate statistical techniques (Piggott, 1986).

Graphical presentation of data is possible by use of a cobweb or radar plot (polar co-ordinated graphs). Meilgaard *et al.* (1991), however, call attention to the fact that such graphical representation can be misleading to some users, to whom the area under the curve usually have some meaning. The sensory dimensions shown in the web may be either unrelated to each other or related in ways that cannot be represented in this manner.

Stone & Sidel (1993) pointed to the important fact that panellists are likely to use different parts of the scale to make their determinations, despite the extensive training. The implication of this is that absolute scale values are not important, while the relative differences among products are. Using the scales as absolute measures of an attribute can bring about disastrous consequences in the decision-making process. This topic has been reviewed by Lawless & Heymann (1998).

Apart from the advantages of QDA, as described by the several authors who reviewed the technique (Lawless & Heymann, 1998; Meilgaard *et al.*, 1991; Powers, 1988; Stone & Sidel, 1993; Stone *et al.*, 1980), it seems to

suffer from the same disadvantage as the flavour and texture profiles, in which panels must be trained for the specific product category. This requires that companies sometimes maintain separate panels for each of their product categories, which is expensive and somewhat limiting in smaller firms. Because panellists depend on the sensory professional for feedback on performance, lack of immediate contact exchange between assessors and experimenter can lead to loss of interest and motivation, as well as to reduction in the opportunity to learn, expand terminology and improve performance (Meilgaard *et al.*, 1991).

2.3.6. Texture

Several researchers have tried, along the years, to create their own definitions for the term texture, at times applying it to specific food products, such as meat (Ball *et al.*, 1957) or cheese (Davis, 1937). More recently, the trend has been to reflect a broader coverage (Bourne, 1982; Corey, 1970; deMan, 1975; Jowitt, 1974; Matz, 1962; Potter, 1968, Szczesniak, 1963b).

The definition used by the International Organisation for Standardisation states that texture of a food product includes all the rheological and structural (geometric and surface) attributes of a product perceptible by means of mechanical, tactile and, where appropriate, visual and auditory receptors (ISO, 1981). Bourne (1982), however, suggested that the term texture, which infers a single parameter, should be replaced by the expression textural properties, more appropriate for the number of different sensations texture consists of.

Food texture can be extremely important to the consumer as an indicator of food quality. One example of its importance, reported by Bourne (1982) and Lawless & Heymann (1998), is a study by Schiffman, from 1973, in which 29 different foods were blended and pureed to eliminate textural clues and served to blindfolded panellists. In that particular case, flavour was to be used as the only basis for identification. It was shown that, in general, no more than 40% and 30% of the foods tested could be correctly identified by young and more aged panellists, respectively. The experimental data helped illustrate the fact

that, in some foods, texture plays a critical role in product quality, acceptance and preference (meat, potato chips), while in others it makes only negligible contribution to the overall quality (beverages, soups) (Bourne, 1982).

Despite the relative, subconscious awareness of food texture by the consumer, as shown by studies carried out by Szczesniak (1971), Szczesniak & Kahn (1971), Szczesniak & Kleyn (1963), Szczesniak & Skinner (1973) and Yoshikawa *et al.* (1970), texture used to be an overlooked food attribute by the food industry. It seems to have gained new importance in more recent years. Some of the reasons why texture had been overlooked as an attribute are summarised and described by Szczesniak (1990b). Among those, one important aspect to be noted is that texture is usually taken for granted and not commented upon. Szczesniak & Kahn (1971) stated that "if the texture of a food is the way people have learned to expect it to be, and if it is psychologically and physiologically acceptable, then it will scarcely be noticed. If, however, the texture is not as it is expected to be, if it is displeasing for psychological reasons, if it is associated with non-edible items, then it becomes a focal point for criticism and rejection of the food". In other words, all being as it should be, food texture often goes unnoticed.

Awareness of and attitudes towards the texture of food products are influenced by a number of factors, including psychological/physiological reasons, socially and culturally learned expectations, socio-economic class and time of day, to name but a few (Szczesniak & Kahn, 1971). Higher awareness of texture and, therefore, appreciation of texture can be observed among those of higher socio-economic classes, which are expected to have had greater exposure to different foods (textures) and willingness to experiment. Women have shown to be more aware of texture than men, in general, and consumers somehow closer to the food industry, regardless of gender, seem to place more emphasis on texture than the general population (Szczesniak & Kleyn, 1963).

Lawless & Heymann (1998) call attention to the importance of texture contrast across food products in a meal. Szczesniak and Kahn (1971), years earlier, had stated that textural contrast is good, pleasurable, necessary to appetite stimulation and is related to the excellence of food preparation. This

appears to be true even in the moment-to-moment change in sensory texture during mastication (Hyde & Witherly, 1993). In the case of children, usually less exposed to a wide variety of textures, or breakfast meals, which are expected to be smooth to eat, textural contrast appears to play a less crucial role.

Most of the sensing of texture occurs in the mouth, during the process of mastication. The oral perception of texture is thoroughly discussed by Bourne (1982), Christensen (1984), Cussler *et al.* (1979) and Heath & Lucas (1988). It is possible, however, by definition, to sense texture nonorally. Some aspects concerning auditory, visual and nonoral tactile texture were reviewed and discussed by Lawless & Heymann (1998).

Although several texture review articles and textbooks have been published (Bourne, 1982; Brennan, 1988; Christensen, 1984; Moskowitz, 1987), the study of food texture is no less difficult than defining it. The identification and differentiation of most textural characteristics of foods occur only after the food is subjected to deformation or manipulation. However, the rate and degree of manipulation or deformation exerted by individuals vary considerably. Variation can even be expected within a single individual in the course of mastication (Christensen, 1984). Szczesniak (1990b) pointed out that two other complications arise in the study of food texture, these being that changes in texture alter flavour perception and appearance, and that changes in intensity of one textural parameter are often followed by changes in other textural parameters.

Early work on texture can be traced back to the second half of the 19th century and many texture-measuring devices/instruments have been developed since. Many of those instruments are described by Bourne (1982) and Szczesniak (1973). It is important to note, however, that instruments generate readings that mean little unless correlated with sensory judgements of quality.

Instrumental evaluation of texture, despite being an objective approach, can never mimic completely the complex sequence of events taking place during mastication. Bourne (1982) stated, in his book, that "no instrument

available has the sophistication, elegance, sensitivity and range of mechanical motions as the mouth or can promptly change the speed and mode of mastication in response to sensations received during the previous chew". His statement holds up to present days. Therefore, any objective instrumental measurement requires calibration against the human senses, which in turn will always dictate the acceptability and preference of a food product.

In view of the above, extensive work has been done in the attempt of correlating data from sensory and instrumental evaluation of food texture (Bourne, 1982; Bourne, 1983; Dijksterhuis, 1994; Friedman *et al.*, 1963; Green *et al.*, 1985; Hough *et al.*, 1996; Jack *et al.*, 1993a; Jack *et al.*, 1993b; Kapsalis *et al.*, 1973; Meullenet *et al.*, 1997; Noble, 1975; Patil *et al.*, 1990; Powers & Moskowitz, 1976; Szczesniak *et al.*, 1963; Szczesniak, 1987; Wium *et al.*, 1997; to name but a few). Positive results were, not surprisingly, only occasionally found. The topic of correlation is reviewed further in section 2.5 of the literature review.

2.3.7. Final remarks

The textural properties of food products have been increasingly recognised as important characteristics that can influence their quality, acceptability and consumption. Due to the dynamic nature of texture perception, sensory evaluation appears to be, at present, the best technique for texture assessment. However, such a process of evaluation tends to be time consuming and expensive, qualities that do not meet the need for a quick, affordable and easy to run methodology for food quality control in the industry.

Lawless & Heymann (1998), quoting Skinner (1989), wrote that the future development in sensory evaluation will depend on several factors, one of the most important being the people and their preparation and training. Sidel & Stone (1993) also emphasized this point. The role of sensory evaluation in the food industry, at the management level, should be a very important one, yet the complete acceptance of sensory evaluation by the business environment has not happened. Great progress has been achieved,

as many food industries around the world already recognise the importance of establishing a sensory laboratory, if they are to succeed in a very competitive market. It is hoped that, more and more, the food, beverages and ingredients industries realise that the complexities of today's technology and the subtleties of the marketplace require that all available resources, including sensory evaluation, be used to their best advantage.

Attempts have been made along the years to find ways to correlate sensory data to instrumental evaluation of texture (rheology and microstructure), but results have proved inconsistent and, at times, coincidental. Major improvement in this field of sensory-instrumental correlation is required, as the gaps in the consumer/texture interface persist.

2.4. RHEOLOGICAL EVALUATION

2.4.1. Introduction

Rheology, as the branch of physics that studies the flow and deformation of materials under well defined conditions of stress and/or strain, is an important and powerful tool for quality assessment and control in the food industry (Matuszek, 1997). It is used in process design and engineering calculations and for investigating how industrial processes influence food structure as well as its sensory and functional properties. Of most importance is its use in providing a better understanding of the structure of the food products themselves, since the rheological behaviour of any material is largely dependent upon its internal architecture (Prentice *et al.*, 1993; Shoemaker *et al.*, 1992; Steffe, 1996; Windhab, 1995).

2.4.2. Stresses and strains

It is the magnitude of the force acting on or within a body per unit of area (stress), rather than the magnitude of the force itself, that determines the deformation produced on that material. Stress, usually measured in pascals

(Pa), can be tensile, compressive or shear (Steffe, 1996; Whorlow, 1992). Shear stresses are parallel to the surface they act on. Normal stresses (compressive and tensile) can, in turn, be presented as engineering stress, when the initial area of the test sample (A_0) is used in the calculation, or as true stress, when the actual cross sectional area of the deformed sample (A_t) is used. The latter is usually preferred due to the little information conveyed regarding the rheological character of the material under investigation when the change in cross sectional area is ignored (Peleg, 1987).

$$\sigma_E = F / A_0 \quad \text{(engineering stress)}$$

$$\sigma_T = F / A_t \quad \text{(true stress)}$$

In rheological studies, the deformation a material is subjected to when a stress is applied is often relative to its undeformed state (strain). Similar to stress, strains can be normal or shear and, by definition, are dimensionless numbers. Normal strains can be presented as engineering/Cauchy strain, defined as the ratio between the deformation (δH) and the initial length of the test sample (H_0), or as true/Hencky strain, defined as the logarithmic ratio between the initial length and the actual length of the sample at time (t) during the test. Dobraszczyk & Vincent (1999) and Steffe (1996) acknowledge the latter is preferred in food studies due to its applicability in large deformation situations. A comparison and discussion of several large strain measures was presented by Peleg (1984).

$$\in_C = \delta H / H_0 \quad \text{(Cauchy strain)}$$

$$\in_H = \ln(H_0 / Ht) \quad \text{(Hencky strain)}$$

Many rheological measurements of viscoelastic materials are carried out in the linear viscoelastic range, generally defined as the range where the material response (strain) is directly proportional to the value of the applied force (stress). Usually this linear range is limited to small strains and stresses (Konstance & Holsinger, 1992; Shoemaker, 1992; Walstra & Peleg, 1991). For larger deformations (non linear behaviour), the structure of the cheese is

altered by the deformation and large stresses can cause the sample to fracture. Although the application of larger strains or stresses may reflect more practically oriented studies (Bagley, 1983; Borwankar, 1992), the mathematics of non linear viscoelasticity are very complex and limited progress has been achieved in this area.

2.4.3. Test methods and viscoelasticity

Among the instrumental rheological measurements, a differentiation can be made between empirical, imitative and those methods that provide fundamental, well defined physical properties of foodstuffs. Empirical methods measure properties that are not well defined and that can not be easily expressed in fundamental units, with the results being characteristic for that particular material, under those particular experimental conditions (Bagley & Christianson, 1987; Konstance & Holsinger, 1992; Sherman, 1969). Reviews of several empirical tests and instruments to perform them was presented by Szczesniak (1963, 1972). Despite the poor definition of the tests, Bourne (1978) emphasised their usefulness in correlations with sensory results.

Imitative methods involve a group of instruments that attempt to simulate, to some degree, the forces and deformations that the food is subjected to while it is being consumed (Bourne, 1982; Lewis, 1990). Because of that, results from these methods are expected by researchers to correlate well with textural attributes perceived in sensory assessment. According to Bourne (1978), Texture Profile Analysis (TPA) would fall into this group.

Extensive research has been done within this group of imitative methods (Bourne, 1968; Friedman *et al.*, 1963; Lucisano *et al.*, 1987; Prentice, 1992; Meullenet *et al.*, 1997; Shama & Sherman, 1973; Szczesniak, 1963a, 1972), and in many publications, some instrumental mechanical parameters are reported in terms of sensory properties without evidence they actually represent such properties (Peleg, 1983).

Recognition of the complexity of mastication processes (Heath & Prinz, 1999; Peleg *et al.*, 1983), where perceived texture is affected by teeth, tongue, cheeks, palate, other hard and soft tissues in the mouth, plus saliva, reinforces the difficulties in developing these types of texture measuring instruments and expressing their results mathematically (Campanella & Peleg, 1988). Like with empirical methods, the results are often characteristic for one particular material, under particular experimental conditions.

Fundamental rheological properties, on the other hand, are independent of the instrument on which they are measured (test geometry) and of the sample size and dimension, thus different instruments will yield the same results, provided the testing conditions (temperature, stresses and strain linear ranges) are comparable (Chakrabarti, 1995; Luyten *et al.*, 1991a). As pointed out by Bagley & Christianson (1987), the advantages of the fundamental approach are that the results can be analysed in a systematic way and predictions of and correlations with other food properties become feasible. This opinion is not shared by Bourne (1982), who concluded that there was generally poor correlation between fundamental and sensory methods. It is important to recognize, however, the fact that numerous foods are so complex that it is not practical, and sometimes not possible, to measure their fundamental rheological properties (Rao, 1992; Steffe, 1996). In such cases, only empirical/imitative testing devices provide a suitable means of characterising the behaviour of the composite material. To date, cheese has often been included in the latter category.

Two major forms of measurement are used to characterise viscoelastic materials. The first of these is dynamic testing, in which the test sample is exposed to applied stresses or strains in a restricted, sinusoidally oscillatory manner (Stanley *et al.*, 1998; Steffe, 1996; van Vliet, 1999). The time scale of the measurement, in this case, can be varied by changing the frequency of the oscillation. The resultant strain or stress amplitude and phase angle are measured and, in turn, separate elastic (G') and viscous (G") components are derived (Figure 5). According to Peleg (1987), the frequency dependency of these components can be treated as a rheological fingerprint of the tested sample.

The storage modulus (G') represent the amount of energy stored and recovered per cycle of deformation, being defined as

$$G'_{(\omega)} = (\sigma_0 / \gamma_0) \cos \delta$$

where σ_0 is the maximum stress, γ_0 is the maximum shear strain and δ is the phase difference between the stress and the strain. In contrast, the loss modulus (G") represents the energy lost per cycle of deformation by viscous dissipation (related to flow of matrix, flow of liquid through the matrix or relative movement of material elements causing friction), which is defined as

$$G''_{(\omega)} = (\sigma_0 / \gamma_0) \sin \delta$$

The ratio between these two moduli gives the loss tangent

$$\tan \delta = G'' / G'$$

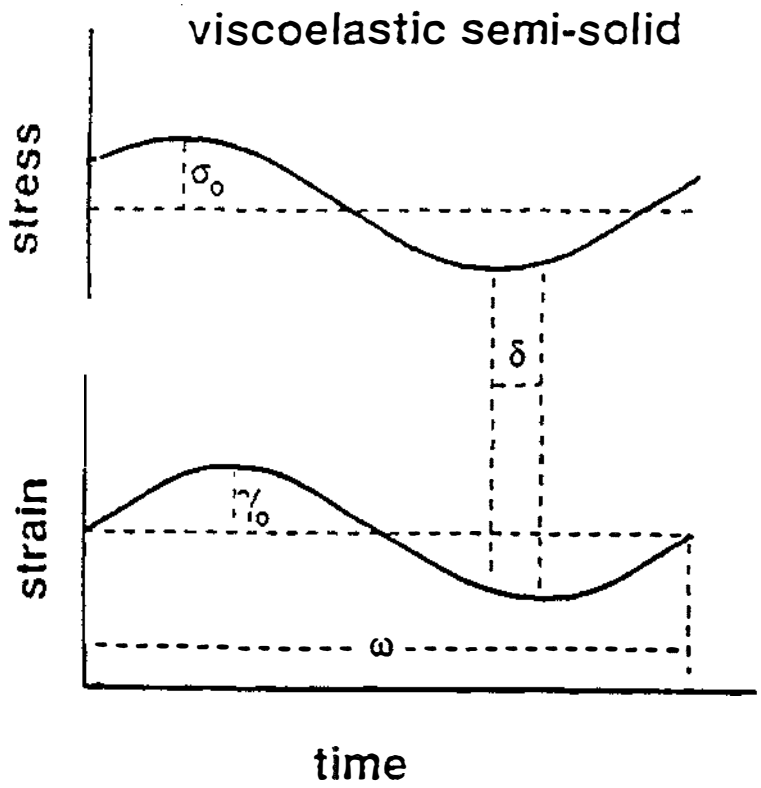


Figure 5. Shear stress response of a viscoelastic material under oscillatory shear strain (Shoemaker *et al.*, 1992)

Dynamic testing provides a way to test materials nondestructively. This is because, for the data to be independent of the magnitude of the applied stress, the elastic limit of the material can not be exceeded (McClements, 1999; van Vliet, 1999). This means the test must be carried out in the linear viscoelastic region, i.e., when the ratio of stress to strain is constant. As pointed out by Stanley *et al.* (1998), the strain limit of the linear viscoelastic region is dependent on the material structure.

The second form of measurements is the transient tests, characterised by the fact that the test sample is stressed constantly in the same direction. Examples of such measurements are the creep test, in which a constant stress is applied to the sample and the resulting strain measured over time (Tunick & Nolan, 1992; van Vliet, 1999), and the stress relaxation. In the latter, an instantaneous and constant strain is applied on the material and the resultant decay of stress required to maintain this strain measured as a function of time (Stanley *et al.*, 1998; van Vliet, 1999).

Stress relaxation tests are usually performed in strain controlled rheometers and show that viscoelastic materials relax gradually with the end point depending on the molecular structure of the material being tested (Steffe, 1996). Peleg (1987) and Shoemaker (1992) draw attention to the fact that instantaneous strain is, in reality, impossible to achieve, which means that relaxation has already started during the very short time required to attain the set deformation.

The increased availability of stress controlled rheometers has greatly facilitated the use of creep tests on a wide range of food materials (Shoemaker *et al.*, 1987). According to Shoemaker (1992), the Rheometrics stress rheometer is a precision built instrument dedicated to creep and recovery measurements.

Viscoelastic materials respond to the applied stress in a non linear pattern (strain), when the sample adapts to the constant stress by a combination or series of overlapping exponential decays, each with a discrete time constant, as bonds break and reform at different rates (Stanley *et al.*, 1998). Typical creep curves are presented in Figure 6. Upon removal of the

stress, the samples show the ability to recover some structure by storing energy and also a permanent deformation due to viscous flow of bond-free constituents of the material through the duration of the test (Steffe, 1996).

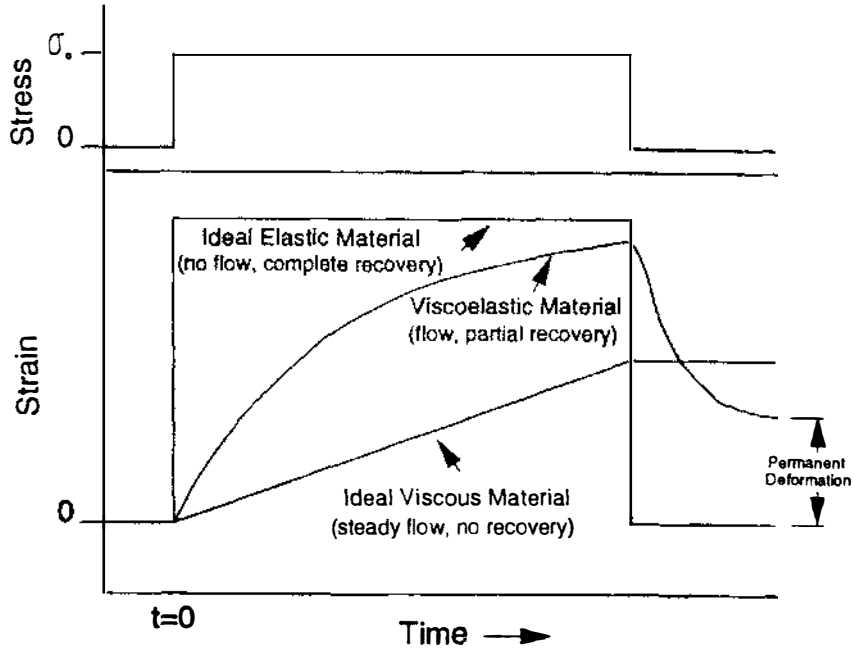


Figure 6. Typical creep curves for elastic, viscous and viscoelastic materials (Steffe, 1996)

Creep tests can be conducted in uniaxial tension or compression, in addition to shear, but Peleg (1987) points out that, in these cases, maintaining a constant stress is difficult because the cross-sectional area of the sample progressively changes. Advantages of performing creep experiments in shear are discussed by Stanley *et al.* (1998). One important aspect reported is that these nondestructive methods, being derived from basic theoretical principles, have predictive capability and are therefore of interest in research and development. Small changes in the test materials can be detected, so the physical quantities measured can lead to a microstructural interpretation.

Results from creep experiments can be presented in the form of deformation (strain) versus time or, more commonly, strain per unit stress

versus time. The latter is called creep compliance, measured in units of stress reciprocal. Higher compliance indicates a greater degree of deformation (Shoemaker *et al.*, 1992). Creep compliance is a better parameter to use to characterise rheological properties of a material because it takes into account the magnitude of the applied stress (McClements, 1999).

Large deformation tests, especially uniaxial compression, are important and useful in dealing with practical problems and providing important mechanical properties (Rao, 1992; van Vliet, 1999), but collection of data relevant to this type of processes (fracture) involves testing in the non linear range of behaviour. Important sensory textural information is obtained during processes such as mastication and swallowing, which are only accomplished with large deformations (Luyten *et al.*, 1992; Steffe, 1996). From a fundamental viewpoint, however, data in the non linear viscoelastic region can only be used for comparative purposes limited to the particular conditions under which they have been determined. This is because the theoretical complexity of non linear viscoelasticity makes the generation of absolute material properties impractical once fracture initiates.

Fracture, as an example, is often taken to occur when all bonds between the structural elements in a certain macroscopic plane break, resulting in an extensive breakdown of the structure and falling apart of the material (van Vliet, 1999). It is accepted, however, that fracture is a process rather than a point in the uniaxial compression test and, as such, initiates and propagates prior to gross mechanical failure of the material (Pollak & Peleg, 1980; Prentice, 1984). Characterising these events mathematically is yet to be achieved. Large deformation and fracture properties are often dependent on the deformation/strain rate (Casiraghi *et al.*, 1989; Culioli & Sherman, 1976), sample size and shape (Chu & Peleg, 1985; van Vliet & Peleg, 1991) and also the size of defects in the material (van Vliet & Peleg, 1991). These tests are, however, very popular among researchers, especially those pursuing correlation of instrumental data with sensory evaluation results.

One common rheological parameter determined from large deformation compressive tests is the elastic or Young's modulus, defined as the slope of

the true stress versus Hencky strain curve at low strains, typically below 0.03. Determination of the modulus at such low strains (linear viscoelastic region) is often not possible, so the term "apparent modulus of deformability" is occasionally used (Peleg, 1987). At relatively small strains, the modulus of deformability and the Young's modulus are, for all practical purposes, straight lines with similar slopes. Other physical characteristics obtained from uniaxial compression tests include the fracture stress, fracture strain and the total energy (work) of fracture (area under the curve) (Ak & Gunasekaran, 1992; McClements, 1999).

2.4.4. Rheology in cheese analysis

The rheological study of cheese is of particular interest as a quality control tool and a means of studying cheese structure as a function of composition, processing techniques and storage conditions (Konstance & Holsinger, 1992; Tunick & Nolan, 1992). In the course of the cheesemaking or the ripening processes, many biochemical and physicochemical changes occur, contributing to the formation of a variety of products with distinct textural behaviour (Prentice, 1984).

The term cheese encompasses a very heterogeneous group of products that differ in composition, sensory characteristics and physical attributes as well as the manufacturing and storage conditions used to make the products (Kosikowski & Mistry, 1997; Olson *et al.*, 1996; Walstra & van Vliet, 1982). In addition to the marked and inherent lack of homogeneity of most cheeses, several cheese varieties exhibit anisotropic behaviour, resulting in an uneven stress distribution in the deformed cheese sample (Peleg, 1987). Such aspects are important when trying to interpret rheological results or to obtain a representative test sample that allow the results of rheological measurements to be extended to the entire cheese product (Walstra & Peleg, 1991; Walstra & van Vliet, 1982).

All different types of cheese, however, exhibit viscoelastic behaviour, i.e., they possess elastic properties as well as viscous properties, with the ratio of elastic to viscous behaviour depending on the time scale of the deformation

(Luyten *et al.*, 1991a; Prentice *et al.*, 1993). At long time scales, the material's behaviour is mainly viscous, with most of the deformation remaining after the stress is removed. This is particularly easier to visualise in soft cheeses such as Brie and Camembert, which clearly flow under the effect of an applied stress (Prentice, 1984). On the other hand, at short time scales, the sample appears more elastic and, so long as the structure is not destroyed, tends to regain its original shape, once the applied stress is removed (Shoemaker, 1992). Elastic behaviour is very easily recognised in cheeses like Parmesan and Italian Grana (Prentice, 1984).

In the case of cheese rheology, empirical and imitative methods have been developed to correlate the sensory perception of texture with test procedures that mimic cheese use. These supposedly provide information on textural attributes such as firmness, hardness, meltability, springiness or rubberiness, stretchability, adhesiveness or stickiness, cohesiveness, chewiness, sliceability and fracturability, all of which are properties normally perceived and evaluated by the senses as cheese is handled or chewed/swallowed (Olson *et al.*, 1996; Prentice *et al.*, 1993; Shoemaker *et al.*, 1992; Steffe, 1996). Empirical methods, however, suffer from limitations, as previously discussed in this review. They lack predictive capability, make the comparison of results coming out of different arbitrary test methods impossible and can give rise to erroneous conclusions from data obtained by methods not fully understood. Notwithstanding these facts, there is no doubt that empirical or imitative tests applied to cheese studies are important.

It is worth noting that the assessment of data reliability, which is important in more fundamental methods, is more or just as important in empirical testing, since for the latter there are no independent criteria to evaluate the results.

2.4.4.1. Factors affecting rheological measurements in cheese

Aspects such as the rheological properties to be measured, the time scale of (or the deformation rate during) the measurement, the measuring method and the measuring apparatus should be properly identified when collecting rheological information from a cheese product. Other important factors

affecting instrumental results are the size, shape and orientation of the test sample (van Vliet, 1991; Luyten *et al.*, 1992), the testing temperature and occurrence of slip during testing. If the goal is to determine the basic material properties of cheese to understand the interaction of components or to measure properties relevant to storage and handling, the test conditions should reflect the quite slow rates of deformation involved. However, when comparing rheological properties of food materials with sensory ones, Bourne (1982) recommended the different test conditions should be used to try and mimic the large strains and multiple fractures that occur during mastication. That recommendation is contested by Thybo *et al.* (2000) based on their results with cooked potatoes.

2.4.4.1.1. Effect of slip and friction

The occurrence of slip during rheological testing of a cheese sample can have a detrimental effect on the results and their interpretation if the testing is being performed in dynamic oscillatory shear mode (Yoshimura & Prud'homme, 1988). This is because, in dynamic experiments, where the stress is continuously changing with time, response of the material may not be able to adjust instantaneously to the current stress. Hence, slip effect and true material property can not be distinguished.

In order to establish occurrence of slip, waveforms must be measured for two different gap separations at the same strain and frequency. Absence of slip will be indicated by identical waveforms, regardless of the gap used (Nolan *et al.*, 1989; Yoshimura & Prud'homme, 1988).

In compression tests, frictional energy losses resulting from slip must be taken into consideration in the calculation of stresses and strains. This is because friction causes the samples to bulge during compression, resulting in uneven stress distribution in the sample and premature fracture of the test piece. Friction in compression experiments has been studied and reported (Casiraghi *et al.*, 1985; Chakrabarti, 1995; Goh & Sherman, 1987; Robert & Sherman, 1988).

In order to minimise barreling or bulging during the compression stroke and to enhance reproducibility of results, it is common for compression tests to be conducted with lubrication of the sample-plate interface or with bonding of the sample to the plate. Ak & Gunasekaran (1992), Brennan & Bourne (1994), Casiraghi *et al.* (1985), Chu & Peleg (1985), Culioli & Sherman (1976), Goh & Sherman (1987), to name but a few, used both types of compression (bonded and lubricated) in their studies. No significant effect of surface lubrication was reported by Ak & Gunasekaran (1992) or, prior, by Casiraghi *et al.* (1985), even though reproducibility of results was improved with lubrication in both studies. Ak & Gunasekaran (1992) also observed that self-lubrication due to fat exudation during compression was responsible for the absence of bulging of the sample in non lubricated experiments.

Brennan & Bourne (1994) tried to relate instrumental compression to that of food materials between the molar teeth and concluded that compression of foods in the mouth follows the non lubricated pattern, despite the lubrication provided by saliva. The mathematical processing of instrumental compression data requires, however, that the effects of friction be controlled or at least minimised.

Slip, however, can also be a positive aspect in rheological testing, as in the case of the squeezing flow technique (Campanella & Peleg, 1987). When studying food materials that are self-lubricating, such as ricotta, melting processed cheese and Mozzarella cheese (Ak & Gunasekaran, 1995a; Apostolopoulos, 1994; Campanella *et al.*, 1987; Suwonsichon & Peleg, 1999), the existence of slip is not only acknowledged in the method but also incorporated in the calculation of results.

2.4.4.1.2. Effect of test method

Reviews of advantages and disadvantages of different testing methods, especially those concerning the evaluation of fundamental rheological properties, are widely reported in the literature (Konstance & Holsinger, 1992; Prentice *et al.*, 1993; Rao, 1992; Shoemaker, 1992; Shoemaker *et al.*, 1987; Steffe, 1996; van Vliet, 1991).

As viscoelastic materials, cheeses can store and dissipate mechanical energy. While the viscous properties are time dependent, the elastic behaviour and fracture properties depend on the deformation or strain rate (Rohm & Lederer, 1992). Hence, in selecting a test method, the properties of interest and the purpose of the instrumental investigation need be considered carefully. Methods well suited for research purposes, for example, are often not so for quality control. In principle, if one measures a well defined, fundamental rheological property by different methods, the result is independent of the methodology used so long as the time scale is the same (van Vliet, 1991). That, however, is not always the case, especially in food studies.

Imoto *et al.* (1979) studied the effect of compression ratio on the mechanical properties of several types of cheese. They observed that these properties changed with compression ratio in a unique pattern for each type of cheese and each instrumentally determined property. It was concluded that optimum compression ratios must be evaluated for each individual property by assessing how well it correlates to the sensory counterpart.

At lower strain rates, more bonds between the structural elements are able to relax during deformation. As a result, corresponding stresses are also lower, which leads to smaller values for the Young's modulus and fracture stress and work (Boyd & Sherman, 1975; Faraay, 1995; Rohm & Lederer, 1992; Thybo *et al.*, 2000). Luyten *et al.* (1991b) observed in Gouda cheese that deformations at lower strain rates also result in higher values of fracture strain.

2.4.4.1.3. Effect of structural defects and sampling method

Structural defects are common occurrence in cheese products and lack of homogeneity can be observed even within the same cheese variety. Defects can include holes, uneveness of network, acid spots, curd junctions, precipitates of salts or differences rind-centre, many of which can be regarded as an inherent property of cheeses. Depending on their size in relation to the sample size, structural defects can lead to poor reproducibility of rheological results (van Vliet & Peleg, 1991). Likewise, interpretation of the rheological

results becomes difficult, particularly at large deformations, since fracture always starts at the weakest point of a structure, near inhomogeneities (Luyten *et al.*, 1992). Hence, these inhomogeneities should be avoided during sampling or, if not feasible, their influence on instrumental results determined first (Walstra & van Vliet, 1982).

It is usually recommended that samples be taken from the middle of the cheese block and, because several cheeses are anisotropic, always in the same direction. These samples should be large enough to be representative of the cheese variety being studied, yet small enough not to include hidden cracks (van Vliet & Peleg, 1991). Being well distributed, the probability of finding structural defects in a sample increases with sample size and/or volume.

Casiraghi *et al.* (1985) reported that samples taken from the surface of a cheese block required higher stresses to achieve a certain deformation than samples from the centre of the same block. They also suggested that more uniform samples are obtained if the cheeses are cooled to 4°C prior to cutting. This is due to the increased firmness of the cheeses at such temperature.

Caution is required to minimise distortion of the samples as they are bored or cut out. For that, it is recommended the process be done slowly and preferably with lubrication of the borer with mineral oil (van Vliet & Peleg, 1991). These practices also help minimise the occurrence of pre-test deformations before the actual rheological test. Since cheese is normally only linearly elastic over small strains, the material properties can be irreversibly affected if the stresses exerted during preparation of the sample deform the test piece to a strain above the limit for linear viscoelastic behaviour. Unless extreme care is taken, it becomes impossible to assess whether the observed yield point is real or an artefact. The problem can be more serious when dealing with spreadable or soft cheeses (van Vliet & Peleg, 1991).

2.4.4.1.4. Effect of sample shape and dimension

Results of instrumental measurements of mechanical properties are largely dependent on the sample geometry and size. Cylindrical samples (cheese plugs) are preferred to minimise irregular crack development in the

sample, even though rectangular samples with precise dimensions are more easily prepared (Prentice *et al.*, 1993). The sample shape, however, depends on the type of test to be done.

The effect of the height to diameter ratio (aspect ratio) of cylindrical samples on their rheological compressive behaviour has been investigated (Chu & Peleg, 1985; Culioli & Sherman, 1976; Peleg, 1987). Chu & Peleg (1985) showed that, when working with small aspect ratios (0.12 to 1.0), flatter samples appeared stiffer. Masi (1987) suggested that the sample height/diameter ratio be greater than unity to avoid excessive effects due to friction between the sample and the compression plates. As the aspect ratio increases, the rheological properties tend to become independent of the sample size. Similar recommendation was given by Peleg (1987), who stated that in food materials, a sample with a larger diameter exhibits higher apparent strength (higher stresses) compared to one with smaller diameter, at the same strain. The reasons for such behaviour are discussed thoroughly in his review.

Theoretically, sample dimension effects are linked to the effect of deformation rate. This was observed by Culioli & Sherman (1976) in their early study, but failed to be observed by Ak & Gunasekaran (1992) when studying Cheddar cheeses in compression.

2.4.4.1.5. Effect of temperature

Temperature has a major influence on the mechanical and textural properties of cheese, which means rheological testing should be performed at a well known constant temperature (van Vliet & Peleg, 1991). This applies not only for the temperature of the testing room but mostly to the sample temperature during testing. Cheese samples to be tested should be allowed enough time to equilibrate to the desired test temperature (Faraay, 1995). This time will vary depending on size, composition and presence of structural defects.

Vernon Carter & Sherman (1978) showed that, the crosshead speed being kept constant, the force required to achieve a certain deformation

decreased as the temperature increased in the range 20°C-36°C. These observations seemed to relate to the fact that at temperatures above 23°C the fat present in the cheeses changes from its solid state to liquid, increasing the fluidity of the cheese. Results from Luyten *et al.* (1991a) supported the idea that the physical state of the fat caused the temperature-related mechanical response.

Nakazawa *et al.* (1993) used a lower range of temperature in their study. Cheeses evaluated rheologically over the range 5°C-18°C showed marked decrease in firmness and increase in the parameters for softness.

More recently, ultrasound techniques have been used to study the rheological properties of cheese (Lee *et al.*, 1992). Mulet *et al.* (1999) investigated ultrasonic velocity in Cheddar cheese as affected by temperature and found that the temperature dependence was linked to fat melting. The most reliable temperature interval to carry out ultrasonic measurements in that variety of cheese was identified as 0°C-17°C, with measurements near 7°C being the most suited ones for studies of structural changes.

2.4.4.1.6. Effect of cheese composition

Langley & Green (1989) and Langley *et al.* (1990) stated that the mechanical properties of a food composite depends on the composition, volume and distribution of each phase as well as on the nature of the interaction between the particles and the matrix.

The qualitative effects of moisture and protein contents, or more specifically the moisture to protein ratio, on the physical properties of cheese are well recognised and reported (Olson *et al.*, 1996; Prentice *et al.*, 1993; Visser, 1991). While the casein matrix is the main structural component and imparts rigidity to the cheese, the moisture reduces the rigidity by acting as a low viscosity lubricant between the surfaces of the fat and the casein (Masi & Addeo, 1986; Prentice, 1992). High moisture to protein ratios imply smaller number of interparticle bonds and consequent more freedom of the particles to move with respect to each other. Casein should be sufficient to form a continuous network around the fat globules, the amount being dependent on

number, size and distribution of fat globules and size of the casein micelles themselves. Once the minimum amount of casein is exceeded, any further casein will serve to strengthen the matrix and chain juctions (Prentice, 1992). The quantitative contribution of these components, however, is yet to be characterised.

Chen *et al.* (1979a) investigated the texture of a wide range of cheeses and found good correlation in linear regression analysis between protein content and hardness as determined by penetration testing. The rigidity of the casein network depends on the degree of openness of the matrix, the amount of bound and free water and the size and size distribution of the fat globules.

Luyten *et al.* (1991a,b) conducted an extensive study on the influence of compositional factors on the rheological properties of Gouda cheese. They showed that increasing moisture levels resulted in decreasing values for Young's modulus and stress at fracture, increasing strain at fracture and, at levels above 44%, increasing loss tangent values. Similar behaviour had been reported by Masi & Addeo for four types of 'pasta filata' cheeses.

Development of reduced fat cheeses has always been a challenge for the cheese industry due to the significant effects that fat has on the physical properties of the low fat products (Guinee et al., 2000; Prentice *et al.*, 1993). Several researchers failed to detect the influence of this component on rheological parameters (Chen *et al.*, 1979a; Marshall, 1990; Olson & Bogenrief, 1995; van Vliet & Dentener-Kikkert, 1982), possibly because fat in cheese is normally held by entrapment within the casein network and the interaction with the protein is solely by friction. Marshall (1990), however, proposed that fat contributes to the fracturability of analogue processed cheeses by forming weak points through which fractures propagate if sufficient strain is applied to the cheese. Such contribution is, according to Langley *et al.* (1990), dependent on the physical state of the fat.

The role of structure promoters or breakers played by emulsion droplets was reported by McClements *et al.* (1993) for polymer network systems. In the case of processed cheese products, fat droplets become an integral part of the structure rather than just a filler and, as such, its contribution to the

physical properties can not be neglected. In protein-stabilised emulsions like processed cheese, where fat-protein interactions are vital for structure formation, displacement of the protein from the oil-water interface using surfactants causes a significant reduction in emulsion strength, as assessed by the storage modulus (G') values (Chen & Dickinson, 1998).

Visser (1991) acknowledged in his review that no systematic study regarding the effect of fat globule size on the consistency of hard and semi-hard cheeses had been made and that the topic required further attention. Recent studies confirm the importance of fat globule size and distribution on the rheological properties of cheeses and emulsified systems like processed cheese (Kim *et al.*, 1996; McClements, 1999).

When relating variations in cheese composition to the rheological properties of cheese, it is often not possible to vary one compositional parameter without varying others. As previously reviewed, the major components of cheese impart structural integrity or discontinuity to cheese depending upon their relative concentrations and state, and the role of each can be altered by factors such as milk pretreatments, pH, temperature, manufacturing conditions, salt content, enzymatic activity and maturation conditions and duration. Therefore, careful design, control and interpretation of the physical properties measurements in cheese are required (Olson *et al.*, 1996; Walstra & van Vliet, 1982).

2.4.5. Rheological studies on natural and processed cheese

A number of studies reported in the cheese literature show the attempt of authors to instrumentally characterise the mechanical properties of cheeses (Ak & Gunasekaran, 1992, 1996; Almena *et al.*, 1998; Ben Omar *et al.*, 1995; Bertola *et al.*, 1995;Korolczuk, 1995, 1996; Raphaelides & Antoniou, 1996). Some developed their research further trying to correlate rheological measurements and microstructural results with chemical characteristics and, ultimately, with sensory textural attributes of the cheeses being evaluated (Antoniou *et al.*, 2000; Drake *et al.*, 1999b; Meullenet & Gross, 1999; Noel *et al.*, 1998). These papers typically address the rheological properties of natural

cheeses, with less numerous research focused on the texture of processed cheese products (Abd El-Salam *et al.*, 1996; Abou El-Nour *et al.*, 1996; Bowland & Foegeding, 1999; Harvey *et al.*, 1982; Lobato-Calleros *et al.*, 1997, 1999).

2.4.5.1. Uniaxial compression tests

Uniaxial compression tests are quite commonly used in cheese research, providing useful information not only about the rheological characteristics but also the fracture properties of the products. The most popular instrument for these types of tests is the Instron Universal Testing Machine (Almena *et al.*, 1998; Ak & Gunasekaran, 1992; Ak & Gunasekaran, 1995b; Green *et al.*, 1985; Masi & Addeo, 1986; Pagliarini & Beatrice, 1994; Raphaelides & Antoniou, 1996; Yun *et al.*, 1994b). A wide range of test conditions has been used and reported in the literature including sample dimensions, strain rates and friction at the contact surface between sample and compression plates. Since these conditions are known to influence the response of cheese to compression, as reviewed in section 2.4.4.1, results from these experiments should always be interpreted with caution.

Many experimental data can not always be directly compared because of the different strain definition chosen by the authors. Peleg (1984) carried out a comparison of different strain definitions and their relationship with true stress. His simulation showed that, for ideal rubbery materials, the experimental stress-strain relationship using the different models is almost linear up to 25% deformation, but not necessarily with the same slope. Beyond this range, the considerable differences observed will have implications in terms of moduli calculation and stress-strain relationship interpretation. For cheeses, true stress-Hencky strain relationships are normally used.

Casiraghi *et al.* (1985) compared the behaviour of three different types of cheese (Cheddar, Mozzarella and spreadable processed cheese) in lubricated and bonded compression. Reproducibility of the data for five replicates of all three types of cheese was found to be good using lubricated, bonded and nonbonded-nonlubricated compression, even though the best results were

obtained with lubrication. Similar results were reported by Ak & Gunasekaran (1992), who attributed the lack of significant differences between lubricated and non lubricated results to fat release from the cheese samples during compression. Even though comparison of the compression curves obtained by Casiraghi *et al.* (1985) for the effect of friction was possible within each cheese variety (for samples tested with the same aspect ratio), comparison between cheeses was not done due to the different strain definitions and crosshead speeds used for each cheese in the test.

While investigating 4 types of 'pasta filata' cheeses differing in moisture content, composition and extent of ripening, Masi & Addeo (1986) determined the elastic (Young's) modulus, the stress and strain at fracture (related to firmness and longness of the cheeses, respectively) and the fracture work per unit of volume (toughness) from the force-displacement curves. The latter was also used by Pagliarini & Beatrice (1994) for Mozzarella cheeses and was found to be inversely correlated to fat content. Masi & Addeo (1986) used prismatic samples as opposed to cylindrical ones, which implies less uniform distribution of stress. True stress-Hencky strain values were not used, so changes in sample shape during compression were not taken into consideration. The implications of this are that the results can not be considered accurate across the whole compression stroke, since the stress evolution as a function of strain can be satisfactorily reproduced using Cauchy' strain definition only for deformation ratios below 20% (Korolczuk, 1995).

In 1987, Masi published the results of a collaborative study on compression tests of cheeses. It was part of a research project to establish the comparability of measurements from different laboratories and to identify the most suitable measurement conditions to generate reproducible mechanical data possible to interpret in fundamental terms on a variety of cheese products. According to the author, the standard deviations obtained between laboratories were similar to those within each laboratory, so long as the results from two laboratories (obtained in tests performed with refrigerated samples) were omitted. The mechanical properties used in the study were the Young's modulus, fracture stress, fracture strain and fracture work per unit of volume. In general, sliceable and spreadable processed cheese showed

uniform results, with some effect of the sample position in the block on the rheological results. The behaviour of samples taken from regions near the surface was slightly different from the behaviour of samples taken from the interior of the block. The lower standard deviation found for processed cheese in relation to Silano cheese was expected, as it is consistent with the fact that processed cheeses are more homogeneous than their natural counterparts. Masi (1987) concluded his research by making suggestions on measurement conditions that facilitate the generation of reproducible data. The aspect ratio of the sample should be equal or larger than unity, since such condition causes the mechanical properties to become almost independent of sample size. In addition, the crosshead speed should be carefully selected, bearing in mind that, most of the times, deformation rates comparable to those found in sensory evaluation are impossible to be reproduced.

Luyten *et al.* (1991a, 1991b) published the results of an extensive study on the rheological and fracture properties of Gouda cheese. When testing under small stresses, the behaviour of the cheese seemed to be linear, with the tan δ values indicating that Gouda is relatively more viscous over longer time-scales. With large stresses, however, the results obtained depended greatly on the strain, making the discrimination of two different types of deformation behaviour possible. Wium & Qvist (1997) observed the same with Feta cheeses and were able to demonstrate that Feta is less viscous than Gouda. Luyten *et al.* (1991a) defined type 1 cheeses as those of low pH as well as those not acid and older than 4-6 weeks, and type 2, cheeses both young and not acid. For type 1 cheeses, the fracture strain seemed to be a kind of material property, the deformation at maximum stress independent of the strain rate in the range studied, just like the shape of the stress-strain curve itself. On the other hand, type 2 cheeses had the fracture strain and curve shape dependent on the strain rate. It was found that the extent to which a cheese can be deformed without fracturing increased with decreased strain rate. This was explained by the occurrence of flow of the casein matrix. The flow also caused the fracture energy to be higher and the difference in deformation between fracture initiation and propagation to be larger.

Ak & Gunasekaran (1992) observed that despite the significant differences in fracture stress and fracture work due to the crosshead speeds

used, the deformation rate did not significantly affect the magnitude of fracture strain in their experimental work. This is in agreement with data obtained by Creamer & Olson (1982) and Luyten *et al.* (1991b). Clear explanation for these observations is not presented. It is important to consider, however, that fracture is usually obtained from the zero-slope point on the stress-strain curve, thus representing gross failure conditions. Because fracture in compression tests starts before the zero-slope location and occur over a region rather than at a point (Luyten *et al.*, 1991b; Pollak & Peleg, 1980), interpretation of fracture results requires caution. Ak & Gunasekaran (1992) also found that the aspect ratio had a strong effect on fracture strain, fracture stress and fracture work.

Working with Mozzarella cheese, Yun *et al.* (1994a) tried to determine the effect of draw pH and storage on the rheological properties of this cheese during 50 days of storage at 4°C. The experiment was performed on an Instron using lubrication and the authors noted that the effect of draw pH on the rheological parameters was relatively small compared to the storage effects. The gross failure point for all cheeses used in the study occurred around a true strain value of 0.5 for cheeses at day 3, but were less discernible after 50 days of storage. The curves (stress x strain) at day 50 were, however, visibly below those obtained at day 3, giving a clear indication of the decreased firmness of the cheeses with proteolysis. Similar studies regarding the effect of maturation on rheological properties were reported by Hort & Le Grys (2000) and Watkinson *et al.* (1997) for Cheddar cheese and Raphaelides & Antoniou (1996) for Teleme cheese. The results reported by Watkinson *et al.* (1997) and Raphaelides & Antoniou (1996) were in agreement with those from Luyten *et al.* (1991a,b) for Gouda cheeses, showing a slight increase in fracture strain during the first month, possibly due to fusion of the curd particles. As a result of proteolysis, however, decreased fracture stress, strain and work followed after the first month.

The studies from Hort & Le Grys (2000), Raphaelides & Antoniou (1996) and Watkinson *et al.* (1997) showed also that the Young's modulus values over the maturation period had a sharp increase in the first 15 days, followed by stabilisation of the values. Watkinson *et al.* (1997) attributed that behaviour mostly to crystallisation of the milk fat rather than to moisture

losses. In contrast, continuous increase of the modulus over storage time was reported by Nunez *et al.* (1991) for La Serena cheeses made with animal rennet. Watkinson & Jackson (1999) presented recently a new procedure for estimating the modulus of deformability, based on the empirical observation that there is an initial concave up region prior to the usually reported concave down region of the stress-strain curve for Cheddar cheeses in compression. Advantages and disadvantages of the new procedure are discussed and comparison with different procedures made. Despite being a real, well defined measurement, the ranking of the modulus of deformability and its relative magnitude for each cheese was the same for each procedure evaluated.

Prediction models for the textural characteristics of Cheddar during maturation were reported by Hort & Le Grys (2000), who claimed those models provided a reliable and objective procedure for mimicking consumer perceptions of textural attributes. However, no information about the quality of the fit was provided.

2.4.5.2. Texture Profile Analysis (TPA)

Texture Profile Analysis is an instrumental method classified as imitative and used to measure textural attributes by means of double compression. In other words, the test consists of compressing a sample twice in such way as to imitate the action of the jaw. By means of strain gauges and a strip-chart recorder, a force-time curve with the entire force history of the simulated masticatory action is plotted. Such a curve provides information on seven textural parameters: fracturability, hardness, cohesiveness, adhesiveness, springiness, gumminess and chewiness, all of them supposedly providing excellent correlation with sensory ratings (Bourne, 1982).

According to Bourne (1978), one great contribution of TPA has been its ability to prove that texture is a multi-point rather than a single-point property of foods. The fact that TPA is an imitative method, however, has to be taken into consideration when interpreting the results. This is because TPA does not provide information on fundamental properties of the food being studied and results can only be considered for the conditions used to obtain

them. This means that once the conditions under which the test is done change, the texture profile will also change.

Several studies on cheese texture have been published using TPA curves since the development of the technique. These studies explored the textural characteristics of Gouda (Chen *et al.*, 1979a; Kanawjia *et al.*, 1995), Cheddar and Cheddar-like cheeses (Attaie *et al.*, 1996; Chen *et al.*, 1979a; Bryant *et al.*, 1995), Feta (Pappas *et al.*, 1994), Mozzarella (Chen *et al.*, 1979a; Tunick *et al.*, 1991) and other Italian cheeses (Chen *et al.*, 1979a; Lucisano *et al.*, 1987) and Reggianito (Bertola *et al.*, 1995), to name but a few. A recent review discussed the evolution of used terminology, testing conditions and sampling methods (Pons & Fiszman, 1996). Studies on processed cheese have also been published (Abou El-Nour *et al.*, 1996; Gupta *et al.*, 1984; Harvey *et al.*, 1982; Lee & Marshall, 1981)

In the present experimental work, fundamental rather than empirical/imitative rheological methods were sought to evaluate the mechanical properties of model analogue processed cheeses. Hence, further review and discussion on Texture Profile Analysis results are not presented.

2.4.5.3. Dynamic tests

These tests offer very rapid results with minimal chemical or physical changes. Among the advantages of dynamic testing, as described by Konstance & Holsinger (1992), mechanical properties may be determined at various frequencies and temperatures within a short time. Moreover, the use of extremely small strains imposed on the sample, usually within 1%, assures linear stress-strain behaviour and thus applicability of mathematical models. By slowly increasing the strain, one can determine at what strain bonds between the structure elements start to break within the time scale.

Some disadvantages of the method, however, are reported by Olson *et al.* (1996) and van Vliet (1991). Among the limitations, the fact that meaningful results are only possible for the so-called linear region (small strains) causes sample slip at interfaces to be a problem, especially at high temperatures (Nolan *et al.*, 1989; Sutheerawattananonda & Bastian, 1998). Besides, when

the food being evaluated is cheese, additional drawbacks exist. It is particularly difficult to obtain samples of sufficiently accurate geometry (such as discs with truly parallel sides) and representative (cheese is neither homogeneous nor isotropic) to suit the small samples required for a typical dynamic test (Olson *et al.*, 1996).

In spite of problems with the nature of cheese and test procedure, the method has been extensively used to study the viscoelastic properties of various cheeses (Ak & Gunasekaran, 1996; Drake *et al.*, 1999b; Ma *et al.*, 1996; Nolan *et al.*, 1989; Rosenberg *et al.*, 1995; Sanchez *et al.*, 1996; Sutheerawattananonda & Bastian, 1998; Taneya *et al.*, 1979; Tunick *et al.*, 1990; Ustunol *et al.*, 1995; Yun *et al.*, 1994a,b).

Nolan *et al.* (1989), while investigating the dynamic rheological properties of natural and imitation Mozzarella cheeses, observed inconsistencies in the measurements that were attributed to slippage of the specimen in the apparatus. Visual observations led them to suggest that the possible cause of slippage was diffusion of milk fat onto the sample surface at room temperature. Among the methods used to solve the problem, bonding of the cheese sample directly onto the aluminium plates with cyanoacrylate ester adhesive produced the best results. Use of serrated plates is also reported to eliminate slippage problems (Rosenberg *et al.*, 1995; Sutheerawattananonda & Bastian, 1998)

In continuation of their research, Nolan *et al.* (1990) compared, also using small amplitude oscillatory shear, some rheological properties of Cheddar and pasteurised process American cheeses. Measurements were made at temperatures up to 60°C, with frequency ranging from 0.10 to 100 rad/s and bonded samples to prevent slippage. An initial strain range of 0 to 20% was used, which showed clear non-linear behaviour for both Cheddar and processed cheese. Strain sweep test indicated that dynamic testing for these types of cheese should be performed over the range 0.55 to 0.70% strain to ensure results in the linear region. Values for G' and G" decreased for both cheeses as the temperature increased and the two parameters were higher for pasteurised processed cheese than for Cheddar. This indicates the less structured body of the latter. Cheddar cheese, when evaluated under strain of

about 3%, underwent body breakdown, suggesting it is crumbly rather than spreadable. This could be seen by the marked inflection points in the curves of shear moduli and viscosity.

Tunick *et al.* (1990) used dynamic testing as a means to distinguishing quite similar cheeses as Cheddar and Cheshire. Compositional differences between these two cheeses are slight and can easily lead to mislabelling. Thus, samples of Cheshire and Cheddar were evaluated rheologically (dynamic testing) and microstructurally (scanning electron microscopy). Scanning electron micrographs of the two cheeses showed that Cheshire has a smooth continuous protein network surrounding irregular lipid inclusions, whereas Cheddar presents a denser protein matrix and smaller and more regular fat globules. Rheological measurements were obtained in a strain sweep test that showed no linear region for the parameters studied. At 0% strain, results show that Cheshire cheese breaks down more easily than Cheddar, the former displaying values of G' and G" almost half those of the latter.

In a study on the effects of composition and storage time on the rheological and textural properties of Mozzarella cheese, Tunick *et al.* (1993) showed that hardness, springiness, complex viscosity and storage and loss moduli all decreased with increasing moisture. They also observed that all parameters but springiness decreased with increasing fat content. Similar results were recently reported by Subramanian & Gunasekaran (1997). Tunick *et al.* (1995), in a subsequent study, showed that homogenisation did not significantly affect hardness or springiness, which indicated these properties in Mozzarella were influenced by fat content rather than fat globule size. In double cream cheese, however, homogenisation led to increased G' and, consequently, a better structured, firmer, more elastic and brittle and less sticky product (Sanchez *et al.*, 1994).

In the initial study (Tunick *et al.*, 1993), fresh low fat cheeses were harder, more viscous, more elastic and less meltable, as evaluated in the Schreiber's test, than fresh full fat cheeses. When analysing the effect of age on the experimental cheeses, the authors observed that allowing the low fat Mozzarella to be stored for 6 weeks produced textural properties similar to

those of full fat Mozzarella at week 1. This was observed so long as the moisture levels were similar. The springiness of full fat cheese was, however, greater than low fat Mozzarella even after 6 weeks of storage. Storage of Mozzarella cheese enhanced proteolysis even at relatively low temperatures and caused meltability to increase, possibly due to the release of fat as a result of protein breakdown (Tunick *et al.*, 1995). Other parameters studied (G' and G") decreased over storage (Ak & Gunasekaran, 1996; Subramanian & Gunasekaran, 1997; Tunick *et al.*, 1995).

Cheddar cheese has also been the subject of extensive research using dynamic rheological properties, mostly regarding the influence of fat content and maturation on cheese functionality. Dynamic testing results have been correlated to empirical tests for meltability, showing that this functional property is significantly influenced by fat content of the cheeses and that the complex modulus G*, defined as $G^* = \sqrt{(G')^2 + (G")^2}$, may be a useful predictor of Cheddar meltability (Ustunol *et al.*, 1994). Sutheerawattananonda & Bastian (1998) were not able to reproduce the meltability x G* correlation in Cheddar with processed cheese, adopting the loss tangent (tan δ) as a better parameter for meltability instead.

Increasing temperature during dynamic testing caused the rheological parameters G' and G" to decrease, indicating softening of the matrix, but the effects on the loss modulus (G") were less pronounced than on G' (Rosenberg *et al.*, 1995; Ustunol *et al.*, 1995). Likewise, increasing fat content and ripening time produced decreased viscoelasticity of the cheeses, as seen from the lower values of G' and G" (Ma *et al.*, 1996; Ustunol *et al.*, 1995). With more fat globule inclusions and increased proteolysis, the resulting protein matrix is less dense and compact, and the cheese is perceived as less elastic and rigid. Drake *et al.* (1999b), however, reported that differences in G' and G" of full fat and reduced fat cheeses within each cheese type studied were not significant.

2.4.5.4. Static transient tests

There have not been many recent publications of results from static transient tests, and, in some of the published material, such tests were

combined with other types of measurements (Ak & Gunasekaran, 1996; Goh & Sherman, 1987; Luyten *et al.*, 1991; Ma *et al.*, 1996).

Studying the influence of surface friction on the stress relaxation of Gouda cheese, Goh & Sherman (1987) observed that the stress relaxation behaviour of this type of cheese is affected by the degree of compression to which samples are initially subjected and also by the presence or absence of friction effects. At compressions above 20%, cracks initiated and propagated in all three test procedures (lubricated, normal non lubricated and with inserted emery paper to exaggerate the frictional effects). Thus, true stress relaxation is not exhibited above 20% compression. Surface lubrication prior to applying the constant strain results in a lower initial stress and lower stress and relaxation time components during stress relaxation. Without lubrication, the force recorded in the test could be divided in two components, one being the force required to overcome the surface friction. This component does not decay with time, such as the component actually involved in maintaining the strain constant. In conclusion, the authors pointed out that true stress relaxation behaviour of Gouda cheese could be studied only if the upper and lower surfaces of the samples are lubricated prior to the initial compression. Further studies on the stress relaxation behaviour of Gouda cheese showed that relaxation time decreased with increasing compression rate and that the stress needed for a certain deformation decreased with time (Luyten *et al.*, 1991).

Ak & Gunasekaran (1996) studied the dynamic rheological properties of Mozzarella cheese and used shear relaxation data to calculate storage moduli (G') and compare them with experimental G' values. According to Zoon *et al.* (1990), if the same rheological property can be calculated from different types of experiments, it provides strong indication that true material properties have been measured. Ak & Gunasekaran (1996) found the agreement between calculated and measured values to be good, even though the calculated values were generally slightly higher than the measured data, especially at low frequencies.

Creep recovery results from a number of cheese types showed, in a study from Drake *et al.* (1999b), that higher compliance values were obtained for

cheeses that were sensorially less firm. In addition, all cheeses showed some recovery of the deformation upon release of the applied stress. Greater recovery was observed in cheeses that were firm, indicating more elastic materials.

2.4.5.5. Meltability/stretchability/spreadability determinations

For many cheeses, spreadability, stretchability and melting characteristics are prime factors for consumer acceptability of the product and for determination of product functionality (Campanella *et al.*, 1987; Kindstedt *et al.*, 1989; Ustunol *et al.*, 1994).

These textural functional properties have been generally investigated by means of empirical methods, as pointed out by Ruegg *et al.* (1991) and shown in several published papers (Abd El-Salam *et al.*, 1996; Abou El-Nour *et al.*, 1996; Apostolopoulos, 1994; Bogenrief & Olson, 1995; Metzger & Barbano, 1999; Park *et al.*, 1984; Pompei *et al.*, 1988a,b; Savello *et al.*, 1989; Strohmaier *et al.*, 1992). However, because of their relative importance in natural and processed cheese functionality, considerable attempts have been made to develop more objective tests for their assessment (Ak & Gunasekaran, 1995a,b; Apostolopoulos, 1994; Mounsey & O'Riordan, 1999; Muthukumarappan *et al.*, 1999; Smith *et al.*, 1980; Sutheerawattananonda & Bastian, 1998; Wang *et al.*, 1998).

Because the properties of spreadability, stretchability and meltability were not investigated in the present experimental work, these properties were not reviewed further.

2.4.5.6. Rheological studies on processed cheese

This section includes studies dealing solely with processed cheese. The rheological behaviour of processed cheese products is very much the result of the compounded rheological contributions of their individual constituents. This includes mainly protein, fat and moisture. Blending materials, processing conditions and compositional parameters are key factors that

influence the rheological properties of processed cheese and were reviewed by Guinee (1987).

Similarly to natural cheeses, processed cheese products have been rheologically evaluated using empirical and imitative tests, especially TPA (Abou El-Nour *et al.*, 1996; Drake *et al.*, 1999b; Gupta *et al.*, 1984; Harvey *et al.*, 1982; Lee & Marshall, 1981; Thapa & Gupta, 1992). However, studies in which fundamental tests are used have also been published.

Gupta *et al.* (1984) evaluated 17 different emulsifier salts used in 3 different concentrations and their effects on processed cheese textural attributes, as assessed by sensory and Texture Profile Analysis. Results from their research show that acidic emulsifying salts, which cause the pH of the product to be less than 5.2, resulted in dry, crumbly products exhibiting no sliceability or meltability. In another study, Thomas *et al.* (1980) had observed that sodium hexametaphosphate resulted in the lowest values for meltability, empirically assessed, and that salt concentration did not significantly affect the results. Salts normally used in processed cheese manufacture, such as disodium phosphate and trisodium citrate, produced good and desirable characteristics in the experimental cheeses (Gupta *et al.*, 1984; Thomas *et al.*, 1980), with higher meltability being found for the products prepared with citrate. Similar results were obtained by Sutheerawattananonda & Bastian (1998) in a fundamental test. The influence of salt concentration on processed cheese hardness was reported by Gupta *et al.* (1984) to be extremely variable, increasing or decreasing product hardness depending on the salt used. In general, data from sensory analysis supported the rheological results in that study, even though statistical correlations between them were not presented.

Meltability has been one of the functional properties most investigated in processed cheese. In an attempt to monitor processed cheese meltability in fundamental terms, Mounsey & O'Riordan (1999) and Sutheerawattananonda & Bastian (1998) tried to use dynamic stress rheometry. The loss tangent (tan δ) was found, in both studies, to be a useful predictor of meltability based on the correlation R-squares with empirical results.

Strohmaier *et al.* (1992) compared empirical, imitative and fundamental tests to determine the spreadability and firmness of processed cheese. Correlation of the results with sensory data from a trained panel was done, showing spreadability can be efficiently determined with a controlled stress rheometer. Spreadability correlated well with the dynamic Weissenberg number ($W'(\omega) = 1/\tan \delta$) from a rotational rheometer (R-square 0.83). Sensory firmness and G' correlated with R-square 0.79.

Marshall (1990) used uniaxial compression to rheologically evaluate processed cheese analogues with different composition. As observed with natural cheeses, the values of Young's modulus increased with increasing deformation rates and with decreasing fat and moisture contents. A similar trend was observed for the maximum stress, strain at maximum stress and work at maximum stress. Strain at maximum stress, however, appeared not to suffer significant increase for deformation rates above 50 mm/min. Fracture evaluation of the samples showed fracture initiated at strains around 1% and, at around 33% deformation, cheeses reached gross failure. Fat was found to be more significant in explaining rheological parameters measured at small deformations than at large deformations.

In a more recent study of the large strain rheological properties of model processed cheeses, Bowland & Foegeding (1999) found that fracture parameters provided good indicators of textural changes associated with ingredient and processing variables. The best indicator was found to be the relationship between fracture strain and fracture modulus (fracture stress/fracture strain), where increased modulus and decreased fracture strain indicate transition from a soft, deformable texture to a firm, brittle one.

Reduced fat processed cheeses and analogues have been, like their natural counterparts, a demand from consumers increasingly concerned about health and nutrition (Kantor, 1990). The textural changes resulting from fat removal, usually excessive firmness and rubberiness, and replacement with fat blends and extenders have been investigated (Drake *et al.*, 1996; Drake *et al.*, 1999c; Lobato-Calleros *et al.*, 1997; Lobato-Calleros *et al.*, 1999).

Soybean fat is reported to confer hardness and adhesiveness to the products while reducing their cohesiveness and springiness, as evaluated using TPA. Butterfat and soybean oil have the opposite effects (Lobato-Calleros *et al.*, 1997). The same rheological method was used to evaluate analogues in which fat had been replaced with pectin and whey protein concentrate (Lobato-Calleros *et al.*, 1999). In addition to TPA, Drake *et al.* (1999c) used creep/recovery testing to assess the textural effects of using soy lecithin in processed cheeses. While fat reduction increases creep recovery by making the cheeses firmer and more elastic, addition of granular soy lecithin decreased the recovery at concentrations of 0.05% (w/w) and above. Hydrogenated soy lecithin was only able to reduce creep recovery at 0.2% (w/w). Percent creep recovery correlated positively with firmness and negatively with slipperiness of the mass.

Drake *et al.* (1999b), in a subsequent study, observed that fundamental tests (frequency sweep and creep/recovery), while revealing important information on network structure and molecular arrangement, can work equally well as empirical testing for predicting sensory texture properties. Such predictions were more accurate for processed cheeses than natural ones, which was attributed to their structural homogeneity and inherent lower sample variability.

2.4.6. Perspectives in cheese rheology research

Cheese rheology goes often beyond the realms of classical rheology. This is because cheeses not only flow and deform, but they also break. In addition, different cheeses break differently (Chakrabarti, 1995). With the recognition that texture is an important characteristic affecting consumer acceptability and perception of quality of a food product, the cheese industry both wants and needs tools to qualitatively and quantitatively evaluate textural attributes. Texture, as described by Dobraszczyk & Vincent (1999), is governed by a combination of mechanical and fracture properties and their modification and expression in the mouth during chewing.

Despite the many instruments available for measuring mechanical properties, none can actually measure texture or analyse all of the parameters responsible for texture and mouthfeel of cheeses. It is possible, however, to identify the main factors that govern cheese texture and measure them (Dobraszczyk & Vincent, 1999). Application of any type of mechanical deformation to a cheese sample generates a response that, to be meaningful, needs to be correlated with results from a sensory panel. Empirical tests have been extensively used for that purpose and provide an excellent tool for quality control. The future trend appears to be, however, the development of fundamental techniques that measure well-defined mechanical properties, taking account of their relationships with the internal structure of the foods and that, ultimately, will strongly correlate to the way food materials respond to applied forces in the mouth during the eating process.

2.5. SENSORY x INSTRUMENTAL TEXTURE CORRELATION

2.5.1. Introduction

It is recognised that sensory evaluation of textural properties of foods is important since it is the primary way consumers judge quality (Bourne, 1983; Green *et al.*, 1985). There is little gain in measuring physical properties that are not perceived by the human senses or that are judged unimportant. If the texture of a food is good, it may go unnoticed, for the awareness of texture is often subconscious. If, on the other hand, the texture is poor, it becomes a point of criticism and rejection no matter what readings an instrument gives or how well it has been calibrated in fundamental units (Bourne, 1983; Moskowitz, 1977; Shoemaker *et al.*, 1992).

Most food scientists, however, prefer to use instrumental data rather than sensory for the characterisation of textural attributes of food materials. As pointed out by Green *et al.* (1985), provided clear relationships can be established with sensory measurements, instrumental methods have numerous advantages. The latter are easier to perform, standardise and reproduce, and require the use of fewer trained people. Additionally, they yield

results more quickly than sensory methods (Bourne, 1983; Green *et al.*, 1985; Szczesniak, 1987). On the other hand, texture is, by definition, a sensory property. As such, it is only the human senses that can truly perceive, describe and quantify texture (Szczesniak, 1987).

2.5.2. Importance of sensory x instrumental data correlation

Szczesniak (1987), in a review on the developments of sensory versus instrumental correlation to that date, emphasised the tremendous effort that had gone into the development of instruments and methodologies for good correlation with sensory assessment of texture. In the course of the years, values for correlation have ranged from excellent to awful. Reasons for pursuing correlation, problems often leading to poor correlation values and current approaches to this issue were discussed by Bourne (1983) and reviewed by Lawless & Heymann (1998) and Szczesniak (1987).

It was pointed out by those authors that the need for quality control instruments, the desire to predict consumer response and to understand what is being perceived in texture evaluation are key elements guiding the research on sensory-instrumental correlation. The need to create or improve a texture testing device to duplicate the sensory assessments has also been of importance. Nevertheless, Bourne (1983) and Szczesniak (1987) pointed out that improper execution of sensory tests, inadequate knowledge of what the instrumental tests actually measure, sampling errors and heterogeneity of food products, and misinterpretation of the meaning of the correlation coefficient are key factors which lead to poor sensory-instrumental correlations. In general, correlation of instrumental and sensory data is limited to a particular product or, in the case of cheeses, a particular variety, and only rarely can they be extrapolated to other products (Bryant *et al.*, 1995; Prentice *et al.*, 1993).

Texture, as previously stated, is a multiparameter attribute (Bourne, 1982). Hence, no one instrument is able to measure all textural parameters adequately or be applied to all food products. On the contrary, instrumental methods usually apply to specific food products and for measuring specific

textural characteristics (Rosenthal, 1999). Such measurements have often been done through an empirical approach (Szczesniak, 1987), measuring not well defined physical properties.

Sensory evaluations, as a rule, involve substantial time and money and are likely to exhibit poor reproducibility, especially when evaluating food acceptability and preference (hedonics), if not carefully controlled and executed (Moskowitz *et al.*, 1979). Instruments, on the other hand, are free from drift and other complicating factors associated with panel testing. Instrumental methods are faster, less expensive to run and easy to standardise and calibrate. Such calibration, however, should be ultimately done against the human senses for the results to be meaningful. Arbitrary standards for calibration, as used years ago (Bourne, 1972), have been tentatively replaced by fundamental internationally recognised standards (fundamental instrumental tests), based on units of mass, length and time (Bourne, 1992). Not much progress, however, seems to have been achieved in finding good sensory-instrumental correlation with the latter approach (Lawless & Heymann, 1998).

As far as prediction and understanding of consumer response are concerned, an important aspect brought up by Szczesniak (1987) is the differentiation between intensity response (quantitative) and hedonic response (qualitative). Correlation is likely to be found when the consumer response of interest deals with the intensity of a given textural attribute. No instrument, however, can predict consumer liking and acceptance of a food product because of the instrument's inability to duplicate the sensory and psychological responses of a human being. This topic had been discussed in detail by Trant *et al.* (1981). In their work, these researchers stated that "intensity and hedonic responses are distinct behaviour which exhibit different functions with stimulus concentration".

2.5.3. Difficulties in sensory x instrumental data correlation

There are many reasons for failure in obtaining good correlation between sensory and instrumental data. The quality of execution of both types of tests

can be pointed out as a key factor (Bourne, 1983; Kapsalis *et al.*, 1973; Szczesniak, 1987). The assumption of good standard practices when running a sensory or instrumental evaluation session does not always hold, in which case poor or no correlation is found and erroneous conclusions are drawn. Proper execution of tests, on the other hand, does not necessarily lead to good correlation results per se.

Texture perception is not comprised of a series of single attributes. It is rather a dynamic process in which the food product is turned around by the tongue and mouth walls to expose the fresh surfaces of the sample to masticatory forces, and in which saliva plays a crucial role in hydrating, lubricating and diluting the bolus (Bramesco & Setser, 1996; Brown *et al.*, 1994; Brown *et al.*, 1996; Hutchings & Lillford, 1988; Szczesniak, 1990a). No instrument simulates those dynamics closely enough.

Lawless & Heymann (1998) and Szczesniak (1987) attributed poor correlation coefficients to factors such as the complexity of sensory terms (making it difficult at times to find meaningful correlation), the existence of different sensory scales (not always one applies the best scale for quantification of sensory perception) or the nature of the test material (possible heterogeneity of samples even from the same source, generating a high degree of scatter).

Just as important is that the instrumental measurement mimics, as much as possible, the conditions used to evaluate the sensory attribute. Thus, the popularity of empirical or imitative instrumental tests when compared to fundamental ones (Lawless & Heymann, 1998). Similarity of the physical aspects of sensory and instrumental measurements is of interest when pursuing good correlation coefficients (some products are usually assessed by flexure, others by puncture and others still by shear or uniaxial compression). Likewise, good understanding of what the instrumental tests actually measure is essential (Szczesniak, 1987).

Many of the poor correlation results found in the literature could be attributed to overlooking the need for data transformation when developing statistical correlation (Lawless & Heymann, 1998). That is because the

determination of correlation coefficients is usually based on linear regressions, not catering for curvilinear relationships, as is often the case with human senses. The aspect of linearity of the psychophysical response was reviewed by Meilgaard *et al.* (1991).

In the context of this discussion, some trends brought up by Szczesniak (1987) are likely to lead to advances in correlating results from sensory and instrumental textural assessments. These trends include the interdisciplinary approach and the pursuit of multiple correlation, i.e., correlating one sensory characteristic with several test types or, conversely, correlating one specific instrumental test with several sensory characteristics (Andersson *et al.*, 1973). Equally important, if progress in this field is to be achieved, is to understand the rheology of the human sensing apparatus, as initially emphasised by Peleg (1980a,b), and to take into careful consideration the rheological nature of foods as well as the engineering treatment of sensory perceptions (Kokini & Cussler, 1983; Kokini & Dickie, 1982).

2.5.4. Research on sensory x instrumental correlation of textural data

In recent years, several studies on sensory-instrumental texture correlation have been carried out using cheese (Drake *et al.*, 1999b; Hort *et al.*, 1997; Jack *et al.*, 1993b; Jack *et al.*, 1994; Meullenet *et al.*, 1998), canned fruit (Apostolopoulos & Brennan, 1994; Meullenet *et al.*, 1998), lemon pie filling (Hill *et al.*, 1995); extruded snack products (Duizer *et al.*, 1998; Reyes-Vega *et al.*, 1998), sweet potato (Truong *et al.*, 1997), to name but a few. Compression using the Instron Universal Testing Machine and instrumental TPA (Texture Profile Analysis) have been used mostly for instrumental characterisation of texture in those studies. Most of them show good correlation values between perceived textural attributes and instrumental measurements. Not many, however, show the use of regression analysis to obtain models for prediction of sensory characteristics. The distinction between correlation and regression models is discussed in detail by Neter *et al.* (1985).

2.5.4.1. Miscellaneous food materials

In 1973, Andersson *et al.* reported the sensory-instrumental correlation results obtained in a study with crisp bread. These researchers used flexure and compression tests using the Instron Universal Testing Machine, together with acoustic measurements, in order to correlate instrumental results with five sensory properties. These were the fracture force by handling, hardness by handling and biting/chewing and brittleness by handling and biting/chewing. Apart from conventional stepwise regression and stepwise discriminant analysis, a new approach checking all possible combinations of regression variables, combined up to 4 at a time, was attempted. A whole set of equally or almost equally well fitting equations was found, contrary to a single best regression equation. Even though the correlation coefficients found were reported, information was not provided about the quality of the fit of the models.

Bread was also the material used by Moskowitz *et al.* (1979) in their study. Correlation, in this particular case, was sought between rheological data and sensory results, as assessed by trained and consumer panellists. Good correlation values were reported for some sensory textural attributes, but not for all. It is worth noting that regression equations produced for trained and consumer panellists were often entirely different, as it would be expected, due to the different approach each panel adopts during evaluation.

Fourteen food products evaluated by an untrained panel and compression tests with an Instron showed that oral evaluation of hardness correlated well with instrumental force x % compression data, provided a critical % compression was exceeded during the instrumental tests (Boyd & Sherman, 1975). This critical value was lower for soft foods than for hard foods. In addition, lower compressive forces were associated with hardness evaluation by squeezing samples between the fingers rather than with oral evaluation. It was also pointed out that the panellists' usage of the term hardness did not correspond exactly to its use by material scientists. The aspect of terminology to be used in rheology, including Texture Profile Analysis, was addressed by van Vliet (1991), while McEwan *et al.* (1989),

Piggot & Mowat (1991) and Szczesniak (1987) discussed the existing gaps in the sensory terminology.

Hamann & Webb (1979) used multiple correlation analysis for the correlation of sensory (trained panel) and instrumental (compression and small strain oscillation) textural properties of heat coagulated fish gels. It was reported that the maximum compression force was a good predictor of sensory springiness, firmness, cohesiveness and gel strength. Gumminess and adhesiveness varied inversely with the compression force and were directly proportional to the loss tangent. The researchers, however, called attention to the fact that their results applied to the specific fish gels they worked with and wider application of the techniques should be made with caution. This is further emphasised by the results of another study (Diehl & Hamann, 1979), in which potatoes, melons and apples were used as testing materials. In this case, instrumental tests and parameters found to correlate well with sensory for one product did not, necessarily, work well for the other food materials.

Tscheuschner & Markov (1986a,b) did a comprehensive research on the textural properties of chocolate. Sensory-instrumental correlation was also sought (Markov & Tscheuschner, 1989). It was stated that good correlation between sensory and instrumental texture characteristics found in the study enabled instrumental texture measurements to be used in routine studies of quality evaluation. Very little information, however, was provided on the sensory protocol and results, as well as on instrumental results. Further, more detailed investigation into those correlation values would be appropriate and advisable.

Meullenet *et al.* (1997) and Meullenet & Gross (1999) studied the relationship of parameters generated in single and double compression testing of several foods with sensory scores for textural attributes. The compressive tests used the Instron fitted with a probe consisting of a set of artificial dentures and built to simulate the chewing action with tri-dimensional movement. Meullenet *et al.* (1997) observed that sensory hardness and springiness correlated well (R-square 0.90) with log-transformed standard parameters (hardness and springiness) from double compression force-deformation curves. Poor univariate models, however, were obtained for the

prediction of cohesiveness and chewiness. The lack of correlation was attributed to inappropriate instrumental capability for measuring those characteristics (Meullenet *et al.*, 1998). It was shown that adequately chosen data transformations can improve correlation coefficients, in agreement with Szczesniak (1987), who noted that failure to recognise non linear relationships where they do exist often results in poor statistical correlation. By using a combination of all instrumental parameters and appropriate data transformation, Meullenet *et al.* (1997) found satisfactory predictive models for cohesiveness (R-square 0.87) and chewiness (R-square 0.88), showing that simple instrumental univariate models not always correlate well with sensory response.

Double compression did not offer significant improvement for the prediction of textural characteristics when compared to single compression (Meullenet & Gross, 1999). The latter enabled accurate prediction of hardness, cohesiveness and fracturability, but failed to generate satisfactory models for springiness, cohesiveness of the mass and chewiness. Predictive models for springiness and cohesiveness of the mass were significantly improved with double compression. Single compression parameters such as the negative area and the maximum negative force were reported, in another study, to be good indices for sensory adhesiveness (Fiszman & Damasio, 2000b), despite the broad agreement that this is a difficult attribute to evaluate (Fiszman & Damasio, 2000a; Hoseney & Smewing, 1999).

Graininess, another important sensory attribute, was investigated by Imai *et al.* (1999). The sensory properties of interest were graininess threshold value and degree of graininess discrimination. The former was found to correlated with degree of circularity of the particles, solubility, water absorption rate-particle size coefficient (ratio of change in water absorption rate to change in particle size) and the ratio of deformation resistance of water absorbed particles under compression in the second stage to that in the first stage. The degree of graininess discrimination, on the other hand, was also correlated with particle size, in addition to the four parameters previously mentioned. It was observed that none of the properties alone was enough to accurately express the degree of graininess. Multivariate regression analysis resulted in R-square values ranging from 0.74 to 0.77 for both sensory

attributes, respectively. In a previous study (Green *et al.*, 1985), graininess correlated significantly with fracture compression of cheeses, even though the level of significance was lower than others textural attributes.

2.5.4.2. Natural and processed cheese products

Sensory evaluation of hardness, chewiness, springiness and adhesiveness of cheeses were correlated with instrumentally determined mechanical properties by Imoto *et al.* (1979). They showed that the correlation changed with the compression ratio used. When trying to correlate sensory hardness with compression force, high correlation was found over all the compression ratios studied (20 to 80%), whereas other parameters could only be satisfactorily correlated within a certain range of compression ratios. The authors suggested that the optimum compression ratio to be used in instrumental evaluation of cheeses should be selected for each property, using the fitness test for correlation between instrumental and sensory data. Results for processed cheese showed distinctly different mechanical responses to changing compression ratios than natural ripened cheeses.

The same textural characteristics studied by Imoto *et al.* (1979), plus cohesiveness and gumminess, were measured by Chen *et al.* (1979a) for 11 types of cheese. Objective measurements were reported to have correlated closely with panel scores, with correlation coefficients ranging from 0.79 to 0.85. The objective measurement of adhesiveness was correlated negatively with the sensory values. This was attributed to the use of the plunger technique instead of the flat plate attachment for determining adhesiveness.

Sensory-instrumental texture correlation was found to be poor in a study with Cheddar and Cheshire cheeses (Green *et al.*, 1985). Sensory data obtained from trained panels proved to be better at discriminating between cheeses than the data from the compression tests. The lack of good correlation (coefficients under 0.70) was attributed to the fact that compression does not really mimic the kind of food failure that occurs in the mouth, which is basically cutting/biting. Biting as opposed to simple compression becomes increasingly important when hard foods are being evaluated (Meullenet *et al.*, 1997). The results from the instrumental tests

reported by Green *et al.* (1985) should be considered with caution. As the authors pointed out, reproducibility among the replicates was fairly low. Besides, no transformation of the force-deformation curves to true stress-Hencky strain was performed to account for the change in shape of the testing samples during compression.

Results from a study on food compression by soft machines (Campanella & Peleg, 1988) indicated that the testing of foods with soft devices can serve as a means to incorporate the effects of the tissue deformability into textural evaluations. As pointed out by the authors, the test conditions, particularly with respect to deformation level and rate, should be as close as possible to those existing in sensory evaluations. Meaningful relationships between sensory and instrumental evaluation require, at least in principle, that the rheological properties of the sensory system be taken into account. It is reported, however, that the mathematical modelling of the sensory processes is very problematic.

A review on the relation between instrumental and sensory evaluation of the rheological and fracture properties of cheese showed that attributes like firmness, cohesiveness, graininess and springiness were correlated with instrumentally accessible parameters (Zoon, 1991). Adhesiveness and chewiness, however, showed no clear reported correlation with instrumental parameters to that date.

Jack *et al.* (1993a) investigated the relationship between rheology and composition of Cheddar cheeses and sensory texture perceived by consumers. Free choice profiling was chosen as the technique for the sensory evaluation, since it requires almost no training time and is cheaper to run than other tests. The research showed that analysis of compositional and instrumental data did not discriminate between 19 samples of cheese in the same way as consumers perceived texture. Therefore, correlation between sensory and instrumental-chemical analyses was found to be limited and of restricted predictive capability. Such lack of agreement could have been caused by the fact that instrumental and sensory tests measured different properties, as mentioned in their discussion. Besides, the kind of association sought, between objective and consumer response, is likely to produce different

outcomes to what would be achieved had a trained, expert panel been used. An example of that can be found in Moskowitz *et al.* (1979).

In another attempt to correlate sensory and instrumental data for Cheddar cheeses, Jack *et al.* (1993b) applied double compression (TPA), electromyography (EMG) and quantitative descriptive profiling to a range of commercial cheeses. Although it is reported that panellists were familiar with the product, little is mentioned about the extent of training they had. EMG traces of masticatory muscle activity were reported to be unique for each subject for different samples, which led to inconsistent correlation with compression results and with sensory responses. The measured Instron variables discriminated between the cheese samples and, according to the authors, were good "predictors" of sensory perception.

The same authors, in a subsequent study, analysed the textural changes perceived in Cheddar cheese during mastication (Jack *et al.*, 1994). Their objective was to develop appropriate sensory methodology to account for mastication, while considering the relationship of such methodology with force-deformation properties. Texture profiles for the different samples changed during chewing in progressive profiling testing, but it was observed that swallowing varied from after 6 chews to 19 chews, depending on the panellists. Instrumental measurements were obtained using double compression to 60% in cycling mode, and, as expected, proved to correlate poorly to sensory data. This study emphasises the fact that mastication is of great importance in relation to texture and its perception, as previously reported by Brown *et al.* (1996), Cardello & Segars (1989) and Christensen (1984).

Instrumental texture profile analysis results and sensory scores for reduced fat Cheddar cheeses correlated well with hardness ($r = 0.95$) and springiness ($r = 0.95$), as reported by Bryant *et al.* (1995). The Instron determination of adhesiveness correlated positively with sensory ratings ($r = 0.73$), but instrumental and sensory determinations for cohesiveness did not correlate ($r = -0.41$). The authors pointed out that uniform lubrication of compression plates and surface friction are critical factors for high correlation. Lubrication with mineral oil was used for the tests. Brennan &

Bourne (1994) reported that compression of foods in the mouth followed the nonlubricated pattern, even though lubrication was provided by saliva. Correlations between sensory and nonlubricated instrumental tests are not reported by Bryant *et al.* (1995).

Reggianito grating cheese was assessed for texture using sensory and instrumental measurements, with good correlations found between sensory results and parameters from a compression test (Hough *et al.*, 1996). Strain at the breaking point was found to be the instrumental parameter that best correlated to visual, manual and oral sensory textural attributes. The correlations were analysed using partial least squares regression. Moisture level in the cheese was considered in the correlation procedure and proved to be a useful parameter for prediction of sensory texture.

Wium *et al.* (1997) characterised Feta cheeses with different textures using uniaxial compression and observed that, among the four rheological parameters derived from the test, stress at fracture, modulus of deformability and work to fracture described the same type of information in the data set. Hencky strain at fracture described a different type of information by itself, as shown by the Principal Component Analysis results. Correlation of the rheological parameters with six sensory attributes, three oral (firmness, stickiness and crumbliness) and three nonoral (firmness, brittleness, spreadability) was reported. Firmness (oral and nonoral) was the sensory attribute best predicted by instrumental measurements, with stress at fracture being the best predictor at all deformation rates. Strain at fracture did not correlate well with data from sensory texture analysis. Combination of all four rheological parameters improved correlation coefficients slightly, but not enough to justify compromising the simplicity of the model with stress at fracture alone. Use of shear testing (frequency sweep, strain sweep and relaxation) to try and improve the predictive precision of the models showed that their contribution was only marginal and that, by themselves, these measurements were not very useful for sensory texture prediction (Wium & Qvist, 1998).

Two different varieties of cheese, one semi hard (Appenzeller) and one very hard (Parmiggiano Reggiano), were used for investigation of sensory

texture and instrumental (rheological and chemical) data in a European interlaboratory experiment (Noel *et al.*, 1998). Even though the results obtained can not be extrapolated to other cheese varieties, they showed that sensory textural attributes can be related to instrumental values when evaluated by partial least square regression. Firmness and friability correlated positively to strain at fracture and dry matter and negatively to the modulus of deformability. Elasticity and deformability correlated to the same parameters in the opposite direction, while dry matter appeared to correlate with adhesiveness. Dry matter, together with the force at peak height of 10% compression, was also found to be an influential objective variable for high correlation with sensory hardness, brittleness, cohesiveness and adhesiveness in several French cheeses (Antoniou *et al.*, 2000).

Pesenti & Luginbuhl (1999) used, among other methods, uniaxial compression and stress relaxation/creep tests to quantitatively assess cohesion in Gruyere-type cheeses of known sensory cohesiveness. Transient tests (relaxation and creep) seemed not to be as powerful as uniaxial compression and tension in distinguishing the two levels of cheese cohesiveness. Within the parameters obtained in uniaxial compression, stress at fracture, strain at fracture and work to fracture depended significantly ($\alpha < 0.01$) on the level of cohesion. Significance was not found, however, for the cohesion-dependency of the modulus of deformability (Young's modulus).

The results of a comprehensive work involving several natural and processed cheeses, evaluated instrumentally (TPA, frequency sweep and creep) and sensorially (in the hand and the mouth) were recently reported by Drake *et al.* (1999b). These authors observed that fundamental and empirical rheological techniques worked equally well in predicting sensory textural response. The use of frequency sweep or creep parameters alone, however, led to very poor prediction of sensory textural attributes in that study. Overall, mouth and hand firmness were the terms best predicted. In a previous study, hand and mouth evaluated sensory attributes correlated highly and worked equally well for differentiation of cheese texture (Drake *et al.*, 1999a). Drake *et al.* (1999b) also reported that instrumental measurements predicted sensory attributes of processed cheeses better than natural cheeses.

2.5.5. Perspectives for the future

Large deformations are known to relate better to the stresses and strains a food is subjected to during the eating process. On the other hand, it has been argued that during mastication, a considerable amount of sensory information is probably obtained well before fracture, allowing for the brain to reach conclusions about texture before gross structural failure.

Rheological testing at large deformations is usually expected to relate to sensory properties, especially when the test method mimics the way consumers appraise texture. The ability of fundamental non destructive small strain tests to predict sensory texture attributes of cheese, however, is yet unknown. Studies on sensory-instrumental correlation have begun to be published more frequently.

As it can be seen, controversial reports are found in the literature concerning those correlations. Some researchers found good correlation (Antoniou *et al.*, 2000; Drake *et al.*, 1999b; Stampanoni & Noble, 1991b; Bryant *et al.*, 1995; Wium *et al.*, 1997), whereas others found poor or no correlation at all (Lakhani *et al.*, 1991; Green *et al.*, 1985; Jack *et al.*, 1993). Therefore, extensive further research is required to establish the key factors for good correlation between instrumental and sensory determinations of textural attributes. Until good sensory-instrumental texture correlation can be established, assessment of product acceptability will continue to depend heavily on consumers' textural appraisal.

3.0. OBJECTIVES and EXPERIMENTAL APPROACH

The development of instrumental methodologies that allow sensory textural responses to be successfully predicted is an important goal for the dairy industry and the food industry as a whole (Moskowitz *et al.*, 1979; Skovgaard, 1995). Availability of such methodologies would result in valuable benefits in product development and quality control by replacement of routine sensory evaluation, usually a time consuming and expensive technique for textural appraisal, with instrumental analyses.

The main objective of the present research work was to study the existence of significant correlations between data from sensory, rheological and microstructural evaluation techniques using a model, controlled food system. The system of choice was a processed cheese analogue. In the event of significant correlations, the ability of the instrumental techniques and compositional data to satisfactorily model and predict individual sensory attributes was to be examined.

The objective of the initial experimental work encompassed the development of homogeneous, stable and reproducible model processed cheese analogues for correlation analysis as well as the determination of the most appropriate piece of equipment and processing parameters for the manufacture of such products. In addition, it was necessary that the specific tests, attributes and parameters within the frame of sensory, chemical, rheological and microstructural techniques be established. Information regarding the preliminary experimental work is presented in the appendix.

Having established equipment, processing conditions and evaluation techniques to be used, a range of textures was created for subsequent study. The different experimental cheeses were characterised by sensory, microstructural and rheological techniques and the quantitative information derived used for correlation and regression analysis.

4.0. MATERIALS and METHODS

4.1. Manufacturing equipment and cheese ingredients

Aspects regarding the definition of the most appropriate piece of equipment for the manufacture of the experimental cheeses as well as the processing conditions to be used are presented in section 9.0. (Appendix).

The experimental cheeses (processed cheese analogues) were manufactured using a 12-kg, pilot plant scale, twin-screw Blentech cooker (Blentech Corporation, Rohnert Park, CA, USA). Figures 7 and 8 show, respectively, an external and an internal view of the equipment used.

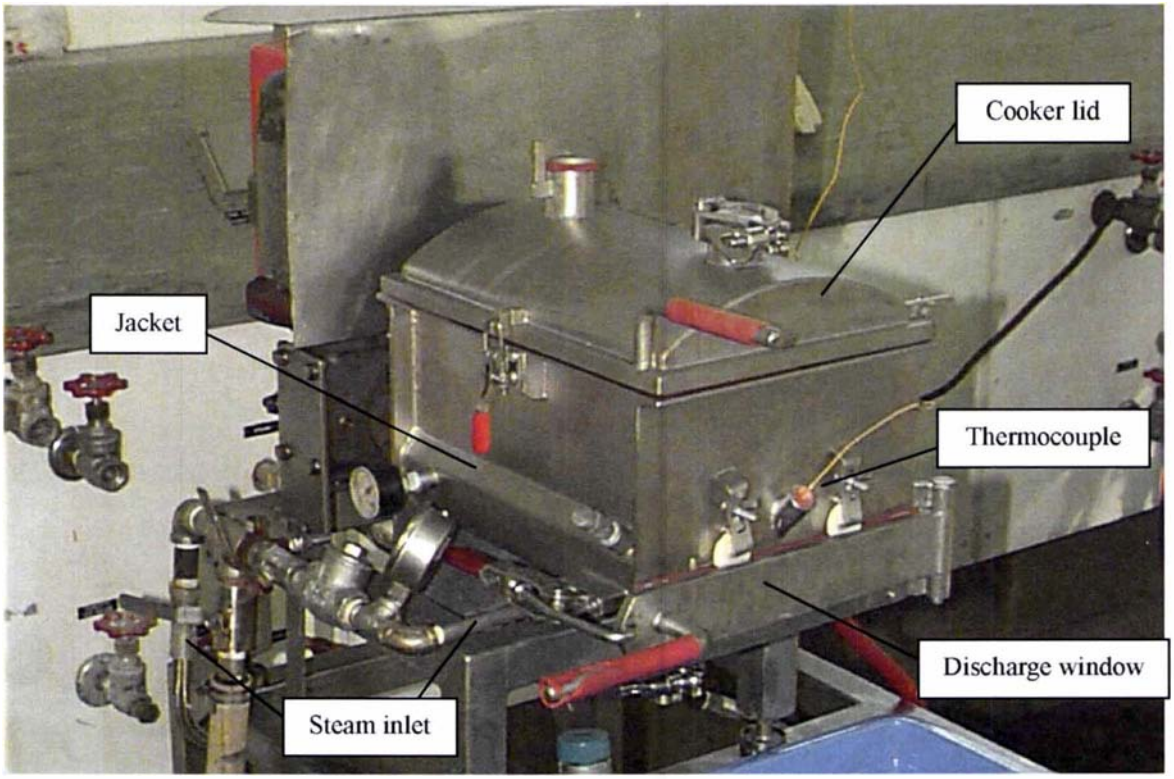


Figure 7. Blentech cooker (external view)

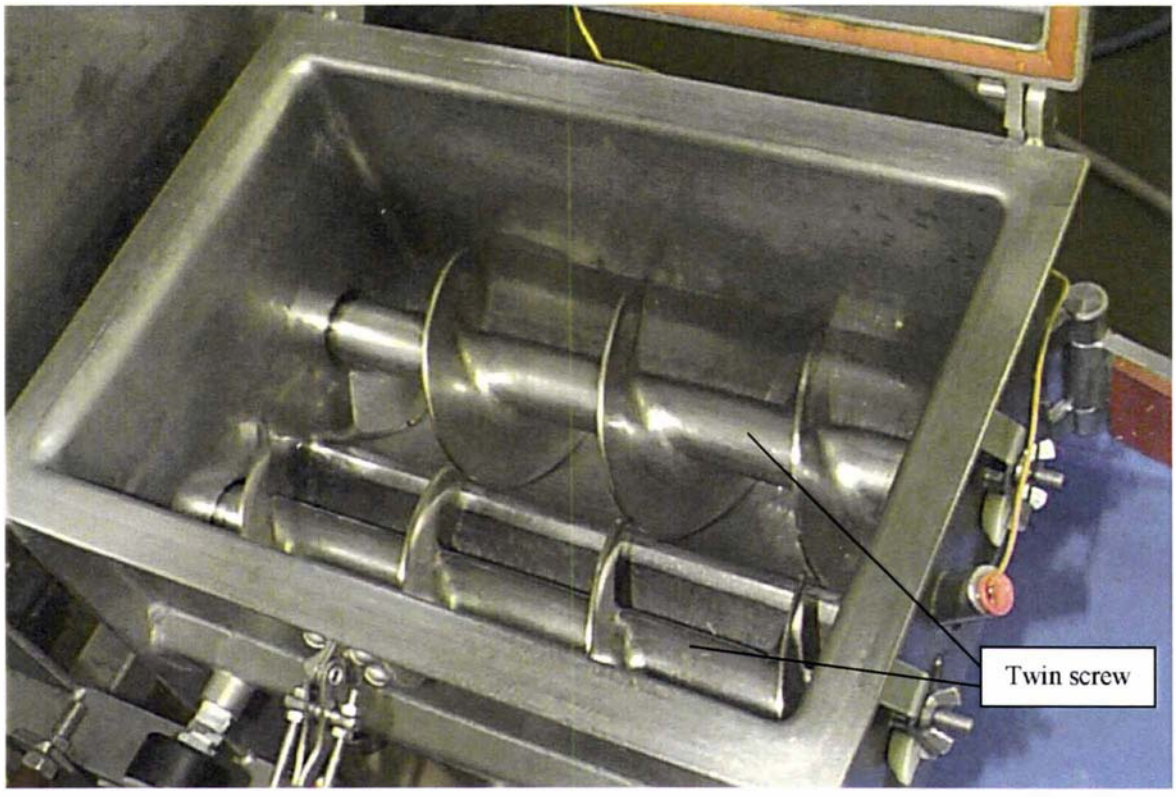


Figure 8. Blentech cooker (twin screw – internal view)

The ingredients used included rennet casein (Alaren 799 edible rennet casein 90 mesh – New Zealand Dairy Board (NZDB), Wellington, New Zealand), fresh frozen milkfat for recombining - FFMR (NZDB, Wellington, New Zealand), water, hydrochloric acid 5 M (Merck, Darmstadt, Germany), Vitraphos - sodium hexametaphosphate $(NaPO_3)_n$ (MedChem, Auckland, New Zealand) and water soluble annatto (New Zealand Dairy Meats, Auckland, New Zealand). The rennet casein and FFMR used were of the same type and the same manufacturer across the different experimental blocks.

Three different mixing speeds were used for cheese manufacture, namely 100, 127 and 155 rpm, chosen in view of the constraints of the equipment and formulations used. The amount of water used in the base formulation (cheeses 6 and 7, Figure 9) was used as a reference (level 0) for the changes in the moisture content of the other experimental cheeses (Table 2). The 'moisture' content includes water plus annatto (aqueous solution). In order to create a range of textures for study, the amount of water added to the

cheese formulations was increased by 5% and 10% (in weight) as well as decreased by 5% and 10% (in weight) in relation to the reference formulation.

4.2. Definition of the experimental design

The combination of the five moisture levels (represented by +10%, +5%, 0, -5% and –10% added water) with the three mixing speeds (100, 127 and 155 rpm) resulted in fifteen different experimental cheeses in a complete factorial (response surface) design. Due to limitations in batch size, production time available and others regarding steam supply to the pilot plant and maximum number of samples evaluated in a single sensory evaluation session, for example, not all fifteen cheeses were used in the final experimental design.

Because the main objective of the study was to correlate textural sensory and instrumental data of the experimental cheeses, it was important that samples of the same batches of each experimental cheese were evaluated chemically, rheologically, microstructurally and sensorially. The Blentech cooker used for cheese manufacture, despite having a capacity of 12 kg, produced batches no bigger than 4.5 kg, due to the only partially jacketed kettle (Figure 7). That meant that at least two batches of each experimental cheese had to be manufactured to provide enough material for all subsequent testing.

Manufacture of 30 batches plus calibration samples for the sensory panel in the time frame of 5 days (steam not available on weekends) was not feasible. In the process of reducing the number of experimental cheeses to allow for manufacture in the shortest time possible (to avoid the risk of day effect on the textural measurements), it was decided to keep the extreme samples, represented by cheeses 1 and 12 in Figure 9. These worked as anchors for the textural range chosen for evaluation. The experimental cheeses selected for examination can be seen in the diagrammatic representation of the final experimental design shown in Figure 9. The reference formulation was replicated in the design to provide for an even

number of experimental samples and also to allow for control of the reproducibility of the cheesemaking process.

The use of replication is known to improve the quality of correlation and regression procedures, as more data points allow for better fit of the predictive models yielded. This was included in the design in the form of different experimental blocks.

As a result, twelve different experimental cheeses were produced, in duplicates, per experimental block, in a partial response surface design (Neter *et al.*, 1985; Wells, 1976). Four experimental blocks were run, even though only three were used for correlation analysis. The first block was used as a dummy one for adjustments in the experimental techniques. The formulations used for these twelve experimental cheeses together with the mixing speed used for each of them can be found in Table 2.

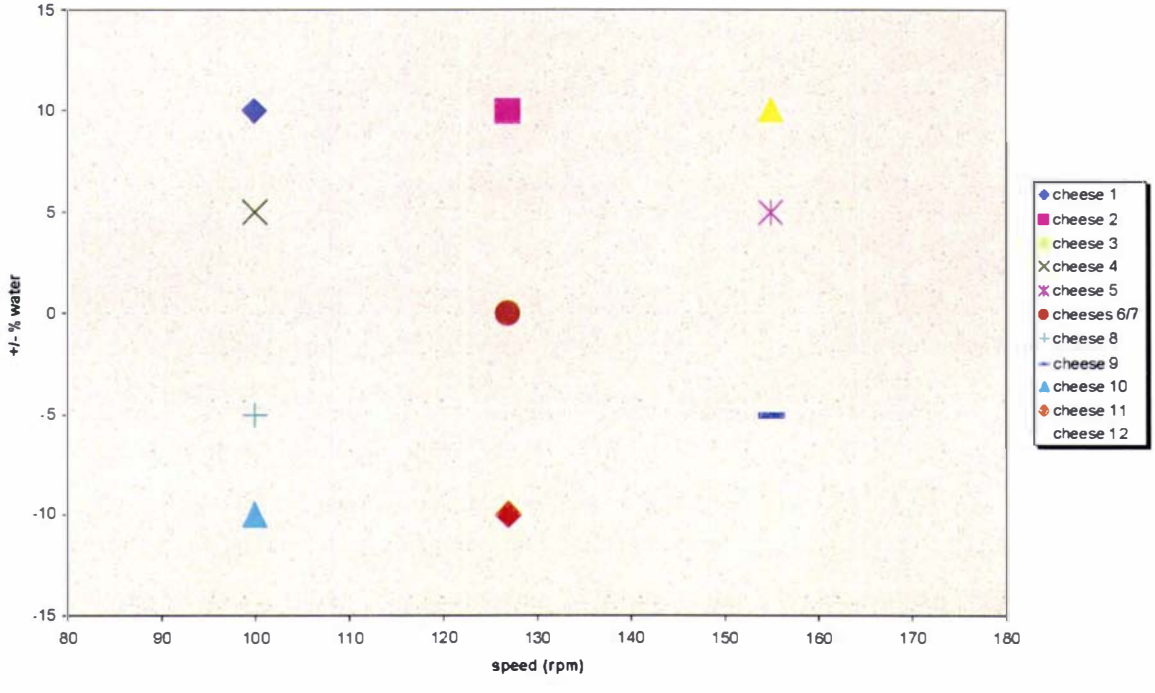


Figure 9. Experimental design used to generate the cheese samples

Table 2. Cheese formulations and mixing speeds used

INGREDIENTS \ CHEESES	RENNET CASEIN (G)	FAT (FFMR) (G)	WATER (G)	HCL 5M (G)	VITRAPHOS (G)	ANNATTO (G)	+/- % WATER	MIXING SPEED (RPM)
1	859	859	2347	50	93	7	*+10*	*100*
2	859	859	2347	50	93	7	*+10*	*127*
3	859	859	2347	50	93	7	*+10*	*155*
4	859	859	2240	50	93	7	*+5*	*100*
5	859	859	2240	50	93	7	*+5*	*155*
6	859	859	2130	50	93	10	*0*	*127*
7	859	859	2130	50	93	10	*0*	*127*
8	859	859	2020	50	93	13	*-5*	*100*
9	859	859	2020	50	93	13	*-5*	*155*
10	859	859	1913	50	93	13	*-10*	*100*
11	859	859	1913	50	93	13	*-10*	*127*
12	859	859	1913	50	93	13	*-10*	*155*

4.3. Cheese manufacture

The ingredients were placed in the cooker in the following order: water plus acid, casein, emulsifying salt and fat. Addition of the ingredients occurred in the course of 2 minutes, during which the cooker was operated at low mixing speed (56 rpm). After the initial 2 minutes, speed was increased to 127 rpm for a further 3-minute period before the cooker was set to the desired speed for cheese manufacture and heating started. Heating of the experimental cheeses was carried out by indirect steam injection, pressurised to 100 kPa, until the cheese temperature reached 86°C. The temperature of the cheeses was recorded throughout the whole manufacture process, at 1-minute intervals, with a thermocouple connected to an EXTECH 421307 thermometer (Gough Technology, Christchurch, New Zealand). The heating curve was kept constant for all experimental cheeses by means of adjustments in the pressure of the steam inside the jacketed cooker. The maximum temperature (86°C) was reached after 4.5 minutes (± 10 s), and kept

at this value for the remaining processing time. Total processing time, from heating to discharge, was 8 minutes.

The molten cheeses were discharged (Figure 10) and poured into 500 g plastic containers/trays (Carter Holt Harvey, Palmerston North, New Zealand) still at temperatures above 73°C (pasteurisation temperature)(Figure 11). The containers were sealed without headspace to avoid loss of moisture by condensation and the cheeses allowed to set on a metal bench, at room temperature (23-25°C), for a further 15 minutes before storage in a cold room at 3-4°C. This was done for all cheese batches, for all blocks, in an attempt to standardise the cooling regime for the experimental cheeses. These cheeses were manufactured in random order within each experimental block.

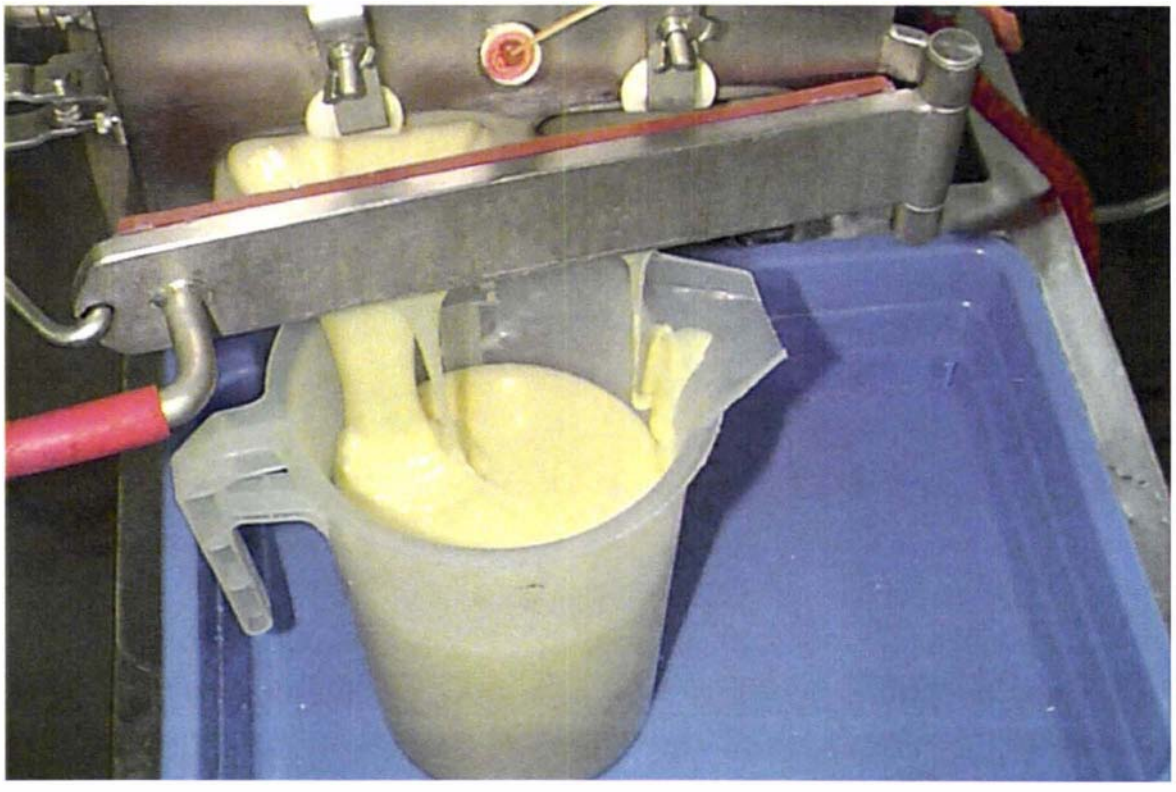


Figure 10. Discharge of molten cheese from Blentech cooker

Figure 11. Experimental processed cheese analogues in tray, set as a block (with addition of annatto)

The kettle was cleaned with caustic solution (NaOH 2% v/v) and hot water (90°C) between each manufacture run to eliminate the effect of rework on the subsequent cheese batches. Acid (HNO_3 2% v/v) and caustic (NaOH 2% v/v) hot cleaning (90°C) were done at the end of each manufacturing day.

Cheeses were stored for one week before chemical analysis (except for pH determinations) and for two weeks before sensory, microstructural and rheological evaluations were performed. These times were used to guarantee a stable product for assessment, as it is standard industry practice to allow a few days (usually three) after manufacture for the product to stabilise and salt hydrolysis/emulsification to cease (Sorley, personal communication).

4.4. Chemical analysis

The experimental cheeses were monitored daily for pH, from the day of manufacture, over a period of one week and every other day for the subsequent two weeks. Measurements were done in quadruplicate, covering different areas of the trays usually affected by slightly different cooling rates.

Moisture, protein and fat content were determined for all samples at one week of age. The samples were kept under refrigeration until immediately prior to the analyses. The chemicals used for the compositional analyses were all analytical grade (ANALAR).

1. Moisture: The moisture content of the experimental cheeses was determined by the oven method (AOAC, 1980). A sample of known weight was placed in a hot air oven and dried at 108°C for about 16 hours. After cooling in a desiccator for a period of 45 minutes, the dried samples were weighed and moisture calculated by subtraction. The samples were returned to the oven for 5 hours and weighed again after that period to confirm all the moisture had been evaporated. Three replicates of each experimental cheese were tested per experimental block.

2. Protein: Protein was determined using the macro-Kjeldahl method (AOAC, 1980). Samples of known weights were digested with concentrated sulphuric acid at 420°C for 1 hour using a Kjeltec 1007 digester (Tecator, Sweden) and subsequently distilled from an alkaline solution using a Kjeltec 1026 Distilling Unit (Tecator, Sweden). The ammonia that distils over was collected in a 2% boric acid solution and titrated directly with hydrochloric acid 0.1 N. Three replicates of each experimental cheese were tested per experimental block.

3. Fat: The Mojonnier gravimetric method was used for fat determination in the experimental cheeses (AOAC, 1980). It is based on the partition of the fat between an organic solvent and the aqueous phase. Three replicates of each experimental cheese were tested per experimental block.

4. pH: pH was determined using a PHM61 standard laboratory pH meter (Radiometer, Copenhagen, Denmark) with a Schott Gerate N6280, spear tip combination electrode (Schott Gerate, Mainz, Germany). Calibration of the electrode was performed daily before measurements, using ColourKey buffer solutions pH 7.00 (± 0.02) and pH 4.01 (± 0.02) (BDH, Poole, England). The electrode was cleaned once a week with a 2% w/v acid pepsin (Sigma Chemical Company, St Louis, USA) solution. Measurements were done by inserting the electrode membrane directly into the cheese blocks. Four measurements in each experimental cheese were done per experimental block, covering different parts of the tray. All measurements were performed at refrigeration temperature (5-6°C).

4.5. Microstructural analysis

The experimental cheeses were dyed using a 1% aqueous solution of Fast Green FCF (Merck, Darmstadt, Germany) and a 1% aqueous solution of Nile Blue (BDH, Poole, England). Fast Green and Nile Blue are specific dyes for protein and fat, respectively. Staining of the samples was done immediately after manufacture, during the filling process. Two trays of each experimental cheese per batch were dyed with 5 ml of the dye solutions. Cheeses were stored in a cold room at 3-4°C for 2 weeks until microstructural evaluation under confocal laser scanning microscopy (CLSM).

Preparation of the samples for microscopic analysis involved cryo-cutting. Small sub-samples of the dyed cheeses were frozen to -20°C and cut to 60 μm slices using a Leica Jung Frigocut 2800 E (Leica Instruments, Nussloch, Germany). The cut slices were assembled on glass slides.

Subsequent to the slides' preparation, the cheese samples were examined using a Leica DM RBE confocal laser scanning microscope LS 510 (Leica Lasertechnik, Heidelberg, Germany), with a Ar/Kr mixed gas laser source (Figure 12). A 10X (magnification 100 times) objective was used for block 1 and a 40X (magnification 400 times) oil immersion objective was used for the subsequent blocks.

Use of Nile Blue was discontinued after unsuccessful dying of the fat particles in the preliminary trial. The excitation wavelength used for Fast Green was 568 nm, with a DD (double dichroic) 488/568 beam splitter and LP 590 detector. For the lower magnification used (block 1), 4 images per sample were collected. For the higher magnification (blocks 2 and 3), 6 images per sample were obtained.

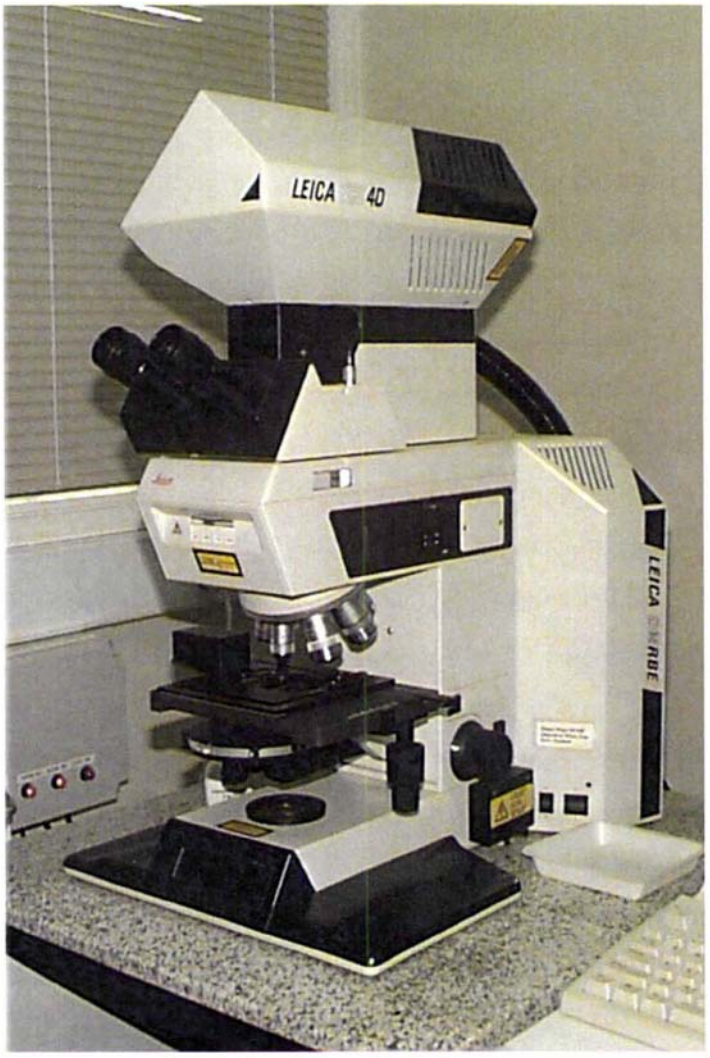


Figure 12. Confocal laser scanning microscope used for microstructural analysis

Calculation of the area occupied by the protein matrix in each scanned section was performed with the image analysis software ImageSpace (Molecular Dynamics, Sunnyvale, USA), in a SiliconGraphics INDY™ workstation (Silicon Graphics Inc., Mt. View, USA). An arbitrary threshold was established for selective removal of artefacts, air bubbles and fat globules. The thresholds varied between experimental blocks, due to differential intensity of dye diffusion into the protein matrix of the cheeses. These threshold ranges were 120-256 grey intensity levels for block 1, 130-256 for block 2 and 150-256 for block 3. The image analysis data were statistically interpreted using each of the three experimental blocks individually, due to the differences in magnifications and thresholds used. Issues regarding calibration references, assessment of precision and repeatability in image processing and analysis are discussed by Stanley *et al.* (1998).

4.6. Rheological evaluation

Rheological assessment of the experimental processed cheese analogues was performed using both small deformation in dynamic oscillatory testing (frequency sweep) and transient testing (creep test), as well as large deformation in compression (compression to 70%).

A. Compression test: Compression of the experimental cheeses to 70% of their original height was performed on a TA-XT2 Texture Analyser (Stable Micro Systems, Surrey, England), using a load cell 250 N (Figure 13). Cylindrical cheese samples for compression were obtained using a 21-mm diameter cork borer lubricated with vegetable oil in order not to cause fracture to the sample during cutting. Cheese plugs of aspect ratio (height:diameter) equal to unity were compressed between a 60-mm diameter acetal (DuPont, USA) upper plate and a square stainless steel bottom plate to 70% of the original height. A crosshead speed of 10 mm/s was used. Both sample surfaces in contact with the plates were lubricated with vegetable oil (canola oil) to overcome friction effects. Cheese temperatures during testing were monitored across the replicates and were in the range of 5-8°C. Collection of data was in the form of

force/time/displacement curves, using the software Texture Expert for Windows, version 1.19 (Stable Micro Systems, Surrey, England). Data were later processed with Excel 97 for Windows (Microsoft, Seattle, USA) and calculations performed to convert the results to true stress and Hencky strain values. Assumptions were made that the cheeses were incompressible materials and that the sample volume was kept constant during compression. Four replicates of each of the experimental samples were tested. Relevant equations for these calculations are

$$\sigma = F_t / \pi r_t^2 = (F_t / \pi r_0^2)(1 - vt/H_0)$$

$$\varepsilon_h = \ln (H_0 / H_t) = \ln (H_0 / [H_0 - vt])$$

where σ = stress from lubricated compression test (Pa)

F_t = force from lubricated compression at time t (N)

r_t = radius of the sample under compression at time t (m)

r_0 = initial radius of the sample (m)

v = speed of compression (m/s)

H_0 = initial sample height (m)

H_t = sample height at time t (m)

ε_h = Hencky strain

t = time (s)

π = 3.141593

Six rheological parameters were obtained from the true stress/Hencky strain curves. These were the initial slope (Young's modulus), the peak stress, the strain at the peak stress, the area under the curve up to the peak stress (work to peak stress), the area under the curve in compression, i.e., the downward movement of the crosshead (total work in compression) and area under the curve during the return of the crosshead to the initial position (total work in decompression). Values for these parameters were obtained using Microcal Origin version 3.5 (Microcal Software Inc., Northampton, MA, USA).

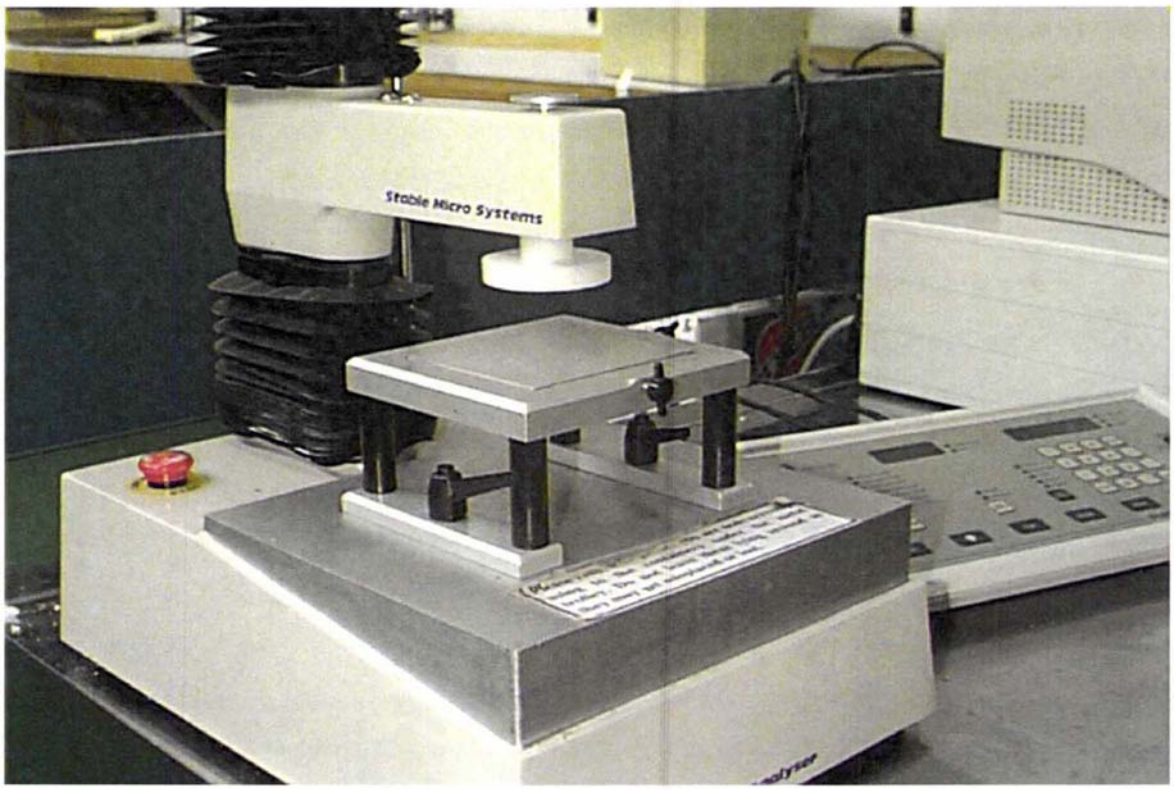


Figure 13. TA-XT2 used for the compression tests

B. Dynamic shear test (frequency sweep): The dynamic oscillatory measurements were performed on a stress controlled SR-5000 Rheometric Scientific rheometer (Rheometrics Inc., Piscataway, USA) fitted with a 25 mm parallel plate with a 2.4 mm gap. A Peltier heating element was used as the bottom plate and set to a temperature of 7°C. In order to prevent slippage, sandpaper was attached to both plates. A humidity cover/solvent trap was used to avoid moisture loss during the tests (Figure 14).

Blocks of each experimental cheese were initially sliced on a Berkel's slicing machine (Berkel & Parnall's MFG Co., Enfield, England) to 2.5 mm thick slices. These were stacked in airtight containers and kept in polystyrene chilly bins filled with ice until the testing. Prior to each run, disc-shaped samples of cheese, 25 mm in diameter, were prepared using a cork borer[c]. Each sample was left undisturbed for 10 minutes, upon compression between the two plates, to allow for sample relaxation before

the test started. Collection of data was performed using the software RSI Orchestrator, version 6.4.3. (Rheometric Scientific, Piscataway, USA).

Figure 14. Stress controlled rheometer SR-5000 (with humidity trap)

The frequency sweep experiments (0.1-22 Hz) were undertaken using a constant stress of 500 Pa (within the linear viscoelastic region). The actual temperature of each sample was measured at the end of each run and recorded. Parameters measured included the storage modulus (G'), the

loss modulus (G") and the loss tangent (tan δ). Four replicates of each experimental processed cheese analogue were assessed in each experimental block.

$$G' = (\sigma_0 / \gamma_0) \cos \delta$$

$$G'' = (\sigma_0 / \gamma_0) \sin \delta$$

$$\tan \delta = (G''/G')$$

where σ_0 = maximum stress

γ_0 = maximum deformation

δ = phase difference between the stress and the shear

(Figure 5, section 2.4, p.76)

C. Static transient test (creep compliance): These tests were also performed on a stress controlled SR-5000 Rheometric Scientific rheometer (Rheometrics Inc., Piscataway, USA) fitted with a 25 mm parallel plate with a 2.4 mm gap (Figure 14). The Peltier heating element was used as the bottom plate and set to a temperature of 7°C. Sandpaper was attached to both plates to prevent slippage. A humidity cover/solvent trap was also used to avoid moisture loss during the tests. Sample preparation was similar to that described for the frequency sweep tests.

Creep tests were undertaken using stresses of 2000 Pa and 3000 Pa for a period of 360 s. During this time, parameters measured were the creep compliance ($J_{(t)} = \gamma_{(t)}/\sigma_{constant}$) and the deformation ($\gamma_{(t)}$). Detailed definition of these parameters can be found in Whorlow (1992). At the end of the initial 360 s, the stress was released and deformation measured for a further 60-second period so that the extent of recovery could be assessed. The actual temperature of each sample was measured at the end of each run and recorded. Four replicates of each experimental cheese were assessed in each experimental block.

With regard to the selected rheological parameters for correlation analysis, the parameters from the frequency sweep curves used for correlation with the sensory textural attributes were the storage modulus (G') and the loss modulus (G") at a selected frequency value. Since the plots of G' and G" versus frequency were linear, values for those parameters at the frequency of 10 Hz were chosen for correlation purposes.

The creep compliance curves were fitted with the Burgers model (Steffe, 1996) shown in Figure 15 using the equation

$$J(t) = P1 + P2\ \{1 - \exp[-t/P3]) + t/P4$$

where J(t) = predicted compliance at time t

P1 = instantaneous compliance, consequence of bond stretching or rupture from the instantaneous stress applied

P2 = time dependent retarded elastic compliance

P3 = retardation time (μ_1 / G_1) of the Kelvin component

P4 = asymptotic Newtonian viscosity of the material (represented by the free dashpot in the mechanical model), result of the viscous flow of bond-free constituents during the test

t = time (in seconds)

Fitting of the model to the deformation curves was also done, but only over the initial 6 minutes, as the Burgers model does not apply to the creep recovery stage (recovery of the strain to the point of permanent deformation).

Both curves (compliance and deformation), after fitting was done, generated 8 rheological parameters (P1, P2, P3 and P4 for each curve), out of 9 in total, used for subsequent correlation. Another parameter used was the relative recovery of the samples five seconds after release of the applied stress (from the deformation curves). The short time (5 s) for the measurement of recovery was selected in order to match the time frame used in sensory evaluation for the scoring of the attribute rubberiness, as presented in section 4.7.

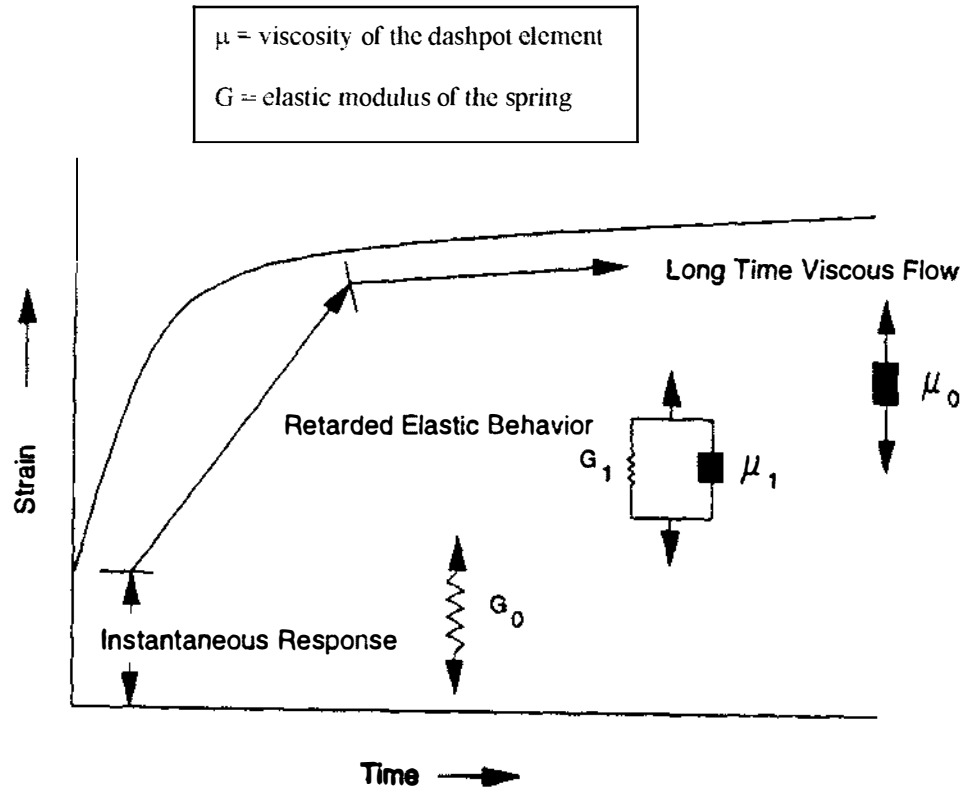


Figure 15. Typical creep curve showing where various elements of the Burgers model describe flow behaviour (Steffe, 1996)

4.7. Sensory evaluation

The ultimate goal of this research work was to find correlation between sensory, microstructural and rheological measurements of a specific model food product, namely processed cheese analogues. Rheological techniques are concerned with the measurement of the mechanical properties of a material, more related to texture. Likewise, microscopy techniques provide information on the organisation of the structural elements, which is related mostly to texture. Hence, the sensory characterisation of the experimental cheeses did not include flavour, more related to chemical composition and more complex in nature (Dravnieks, 1976), even though flavour release and perception is linked, to a significant extent, to the texture of the food material. Instead, this study was restricted to textural evaluation, more likely to physically relate to

those instrumental techniques. For that purpose, no cheese flavour was included in the formulations of the model cheese analogues.

A trained panel of 8-10 panellists was used to evaluate the sensory textural attributes of fracturability, firmness in compression, firmness in cutting, rubberiness, stickiness, curdiness and greasiness upon handling. These characteristics were assessed in the hand (Drake *et al.*, 1999a) and were determined after a sensory profiling session with the panellists. Mouth evaluated characteristics were used in the preliminary profiling session, but they were not used further due to oral fatigue of the panellists. All panellists were experienced in sensory evaluation of cheese texture. Based on the results reported by Chambers IV & Smith (1993), the extent of the panellists' testing experience was not verified. Prior to each evaluation session, reference samples representing the extremes among the experimental processed cheese analogues were used for calibration and familiarisation with the products to be evaluated and the testing conditions. The testing samples were presented in slices (40 mm x 100 mm), 10 mm thick, on trays lying on ice bags. The slices were prepared using a wire cheese cutter (Nemco model N55300A, Hicksville, USA). The attributes were assessed using different parts of the slices. The temperatures of the samples when served to the panellists were recorded. Evaluations were made using a 150-mm line scale labelled with anchors appropriate for each sensory characteristic. Definitions for the characteristics evaluated are:

- Fracturability: extent to which a cheese slice (1 cm thick, 9 cm long) can be bent between the thumb and the index and middle fingers, until the ends touch, without breaking.
  low score = does not bend much/easy to fracture
  high score = hard to fracture

- Firmness in compression: amount of resistance to compression offered by a 1 cm thick slice of cheese, when pushed between the thumb and index finger, until fingers touch each other (force required to deform the cheese structure).
  low score = less firm
  high score = very firm

- Firmness in cutting: force required to cut through a 1 cm thick slice of cheese with a knife (pushed down on an angular, guillotine-like movement, from tip to full length of the knife).
  low score = less firm to cut
  high score = very firm to cut

- Stickiness: extent to which the cheese samples stick to the fingers (thumb and index finger) when compressed until fingers touch each other (residual on fingers).
  low score = not sticky
  high score = very sticky

- Rubberiness: amount of recovery to the original shape, after 2-3 seconds, after slightly compressing the sample to form a visible dent.
  low score = less rubbery
  high score = very rubbery

- Curdiness: extent to which the original sample produces curdy lumps after being kneaded 7 times between thumb and index and middle finger
  low score = smooth, not curdy
  high score = curdy

- Greasiness during handling: degree of slipperiness on the top surface of the sample when rubbed with the thumb across the surface (how slippery it feels during rubbing).
  low score = not greasy
  high score = greasy

All twelve cheeses were randomly presented to the panellists at each session and evaluated in three test sessions (3 replications) held on separate days, per experimental block. On each test day, the presentation order for all twelve cheeses was randomly assigned by computer (CSA version 4.2, Compusense, Inc., Guelph, Canada) to the panellists. Reproducibility within a

session was monitored by inclusion of 4 repeats from within the twelve cheeses on each test day. Panellists were presented with 4 sets of 4 samples in a complete block partial presentation design in each session. No break was allowed between each test set, as all characteristics were hand evaluated and oral fatigue was not of concern (Drake *et al.*, 1999a,b).

4.8. Statistical analysis

The statistical analysis of the data was performed using MINITAB® release 12.2.3 statistical software (Minitab Inc, State College, PA/USA) and SAS version 6.12 (SAS Institute Inc, Cary, NC/USA) statistical software. The latter was used for canonical correlation analysis only. The chemical, rheological, microstructural and sensory data were independently statistically analysed using analysis of covariance (Cliff, 1987), analysis of variance (Kleinbaum & Kupper, 1978; Neter *et al.*, 1985) and Tukey's HSD significance test. In addition, response surface regression analysis (Neter *et al.*, 1985; Wells, 1976) was performed on these sets of data. For the rheological and sensory data sets, the effect of testing temperature on the results was assessed using analysis of covariance (ANCOVA). Adjustments in the data were performed, when and where necessary, and the three different experimental blocks averaged and reanalysed using analysis of variance (ANOVA). The microstructural results across the blocks were not averaged due to differences in the magnification and threshold used for image analysis. Statistical analysis, in this case, was conducted using each experimental block separately.

Chemical results

Chemical results were analysed by ANOVA to determine whether significant differences existed between the twelve experimental cheeses. A Tukey's HSD test (α = 5%) was used to indicate where there were significant differences. Correlation of the chemical data with the sensory scores was done using different correlation and regression techniques. For the canonical correlation technique, the chemical parameters were combined with the rheological ones prior to the statistical procedure.

Microstructural results

The results (area occupied by the protein matrix) obtained from the image analysis were analysed by ANOVA and Tukey's HSD test to ascertain the existence of significant differences between the several experimental cheeses and where they occurred. A response surface regression analysis (Minitab) was performed in an attempt to model the variable "area" as a function of moisture content and mixing speed. The calculated areas, for each cheese, in each experimental block, were correlated to the sensory scores and the rheological parameters in the corresponding experimental block. Pairwise correlation (MacFie & Hedderley, 1993; Neter *et al.*, 1985) was used.

Rheological results

The rheological parameters generated by the three different tests performed (frequency sweep, creep and compression to 70%) were analysed by analysis of covariance to ascertain the significance of temperature on the values, across the different cheeses and experimental blocks. The assumptions of homogeneity of variances and normality of residuals were tested for all parameters. For those for which the assumptions did not hold, appropriate data transformation was done and the transformed data reanalysed. A Tukey's HSD test was used to detect where the significant differences between cheeses were.

The response surface regression analysis was performed on the back-transformed data to try and model the several rheological parameters as a function of moisture content and mixing speed. Correlation of the rheological data with the sensory scores was performed to find satisfactory prediction models for individual sensory attributes.

Sensory results

The statistical analysis of the sensory data was performed in two stages. Initially, an analysis of covariance was done so that the effect of temperature on the sensory scores for the several attributes under study could be assessed. This analysis used as the error term the pooled duplicates plus the interactions of blocks/sessions/cheeses with judges (panellists) to estimate the noise of the panel over experimental blocks, sessions and duplicates.

The assumptions of homogeneity of variances and normality of residuals were tested for all attributes (Hair *et al.*, 1998). For those for which the assumptions did not hold, weighted least squares (WLS) regression (Myers, 1990) was considered as an alternative to overcome the problem. The technique was applied using as weight the inverse of the estimate of variance (standard deviation^2) within each of the three individual sessions to evaluate the 'samples' (cheeses) from an experimental block. Each of the 108 (3 blocks x 12 cheeses x 3 sessions) individual estimates (stdev^2) was calculated using the data from between the 7-9 panellists present at the session.

In the analysis of covariance, the factor 'samples', representing the experimental cheeses, were tested for significant differences over and above the noises inherent to the judges (panellists) over experimental blocks, sessions and duplicates.

For those attributes in which temperature was found to be a significant covariate, the sensory scores were adjusted for temperature effect and the adjusted values reanalysed by ANOVA, in a second stage, to ascertain the existence of significant differences between 'samples' (cheeses) over and above the experimental error. Suspected outliers among the sensory results were checked according to the outlier test described by Sachs (1984) and those points that failed the test were set to missing.

Response surface regression analysis (Wells, 1976) was performed to model the sensory response as a function of moisture content and mixing speed. Correlation of the sensory response with the chemical and rheological results, both as individual data sets and combined as a single set of instrumental data, was performed.

Correlation Analysis

The correlation analysis between the sensory textural attributes and the chemical and rheological data was done in four stages. Initially, pairwise correlation was performed, in which each individual sensory attribute was correlated with each individual chemical and rheological parameter (MacFie &

Hedderley, 1993; Neter *et al.*, 1985). MINITAB® release 12.2.3 (Minitab Inc, State College, PA/USA) was used for this analysis.

Subsequently, stepwise regression was used to try and find the best single instrumental parameter, if available, that could model individual sensory response (Kleinbaum & Kupper, 1978; Neter *et al.*, 1985). For those sensory attributes that could not be modelled efficiently with a single rheological or chemical parameter, the regression analysis (stepwise estimation) proceeded to add terms or parameters to the model equation in order to improve the quality of the fit. Additional independent parameters are selected in terms of the incremental explanatory power they can add to the regression model (Hair *et al.*, 1998). MINITAB® release 12.2.3 (Minitab Inc, State College, PA/USA) was used for this regression analysis.

In a third stage, sensory attributes, rheological parameters and chemical parameters were subjected to principal component analysis (Giri, 1996; Hair *et al.*, 1998; Johnson & Wichern, 1992) and regression (Dijksterhuis, 1995; MacFie & Hedderley, 1993). Individual sensory attributes, as well as sensory principal component 1 (PC1), were correlated with rheological PC1 and chemical PC1. The principal components correlation, like the pairwise correlation and stepwise regression, was performed using MINITAB® release 12.2.3 (Minitab Inc, State College, PA/USA).

Finally, canonical correlation (Cliff, 1987; Hair *et al.*, 1998; MacFie & Hedderley, 1993) was used to establish the best possible correlation between sensory and instrumental evaluation of texture. Individual sensory attributes were correlated with rheological canonical variables and, subsequently, sensory canonical variables were correlated with rheological canonical variables. In order to verify any improvement in the precision of the predictive models, the rheological and chemical parameters were combined and new canonical correlation performed for individual sensory attributes. SAS version 6.12 (SAS Institute Inc, Cary, NC/USA) was used for the canonical correlation procedure.

With regards to the microstructural information, the impossibility of combining the three experimental blocks limited the use of the data in

correlation analysis. Pairwise correlation, however, was done against sensory attributes and chemical and rheological parameters, across each experimental block, as previously described. The procedure was run using MINITAB® (Minitab Inc, State College, PA/USA).

5.0. RESULTS and DISCUSSION

The development of the model systems used in this research project was an important part of the experimental work. Different pieces of equipment were investigated to determine which was able to produce consistently reproducible products. Likewise, different compositional and processing parameters were explored in order to assess those that generated the widest range of textures for subsequent evaluation using sensory, microstructural and rheological techniques. Those findings are presented in the Appendix.

A brief discussion is presented here regarding the cheesemaking process, followed by the chemical, microstructural, sensory and rheological assessment of the different formulations used. A final section is presented regarding the correlation between the various instrumental techniques and sensory evaluation of textural attributes.

5.1. Cheesemaking

Apart from the different mixing speeds used, all other processing parameters that usually affect the quality of processed cheeses (mixing time, cooking temperature, rate of heating, use of rework, applied vacuum, type of emulsifying salts used and cooling time) were kept constant for the different experimental cheeses. This was done in order to minimise and control the sources of textural variability.

It is known that all the factors mentioned above can influence the textural properties of the final processed cheese products (Caric & Kalab, 1993; Sorley, 1997). Mixing time, cooking temperature, rework and type of emulsifying salts were used in the preliminary phase of the experimental work to try and generate the range of textures for study, but most of them showed practical limitations that resulted in them not being used further. Hence, in

this research project, only mixing speed (3 levels) and moisture content (5 levels) were used to generate the textural range for subsequent investigation with sensory and instrumental techniques.

It was observed during the preliminary runs that an almost linear pattern of colour existed between the cheeses. A gradual change of colour, from yellow to white, was seen from sample 1 through to sample 12. Cheeses with higher moisture content (cheeses 1 to 3, Figure 9) were yellow, possibly derived from the dispersion of the milk fat, i.e., the fat globule size distribution in the formulation. Those cheeses presented relatively large fat globules, regardless of the mixing speed used in the manufacture (Figure 16).

The fat globules decreased in size as the moisture content was reduced. Mixing speed, as a processing parameter (Figure 9), has not influenced the final colour of the products as much as moisture content. This is shown by the micrographs from the confocal microscope in Figures 16 and 17. In these figures, the protein matrix appears in bright yellow colour while the fat globules, together with some air bubbles, appear as dark, round structures.

Cheese 4, with less moisture and smaller fat globules than cheeses 1 to 3 (Figure 16), displayed a lighter colour than the latter under visual examination. Cheeses with lower moisture (cheeses 8 to 12), on the other hand, were visibly whiter and pale. Intermediate cheeses showed intermediate colours between these two groups.

Sorley (1997) reported a decrease in colour intensity in processed cheeses products subjected to high speed/shear pumps and extensive pipework during transport of the molten mass. That was attributed to the homogenising effect (reduction of fat globule size) of those pumps, which added to the effects of emulsification. Different mixing speeds used in this experimental work seemed not to be able to generate significant globule size differentiation within each moisture level (cheeses 1-3 and 10-12). The results obtained regarding the colour intensity of the final products, however, seem to be in agreement with the observations from Sorley (1997).

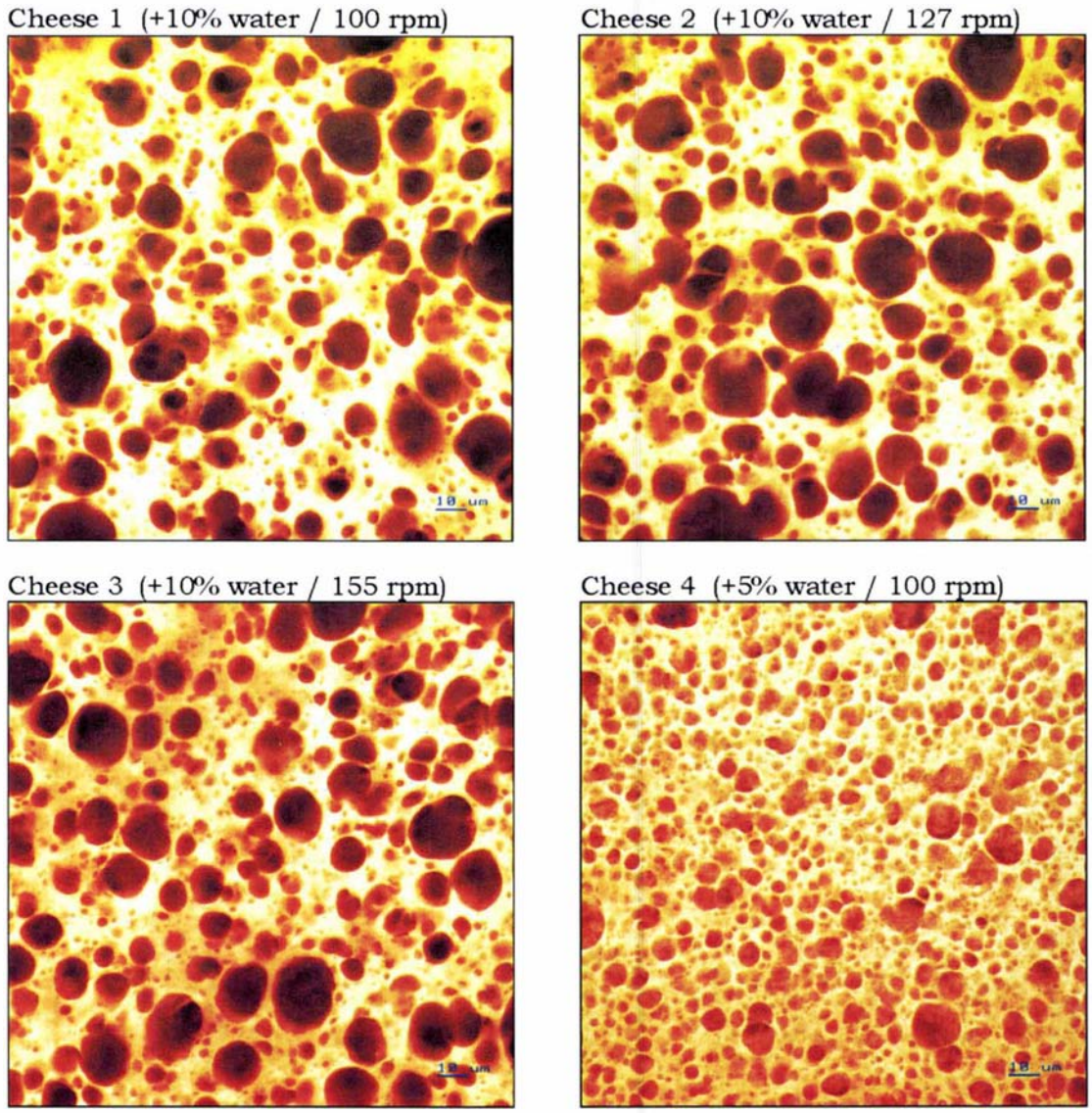


Figure 16. Confocal micrographs of experimental cheeses 1 to 4, with higher moisture content and larger fat globules (magnification 400 X)

Tentative observation of the experimental cheeses in a sensory evaluation session, under red light, showed that the colour differences could not be entirely masked with the light and would potentially be used by panellists as a cue during their evaluation of the products. This, in turn, is likely to generate biased sensory responses, difficult to be objectively analysed and interpreted.

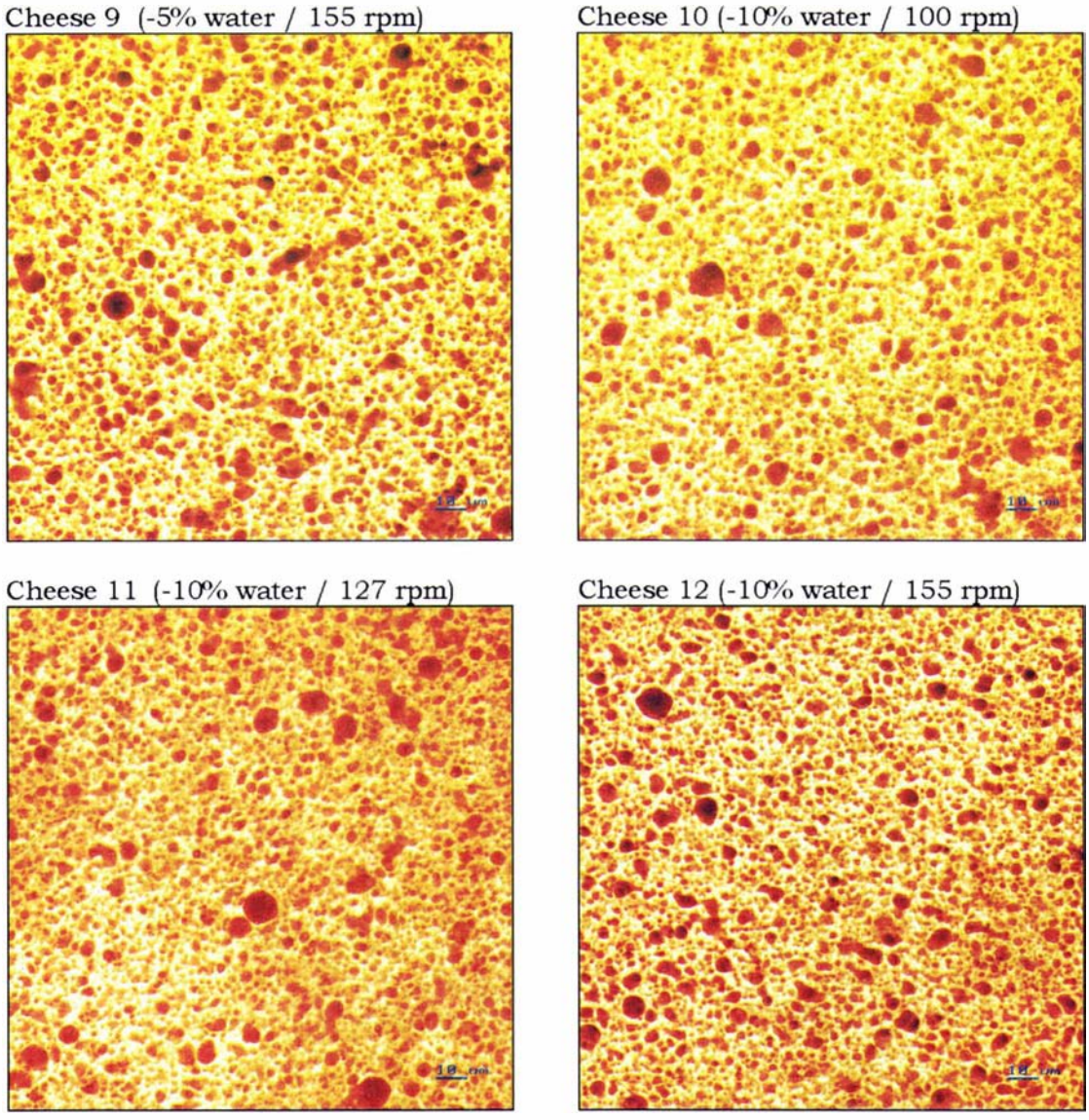


Figure 17. Confocal micrographs of experimental cheeses 9 to 12, with lower moisture content and smaller fat globules (magnification 400 X)

The use of colouring (annatto) is a standard industrial practice and was introduced as an alternative to eliminate that problem. Since the final colour of the product was the result of several factors combined (mixing speed, amount of annatto added, cooking temperature, cooling rate, pH and moisture content), exact matching of the colour of the twelve experimental cheeses was not possible. The use of the colouring agent was retained, however, as it proved useful in breaking the colour sequence across cheeses. Colour changes

between the twelve experimental cheeses with annatto were more discreet and not linear, i.e., no clear trend from cheeses 1 to 12 could be detected.

All cheeses had their heating curves monitored during the 8 minutes of processing. These curves were kept the same for the various experimental cheeses, as described in section 3.0. The maximum temperature of processing (86°C) was reached at about 4.5 minutes (± 10 s) for all cheeses, allowing for at least 3 minutes of further mixing at constant temperature. Caric & Kalab (1993) reported results from a study of Kostermeyer & Buchheim (1988) showing that 4 minutes of processing was not enough for creaming to occur. According to those researchers, optimal creaming and production of a uniform protein matrix was only obtained with a 9-minute creaming time. In this study, processing beyond 8 minutes caused the samples with low moisture content and high mixing speed (samples 11 and 12) to become very thick and viscous. They were difficult to discharge from the cooker and pour into the plastic containers/trays. Processing for less than 8 minutes led to insufficient hydration of the casein and emulsification of the fat for some of the experimental cheeses (samples 1 to 5), as seen in the preliminary runs.

Pre-mixing (3 minutes) of the ingredients before heating was used to allow for hydration of the rennet casein prior to cooking and emulsification. The presence of emulsifying salts is required during the process of hydration in order to decrease the extent of calcium phosphate cross-linking in the casein and to enhance the emulsifying ability of the protein.

It has been reported that significant variations in hydration times and solution viscosity after hydration exist between rennet casein from different manufacturers and even different batches from the same manufacturer (Ennis & Mulvihill, 1996; Ennis et al., 1998). In this study, all the experimental cheeses within each block of the experimental design were manufactured using the same 25-kg bag of rennet casein. Even though care was taken to use rennet casein bags of the same type and from the same manufacturer across blocks, a new bag was used for each of the three experimental blocks to account for the possibility of variation in the hydration times across bags.

At discharge of the cheeses from the cooker, some lumps of undissolved casein could still be seen in the molten cheese mass, especially in the cheeses with higher moisture content (samples 1, 2, 3). The glassy-looking lumps are described in the literature as "fish eyes" (Aimutis, 1995) and are described as a common occurrence when the casein has undergone fast or insufficient, poor hydration.

For analogue processed cheeses with high moisture content, such as those represented by cheeses 1, 2 and 3, the speed of mixing seems to be a determinant factor in the occurrence of "fish eyes". All three cheeses had similar moisture content in their formulation, but the high mixing speed used for cheese 3 contributed more lumps to this sample when compared to cheeses 1 and 2. This is an indication that, in dealing with high moisture formulations, less intensive mechanical energy input is crucial to allow slower and better casein hydration. This is in agreement with observations reported by Aimutis (1995). Cheeses 10, 11 and 12, all with lower moisture content, showed almost no presence of casein lumps, regardless of the mixing speed.

The presence of "fish eyes" in the experimental cheeses could have been used as a cue to the panellists in the sensory evaluation sessions. Because their presence in the cheeses could not be avoided or masked, the samples provided for training and panel calibration were selected in such way as to show the panellists that the "fish eyes" could be equally present or absent in any cheese of the range studied.

Vacuum is usually utilised in commercial processed cheese manufacture to help control the final moisture content of the product and also to prevent the presence of air trapped in the molten mass. It could not, however, be applied to the experimental cheeses because the piece of equipment used (pilot plant scale Blentech) did not allow for the use of vacuum. In view of that, the presence of air bubbles was evident in all cheeses, being smaller in size in those that had higher moisture levels.

Cheeses with high moisture content (cheeses 1, 2, 3) had lower viscosity immediately after manufacture (molten mass), which enabled part of the trapped air to migrate to the surface of the tray after filling. Consequently, the

centre of the cheese blocks appeared smoother and less porous than the surface of the corresponding blocks under visual examination. In Cheeses 10, 11 and 12, all more viscous, the presence of the bubbles was uniform throughout the cheese block. These bubbles, smaller in size than the ones observed in the high moisture cheeses, could still be observed with the naked eye and provided these experimental cheeses with a porous structure resembling high density foam.

Samples prepared for sensory evaluation were consistently taken from the centre of the cheese block, after trimming, so that the higher concentration of air bubbles on the surface of the trays were not used as a cue to the panellists scores.

5.2. Chemical evaluation

The effect of chemical parameters such as moisture content, usually expressed as moisture in the non fat substance (MNFS), fat and pH of the curd on the textural properties of cheeses has been reported in the literature (Lawrence *et al.*, 1983; Creamer *et al.*, 1988; Olson & Johnson, 1990). MNFS is in essence a ratio of water to protein. In this study, the moisture content of a singular processed cheese analogue formulation was manipulated, together with one processing parameter (mixing speed), to create the range of textures for subsequent sensory and instrumental evaluation. The composition of the different experimental cheeses in terms of total moisture, MNFS, total protein and fat content is shown in Table 3.

The twelve experimental cheeses can be divided in 5 different groups based on their compositional analysis (Table 3). These compositional differences are in line with the expected differences from the formulations used (Table 2) and resulted in cheeses that were clearly dissimilar in textural attributes, especially firmness and stickiness, under preliminary tactile examination.

Since the type and amount of emulsifying salt as well as the amount of acid added to each experimental cheese were the same, the observed differences in pH values between the twelve experimental cheeses resulted basically from their different moisture contents (dilution factor). This can be observed in Table 4, which shows that cheeses with similar moisture content had similar, not significantly different pH values (α = 95%).

Cheeses with high moisture content and pH around 5.70 (cheeses 1, 2, 3) were in general softer and stickier, while those with lower moisture and pH values, around 5.60 (cheeses 10, 11, 12), were firmer and easier to handle. The decrease in firmness caused by the increase in the moisture content of the cheeses was expected. It occurs due to the greater hydration and consequent weakening of the casein network. Similar behaviour was reported by Taranto *et al.* (1979), Lawrence *et al.* (1983) and Tunick *et al.* (1993) for natural cheeses and by Fox *et al.* (1996) for processed cheese products.

Table 3. Total moisture, MNFS, total protein and fat content (in %) of the experimental processed cheeses analogues (experimental values) and corresponding *p*-values[1]

CHEESE	MOISTURE (%)	MNFS (%)	PROTEIN (%)	FAT
1	58.02 [a 2]	69.69 [a]	16.63 [a]	16.75 [a]
2	58.00 [a]	69.64 [a]	16.66 [a]	16.72 [a]
3	58.02 [a]	69.70 [a]	16.68 [a]	16.75 [a]
4	56.81 [b]	68.63 [b]	17.31 [b]	17.23 [b]
5	56.77 [b]	68.60 [b]	17.34 [b]	17.24 [b]
6	55.69 [c]	67.72 [c]	17.75 [c]	17.77 [c]
7	55.66 [c]	67.67 [c]	17.70 [c]	17.75 [c]
8	54.68 [d]	66.90 [d]	18.25 [d]	18.26 [d]
9	54.49 [d]	66.67 [d]	18.28 [d]	18.27 [d]
10	53.35 [e]	65.62 [e]	18.68 [e]	18.71 [e]
11	53.34 [e]	65.65 [e]	18.69 [e]	18.75 [e]
12	53.28 [e]	65.57 [e]	18.71 [e]	18.75 [e]
p-value [1]	**0.0001**	**0.0001**	**0.0001**	**0.0001**

[1] probability associated with the F-test of factor "sample"

[2] means within a column with no common letters differ significantly ($p<0.05$, n=9)

The pH of the different experimental cheeses was followed daily for the first week after manufacture and at two-day intervals for the subsequent 2 weeks, so that the changes of pH values over time could be assessed. Care was taken to guarantee that the rheological, microstructural and sensory evaluations of the samples were performed after the pH values had stabilised. It was observed that stabilisation was achieved after one week of manufacture, when the variability of readings within a cheese block was considerably reduced (raw data not shown). No major differences in the pH values were noticeable between days 14 and 21, supporting the decision to run the textural evaluation on two week old cheeses.

Table 4. Values of pH at days 1, 3, 7, 14 and 21 after manufacture for the experimental processed cheese analogues and corresponding *p*-values[1]

CHEESE	pH DAY 1	pH DAY 3	pH DAY 7	pH DAY 14	pH DAY 21
1	5.69 a 2	5.68 a	5.68 a	5.67 a	5.67 a
2	5.68 ab	5.68 a	5.68 a	5.67 a	5.67 a
3	5.69 a	5.68 a	5.68 a	5.68 a	5.68 a
4	5.65 cd	5.65 bc	5.64 b	5.64 bc	5.63 bc
5	5.66 bc	5.65 ab	5.64 b	5.64 bc	5.64 bc
6	5.64 cde	5.64 bcd	5.63 bc	5.62 cd	5.62 cd
7	5.64 cdef	5.63 bcd	5.60 bc	5.62 cd	5.62 cd
8	5.62 def	5.62 cd	5.61 c	5.60 d	5.60 d
9	5.61 ef	5.61 d	5.60 c	5.60 d	5.60 d
10	5.61 f	5.61 d	5.60 c	5.59 d	5.60 d
11	5.61 f	5.61 d	5.60 c	5.60 d	5.60 d
12	5.61 f	5.61 d	5.60 c	5.60 d	5.60 d
p-values [1]	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**

[1] probability associated with the F-test of factor "sample"

[2] means within a column with no common letters differ significantly ($p<0.05$, $n=12$)

The pH values for each experimental cheese were obtained from several readings across the trays (cheese blocks). This was done to account for the variability between the centre of the cheese block (usually with slightly lower pH) and the extremities of the block (usually higher readings for pH). The pH gradient from the outside to the interior of a cheese block is known to result in a change of consistency across the product (Noomen, 1977). Such a gradient, in a product like processed cheese analogue, is likely to be the result of different cooling regimes at different parts of the cheese blocks, where the centre cools more slowly than the borders/extremities.

It was noted in this study that the variability of readings within a tray was much less ($\approx$ 0.01) among those experimental cheeses with lower moisture content, during the first week of measurement, in comparison to the cheeses with high moisture content ($\approx$ 0.03). Despite the slightly larger variation for the high moisture cheeses, it is assumed the value was not large enough to cause the properties of one cheese to be different between the centre and edges.

The importance of pH as a determinant of texture in natural cheeses such as Cheddar, Gouda, Mozzarella and Colby has been described in the literature (Schultz, 1952; Lawrence *et al.*, 1983; Creamer *et al.*, 1990; Ramkumar, 1997). In processed cheese manufacture, pH is an important factor during the cooking process and plays a major role in the final texture of the product (Karahadian, 1984; Shimp, 1985). The calcium sequestration efficiency of the emulsifying salts is strongly influenced by pH. Likewise, pH has significant effect on protein configuration, solubility and hydration/emulsification capability.

The production of a stable processed cheese product relies on the dissociation of individual calcium binding groups in the *para*-casein network, which is pH dependent. Thus, the effect of the emulsifying salts as ion exchangers is also pH dependent, leading to textural characteristics that can range from very soft cheeses (high pH values, close to 6.0) to dry, crumbly cheeses, when the product pH is low, around 5.2 (Guinee, 1987).

The range of pH measured for the experimental cheeses in this study was well within the usual values recommended for processed cheese blocks (5.4 to 5.7), as described by Berger *et al.* (1989) and Caric & Kalab (1993). It is difficult to assess, with the used experimental design, the extent of the role of pH itself on the textural differences between the experimental cheeses, but it is known to affect protein-protein and protein-moisture interactions. It is assumed that the near 0.1 difference in pH measured between the extreme samples, cheeses 1 and 12, could have only marginally added to the moisture and fat globule size effects in yielding cheeses that were soft and sticky (samples 1, 2, 3) at one end and firmer/only slightly sticky (samples 10, 11, 12) at the other end. Further experimental work would be required to verify this assumption.

An increase in fat content, like moisture, tends to produce processed cheeses that are softer and more plastic (Olson *et al.*, 1996; Stampanoni & Noble, 1991b; Subramanian & Gunasekaran, 1997). Such an effect of fat content was also reported for Cheddar cheese (Guinee *et al.*, 2000). This is due to the fact that the fat globules disrupt the continuity of the protein matrix, providing less resistance for one plane of the product to slide over the other when the sample is subjected to external stress (either shear or compression). The disruptive effect of the fat droplets on the protein network had been reported by Jost *et al.* (1986) and was confirmed by Kim *et al.* (1996) in a study with emulsion gels. It is a particularly significant effect in poorly emulsified systems, where the fat globules are large and the structural role of this component is great.

In a perfectly emulsified system, the fat globules are small and almost lost in the protein network, which forms a structure of its own (Kim *et al.*, 1996; Shimp, 1985). Protein-protein interactions, like protein-fat ones, are increased in well emulsified products and provide stronger resistance to applied forces. The cheese, in this case, appears much more stable and firm as a product.

The fat content of the experimental cheeses in this research (16.7% to 18.8%) was not high, compared to a commercial, full fat processed cheese product. Despite the significant differences found between the 12 cheeses, the

fat content might not have, by itself, affected the textural characteristics of the products to a great extent. Chen *et al.* (1979) concluded from their study that fat had little influence on the hardness of a wide variety of natural and processed cheeses. Likewise, Marshall (1990) found that fat had little direct effect on the mechanical properties of processed cheese analogues. Muir *et al.* (1997), on the other hand, found that fat content influenced the sensory dimensions associated with texture and mouthfeel in commercial processed cheese spreads. Guinee *et al.* (2000) also found that fat content was inversely related to firmness and fracture stress in Cheddar cheese.

It is believed that rather than the fat content, the fat globule size and distribution, caused as a result of the changes in moisture content, played a more significant role in yielding textural differences between experimental cheeses. Figure 18 illustrates the differences in fat globule size and microstructure shown by the extreme samples (cheeses 1 and 12). The protein:fat ratio, known to determine the limits to which texture can be modified (Shimp, 1985), was kept constant for all cheeses.

Moisture content seems to be the main factor that influences the fat globules size in this particular experimental work (Figures 16 and 17), instead of mixing speed. The range of mixing speeds used was possibly not wide enough to illustrate the effect of this processing parameter on the microstructure of the processed cheese analogues.

Small fat globules like the ones observed in sample 12 (Figure 18 B) yield a more compact, homogeneous and stable structure, responsible for the increased firmness of this experimental cheese in relation to cheese sample 1 (Figure 18 A). Shimp (1985) and Caric & Kalab (1993) also reported that if other parameters (moisture, total fat and pH) are the same, processed cheese in which the fat occurs as large particles is softer than processed cheese containing small fat globules. Similar results were reported by Kim *et al.* (1996), who observed that emulsion gels containing smaller oil droplets were perceived as harder and displayed higher values for compressive stress, strain and energy (work).

A (+10% water / 100 rpm) B (-10% water / 155 rpm)

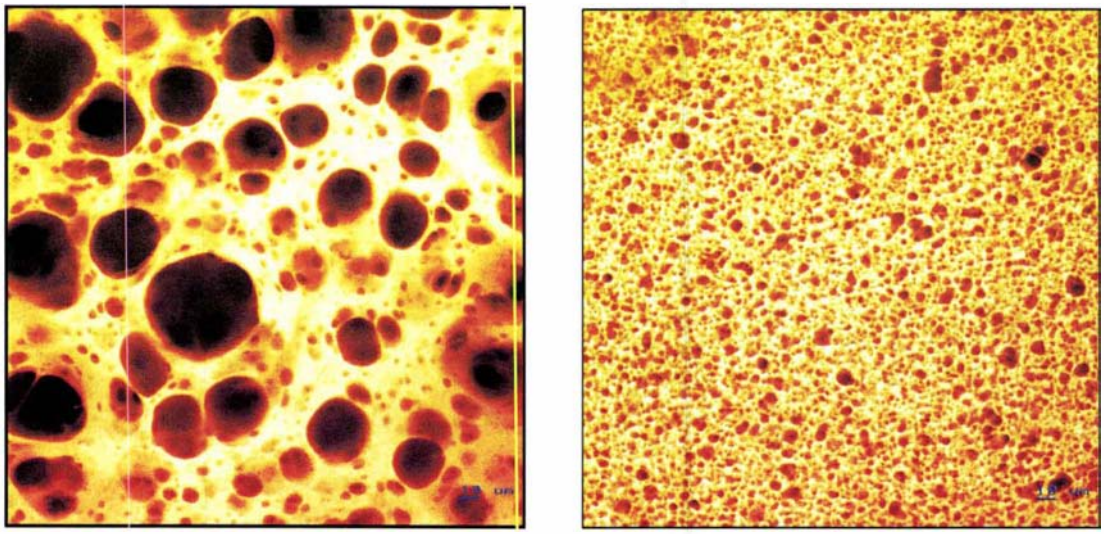


Figure 18. Confocal micrographs of experimental cheeses 1 (A) and 12 (B) (magnification 400 X)

The correlation of chemical (compositional) parameters, both individually considered, grouped in principal components or combined to rheological parameters in canonical variables, with individual sensory attributes was studied and is reported in section 5.6.

5.3. Microstructural evaluation

The microstructural investigation of the experimental cheeses was done using confocal laser scanning microscopy (CLSM). The technique was chosen for the ease of sample preparation and the good resolution obtained (Blonk & van Aalst, 1993; Aguilera & Stanley, 1999). Electron microscopy was not considered due to its high cost and the time required for sample preparation.

Studies on the microstructure of natural and processed cheeses reported in the literature commonly use double staining for the protein matrix and the fat phase (Blonk & van Aalst, 1993; Everett *et al.*, 1995; Faraay, 1995, Ramkumar, 1997). In this research, only the protein matrix was stained. Preliminary attempts to dye the fat phase with a 1% aqueous solution of Nile Blue, a specific dye for lipids, proved unsuccessful.

Microscopic examination of the samples that received Nile Blue by itself showed that the dye stained the protein matrix instead of the fat droplets. This has been observed and reported in a previous study, when Heertje *et al.* (1987) used this dye to examine the microstructure of mayonnaise. Cheeses that received Nile Blue and FastGreen together showed, under confocal microscopy analysis, that the Nile Blue was unable to penetrate the fat droplets. Instead, crystals of the dye could be detected immersed in the protein matrix, with very intense fluorescence, even though FastGreen and Nile Blue fluoresce at different wavelengths. The use of Nile Blue was discarded and further microscopic investigation carried on using solely the stain for protein, FastGreen.

Micrographs of the twelve experimental samples are shown in Figures 19 to 23. The protein matrices appear in bright yellow colour, while the dark round structures immersed in them correspond to fat globules and entrapped air bubbles. Differentiation between the fat droplets and the bubbles could not be achieved due to the lack of success in staining the fat phase.

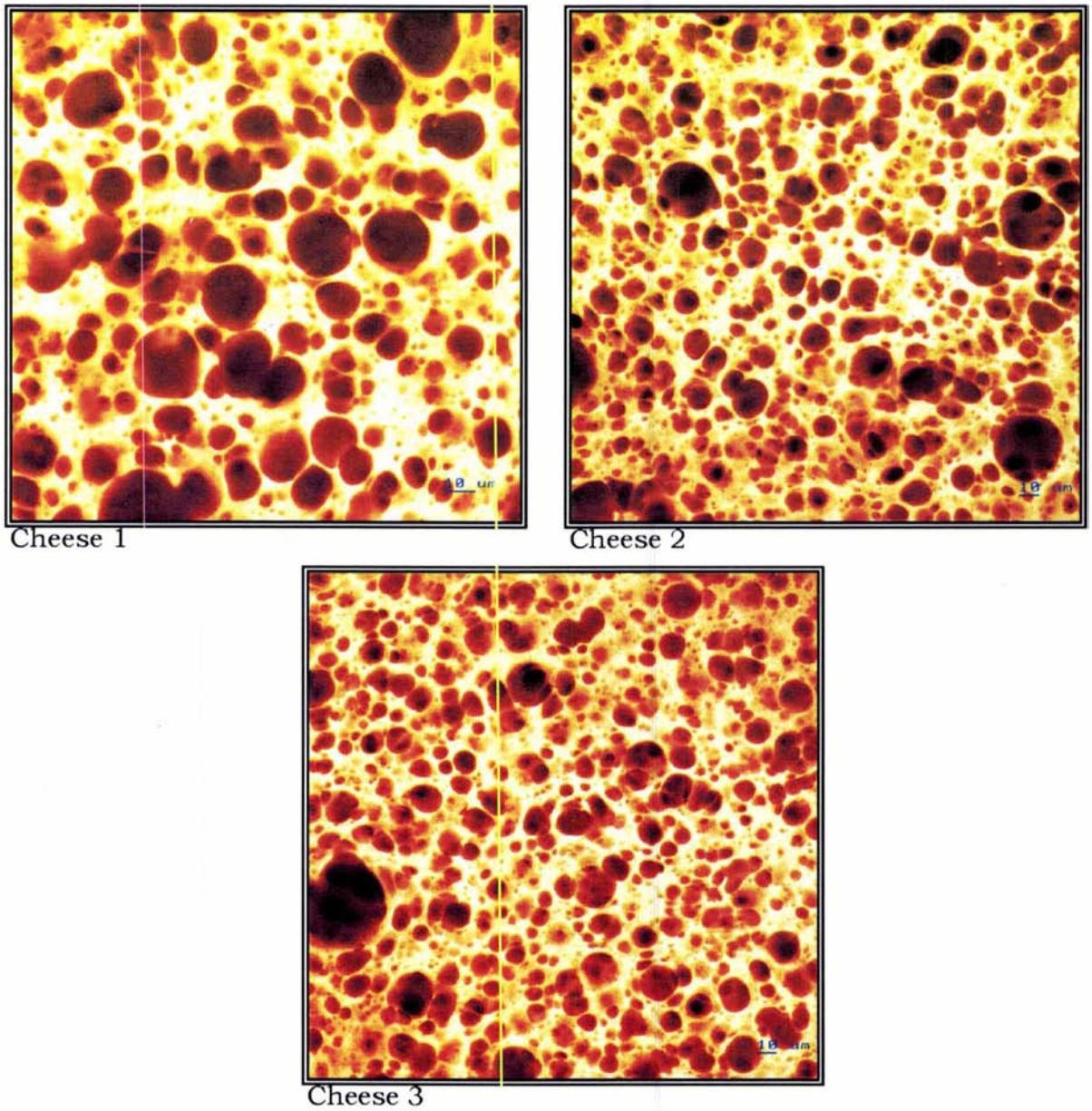


Figure 19. Confocal laser scanning micrographs of experimental cheeses 1 (+10% water, 100 rpm), 2 (+10% water, 127 rpm) and 3 (+10% water, 155 rpm), stained with FastGreen FCF, obtained with a 40x oil immersion objective (magnification 400 X)

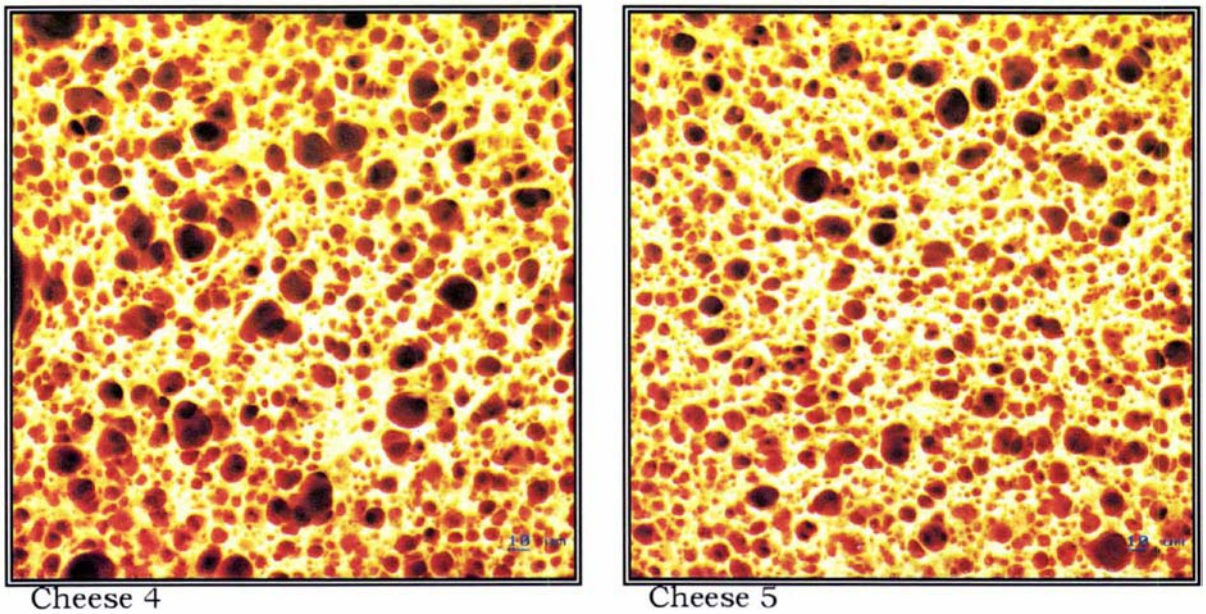


Figure 20. Confocal laser scanning micrographs of experimental cheeses 4 (+5% water, 100 rpm) and 5 (+5% water, 155 rpm), stained with FastGreen FCF, obtained with a 40x oil immersion objective (magnification 400 X)

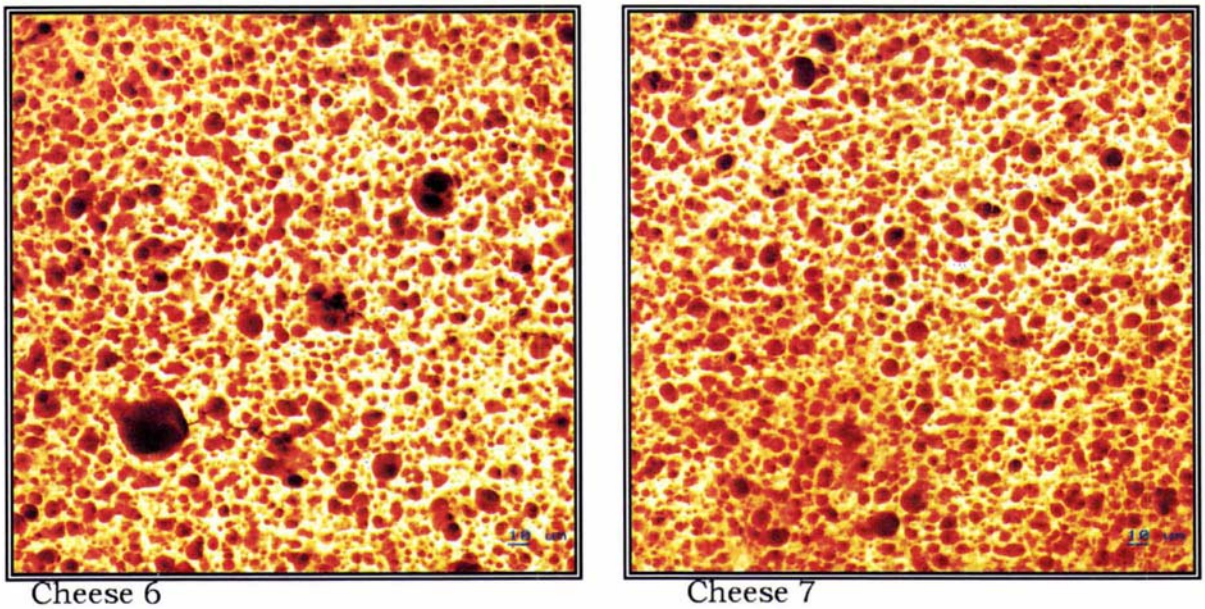


Figure 21. Confocal laser scanning micrographs of experimental cheeses 6 and 7 (base formulation/no added water, 127 rpm) stained with FastGreen FCF, obtained with a 40x oil immersion objective (magnification 400 X)

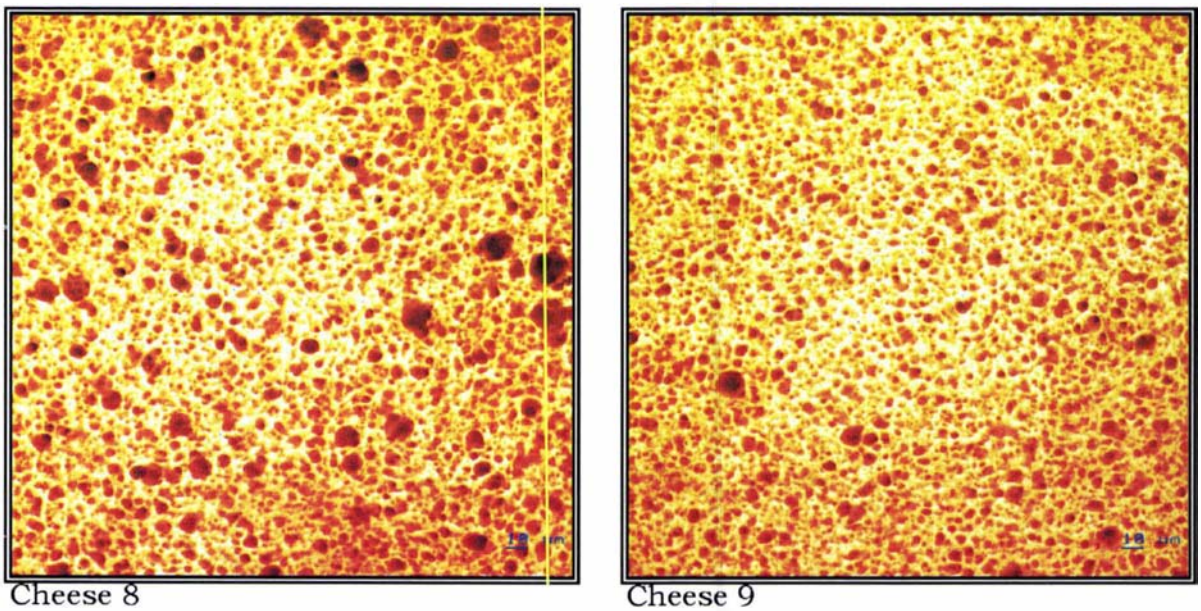


Figure 22. Confocal laser scanning micrographs of experimental cheeses 8 (-5% water, 100 rpm) and 9 (-5% water, 155 rpm), stained with FastGreen FCF, obtained with a 40x oil immersion objective (magnification 400 X)

The micrographs in Figures 19 to 23 show that the range of mixing speeds used for sample manufacture was not wide enough to yield visible differences between the experimental cheeses in the same moisture content group. Apart from cheese 1, shown in Figure 19 to have slightly bigger fat globules than cheeses 2 and 3 (all with the same moisture content and different mixing speeds), all other experimental cheeses inside each level of moisture do not differ, regardless of the mixing speed used (Figures 20 to 23).

Moisture content, contrary to mixing speed, seemed to play a major role in yielding different microstructures among the experimental cheeses. As the moisture content of the samples decreased (cheeses 1 to 12), the viscosity of the formulations increased. The same mixing speed used in a more viscous, less fluid formulation resulted in this formulation experiencing higher shear stress during manufacture. This, in turn, resulted in decreasing fat globule sizes.

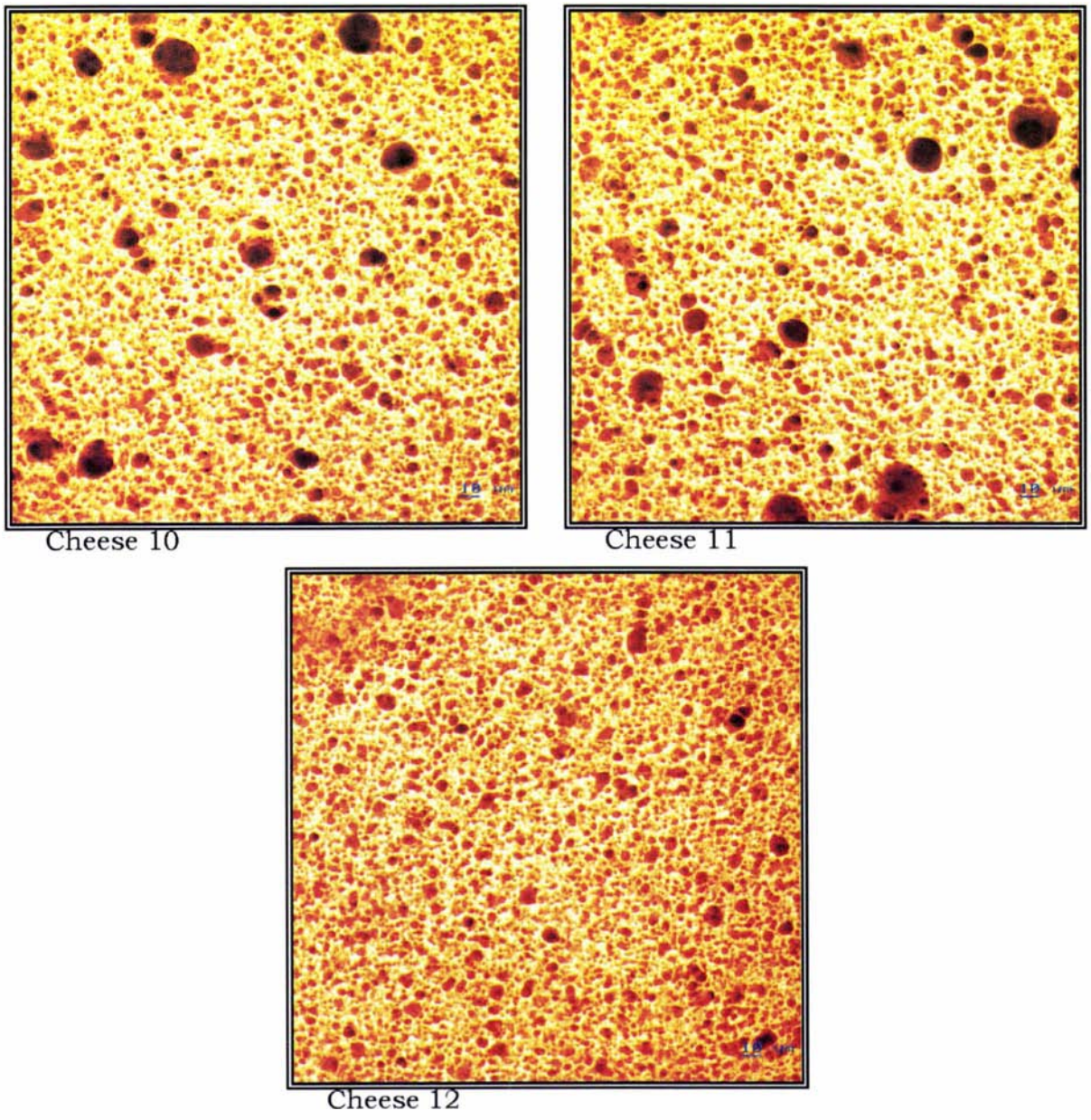


Figure 23. Confocal laser scanning micrographs of experimental cheeses 10 (-10% water, 100 rpm), 11 (-10% water, 127 rpm) and 12 (-10% water, 155 rpm), stained with FastGreen FCF, obtained with a 40x oil immersion objective (magnification 400 X)

The area occupied by the protein matrix in each micrograph was determined by image analysis. Because this procedure requires the establishment of an arbitrary pixel threshold to remove the dark areas from

the calculations, variability in the values of "area" between the three experimental blocks was observed. Differences in the intensity of protein staining, possibly caused by the different bags of casein used across the experimental blocks, also contributed to the variability found in the data. The image analysis results were, therefore, subjected to analysis of variance one experimental block at a time, using the pooled replicates as the error term (Table 5).

Table 5. Analysis of variance (*p*-values[1]) and Tukey's HSD results for the variable "area of protein matrix" obtained from the micrographs from the confocal laser scanning microscope, across individual experimental blocks

	AREA OF THE PROTEIN MATRIX (μm²)		
CHEESE	**Exp block 1 [2]**	**Exp block 2 [3]**	**Exp block 3 [3]**
1	6.48 E5 a[4]	11911 a	27890 a
2	6.72 E5 a	11237 a	31919 b
3	6.59 E5 a	10918 a	32285 bc
4	7.27 E5 b	13801 b	33599 c
5	7.28 E5 b	13653 b	36509 d
6	7.76 E5 c	14504 bc	35959 d
7	7.70 E5 c	14185 bc	35784 d
8	8.58 E5 d	14439 bc	38644 e
9	8.73 E5 de	14842 cd	39806 ef
10	8.36 E5 d	14599 bcd	41067 fg
11	9.02 E5 ef	15877 e	41475 g
12	9.23 E5 f	15537 de	43481 h
p-values	**0.000**	**0.000**	**0.000**

n = 6

[1] probability associated with the F-test of factor "sample", representing experimental cheeses

[2] magnification of 100 X

[3] magnification of 400 X

[4] means within a column with no common letters differ significantly ($p<0.05$)

It can be seen from the *p*-values in Table 5 that significant differences in the protein area per micrograph existed between the experimental samples across each of the experimental blocks. The results from the Tukey's HSD significance test at the 95% confidence level illustrate the fact that the area occupied by the protein matrix in the micrographs increases, in general, from cheese 1 to cheese 12. This is mainly due to the decrease in fat globule and air bubble sizes as the moisture of the cheeses is reduced. In experimental cheeses 1 to 3, large dark areas corresponding to fat and air are "erased" from the confocal image during the image analysis and area calculation procedures. These areas are of intermediate size in cheeses 4 to 7 and much smaller in the lower moisture cheeses (cheeses 8 to 12).

Response surface regression analysis was performed on the microstructural data to try and model the variation in the "area of protein matrix" as a function of moisture content and mixing speed. The coefficients obtained from the regression analysis of each individual experimental block and the respective plots are shown in Table 6 and Figure 24. The magnitude of these coefficients provides an indication of the relative importance and contribution of each term of the model in the quality of the fit.

Table 6. Response surface regression coefficients[1] and R-square values for the microstructural parameter "area of protein matrix", for each experimental block, as a function of moisture content and mixing speeds

AREA OF PROTEIN MATRIX	CONST	M	M^2	S	S^2	M X S	R-SQUARE (ADJ) [2]
Exp. block 1	7.8096 (e +5)	-0.1170					93.0 %
Exp. block 2	14406.1	-183.7	-10.5				89.1 %
Exp. block 3	30227.9	-544.3		49.5			94.9 %

[1] coefficients are: constant (CONST), moisture (M), moisture squared (M^2), speed (S), speed squared (S^2) and moisture x speed (M X S)

[2] R-square values were adjusted for the number of terms in the model. R-square values < 80% indicate inability to model the sensory response. R-square values > 90% indicate good, satisfactory fit of model.

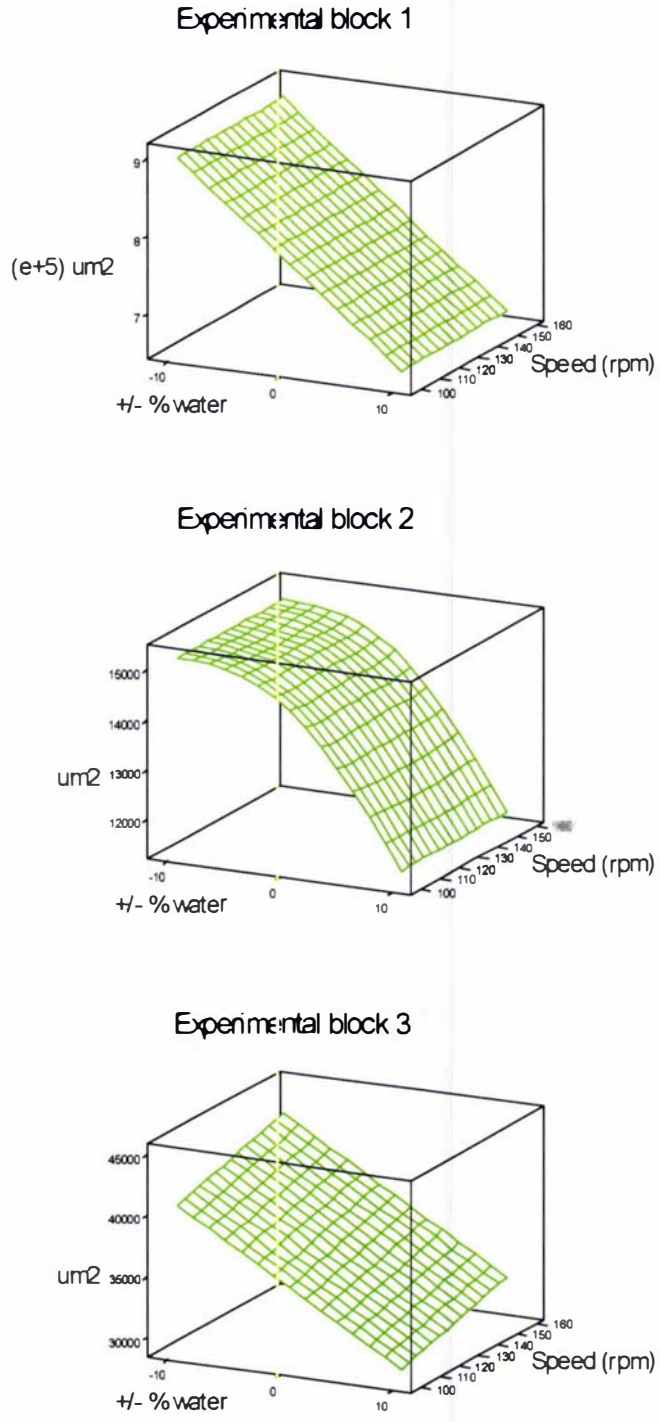


Figure 24. Response surface plot for the parameter "area of protein matrix" as a function of moisture content and mixing speed

It can be seen in Table 6 that, despite the good fit obtained for each individual experimental block shown by the R-square values, the use of different bags of casein appears to have generated different structures that were modelled differently. In the case of experimental block 1, moisture plays a major role in explaining the variation in the data. In block 2, moisture continues to play a major role, but a small quadratic effect of this factor is observed. In experimental block 3, unlike in the previous ones, mixing speed appears to model the variation in the data together with moisture content, even though the contribution of the former is small compared to the latter.

The lack of significance or, in the case of experimental block 3, the small significance found for mixing speed in the response surface regression analysis indicates, once again, that the range explored in this study was not wide enough for differences between formulations to be detected. With regards to moisture content, reduction in the moisture of the experimental cheeses caused them to become gradually more viscous and thicker. This, in turn, resulted in these cheeses experiencing larger mechanical work during manufacture, even at the low mixing speed used. More mechanical work translated, texturally, into cheeses with smaller fat globules that were more uniform in size and distribution (cheeses 10 to 12, Figure 23).

The ability of microstructural information to correlate to sensory textural perception (Langton *et al.*, 1996) and to rheologically assessed functional properties of food materials (Holcomb *et al.*, 1992) has been reported. In this study, differences in the magnification used and threshold selected for image analysis across the experimental blocks prevented the use of the microstructural data in all the correlation procedures described in section 4.6. However, correlation of the microstructural results (area of protein matrix) for each individual experimental block with their respective sensory scores and rheological and chemical parameters are presented and discussed in section 5.6. Pairwise correlation was used.

5.4. Sensory evaluation

Texture is an important quality factor in cheese and it is, according to many food researchers, essentially a sensory attribute (Bourne, 1982, 1983; Christensen, 1984; Heath & Lucas, 1988; Lawless & Heymann, 1998). It is not, however, a single parameter. It consists of a number of different sensations that can be perceived by the senses of touch (tactile and mouthfeel) and sight.

The currently used definition for texture, from the International Organisation for Standardisation (ISO, 1981), includes all attributes of a product perceptible by means of mechanical, tactile and, where appropriate, visual and auditory receptors. It also implies texture is essentially a sensory attribute or group of attributes, even though instruments have been used to measure the mechanical properties, often related to texture, of a food product. It is important to bear in mind, however, that regardless of the instrumental reading for a textural attribute of a cheese, it is ultimately the consumer acceptance of the attribute or attributes that will define the cheese's quality.

In this research project, acceptance and quality of the experimental cheeses were not an issue. Due to the ingredients used in the formulation, the processed cheese analogues manufactured were model systems, having no appealing or added cheese flavour. The ultimate goal was to reproduce the texture of a processed cheese block to be evaluated through instrumental and sensory techniques and to explore the correlation between these techniques.

In a preliminary sensory evaluation session, the experimental cheeses were presented, blind coded, to a panel of about 9 trained panellists for a profiling tasting. The panellists were exposed to all the different cheeses and were requested to come up with a number of different textural attributes that they could identify in the products, using both the hands and the mouth. Fracturability, firmness in compression, rubberiness, stickiness, curdiness, greasiness (for the hand evaluated attributes) and firmness in chewing, cohesiveness, mouthcoating (for the mouth evaluated attributes) were mentioned. A definition for each of these attributes as well as the protocol for

consistent evaluation of each of them was discussed and established with the panellists.

A common observation from the panellists during mouth evaluation of the cheeses was the coating of the mouth caused by the fat and especially the rennet casein. This caused a serious problem of fatigue during the evaluations, as panellists were able to test no more than 4 cheese samples at any one time (session). In view of that limitation, the mouth evaluated attributes were eliminated and only the hand evaluated ones were used for subsequent quantitative descriptive analyses (QDA) testing. The ability of hand evaluation to differentiate texture of cheese in comparison to mouth evaluation has recently been investigated by Drake *et al.* (1999a). It has been reported that either can be used to efficiently discriminate cheese texture and that hand evaluation has the advantage of eliminating panellist oral fatigue. This provides support to the decision made in the course of this study to eliminate the mouth evaluated attributes in the tasting sessions and use only the hand evaluated ones.

All the cheese samples were cut into slices (consistent with processed cheese slice testing protocols) and kept in a fridge until evaluation. Each tray was presented to the panellists over a bag of ice, in order to keep the temperature of the cheeses as close as possible to refrigeration temperature. Szczesniak (1975b) discussed the effect of temperature on the textural characteristics of some thermally sensititve solid and semi-solid foods. Changes in the perception of texture at different temperatures were also reported by Nakazawa *et al.* (1993). For this research, the choice of using lower temperatures was based on the ease for sample preparation and ease of comparison to the samples tested under rheological techniques, as instrumental evaluation at room temperature proved difficult and troublesome. The temperatures of the experimental cheeses upon serving to the panellists were measured for the various samples and averaged over the different trays. These measurements were performed throughout the three experimental blocks and the results are displayed in Table 7.

Even though the ice bags helped keep the samples temperature below room temperature, considerable variation still occurred across the blocks and

trays in a session, as seen in Table 7. This happened mainly due to the time required for preparing the trays for serving, during which the samples had to be outside the fridge, on the bench. In view of this variation of cheese sample temperature, the effect of this factor over the sensory scores was included as a covariate in the statistical covariance analysis of the data. For those attributes in which the covariate temperature was significant, the sensory scores were adjusted before further analysis.

Table 7. Cheese temperatures (°C) during the sensory evaluation sessions, across experimental blocks and across trays within a session

		TEMPERATURES (°C) ± ST. DEV.			
		Tray 1	**Tray 2**	**Tray 3**	**Tray 4**
Block 1	**Session 1**	9.80[1] ± 0.61	11.23 ± 0.72	11.20 ± 0.47	12.57 ± 0.79
	Session 2	9.93 ± 0.30	10.73 ± 0.59	10.31 ± 0.29	12.17 ± 0.54
	Session 3	11.30 ± 0.44	11.94 ± 0.48	11.67 ± 0.62	12.60 ± 0.30
Block 2	**Session 1**	17.24 ± 0.40	17.68 ± 0.54	17.58 ± 0.50	17.72 ± 0.36
	Session 2	16.58 ± 0.33	17.74 ± 0.48	17.42 ± 0.54	18.02 ± 0.28
	Session 3	11.10 ± 1.50	11.04 ± 1.04	12.96 ± 0.57	12.20 ± 0.41
Block 3	**Session 1**	9.63 ± 0.47	10.25 ± 0.39	10.60 ± 0.63	11.76 ± 0.84
	Session 2	11.95 ± 0.60	14.03 ± 1.47	15.25 ± 0.59	15.91 ± 0.44
	Session 3	11.95 ± 0.42	13.16 ± 0.46	15.49 ± 0.40	15.79 ± 0.29

[1] N = 7 (number or readings per tray, per session)

Table 8 shows the probability values for the sensory attributes evaluated and the covariate temperature. Of all the attributes tested, temperature was found not to significantly affect rubberiness (Table 8), in which case the analysis was repeated without the covariate to establish the existence of significant differences between 'samples' (represented by the cheeses).

Fracturability, firmness during compression, firmness in cutting, rubberiness, stickiness and curdiness were different between the twelve

experimental cheeses when evaluated against the panel noises (Table 8). This means that significant differences between the cheeses could be detected over and above the noises inherent to the judges (panellists) over experiments (blocks), sessions and duplicates.

Table 8. Probability scores for seven hand evaluated sensory attributes, tested against panel noises and adjusted for the effect of temperature (covariate)

ATTRIBUTES	TEMPERATURE	JUDGE	'SAMPLE'
Fracturability	**0.0004** [1]	**0.0001**	**0.0310**
Firmness (compression)	**0.0090**	**0.0001**	**0.0001**
Firmness (cutting)	**0.0358**	**0.0001**	**0.0001**
Rubberiness	0.4136 [3]	**0.0001**[2]	**0.0055**[2]
Stickiness	**0.0218**	**0.0001**	**0.0001**
Curdiness	**0.0230**	**0.0001**	**0.0001**
Greasiness	**0.0095**	**0.0001**	0.1654

[1] probability values in bold are significant at $p<0.05$

[2] the probability for the judge and sample effects were obtained without the covariance analysis

[3] obtained over the covariance analysis

Differences in greasiness were not observed between the cheese samples (p = 0.1654, Table 8). Setser *et al.* (1996) reported that relatively large changes in oil levels were required, in a sensory study, for discrimination of differences between samples of oil/water emulsions. In this study, the variations in fat content of the experimental cheeses were not large, despite being significant between cheeses (Table 3).

Adjustments to the 'sample' means were made, for each attribute, according to the significance of the covariate temperature and compliance of the data to the assumptions of homogeneity of variance and normality of residuals.

The previously mentioned assumptions were found not to hold for the attributes fracturability and stickiness. In this case, weighted least squares (WLS) regression was considered (Myers, 1989). WLS was able to eliminate the problem of heterogeneous variance, but not the lack of normality of the residuals for those attributes. Because the residuals for fracturability were not normally distributed, the test of significance of the sample effect may not be accurate for this particular attribute (p = 0.0310, Table 8). With regards to stickiness, the highly significant p-value obtained (p = 0.0001, Table 8) makes the inference on sample (cheese) effects at 5% significance level unlikely to be affected by the lack of normality.

The adjusted means for each sensory attribute, for the twelve experimental cheeses, can be found in Table 9. These results were subjected to an analysis of variance (ANOVA) to determine the existence of significant differences between the experimental samples against the experimental error, i.e., differences over and above the experimental noise. The probabilities (p-values) for each attribute are also included in Table 9.

It can be seen from the results in Table 9 that no significant differences between the experimental cheeses were detected for the attributes fracturability and greasiness (p-values 0.2120 and 0.2209, respectively). This is illustrated in Figure 25 (A and G), in which an almost horizontal line is displayed across cheeses 1 to 12 for these attributes.

Firmness, both in compression and cutting, stickiness and curdiness were found to be highly significant at the 95% confidence level (p-values 0.0001, Table 9), indicating clear differences between the experimental cheeses for these attributes. Cheeses with high moisture content, regardless of the mixing speed used (cheeses 1, 2, 3), were less firm to compress and to cut than those cheeses with lower moisture levels (cheeses 8 to 12). Jack & Paterson (1993) reviewed the effect of increasing moisture content of cheeses on the texture and quality of the products, reporting the weakening of the protein structure and reduction of the resistance to deformation in high moisture cheeses. Similar findings had been reported by Gupta *et al.* (1984),

Tunick *et al.* (1991) and Fox *et al.* (1996), who indicated that water could act as a lubricant or plasticiser for the movement of casein in relation to the fat.

Table 9. Adjusted score means (for temperature) for seven hand evaluated sensory attributes and corresponding *p*-values[1]

	ATTRIBUTES[2]						
CHEESE	**FRACT**	**FIRM (CP)**	**FIRM (CT)**	**RUBBER[3]**	**STICK**	**CURD**	**GREASE**
1	13.10	3.80	5.57	4.65	4.81	6.93	4.22
2	13.51	3.95	5.69	4.39	4.57	7.47	4.37
3	12.85	3.87	5.76	4.30	4.50	7.39	4.26
4	12.94	4.39	6.44	4.47	4.03	7.81	4.01
5	12.96	4.73	6.59	4.48	3.83	7.86	4.03
6	13.07	5.29	7.16	4.52	3.67	8.85	3.95
7	12.99	5.13	7.05	4.43	3.84	8.43	3.82
8	12.98	6.50	7.94	4.82	3.06	9.15	3.96
9	12.44	6.37	8.12	4.92	2.79	9.79	3.77
10	13.36	6.83	8.18	4.86	2.76	10.04	3.88
11	13.02	6.96	8.45	4.71	2.52	10.19	3.98
12	13.32	6.96	8.36	4.70	2.59	10.38	4.10
MSD[4]	1.1572	**0.8576**	**0.9155**	**0.6594**	**0.6708**	**1.0931**	0.7711
***p*-value**	0.2120	**0.0001**	**0.0001**	**0.0352**	**0.0001**	**0.0001**	0.2209

** all values are input onto a 15 cm line scale

[1] probability associated with the F-test of factor "sample"

[2] sensory attributes are fracturability (FRACT), firmness in compression (FIRM CP), firmness in cutting (FIRM CT), rubberiness (RUBBER), stickiness (STICK), curdiness (CURD) and greasiness (GREASE)

[3] means for rubberiness were not adjusted for temperature

[4] Minimum Significant Difference for Tukey's HSD significance test

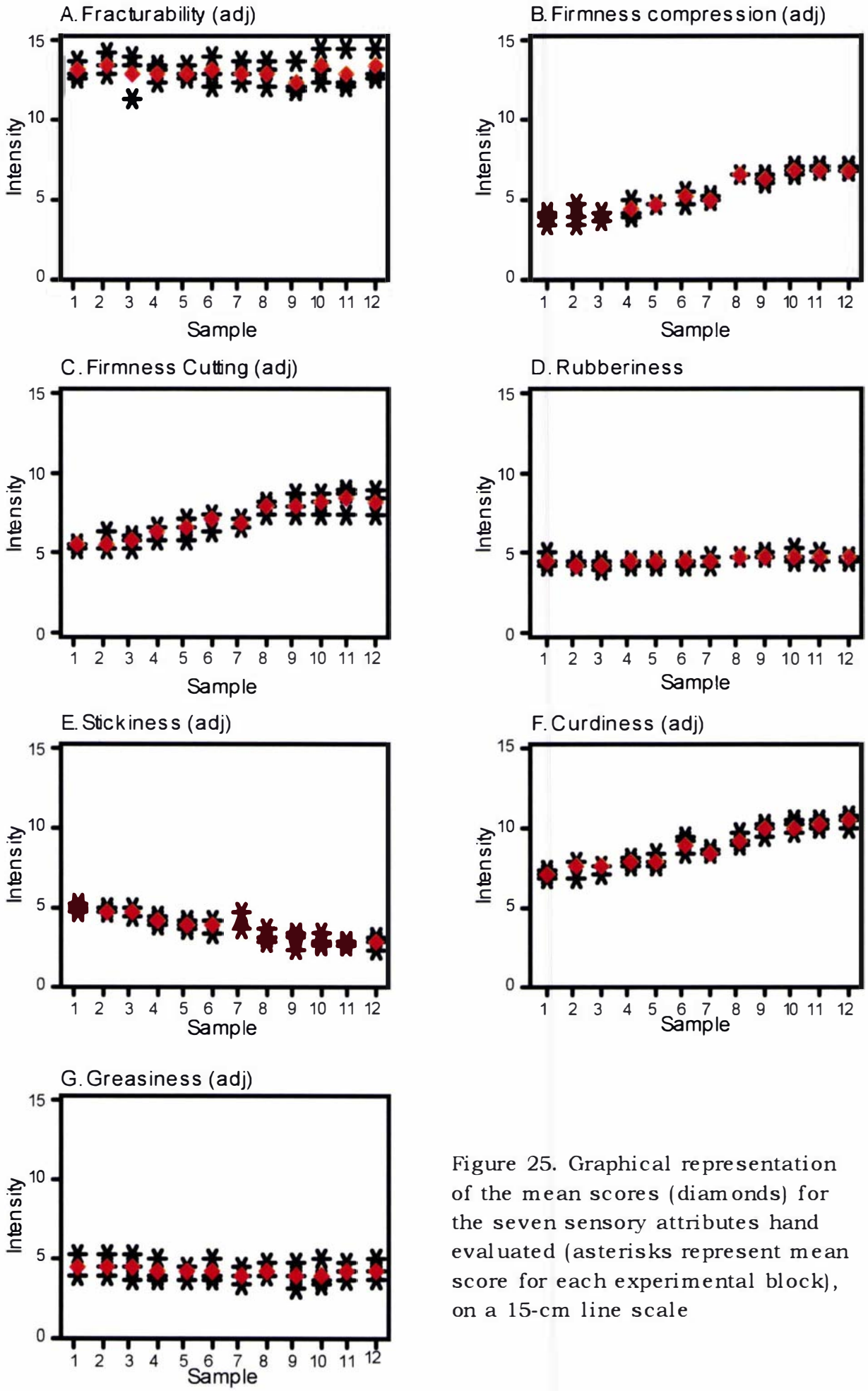


Figure 25. Graphical representation of the mean scores (diamonds) for the seven sensory attributes hand evaluated (asterisks represent mean score for each experimental block), on a 15-cm line scale

As observed in the initial visual appraisal of the molten cheese masses after manufacture, cheeses with higher moisture content (1, 2, 3) were stickier, at two weeks of age, than those with less moisture (cheeses 8 to 12). Kairyukshtene & Zakharova (1982) observed similar behaviour when studying the consistency of low fat cheeses. Their results showed that increasing moisture content in cheese manufacture yielded sticky products.

The adjusted scores reported for curdiness increased as the moisture content of the cheeses decreased. This shows that in spite of the higher incidence of casein lumps in the high moisture cheeses (cheeses 1, 2, 3), these were perceived by the panellists as being smoother, almost pasty in texture. Cheeses 10, 11 and 12, on the other hand, were curdy after kneading. Kairyukshtene & Zakharova (1982) have also indicated a relation between increased moisture content and spreadability/smoothness of the resulting cheeses.

Differences in rubberiness between the twelve experimental cheeses were significant at α=5% (p-value 0.0352, Table 9). Such differences were not, however, as clearly detectable as for the other significant sensory attributes. The mean scores for rubberiness across the experimental samples, graphically shown in Figure 1d, follow a slightly sigmoidal, almost horizontal curve, with very small variability across the different experiments/blocks. Despite the use of a 15 cm line scale for the evaluation, the mean scores for all twelve cheeses ranged from 4.30 (cheese 3) to 4.92 (cheese 9), as seen in Table 9.

Rubberiness was a sensory attribute difficult to evaluate in this study. As it was defined, it involved the compression of the sample to produce a visible dent and subsequent recovery of the sample to the initial shape upon removal of the applied stress. The extent of compression becomes, by the attribute's own definition, subjective. It is quite possible that, in many evaluations, the compression exerted was enough to cause minute, "invisible" fractures or cracks in the structure. This, in turn, would lead to poor recovery of the initial shape and, consequently, lower scores for the attribute.

The slices used for sensory evaluation were prepared using a wire cheese cutter to guarantee uniformity of slice thickness. It is possible, however, that the thickness used (0.9 cm) was not adequate for reasonable differentiation between the fracture properties of the experimental cheeses. Evaluation of fracturability in processed cheese products is usually performed using plugs (for blocks) or 0.2 cm thick slices (for IWS – individually wrapped slices or SOS – slice on slice cheeses)(A.P.E.C., personal communication). It was observed that, in the preparation of the samples for evaluation, some samples fractured during the wire cutting. When this early fracturing was detected, the sample was discarded and a new one used for evaluation. It is possible, however, that "invisible" other fractures might have occurred in the samples presented to the panellists. This could have been the reason for the relatively big differences across the experimental blocks, especially for the lower moisture cheeses (less plastic), for this attribute. This can be seen in Figure 25A.

The panellists rated all cheese samples as being equally very greasy. Even though significant differences were found for the fat content of the experimental cheeses, the differences were difficult to be detected through tactile evaluation. This particular attribute is very prone to individual variability since its perception in the hand depends on the type and dryness of skin of each panellist. Even though the panellists were instructed not to put moisturiser on prior to a session, natural oiliness/dryness is a factor difficult to control and might have contributed to the lack of significance observed for the attribute.

In view of the results presented, fracturability, greasiness and, to some extent, rubberiness were found not to be appropriate textural attributes to differentiate between rennet casein based processed cheese analogues such as those used in this research. It is possible that the range of textures used was not wide enough for differences in these attributes to be detected in a significant way.

All the sensory attributes investigated in the course of this research were subjected to a response surface regression analysis. The purpose of this analysis was to attempt to model sensory response as a function of moisture

content and mixing speed, the parameters used to generate the textural differences in the experimental cheeses. The results (coefficients) of the response surface regression are shown in Table 10. For those attributes in which the block effect was found to be significant (Table 10), coefficients are presented for blocks 1 and 2, but not for block 3. This is because block 3 was used as a reference for the fitting procedure and, as such, any fitted value belonging to block 3 does not require a block coefficient (B1 and B2, in this case, equals zero). Figures 26 to 29 show the response surface graphs for those sensory attributes that were considered satisfactorily modelled by the parameters used (moisture and speed).

Table 10. Response surface regression coefficients[1] and R-square values for seven sensory attributes as a function of experimental blocks, moisture content and mixing speeds

RESPONSE (ATTRIB) [2]	CONST	M	M^2	S	S^2	M X S	B1	B2	R-SQUARE (ADJ) [3]
FRACT	12.8629	-0.0001	0.0031				-0.7368	-0.1493	73.5%
FIRM (CP)	5.3986	-0.1572					-0.0939	0.2116	94.5%
FIRM (CT)	7.2388	-0.1354	-0.0022				0.3478	0.2282	93.7%
RUBBER	7.6742	-0.0189		-0.0478	0.0002				32.4%
STICK	4.1327	0.1003		-0.0043			0.3344	-0.2735	92.2%
CURD	7.8364	-0.1495		0.0067					91.4%
GREASE	3.8819	0.0150	0.0025				-0.5088	-0.2004	84.5%

[1] coefficients are: constant (CONST), moisture (M), moisture squared (M^2), speed (S), speed squared (S^2), moisture x speed (M X S), block 1 (B1) and block 2 (B2)

[2] sensory attributes are: fracturability (FRACT), firmness in compression (FIRM CP), firmness in cutting (FIRM CT), rubberiness (RUBBER), stickiness (STICK), curdiness (CURD) and greasiness (GREASE)

[3] R-square values were adjusted for the number of terms in the model. R-square values < 80% indicate inability to model the sensory response. R-square values > 90% indicate good, satisfactory fit of model.

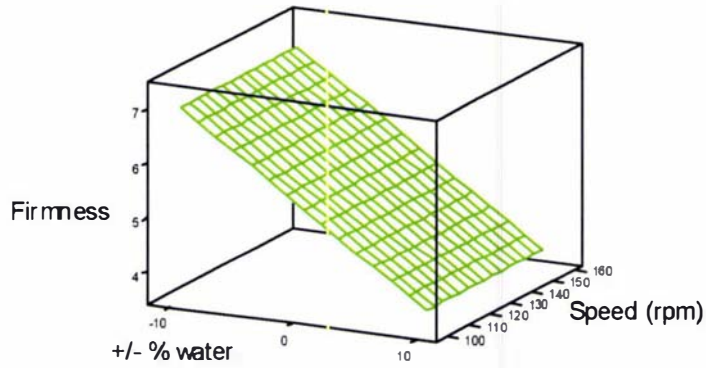


Figure 26. Response surface plot for the attribute firmness in compression as a function of moisture content and mixing speed

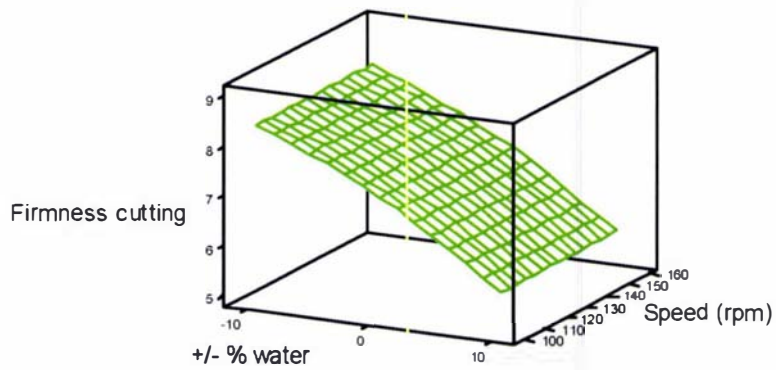


Figure 27. Response surface plot for the attribute firmness in cutting as a function of moisture content and mixing speed

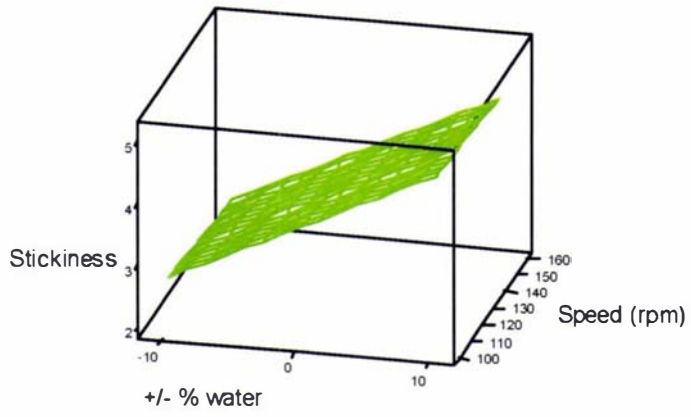


Figure 28. Response surface plot for the attribute stickiness as a function of moisture content and mixing speed

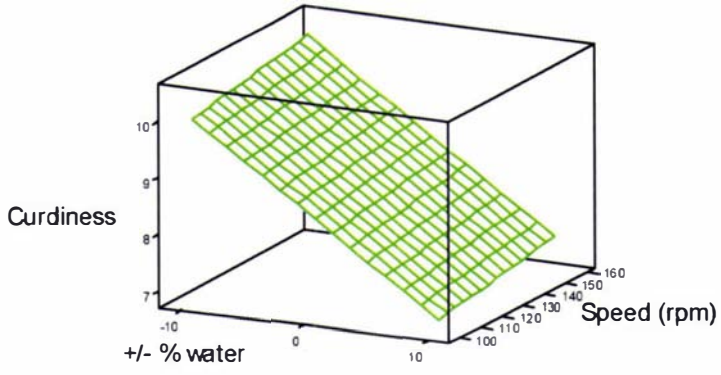


Figure 29. Response surface plot for the attribute curdiness as a function of moisture content and mixing speed

It can be seen in Table 10 that rubberiness could not be properly modelled as a function of moisture content and mixing speed (R-square 32.4%). Fracturability and greasiness were not as efficiently modelled as those attributes with R-square above 90% (firmness in compression, firmness in cutting, stickiness and curdiness). This was expected since fracturability, rubberiness and greasiness were found not to be good discriminators between the twelve experimental processed cheese analogues in this study. The results also suggest that other compositional and/or processing parameters, either isolated or in combination with moisture and/or speed, would have to be considered in the attempt to model sensory response for these attributes.

Firmness of the experimental cheeses, both under compression and upon cutting, was found to be negatively related to moisture content, but no effect of the mixing speeds used on these attributes was detected (Figures 26 and 27). For both attributes, the effect of different experimental blocks was found to be significant and coefficients were presented for the term 'block' within the fitting model. The significance of 'block' could be due to the different bags of rennet casein used for sample manufacture in each experimental block. Ennis & Mulvihill (1997) reported differences in the hydration behaviour of rennet casein from different manufacturers and even different batches from the same manufacturer. Other compositional and processing parameters were kept constant for all the manufactured cheeses, regardless of the experimental block. In addition, the sensory scores were all adjusted for the effect of temperature. That suggests that differences in the properties of the rennet casein used are the most likely explanation for the significant block effect for these attributes.

Unlike firmness, stickiness and curdiness were affected by both moisture content and mixing speed, with the two factors being significant under the response surface regression (Table 10). The coefficient values for speed were, however, much smaller than those for moisture, indicating only a minor influence of the former if compared to the moisture content.

Stickiness is positively affected by moisture and negatively affected by mixing speed. These results were expected, as moisture is known to increase the stickiness of cheese products (Kairyukshtene & Zakharova, 1982) and

higher mechanical agitation leads to improved emulsification process and a more stable product that is sliceable and not sticky (Sorley, 1997). The effect of mixing speed on the stickiness of the experimental cheeses was, however, negligible, as observed in Figure 28. The effect of blocks, for this particular attribute, was also found to be significant, possibly due to differences in the hydration properties of the rennet casein used in each experimental block.

Curdiness is negatively affected by moisture content, which means that lumpy, curdy products are produced with a decrease in moisture in the formulation. On the other hand, increasing the mixing speed for manufacture of the cheeses significantly increased the perceived curdiness, which is likely to be caused by the positive effect of agitation on the formation of a stable analogue processed cheese.

Model systems like the ones used in this study require extensive mixing to be able to firm as a block-type product due to the high content of moisture (Sorley, 1997). Reduced mixing speed will result in a softer final product that will appear pastier, hence smoother, during sensory appraisal. The block effect for the attribute curdiness was found not to be significant at the 95% confidence level. Since the block effect was significant for the other attributes modelled, it was expected it would also appear as a significant effect for curdiness. Any difference in the hydration properties of the rennet casein used for each experimental block could cause differences in curdiness due to inconsistent absorption of water. It is possible that sensory evaluation, in this case, was not sensitive enough a technique to detect any differences that might have occurred between the cheese samples.

Correlation of the sensory textural attributes with instrumental measurements was performed. The results from the pairwise correlation, stepwise regression, principal component analysis and canonical correlation are presented and discussed in section 5.6.

5.5. Rheological evaluation

5.5.1. Preliminary considerations

Preliminary rheological measurements (creep test) on the experimental cheeses 1 and 12 showed that the creep compliance curves were sensitive to testing temperature variation during evaluation at room temperature (Appendix). Differences seemed to be slightly larger for the cheese sample with higher moisture content (cheese 1). Variations of less than 1°C in the cheese sample temperature caused the rheological response to vary slightly, around 3-8%. The significance of these differences was not verified through statistical analysis; however, measurements lay in general within the acceptable variation due to the precision of the instrument. Variation larger than 1°C, on the other hand, caused differences to be larger than 10%.

Despite the possibility of instrument precision having caused the differences in the creep test curves, a decision was made to ensure all testing was carried out under cold temperature in the range of 5°C to 8°C rather than at room temperature.

The rheological properties of the cheeses in the study are not expected to change much at lower temperatures, such as those used in refrigeration. Temperature values around 4°C to 8°C are far from the "fat melt transition" range, which is around 20°C to 25°C. High temperature dependence of rheological response due to fat melting was reported by van Vliet & Peleg (1991) and Visser (1991). Nakazawa *et al.* (1993) analysed commercial cheeses using Texture Profile Analysis (TPA) at 5°C and 18°C and reported on the importance of temperature control for textural assessment.

Sampling at refrigeration temperatures was also a lot easier to achieve, with less risk of damage (fracture) to the samples prior to the tests. In addition, the different moisture content of the twelve cheeses in this study caused them to adjust differently to the external temperature. It was observed that 2 hours of tempering in a temperature controlled room (22-24°C) were

not sufficient to bring all the samples from refrigeration temperature (4°C) to room temperature. The effect of temperature equilibration on test results was briefly reviewed by van Vliet & Peleg (1991). Hence, in this study, measurements at room temperature were discarded and all subsequent rheological testing was performed within the range 5°C to 8°C.

The temperature of each sample was measured and recorded during the textural evaluation to enable the assessment of the significance of temperature effect on the test results and, if and when necessary (covariance analysis), adjustments in the textural responses for that effect to be made.

The model systems used in this research work are, from a rheological viewpoint, viscoelastic solids in nature. This means that a partial elastic recovery is observed in these materials upon removal of an applied force (Stanley *et al.*, 1998). The rheological responses of the cheese analogues investigated here to an applied force or stress depend on the magnitude of the stress and the time over which it is applied.

Small stresses and short times may cause slow and reversible flow, while larger stresses over longer periods can lead to some permanent deformation. Even larger stresses can lead to destruction of the structure and fracture of the samples, also a rheological fingerprint of viscoelastic materials, even though fracture properties are often rate dependent. Hence, the rheological tests performed in the course of this study included a frequency sweep (small stress/short time) and creep compliance (large stress/long time) on a dynamic stress controlled rheometer and compression to 70% (very large stress to fracture) on the TX2-Texture Analyser.

Experimental cheeses representing the extremes of the textural range studied (cheeses 1 and 12) were initially subjected to a strain sweep to determine the linear viscoelastic region.[1] Validity of the rheological results (data independent of the magnitude of the stress applied) requires that the elastic limit of the tested material be not exceeded (Stanley *et al.*, 1998). The values of stress chosen for the frequency sweep test (500 Pa) and creep compliance tests (2000 Pa) were within the linear region. Stress of 3000 Pa,

[1] in Appendix, section 9.0.

also used for the creep experiments, was very close to the limit of the region (critical strain) and was therefore discarded.

5.5.2. Frequency Sweep

The frequency sweep test yielded curves of storage and loss moduli (G' and G", respectively) as a function of frequency. The range of frequency used was 0.1 Hz to 22 Hz. The curves shown in Figure 30 (results from experimental block 3 were used for illustration purposes) show, respectively, the variation in G' and G" for the twelve experimental samples over the specified frequency range. Curves for the loss tangent (tan δ) are not presented, as they were reasonably similar for all the experimental samples and did not allow for proper differentiation between them.

It is possible to observe, from the results in Table 11, that the experimental cheeses were significantly different from each other ($p < 0.001$). Only marginal significance of the experimental blocks was found for both parameters (G' and G"), indicating good reproducibility of results across blocks. The storage modulus (G') is a measure of the energy stored in a material and is related to the elasticity of the material. The loss modulus, on the other hand, is a measure of the energy dissipated in the material, which is related to viscosity (Stanley *et al.*, 1998; Whorlow, 1992). Working with strong gels, Lapasin & Pricl (1995) stated that the dynamic analysis of such materials is quite often restricted to the elastic component, G', which is typically 1 to 2 orders of magnitude greater than G". It is reasonable to assume, from the behaviour shown in Figure 30, that the model cheese analogues in this study are relatively strong gelled emulsions (Lapasin & Pricl, 1995).

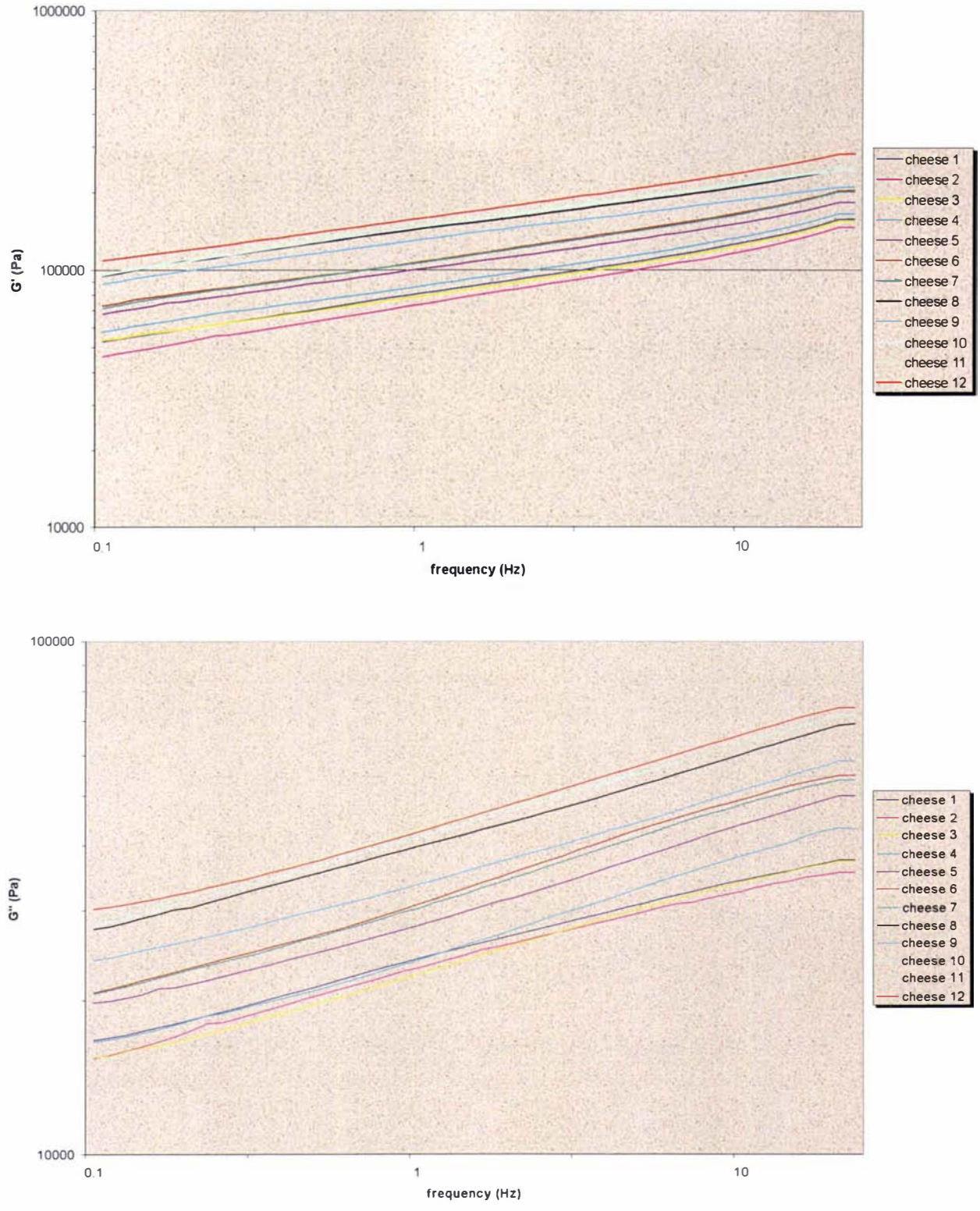


Figure 30. Storage moduli (G') and loss moduli (G") for the experimental cheeses from a frequency sweep test (frequency range 0.1 to 22 Hz)

Table 11. Probability values (*p*-values)[1] from the analysis of covariance for the results of the frequency sweep tests on the twelve experimental cheeses and required transformations for data analysis

PARAMETERS	TEMPERATURE	EXPERIMENTAL BLOCK	SAMPLE (CHEESE)	DATA TRANSFORM
G'	0.638	0.042	**0.000**	--
G"	0.652	0.070	**0.000**	--

[1] p-values in bold are significant at $p<0.05$

The Tukey's HSD significance test results (Table 12), at $\alpha = 5\%$, showed that experimental cheeses with lower moisture content (cheeses 10 to 12) have the highest values of G' and G" and were significantly different to the other cheeses. This means that cheeses 10 to 12 are more elastic than the others (cheeses 1 to 7). Cheeses 8 and 9 were not significantly different to cheeses 10 and 11, but were clearly less elastic than cheese 12 (Table 12).

Table 12. Mean values* and Tukey's HSD test results for G' and G" for the twelve experimental cheeses across the experimental blocks

CHEESES	STORAGE MODULUS (G') (Pa)	LOSS MODULUS (G") (Pa)
1	111863 a [1]	30047 a
2	121406 a	34383 a
3	119687 a	34474 a
4	117911 a	33737 a
5	130951 a	37391 a
6	164699 b	48015 b
7	163996 b	48647 b
8	192047 bc	55017 bc
9	193438 bc	55008 bc
10	213700 cd	61635 cd
11	214770 cd	62260 cd
12	233979 d	66026 d

[1] means within a column with no common letters differ significantly ($p<0.05$, $n=36$)

Those experimental cheeses in which the amount of water was increased from the base formulation (cheeses 1 to 5) showed the lowest values for G' and G", as seen in Table 12. This is an indication that these cheeses are the least elastic of all cheeses studied, i.e., they are the softest within the range of experimental cheeses evaluated. No significant differences were detected between cheeses 1 to 5 at the 95% confidence level. Cheeses 6 and 7 (repeats), as expected, did not differ between them and showed intermediate values of G' and G". Experimental cheeses 8 and 9 were not significantly different to cheeses 6-7 in the frequency sweep test, in spite of the higher G' and G" numerical values.

The storage and loss moduli of ideal solid materials do not vary with frequency, which means the slopes of the G' and G" curves as a function of frequency are very close or equal to zero. Increasing values for the slopes of G' curves mean more fluid, softer materials. Thus, the determination of the slopes of the G' curves in this study should provide an indication of the strength of the experimental cheeses within the frame of viscoelastic materials. The values for the slopes of G' and G" curves for each experimental cheese, across blocks, were determined and are presented in Table 13.

It can be seen that for G', the slopes range from 0.19 for cheese sample 1 (high moisture/low speed) to 0.16 for cheese sample 12 (low moisture/high speed). Values of slope (G') for Feta cheeses were reported by Wium & Qvist (1997) and were in the range 0.12-0.14. The values obtained in this study show that the experimental cheeses are not as elastic as Feta cheeses, albeit the slopes are relatively low and indicate the experimental cheeses are strong, homogeneous gelled emulsions. The decrease in the value of the slopes with decreasing moisture content confirms the higher strength or elasticity of the low moisture cheeses when compared to the high moisture ones. This observation could be attributed to the plasticising effect of increased moisture.

Table 13. Mean values* for the slopes of G' and G" for the twelve experimental cheeses across the experimental blocks

CHEESES	SLOPE (G' CURVES) (Pa.s)	SLOPE (G" CURVES) (Pa.s)
1	0.19	0.16
2	0.19	0.17
3	0.18	0.18
4	0.18	0.18
5	0.18	0.18
6	0.17	0.19
7	0.17	0.19
8	0.17	0.18
9	0.16	0.18
10	0.17	0.18
11	0.16	0.18
12	0.16	0.18

The slopes for the curves of G" were in the range 0.16-0.19, with cheeses 1 and 2 (high moisture/ low and intermediate speeds, respectively) displaying the lowest slope values (Table 13). These values are much higher than those reported by Wium & Qvist (1997) for Feta cheeses, in the range 0.07-0.11.

The results in Table 12 are complemented by the outputs of the response surface regression analysis shown in Table 14. Results from this regression show that moisture content plays a more important role than mixing speed on the storage (G') and loss (G") moduli values, as seen by the relative magnitude of the coefficients for both factors (moisture and speed). Figures 31 and 32 show graphically the relative importance of each factor on the response surface analysis, reinforcing the stronger contribution of moisture content on the parameters G' and G" when compared to mixing speed. A small quadratic moisture effect can be observed for G', but only a linear effect could be found for G".

Table 14. Response surface regression coefficients[1] and R-square values for the frequency sweep parameters G' and G" (storage and loss moduli, respectively) as a function of experimental blocks, moisture content and mixing speeds

PARAMETER	CONST	M	M^2	S	S^2	M X S	B1	B2	R-SQUARE (ADJ) [2]
G'	134996	-5397	92	193			-4502	7137	92.6%
G"	40054	-1578		56			-2056	1439	91.4%

[1] coefficients are: constant (CONST), moisture (M), moisture squared (M^2), speed (S), speed squared (S^2), moisture x speed (M X S), block 1 (B1) and block 2 (B2)

[2] R-square values were adjusted for the number of terms in the model.. R-square values < 80% indicate inability to model the rheological parameters. R-square values > 90% indicate good, satisfactory fit of model.

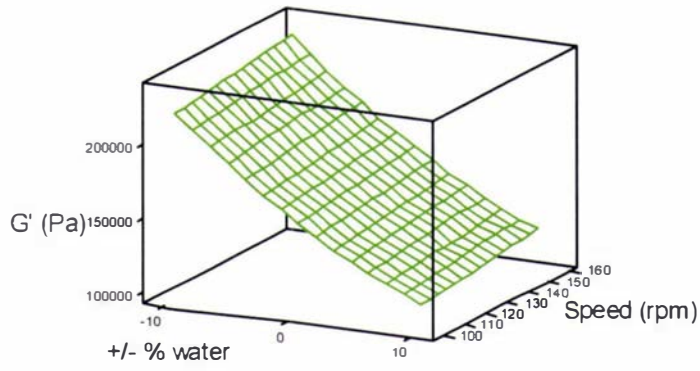


Figure 31. Response surface plot for the parameter G' (storage modulus) as a function of moisture content and mixing speed

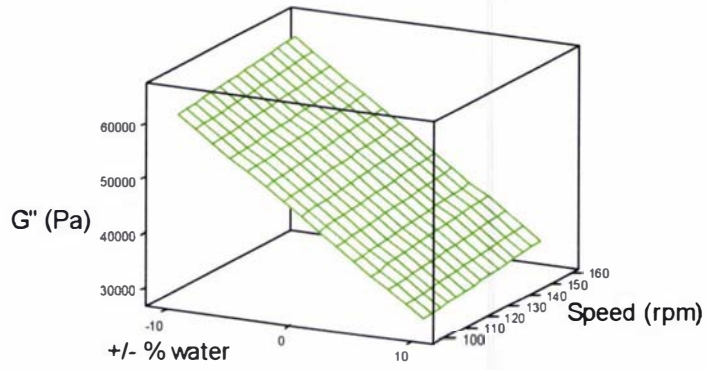


Figure 32. Response surface plot for the parameter G" (loss modulus) as a function of moisture content and mixing speed

Both parameters, G' and G", increased as the moisture content of the cheeses decreased. Micrographs from the confocal laser microscope (Figures19-23) shown in section 5.3 illustrate the fact that decreasing moisture content led to a decrease in the size and increase of the number of fat globules in the experimental cheeses. This, in turn, is assumed to have promoted a non linear increase (in relation to the changes in moisture content) in the occurrence of protein-protein, protein-fat and fat-fat links. More links and particle interactions, together with the lower plasticising effect of reduced moisture, increased the elasticity and therefore G' values of the experimental cheeses.

The increase in the elasticity of the experimental cheeses, however, was more pronounced than the changes in the viscous dissipation resulting from moisture content variations. It is known that it is the volume fraction of the particles in a dilute emulsion (such as the cheeses in this research), rather than the size and shape of these particles, which influences the viscosity of a system (McClements, 1999). In this study, the volume fraction of the fat globules is inversely proportional to the total fat and moisture content of the

cheeses, and resulted in the linear effect of moisture on G" found in Table 14 and Figure 32.

A small contribution of mixing speed on G' and G" can be observed from the response surface plots, even though such effect was not detected in the Tukey's HSD test results (Table 12). This is mainly due to the higher cut off level for the *p*-values in the regression analysis (10%, instead of 5%). It is likely that the range of mixing speeds used for the experimental cheeses in this study was not wide enough to yield significant differences between cheeses. The high values for R-square obtained indicate that more than 90% of the variance in the data (G' and G") can be accounted for by the two factors considered (moisture and speed).

5.5.3. Creep Test

The creep test used a constant stress of 2000 Pa and generated two curves, creep compliance and deformation, as a function of time (Figures 33 and 34, respectively). Similarly to the frequency sweep curves, data from experimental block 3 were used, instead of an average of all three blocks, to illustrate the creep test curves shown in Figures 33 and 34.

The value of stress used (2000 Pa) was selected from a strain sweep experiment (Appendix, section 9.0) and falls in the linear viscoelastic region for the experimental cheeses. It is worth noting that both curves (compliance and deformation) are actually the same, except for the recovery stage, as per the definition of compliance, shown in equation (2) below. A higher value of stress (3000 Pa) was also used in the preliminary runs, but such stress proved to cause the samples to fracture, yielding irregularly shaped curves, atypical of a creep compliance test.

The fitting of the compliance and deformation data with a four element Burgers model (Steffe, 1996), shown in Figure 35, yielded the parameters P1, P2, P3 and P4 (for each curve) shown in equation (1)

$$J(t) \text{ or } \gamma(t) = P1 + P2\,(1 - \exp[-t/P3]) + t/P4 \qquad \text{equation (1)}$$

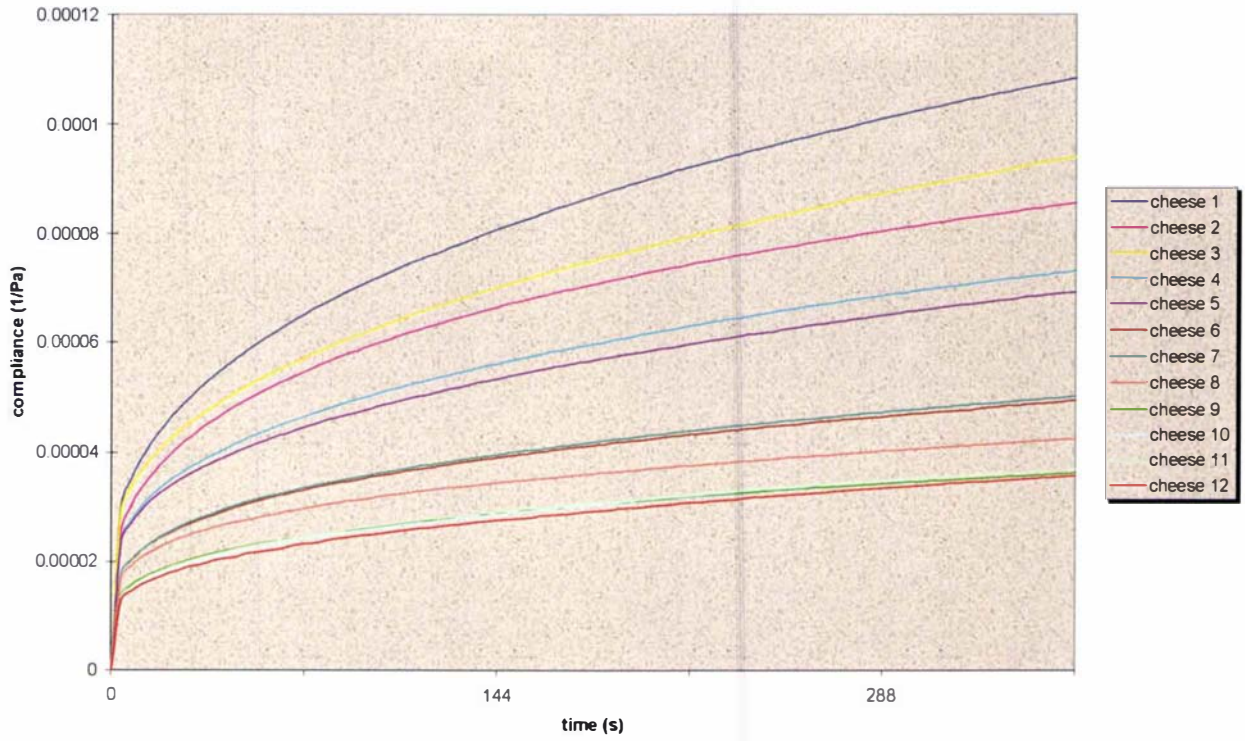


Figure 33. Creep compliance curves for the experimental cheeses from a creep test (stress 2000 Pa) over a period of 6 minutes (block 3)

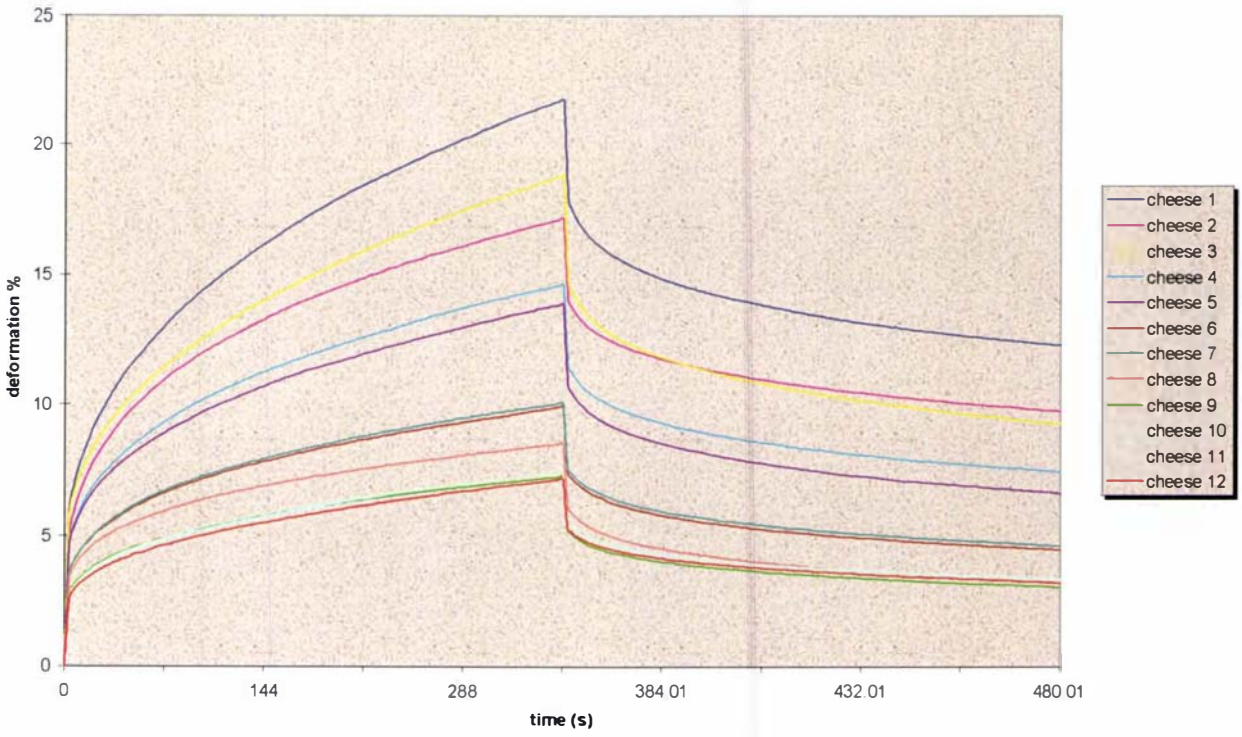


Figure 34. Deformation curves for the experimental cheeses from a creep test (stress 2000 Pa) over a period of 8 minutes (block 3)

where the compliance J is related to the shear deformation γ according to

$$J(t) = \gamma / \sigma_{constant} \qquad \text{equation (2)}$$

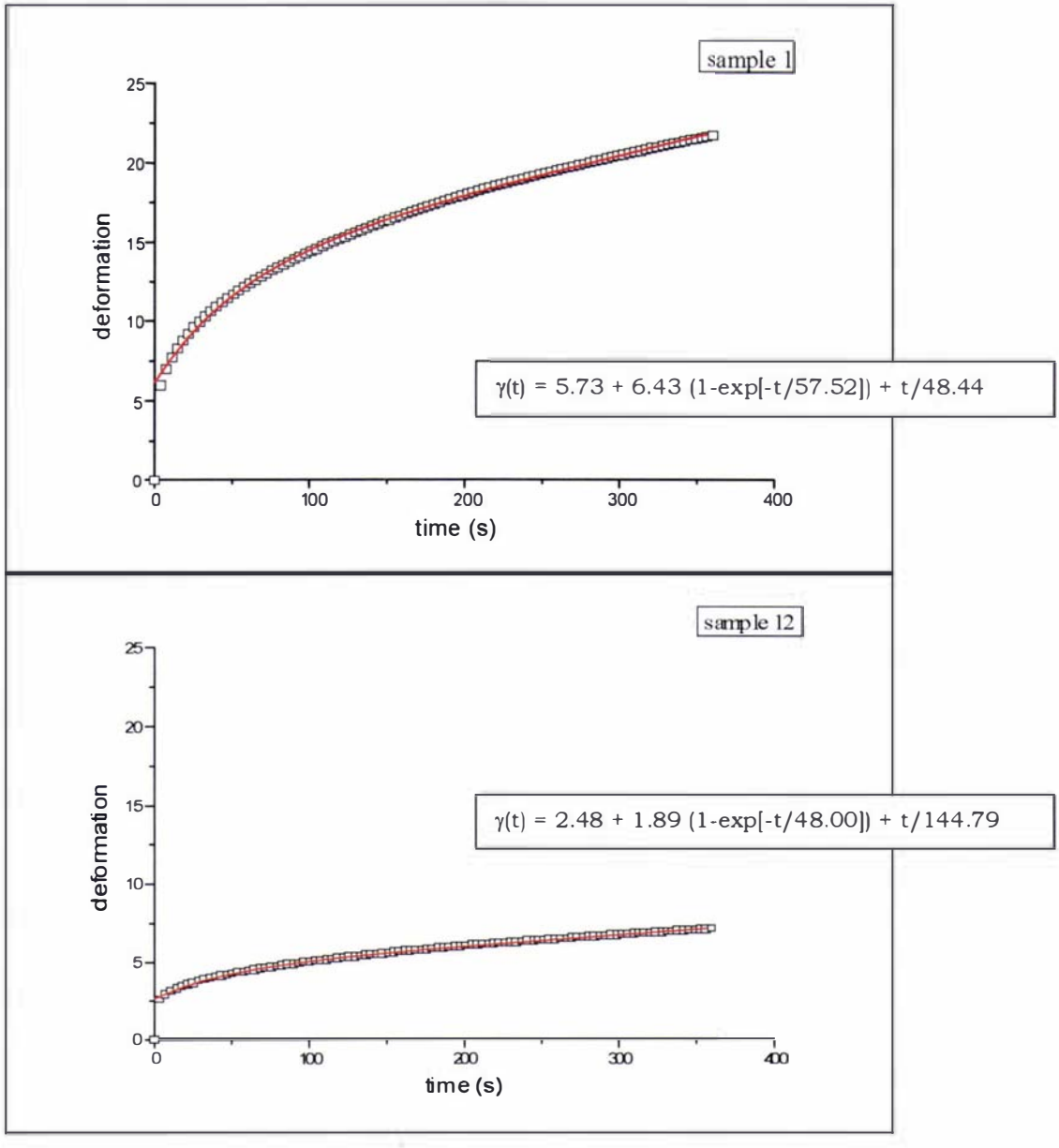


Figure 35. Fitting of the Burgers model (with equations) to experimental samples 1 (high moisture/low speed) and 12 (low moisture/high speed)

Fitting of the model to the deformation curves was done over the initial 6 minutes only (Figure 35), as the Burgers model does not apply to the creep recovery stage (recovery of the strain to the point of permanent deformation). Release of the applied stress and continuous measurement of the deformation for a further two minutes allowed this other parameter, the relative recovery of the sample after deformation, to be established.

The fitted equations for the different experimental samples can be found in Table 15, for both compliance and deformation, while the results of the analysis of covariance for each of the parameters from the creep test are shown in Table 16.

Table 15. Fitted equations, based on the Burgess model, to the creep compliance and deformation curves for the twelve experimental cheeses

CHEESE	FITTED EQUATIONS
1	$J(t) = 0.000027 + 0.005760\ (1\text{-}\exp[-t/56.94]) + t/9530426.2$ $\gamma(t) = 5.73 + 6.43\ (1\text{-}\exp[-t/57.52]) + t/48.44$
2	$J(t) = 0.000030 + 0.005477\ (1\text{-}\exp[-t/51.88]) + t/10638605.8$ $\gamma(t) = 5.62 + 5.99\ (1\text{-}\exp[-t/52.36]) + t/53.99$
3	$J(t) = 0.000027 + 0.005477\ (1\text{-}\exp[-t/53.52]) + t/11184058.8$ $\gamma(t) = 5.57 + 5.19\ (1\text{-}\exp[-t/53.67]) + t/56.67$
4	$J(t) = 0.000023 + 0.004807\ (1\text{-}\exp[-t/50.60]) + t/13124614.6$ $\gamma(t) = 4.94 + 4.66\ (1\text{-}\exp[-t/50.97]) + t/66.09$
5	$J(t) = 0.000020 + 0.004472\ (1\text{-}\exp[-t/51.62]) + t/14360615.8$ $\gamma(t) = 4.46 + 4.03\ (1\text{-}\exp[-t/51.93]) + t/71.94$
6	$J(t) = 0.000020 + 0.004236\ (1\text{-}\exp[-t/49.65]) + t/18255920.6$ $\gamma(t) = 3.64 + 3.24\ (1\text{-}\exp[-t/48.56]) + t/91.98$
7	$J(t) = 0.000020 + 0.004472\ (1\text{-}\exp[-t/50.20]) + t/18074271.1$ $\gamma(t) = 3.66 + 3.41\ (1\text{-}\exp[-t/48.80]) + t/90.26$
8	$J(t) = 0.000017 + 0.003162\ (1\text{-}\exp[-t/48.18]) + t/23676652.6$ $\gamma(t) = 3.09 + 2.51\ (1\text{-}\exp[-t/47.68]) + t/118.07$
9	$J(t) = 0.000013 + 0.003162\ (1\text{-}\exp[-t/49.55]) + t/26429728.1$ $\gamma(t) = 2.87 + 2.28\ (1\text{-}\exp[-t/48.33]) + t/131.64$
10	$J(t) = 0.000010 + 0.003162\ (1\text{-}\exp[-t/50.00]) + t/26429728.1$ $\gamma(t) = 2.69 + 2.20\ (1\text{-}\exp[-t/48.74]) + t/131.56$
11	$J(t) = 0.000010 + 0.003162\ (1\text{-}\exp[-t/48.86]) + t/22976901.8$ $\gamma(t) = 2.67 + 2.20\ (1\text{-}\exp[-t/48.17]) + t/135.29$
12	$J(t) = 0.000010 + 0.003069\ (1\text{-}\exp[-t/49.30]) + t/25393403.7$ $\gamma(t) = 2.48 + 1.89\ (1\text{-}\exp[-t/48.00]) + t/144.79$

Table 16. Probability values (*p*-values)[1] from the analysis of covariance for the results of the creep tests on the twelve experimental cheeses and required transformations for data analysis

PARAMETERS	TEMPERATURE	EXPERIMENTAL BLOCK	SAMPLE	DATA TRANSFORM
P1 (compliance)	0.187	**0.015**	**0.000**	--
P2 (compliance)	*	*	*	--
P3 (compliance)	**0.004**	**0.030**[2]	**0.023**[2]	log e
P4 (compliance)	0.991	0.136	**0.000**	log e
Recovery	0.221	0.056	**0.001**	exp
P1 (deformation)	0.103	**0.005**	**0.000**	--
P2 (deformation)	0.351	**0.031**	**0.000**	log e
P3 (deformation)	**0.033**	**0.000**[2]	**0.000**[2]	--
P4 (deformation)	0.382	0.131	**0.000**	--

* ANCOVA could not be performed because the assumptions of homogeneity of variance and normality of residuals did not hold, even after data transformation

[1] p-values in bold are significant at $p<0.05$

[2] p-values after adjustments of the values for the effect of temperature

The analysis of covariance showed that the assumptions of homogeneity of variance and normality of residuals did not hold for the parameter P2 (compliance), even after data transformation. The *p*-value obtained for "sample", however, was highly significant ($p = 0.000$). Hence, the inference that significant differences exist between the experimental cheeses for this parameter is unlikely to be affected by the lack of compliance to the assumptions, and Tukey's HSD test was performed to ascertain where the differences were (Table 17).

It can be seen from equation (1) that for time 0, both the predicted compliance and deformation are given by the parameter P1, which is the intercept of the curves. This parameter is the instantaneous response of the material to the applied stress and is expected to be larger for less elastic cheeses. In other words, for the same applied stress, less elastic cheeses will undergo higher deformation. Confirmation of this fact is obtained from the

results shown in Table 17, in which the values for the parameter P1 decrease with decreasing moisture content of the experimental cheeses.

For very short times, equation (1) can be expressed as a series of terms as a function of time (t), represented by

$$J(t) \text{ or } \gamma(t) = P1 + (P2/P3 + 1/P4)\, t - (P2/2\, P3^2)\, t^2 + 0\, t^3 \qquad \text{equation (3)}$$

At short times, i.e., in the initial stage of the creep test, the slopes of the creep compliance and deformation curves are given by the term (P2/P3 + 1/P4). It can be seen, from equation (3), that for fixed values of P3 and P4, the slope of the curves will increase with increasing values of P2. When P2 and P4 are fixed, the slopes decrease with increasing P3; similar behaviour is observed when P2 and P3 are the fixed terms.

For an ideal, Hookean solid, the parameter P3 (retardation time of the Kelvin component) equals zero, while it tends to infinity for a perfect, Newtonian liquid (Steffe, 1996). When evaluating viscoelastic materials, therefore, the value of P3 provides an indication of how solid or strong the material is. The results in Table 17 indicate that a clear differentiation between the experimental cheeses regarding the parameter P3 was difficult. A slight indication is observed, however, that cheeses with low moisture content are stronger, more solid-like than those with high moisture content, when the values of P3 from the deformation curves are considered. Cheese 1 (high moisture/low speed) is significantly different to cheeses 6 to 12, all with lower moisture content.

In this study, values of P2, P3 and P4 were all variable, as observed in Table 17. Both P2 and P3 decrease from sample 1 to sample 12, while P4 increases substantially. Hence, the slopes of the curves decrease from cheese 1 (high moisture/low speed) to cheese 12 (low moisture/high speed). In a creep test, high values for the initial slope are associated with less elastic samples. Hence, experimental cheeses 9 to 12 were the strongest ones among those evaluated, while cheese 1 was the least elastic, more fluid one.

Table 17. Mean values for 9 parameters obtained from the creep compliance test, for the twelve experimental cheeses, across the experimental blocks

	COMPLIANCE (1/Pa)					DEFORMATION (%)			
CHEESE	**P1 (1/Pa)**	**P2 (1/Pa)[2]**	**P3 (s)**	**P4 (Pa.s)**	**Recovery**	**P1 (%)**	**P2 (%)**	**P3 (s)**	**P4 (s)**
1	27E-6 ab[1]	57.6E-4 a	56.94 a	953E4 a	0.26 a	5.734 a	6.43 a	57.52 a	48.44 a
2	30E-6 a	54.8E-4 ab	51.88 ab	1064E4 a	0.26 a	5.615 a	5.99 a	52.36 ab	53.99 a
3	27E-6 ab	54.8E-4 ab	53.52 ab	1118E4 a	0.29 ab	5.570 a	5.19 ab	53.67 ab	56.67 a
4	23E-6 abc	48.1E-4 bc	50.60 ab	1312E4 ab	0.29 ab	4.943 ab	4.66 bc	50.97 b	66.09 ab
5	20E-6 bcd	44.7E-4 c	51.62 ab	1436E4 ab	0.29 ab	4.463 bc	4.03 cd	51.93 ab	71.94 ab
6	20E-6 bcd	42.4E-4 cd	49.65 ab	1826E4 bc	0.30 ab	3.636 cd	3.24 d	48.56 b	91.98 bc
7	20E-6 bcd	44.7E-4 cd	52.20 ab	1807E4 bc	0.30 ab	3.661 cd	3.41 d	48.80 b	90.26 bc
8	17E-6 cde	31.6E-4 d	48.18 b	2368E4 c	0.32 b	3.089 de	2.51 e	47.68 b	118.07 cd
9	13E-6 de	31.6E-4 d	49.55 ab	2643E4 c	0.33 b	2.866 de	2.28 ef	48.33 b	131.64 d
10	10E-6 e	31.6E-4 d	50.00 ab	2643E4 c	0.32 b	2.689 de	2.20 ef	48.74 b	131.56 d
11	10E-6 e	31.6E-4 d	48.86 b	2298E4 c	0.32 b	2.668 de	2.20 ef	48.17 b	135.29 d
12	10E-6 e	30.0E-4 d	49.30 ab	2539E4 c	0.33 b	2.478 e	1.89 f	48.00 b	144.79 d

[1] means within a column with no common letters differ significantly ($p<0.05$, $n=12$)

[2] the parameter P2 compliance was subjected to Tukey's HSD test despite the fact the assumptions of ANOVA did not hold

According to the model used, the parameter P4 is the asymptotic, Newtonian viscosity of the free dashpot. As described by Steffe (1996), after sufficient time has passed, the independent dashpot generates a purely viscous response in the material. This can be seen in equation (1) for values of time (t) tending to infinity, in which case the curves are linear and governed by the slope 1/P4.

The parameter P4 (viscosity) will be very large for elastic, solid-like materials and small for less viscous, more fluid-like materials. It is expected, therefore, that firmer cheese samples tested under creep have smaller values for slopes than softer cheeses. In this study, cheeses 8 to 12 (lower moisture contents when compared to base formulation) show the highest values for P4 among the experimental cheeses tested, while those cheeses presenting higher moisture content (cheeses 1 to 5) displayed larger slopes. This is confirmed by the results in Table 17.

The results of the creep test are in agreement with those yielded by the frequency sweep test, which indicated that the experimental cheeses with lower moisture content were stronger and more elastic than the other cheeses in the range studied. Drake *et al.* (1999b) used frequency sweep and creep tests on several cheese varieties and reported that stronger, firmer cheeses such as Parmesan and Cheddar showed higher values of G' and G" and lower values of creep compliance than cheeses such as Brie and Velveeta.

With regards to the creep recovery, the results presented in Table 17 show that this parameter increased from cheeses 1 to 12, as the moisture content decreased. Cheeses 8 to 12 were significantly different to cheeses 1 and 2. Intermediate recovery was shown by the experimental cheeses 3 to 7. This behaviour of the cheeses studied was expected, as more solid-like cheeses have an enhanced ability to store energy and recover their structure upon release of an applied stress, suffering smaller permanent deformations. This was also observed by Drake *et al.* (1999b) in their research.

The effects of changes in moisture content and mixing speed of the experimental cheese samples on the parameters from the creep test were

evaluated by response surface regression analysis. These results are summarised in Table 18 and Figures 36 and 37.

It can be seen in Table 18 that the R-square values obtained for the parameters P1 to P4 in deformation are mostly above 90%, indicating good fit of the model to explain the variation in the data. Fitting of P3 (deformation) was not as satisfactory, but still good, with an R-square value of 84%.

Table 18. Response surface regression coefficients[1] and R-square values for the creep compliance and deformation parameters as a function of experimental blocks, moisture content and mixing speeds

PARAMETER	CONST	M	M^2	S	S^2	M X S	B1	B2	R-SQUARE (ADJ) [2]
P1 compliance	0.000019	0.000001					0.000002	-0.000002	82.8 %
P2 compliance	0.000016	0.000001	0.000000						88.5 %
P3 compliance	30.2295	0.2519	0.0146				-0.7375	2.6033	64.1 %
Loge P4 compliance	16.7492	-0.0458	-0.0013				0.0104	-0.0727	84.4 %
Recovery	0.26994	-0.00278		0.00025			0.0033	-0.01153	58.0 %
P1 deformation	3.6986	0.1544	0.0043				0.1844	-0.3076	93.0 %
Loge P2 deformation	1.1706	0.0524	0.0008				0.0547	-0.0405	93.5 %
P3 deformation	52.0970	0.3230	0.0190	-0.0210			-1.8970	3.1470	84.1 %
P4 deformation	95.060	-4.405							90.6 %

[1] coefficients are: constant (CONST), moisture (M), moisture squared (M^2), speed (S), speed squared (S^2), moisture x speed (M X S), block 1 (B1) and block 2 (B2)

[2] R-square values were adjusted for the number of terms in the model.. R-square values < 80% indicate inability to model the rheological parameters. R-square values > 90% indicate good, satisfactory fit of model.

Values of R-square for the same parameters (P1-P4) in compliance were mostly around 80-90%, but the parameter P3, in this case, could not be satisfactorily modelled as a function of moisture content and mixing speed (R-square < 80%). Differences in the quality of the fit between parameters from

the compliance and deformation curves are likely to have been caused by the numerical values for these parameters in each case. Table 17 shows that, in compliance, values of P1 and P2 are very small, while P4 values are quite large. In contrast, values obtained for the deformation parameters are less extreme and easier to work with in regression analysis.

"Recovery" of the cheeses to their initial state after release of the stress could not be explained and modelled as a function of moisture and speed either, with an R-square of 58% indicating a poor fit of the response surface model (Table 18).

Mixing speed, as a factor, played a much less significant role in explaining the variation in the data than moisture content, regardless of the parameter considered. This is evident from the low coefficient values for speed in relation to those for moisture (Table 18). In addition, mixing speed was found to be significant only for two parameters, namely creep recovery (which showed poor fit of the model) and P3 in deformation.

The plots presented in Figures 36 and 37 take into account the different experimental blocks (n = 36). Table 18, however, illustrates the significance of the "block" effect on the data, using block 3 as the reference. Different experimental blocks affected the values of all parameters except P2 compliance and P4 deformation. The block differences are possibly due to the different bags of rennet casein, with different hydration properties, used for cheese manufacture in each individual block. Lack of significance for block in P2 compliance and P4 deformation could have been coincidental.

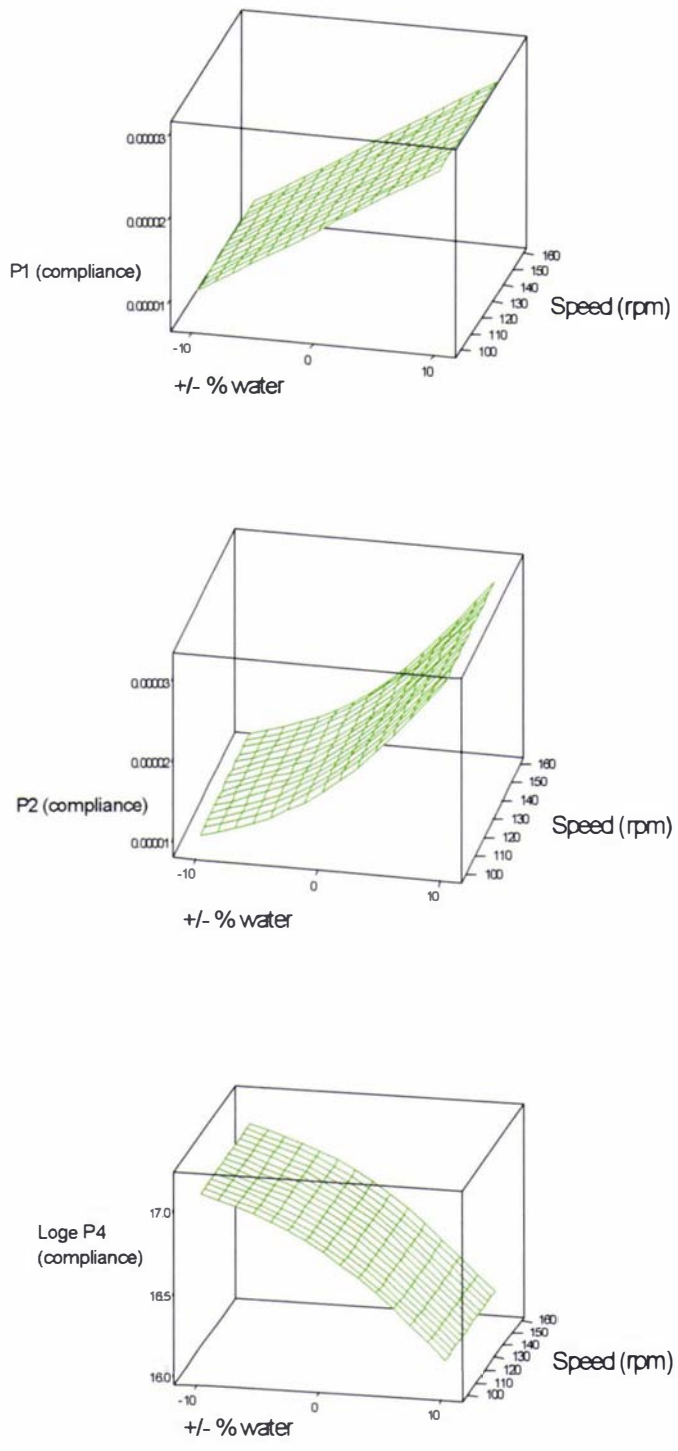


Figure 36. Response surface plot for the parameters P1, P2 and P4 (creep compliance) as a function of moisture content and mixing speed

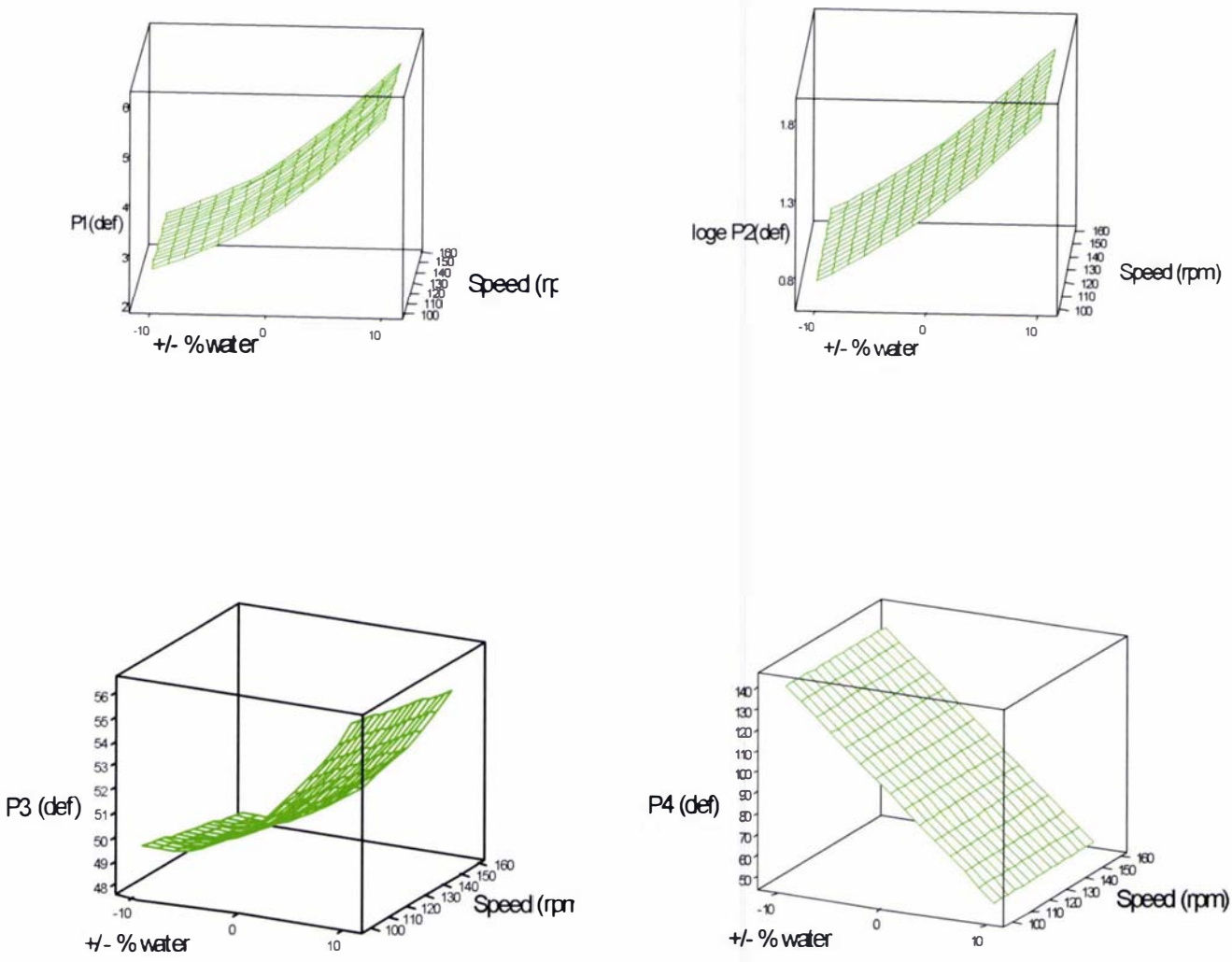


Figure 37. Response surface plot for the parameters P1, P2, P3 and P4 (shear deformation) as a function of moisture content and mixing speed

5.5.4. Compression to 70%

Compression tests are often used in texture research and results have been reported extensively (Ak & Gunasekaran, 1992; Ak & Gunasekaran, 1995b; Green *et al.*, 1985; Masi & Addeo, 1986; Pagliarini & Beatrice, 1994; Raphaelides & Antoniou, 1996; Yun *et al.*, 1994). In this study, cylindrical samples of the experimental cheeses with aspect ratio equal to unity were compressed to 70% of their original height. Curves of force versus time/displacement were generated for an entire cycle, which means the compression and the return of the probe to the initial position (decompression). With the calculations to convert the force versus displacement curves into true stress versus Hencky strain (Figure 38), six parameters were generated. These parameters and the *p*-values associated with the significance of the covariate temperature and the effect of experimental blocks and 'samples' (cheeses) can be found in Table 19.

Data from block 3 were used to illustrate the compression curves shown in Figure 38. It can be seen that stress increases linearly with compression until strains around 0.5, for all samples. Continuous compression of the samples seems to have caused initial fractures to develop in the cheese structure at strains above 0.04 (4% deformation), which is illustrated by a slight change in the slope of the stress x strain curves (structural rearrangement). Another very visible change in slope can be seen at strains above 0.5, until complete destruction of the integrity of the sample (peak stress). The strains at which the peak stresses occur are different for the different experimental cheeses (p = 0.038, Table 19), but the output of the Tukey's HSD test (Table 20) failed to detect where the differences were. This can be attributed to the fact that the Tukey's HSD test is quite a conservative type of test. It can be seen, however, that the lowest strains (at peak stress) were shown by the base formulation (cheeses 6 and 7).

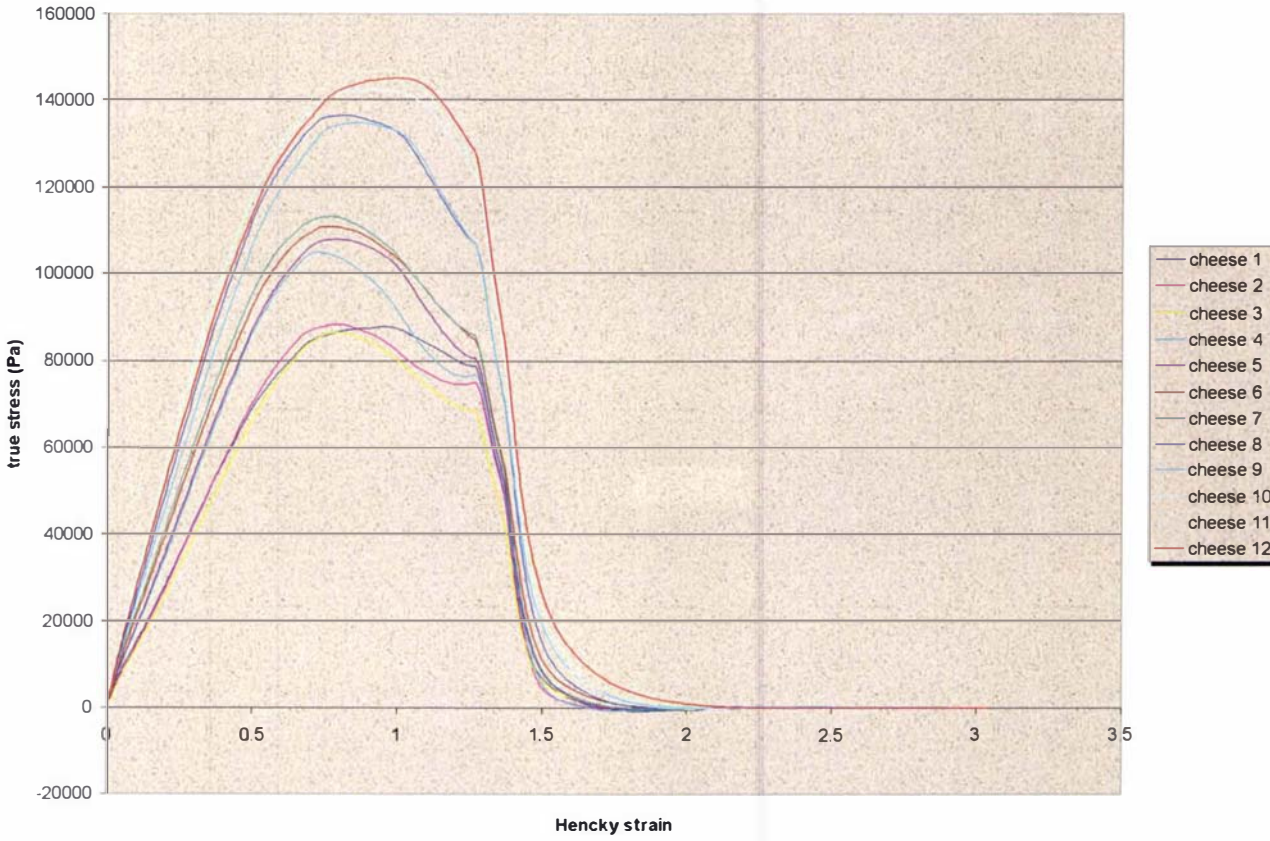


Figure 38. Compression curves for the experimental cheeses from the TA-XT2 Texture Analyser at a crosshead speed of 10 mm/s and load force of 250 N (testing temperature 6-7°C) (block 3)

As can be observed in Table 19, none of the parameters from the compression test were affected by the testing temperature (6-7°C), which means no adjustment to the values of those parameters had to be made. Highly significant *p*-values were found for the compression parameters other than the "strain at peak stress", indicating that differences between the experimental cheeses were clearly detectable.

Table 19. Probability values (*p*-values)[1] from the analysis of covariance for the results of the compression tests on the twelve experimental cheeses and required transformations for data analysis

PARAMETERS	TEMPERATURE	EXPERIMENTAL BLOCK	SAMPLE	DATA TRANSFORM
Young's modulus	0.555	**0.029**	**0.000**	--
Peak stress	0.956	**0.001**	**0.000**	log e
Strain peak stress	0.413	0.603	**0.038**	--
Work peak stress	0.739	0.304	**0.000**	--
Work (compression)	0.834	**0.000**	**0.000**	--
Work (decompression)	0.671	**0.000**	**0.000**	--

[1] p-values in bold are significant at $p<0.05$

The values obtained for Young's modulus or modulus of deformability (Table 20) show that cheeses with maximum moisture content among those tested (cheeses 1 to 3) were significantly different from the other cheese samples. Cheeses 8 to 12 were the firmest, most elastic ones within the range studied, showing the highest values for Young's modulus. Cheeses 4 to 7 were not significantly different from each other and showed intermediate elasticity as assessed by the Young's modulus.

The parameter "peak stress" provides also an indication of the elasticity of the different cheeses. It shows that cheeses with lower moisture content (cheeses 8 to 12) display the highest values of stress at the point of gross structural failure, regardless of mixing speed, being the firmest, most elastic cheeses among those studied. These observations are consistent with those obtained based on Young's modulus. Cheeses 1 to 3 were the softest of all experimental cheeses, with the lowest values of peak stress (Table 20).

With regards to the work each experimental cheese was subjected to in the compression cycle, it is possible to observe from Table 20 that both in compression (area under the curve during the downward movement of the crosshead) and decompression (area under the curve during the upward movement of the crosshead), cheeses with low moisture content (cheeses 10 to

12) showed the highest values for these parameters, regardless of mixing speed. Cheeses with higher moisture content (cheeses 1 to 3) were clearly the ones subjected to less work during compression due to their decreased firmness, but did not differ from cheeses 4 to 7 in decompression. "Work in decompression" was determined in an attempt to pick up any differences between experimental cheeses regarding the adhesiveness of these to the compression plate during decompression.

Table 20. Mean values for six parameters obtained in a compression to 70% test for the twelve experimental cheeses across the experimental blocks

CHEESE	Young's modulus (Pa)	Peak stress (Pa)	Strain peak stress (%)	Work peak stress (J)	Work compression (J)	Work (decompr) (J)
1	129022 a [1]	84965.5 a	0.9492 a	51729.8 a	78655 a	10634 ab
2	133115 a	87553.0 a	0.8651 a	46436.6 a	79740 a	10543 ab
3	134287 a	85819.4 a	0.7967 a	40520.1 a	78134 a	9203 a
4	163573 b	102744.4 b	0.7968 a	48825.2 a	92814 b	11557 ab
5	170042 bc	102744.4 b	0.8111 a	51492.0 a	95474 bc	11464 ab
6	189082 c	112420.3 c	0.7828 a	53825.1 ab	104474 d	12666 bc
7	187238 bc	110194.3 bc	0.7900 a	54015.5 ab	102682 cd	12274 b
8	223912 d	129314.2 d	0.8109 a	66534.0 bc	122284 e	15199 cd
9	227976 de	133252.4 de	0.8260 a	69971.7 bc	125842 e	16527 d
10	249075 e	142914.2 e	0.8754 a	82623.2 cd	137442 f	19404 e
11	243356 de	142914.2 e	0.8751 a	81654.1 cd	135746 f	19436 e
12	237675 de	141492.2 e	0.9541 a	90967.8 d	134472 f	20391 e

[1] means within a column with no common letters differ significantly ($p<0.05$, $n=36$)

The calculated areas are related to the energy stored by the samples during compression, i.e., they are the work the samples are subjected to per unit of volume. More elastic, firmer cheeses (cheeses 10 to 12) are expected to store more energy than the less elastic ones (cheeses 1 to 3), which will tend to dissipate part of the energy applied to them through flow.

The work to the gross failure point, represented by "work to peak stress", showed that cheeses 1 to 7 did not differ significantly from each other (Table 20). In agreement with the results for total work in compression, cheeses with low moisture (10 to 12) had the highest values for this parameter and did not differ from each other. Cheese 12 (low moisture/high speed) was significantly different from all remaining experimental cheeses in the range (cheeses 1 to 9), with the highest absolute value for this parameter.

The response surface regression analysis for the compression test (Table 21) showed that all parameters except "strain at peak stress" could be efficiently modelled as a function of moisture content. The value for R-square for the parameter "strain at peak stress" in the response surface regression (27.5%) indicates that the fit of the regression model to those data is quite poor. This observation confirms the previous findings regarding this parameter shown in Table 20. The plots for each modelled parameter obtained from the response surface regression are shown in Figures 39 to 43.

Table 21. Response surface regression coefficients[1] and R-square values for the six parameters obtained in the compression test as a function of experimental blocks, moisture content and mixing speeds

PARAMETER	CONST	M	M^2	S	S^2	M X S	B1	B2	R-SQUARE (ADJ) [2]
Young's modulus	190696	-5612					1830	-5492	96.2 %
Peak stress	114850	-2814					967	-3173	97.1 %
Strain to peak stress	0.78635	-0.0015	0.0010						27.5 %
Area to peak stress	55548	-1924	103						84.5 %
Area Up (total)	107313	-2872					2564	-3771	97.7 %
Area down	12886	-474	21				2236	-1451	94.5 %

[1] coefficients are: constant (CONST), moisture (M), moisture squared (M^2), speed (S), speed squared (S^2), moisture x speed (M X S), block 1 (B1) and block 2 (B2)

[2] R-square values were adjusted for the number of terms in the model.. R-square values < 80% indicate inability to model the rheological parameters. R-square values > 90% indicate good, satisfactory fit of model.

As in the previous response surface regressions, for the other rheological tests, the coefficients obtained for moisture are large and significant. This shows that, also in compression, differences in moisture content in the cheeses play a major role in yielding different rheological responses when compared to mixing speed. The effect of mixing speed was found not to be significant for any parameters in compression and its contribution was assumed to be negligible in yielding detectable differences between the range of textures chosen for the experimental cheeses.

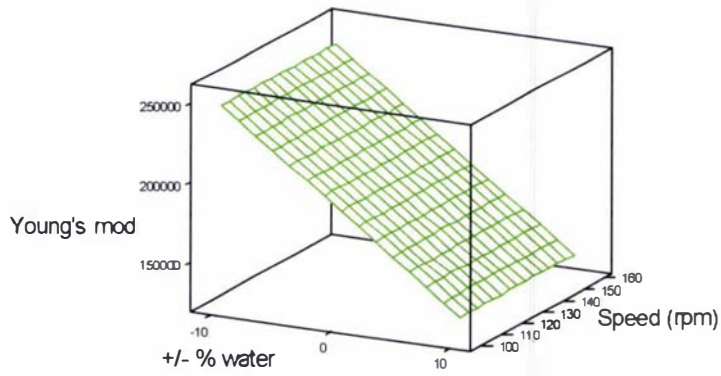


Figure 39. Response surface plot for the parameter "Young's modulus" as a function of moisture content and mixing speed

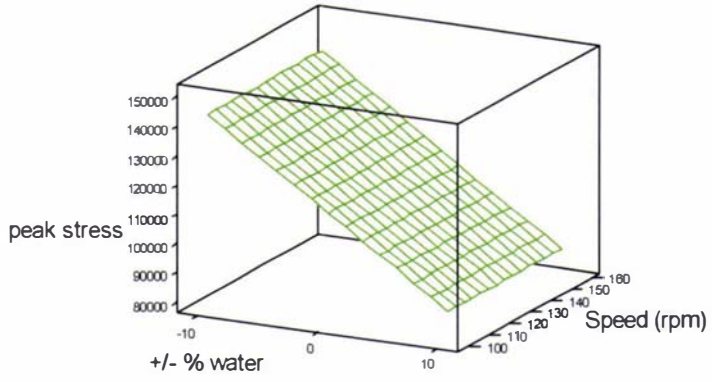


Figure 40. Response surface plot for the parameter "peak stress" as a function of moisture content and mixing speed

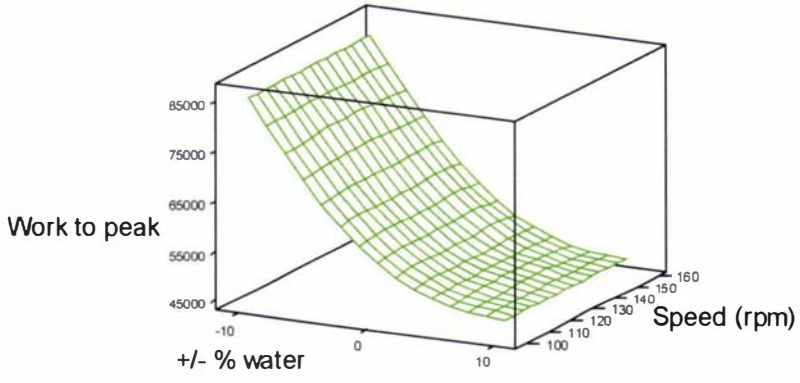


Figure 41. Response surface plot for the parameter "work to peak stress" as a function of moisture content and mixing speed

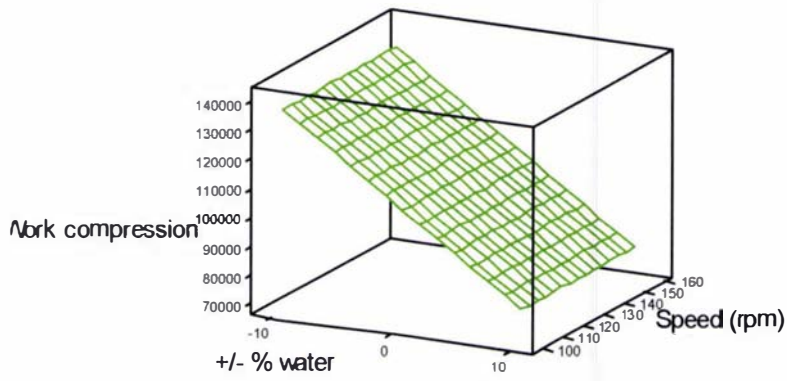


Figure 42. Response surface plot for the parameter "work in compression" as a function of moisture content and mixing speed

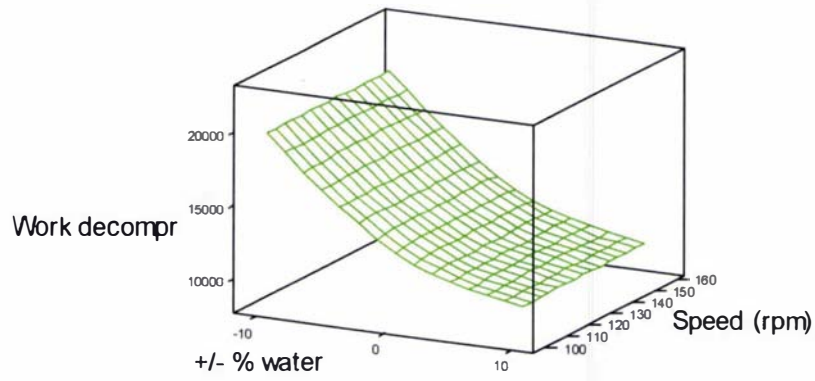


Figure 43. Response surface plot for the parameter "work in decompression" as a function of moisture content and mixing speed

The fact that no significance was obtained for mixing speed when the full regression model was used in the response surface analysis does not mean that the mixing speed used in processed cheese manufacture is not a contributing factor for the textural attributes of the finished product. Instead, it only suggests, once again, that the range of mixing speeds explored in this study was not wide enough to generate significant, detectable textural differences in the products, at the 95% confidence level. The possibility can not be ruled either that the effect exerted by moisture content in yielding textural differences, being so significant, might have masked any slight differences that the narrow range of mixing speed could have yielded.

It is important to note that all parameters from the compression tests seem to behave in the same direction, which means higher values for those parameters result from decreasing moisture content in the cheeses.

5.5.5. Interrelationship between rheological methods

Even though three different rheological tests (frequency sweep, creep and compression) were used to try and characterise the experimental processed cheese analogues in terms of textural parameters, a pairwise correlation procedure between all the rheological parameters yielded showed that all except "strain at peak stress" are highly correlated (Table 22).

The definition of several parameters from one single rheological test does not imply these parameters are necessarily independent from each other. In addition, parameters from different tests can actually measure the same or equivalent properties in the experimental samples, only in different ways. One example would be the correlation between Young's modulus or modulus of deformability from the compression test and the storage modulus (G') from the frequency sweep. Both of these parameters provide an indication of the strength or elasticity of the materials being tested and should be large for more solid-like cheeses (Drake *et al.*, 1999b).

Table 22. Probability values [1] (*p*-values) for the pairwise correlation between the rheological parameters

	G'	G"	P1 comp	P2 comp	P3 comp	P4 comp	Recov	P1 def	P2 def	P3 def	P4 def	Young mod	Peak stress	Strain peak	Area peak	Area up
G"	0.000	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
P1 comp	0.000	0.000	--	--	--	--	--	--	--	--	--	--	--	--	--	--
P2 comp	0.000	0.000	0.000	--	--	--	--	--	--	--	--	--	--	--	--	--
P3 comp	0.018	0.011	**0.085**	0.001	--	--	--	--	--	--	--	--	--	--	--	--
P4 comp	0.000	0.000	0.000	0.000	0.001	--	--	--	--	--	--	--	--	--	--	--
Recov	0.000	0.000	0.000	0.000	0.000	0.000	--	--	--	--	--	--	--	--	--	--
P1 def	0.000	0.000	0.000	0.000	0.038	0.000	0.000	--	--	--	--	--	--	--	--	--
P2 def	0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.000	--	--	--	--	--	--	--	--
P3 def	0.002	0.001	0.029	0.000	0.000	0.000	0.000	0.004	0.000	--	--	--	--	--	--	--
P4 def	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	--	--	--	--	--	--
Young mod	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	--	--	--	--	--
Peak stress	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	--	--	--	--
Strain peak	**0.370**	**0.534**	**0.587**	**0.827**	**0.562**	**0.622**	**0.585**	**0.938**	**0.930**	**0.907**	**0.372**	**0.776**	**0.442**	--	--	--
Area peak	0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000	--	--
Area up	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.372**	0.000	--
Area down	0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.000

[1] *p*-values in bold are **NOT** significant (rheological parameters do not correlate)

For the experimental cheeses used in this study, the plot of G' versus Young's Modulus is presented in Figure 44 and shows that the line of best fit has a slope very close to unity. The R-square for the linear regression was 0.82, which indicates the fit of the linear model is good. In this case, it is valid to infer that these two parameters are not truly independent and can lead to a problem of multicollinearity (Myers, 1990) in multiple linear regression. Multicollinearity is an important aspect to consider when correlating instrumental parameters to the sensory attributes and statistical techniques exist to account for its occurrence. Principal Component Analysis (PCA) and Canonical Correlation, used in this study, are examples of these techniques.

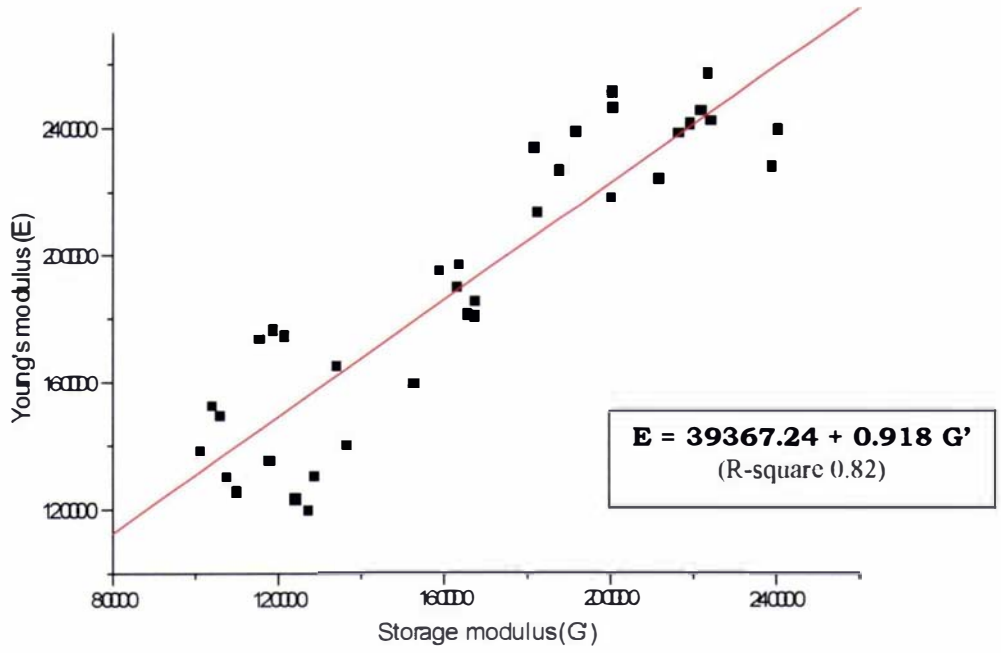


Figure 44. Linear regression between the storage (G') and Young's (E) moduli from the frequency sweep and compression tests, respectively (n = 36)

Multicollinearity must be diagnosed and dealt with if a model for prediction purposes is desired. Myers (1990) pointed out that a model with good fit does not necessarily behave as a good predictor if the regressor variables display redundant information, i.e., they inherently overlap in their

influence on a dependent response. Taking the parameters from the compression test as an example, the fact that all change in the same direction raises doubt about how orthogonal or independent they are from each other. Hence, their inclusion in a predictive model would raise doubt about the stability of the coefficients.

Principal component analysis can be used, in situations like this, to group those parameters that are or could be measuring the same material property, i.e., those parameters that are not truly independent. When performed on the rheological parameters used in this experimental work, three principal components were found. Principal component 1 (PC1), however, proved to explain more than 77% of the variance in the rheological data alone and is by far the most important one, if compared to the percentage of variance explained by the second and third principal components.

Out of the entire set of 17 rheological parameters considered in this study, 14 appear in principal component 1 (PC1) with coefficients of very similar magnitude. These values are shown in Table 23. The similarity in the coefficients displayed is an indication that these parameters are not truly independent from each other and that they can satisfactorily be summarised as one single variable called PC1. This provides evidence of the occurrence of multicollinearity in the data, as previously discussed. It is also observed that the parameters P3, both in compliance and deformation, display slightly smaller coefficients. Since the numerical values of P3 are not too far apart from the other parameters, with the exception of "strain at peak stress", it would be reasonable to infer that P3 is not an independent parameter and is also related to the others that constitute PC1.

Strain at the peak stress, however, has a very distinct coefficient (0.042), smaller than any other parameter in PC1. This indicates that the latter is an independent parameter, orthogonal to the combination of the others. Such assumption is reinforced by the high coefficient for this parameter (strain at peak stress) in PC2, which indicates that "strain at peak stress" is the main factor explaining the variance in the data not already explained by PC1. Details of the third principal component, PC3, are not

presented due to the small amount of variance explained and its relative importance in comparison to PC1 and PC2.

Table 23. Principal component results for the set of rheological parameters

VARIABLE	PC1	PC2
G'	-0.259	0.106
G"	-0.260	0.122
P1 compliance	0.241	-0.215
P2 compliance	0.259	-0.191
P3 compliance	0.184	0.305
P4 compliance	-0.259	0.026
Recovery	-0.222	-0.121
P1 deformation	0.256	-0.247
P2 deformation	0.264	-0.170
P3 deformation	0.205	0.251
P4 deformation	-0.270	0.019
Young modulus	-0.269	0.071
Peak stress	-0.272	0.007
Strain peak	-0.042	-0.686
Area peak	-0.245	-0.291
Area up	-0.271	-0.012
Area down	-0.242	-0.263
Explained variance	77.1 %	8.3 %

The grouping of parameters in canonical variables was also performed, for the purpose of correlating the instrumental results to the sensory textural attributes. Unlike PCA, canonical correlation is a powerful technique to maximise the correlation of two sets of independent data. Further discussion on the use of these techniques, in addition to using pairwise correlation and stepwise regression, is presented in section 5.6.

5.6. Correlation of sensory, chemical and instrumental data

The sensory, chemical and instrumental (microstructural and rheological) evaluation results obtained for the different experimental processed cheese analogues used in this study were adjusted for the influence of testing temperature, when and where necessary, before the correlation procedures were initialised.

In the case of the sensory scores and chemical and rheological parameters, the three experimental blocks were averaged for the correlation analysis. The microstructural data obtained through image analysis, on the other hand, could not be averaged across experimental blocks because of the different magnifications and thresholds used, as discussed in section 5.3. Consequently, the microstructural results for each block were subjected only to pairwise correlation with the respective sensory scores (Table 24) and chemical and rheological parameters (Table 25 and 26, respectively), but not carried onto the Principal Component Analysis (PCA) and Canonical Correlation (CC) procedures.

Pairwise correlation is a useful exploratory statistical technique to gather information on the significance of the relationship or association between two individual random variables. However, according to MacFie & Hedderley (1993), the researcher must bear in mind that the usual practice of computing the correlation using the mean of a number of replicates of a treatment is likely to overestimate the true correlation. In addition, simple correlation does not have predictive ability by itself since it does not set one of these random variables as a function of the other. This effect was shown by Roberston *et al.* (1984) when correlating sensory and instrumental firmness in kiwifruit.

The degree of linear association between the variables can only be ascertained by regression analysis, which involves estimating the best straight line to summarise a significant association. Predictive ability through regression is often improved by the use of multiple linear regression or, at times, nonlinear regression. In this study, multiple linear regression procedures were used. The theoretical basis of the statistical techniques used

can be found elsewhere (Myers, 1990; Neter *et al.*, 1985) and hence are not presented here.

5.6.1. Microstructural versus sensory/rheology pairwise correlations

It can be seen in Table 24 that the parameter "area of protein matrix" obtained from the confocal micrographs correlates highly with the sensory attributes "firmness in compression, firmness in cutting, stickiness and curdiness" (p = 0.000), regardless of the experimental block. Fracturability, rubberiness and greasiness showed not to correlate with the microstructural data. As discussed in section 5.4, these attributes were not efficient for discriminating between the cheese samples used in this study, possibly due to the narrow range of textures investigated. Hence, lack of significant correlation for these attributes was expected.

Table 24. Correlation coefficients[1] and probability values[2] for the pairwise correlation between the microstructural results for each experimental block and the respective sensory scores

ATTRIBUTES	AREA PROTEIN exp. Block 1	AREA PROTEIN exp. block 2	AREA PROTEIN exp. block 3
Adj Fracturability	-0.176 [1] 0.584 [2]	-0.447 0.146	0.400 0.198
Adj Firmness (compression)	0.962 **0.000** [3]	0.852 **0.000**	0.938 **0.000**
Adj Firmness (cutting)	0.963 **0.000**	0.894 **0.000**	0.910 **0.000**
Rubberiness	0.129 0.690	0.422 0.172	0.809 **0.001**
Adj Stickiness	-0.963 **0.000**	-0.920 **0.000**	-0.938 **0.000**
Adj Curdiness	0.946 **0.000**	0.857 **0.000**	0.935 **0.000**
Adj Greasiness	-0.299 0.345	-0.470 0.123	-0.525 0.079

[3] p-values in bold are significant at $p<0.05$

The significant *p*-value (p = 0.001) observed for the correlation of "rubberiness" and "area of protein matrix" in experimental block 3 (Table 24) is likely to have been coincidental, since the *p*-values for the same variables in the other blocks were largely non significant.

The results in Table 25 show that all chemical parameters are highly correlated to the microstructural results. Protein and fat content were both positively correlated to the area of the protein matrix, indicating that an increase in those parameters is associated with increasing area. Moisture content and pH values, on the other hand, are negatively correlated to the area of the protein matrix.

Table 25. Correlation coefficients[1] and probability values[2] for the pairwise correlation between the microstructural results for each experimental block and the respective chemical results

PARAMETERS	AREA PROTEIN exp. Block 1	AREA PROTEIN exp. block 2	AREA PROTEIN Exp. block 3
Moisture	-0.974 [1] **0.000** [2, 3]	-0.901 **0.000**	-0.946 **0.000**
Protein	0.975 **0.000**	0.935 **0.000**	0.955 **0.000**
Fat	0.968 **0.000**	0.921 **0.000**	0.952 **0.000**
pH day 1	-0.940 **0.000**	-0.950 **0.000**	-0.898 **0.000**
pH day 3	-0.939 **0.000**	-0.970 **0.000**	-0.911 **0.000**
pH day 7	-0.929 **0.000**	-0.966 **0.000**	-0.911 **0.000**
pH day 14	-0.910 **0.000**	-0.949 **0.000**	-0.906 **0.000**

[3] *p*-values in bold are significant at $p<0.05$

An increase in fat and protein content implies a decrease in moisture content. The relationship between the area of the protein matrix and moisture content was already shown in section 5.3. It was observed that decreasing moisture content caused the molten mass in the cooker to be thicker and subjected to higher shear stresses, which ultimately led to decreasing fat globule size and increasing protein matrix area. In view of that, the effect of protein and fat content variations on the area of the protein matrix of the cheeses in this study appears to be indirect.

Table 26 presents the correlation coefficients and probability values for the correlation of the microstructural data with the rheological parameters. It shows that the area of protein matrix is also highly correlated to almost all rheological parameters. The rheological parameter "strain at peak stress" proved not to correlate with the microstructural results, similarly to "P3 compliance" in experimental block 1 (p-value 0.104).

Existence of significant statistical correlation between two variables does not necessarily imply, as mentioned previously, that when regressed one against the other, a satisfactory line of best fit will be found. In general, however, it is possible to infer that great potential exists for the use of structural data obtained from the confocal microscope in correlation with sensory, rheological and chemical parameters for the range of processed cheese analogues investigated in this research. As an example, based on the results presented this far, it would be expected that cheeses in which the area of the protein matrix was large had higher scores for sensory firmness and curdiness, lower scores for sensory stickiness, higher relative protein content and higher values for the storage modulus (G'), Young's modulus and work in compression.

The fact that significant probability values were obtained for most parameters in Tables 24, 25 and 26, regardless of the differences in magnification and pixel threshold for the generation of the "area" values, supports the relevance of the quantitative structural information from image analysis techniques as a means of correlation to textural measurements in sensory and rheological analysis

Table 26. Correlation coefficients[1] and probability values[2] for the pairwise correlation between the microstructural results for each experimental block and the respective rheological results

PARAMETERS	AREA PROTEIN exp. Block 1	AREA PROTEIN Exp. block 2	AREA PROTEIN exp. block 3
G'	0.937 [1] **0.000** [2,3]	0.868 **0.000**	0.918 **0.000**
G"	0.935 **0.000**	0.884 **0.000**	0.917 **0.000**
P1 compliance	-0.874 **0.000**	-0.770 **0.003**	-0.929 **0.000**
P2 compliance	-0.948 **0.000**	-0.938 **0.000**	-0.955 **0.000**
P3 compliance	-0.493 0.104	-0.845 **0.001**	-0.823 **0.001**
P4 compliance	0.950 **0.000**	0.736 **0.006**	0.912 **0.000**
Creep recovery	0.603 **0.038**	0.883 **0.000**	0.826 **0.001**
P1 deformation	-0.971 **0.000**	-0.902 **0.000**	-0.934 **0.000**
P2 deformation	-0.968 **0.000**	-0.921 **0.000**	-0.951 **0.000**
P3 deformation	-0.827 **0.001**	-0.853 **0.000**	-0.887 **0.000**
P4 deformation	0.946 **0.000**	0.898 **0.000**	0.912 **0.000**
Young's mod	0.943 **0.000**	0.907 **0.000**	0.932 **0.000**
Peak stress	0.944 **0.000**	0.910 **0.000**	0.951 **0.000**
Strain peak stress	-0.512 0.089	0.317 0.315	0.389 0.211
Work peak stress	0.854 **0.000**	0.789 **0.002**	0.854 **0.000**
Work compression	0.949 **0.000**	0.901 **0.000**	0.941 **0.000**
Work decompression	0.877 **0.000**	0.832 **0.001**	0.915 **0.000**

[1] *p*-values in bold are significant at $p<0.05$

5.6.2. Sensory versus rheological and chemical correlations

5.6.2.1. *Pairwise correlation (sensory x chemical x rheological)*

Each individual sensory attribute or instrumental parameter was correlated with each other in this initial stage of the correlation procedure. The objective of the pairwise correlation was to ascertain if any individual instrumental parameter (chemical or rheological) could be used to establish significant association with any one sensory response. Correlations within the rheological parameters are not presented here, as they have been reported and discussed previously in section 5.5.

The correlation coefficients and probability values for the pairwise correlation between the sensory attributes and the chemical/rheological parameters are found in Tables 27, 28 and 29.

The results displayed in Table 27 show that all chemical parameters are highly correlated with the sensory attributes "firmness" (both in compression and cutting), "rubberiness", "stickiness" and "curdiness", when individually considered. It is possible to observe that the correlation coefficients obtained for rubberiness are relatively low, despite the significance found for the correlation. This is an indication that the accuracy of the association between sensory rubberiness and the chemical results is not satisfactory for predictive purposes. Similarly to the microstructural results, no significant correlation was found for fracturability and greasiness in relation to the chemical parameters. For those highly correlated attributes, the pairwise correlation does not allow for the best predictor to be detected nor does it provide any information regarding how independent each sensory attribute or instrumental parameter is from others.

The same was observed when the sensory attributes were correlated to individual frequency sweep and creep test parameters (Table 28). Fracturability and greasiness showed poor correlation with any sensory attribute. This is consistent with the results from the sensory analysis of variance (section 5.4, Table 9), which showed that, for those parameters, the range of textures studied was not wide enough for the response differences

between the experimental cheeses to overcome the panel noise. Once again, all significant correlations were highlighted without an indication of which rheological parameter was the best single predictor of the sensory responses.

With regards to the compression test (Table 29), "firmness" (compression and cutting), "rubberiness", "stickiness" and "curdiness" highly correlate to most of those rheological parameters. Rubberiness was the only one sensory parameter that showed significant correlation to the "strain at peak stress" (p = 0.023), even though the correlation coefficient is low (0.378). Thus, such correlation might not have practical significance. This rheological parameter (strain at peak stress) appears not to be an appropriate one to discriminate between the experimental samples. "Strain at the peak stress" was shown in section 5.5.5 to be a poor parameter even in pairwise correlation within the rheological group of parameters. These results are in agreement with those from Wium *et al.* (1997) for this parameter, but differ from those reported by Hough *et al.* (1996) for Reggianito cheese, which indicated that strain at breaking point was the best instrumental parameter to correlate with sensory attributes.

Table 27. Correlation coefficients[1] and probability values[2] for the pairwise correlation between the sensory scores and the chemical parameters

SENSORY ATTRIBUTE	CHEMICAL PARAMETERS						
	Moisture	protein	fat	pHday1	pHday3	pHday7	pHday14
Adj Fracturability	-0.027 [1] 0.877 [2]	0.127 0.462	0.113 0.513	-0.305 0.070	-0.305 0.070	-0.241 0.157	-0.252 0.138
Adj Firmness (compression)	-0.963 **0.000** [3]	0.935 **0.000**	0.950 **0.000**	-0.795 **0.000**	-0.781 **0.000**	-0.821 **0.000**	-0.824 **0.000**
Adj Firmness (cutting)	-0.889 **0.000**	0.825 **0.000**	0.840 **0.000**	-0.648 **0.000**	-0.627 **0.000**	-0.698 **0.000**	-0.707 **0.000**
Rubberiness	-0.548 **0.001**	0.561 **0.000**	0.571 **0.000**	-0.490 **0.002**	-0.482 **0.003**	-0.503 **0.002**	-0.456 **0.005**
Adj Stickiness	0.903 **0.000**	-0.908 **0.000**	-0.919 **0.000**	0.830 **0.000**	0.834 **0.000**	0.853 **0.000**	0.837 **0.000**
Adj Curdiness	-0.948 **0.000**	0.917 **0.000**	0.935 **0.000**	-0.802 **0.000**	-0.773 **0.000**	-0.803 **0.000**	-0.784 **0.000**
Adj Greasiness	0.169 0.325	-0.062 0.718	-0.077 0.654	-0.163 0.342	-0.190 0.267	-0.089 0.605	-0.121 0.482

[3] The p-values in bold are significant at $p<0.05$

Table 28. Correlation coefficients[1] and probability values[2] for the pairwise correlation between the sensory scores and the rheological parameters from dynamic testing (frequency sweep and creep compliance test)

SENSORY ATTRIBUTE	RHEOLOGICAL PARAMETERS										
	Frequency sweep		Creep compliance/deformation								
	G'	G"	P1 (compl)	P2 (compl)	P3 (compl)	P4 (compl)	Recovery	P1 (def)	P2 (def)	P3 (def)	P4 (def)
Adj Fracturability	0.023 [1]	0.084	-0.051	0.050	-0.201	0.080	0.041	0.035	0.027	-0.016	0.061
	0.893 [2]	0.625	0.767	0.770	0.240	0.643	0.811	0.839	0.874	0.926	0.723
Adj Firmness (compression)	0.945	0.934	-0.895	-0.927	-0.452	0.860	0.652	-0.942	-0.909	-0.554	0.922
	0.000 [3]	**0.000**	**0.000**	**0.000**	**0.006**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
Adj Firmness (cutting)	0.868	0.839	-0.795	-0.870	-0.396	0.764	0.585	-0.884	-0.850	-0.550	0.830
	0.000	**0.000**	**0.000**	**0.000**	**0.017**	**0.000**	**0.000**	**0.000**	**0.000**	**0.001**	**0.000**
Rubberiness	0.471	0.464	-0.497	-0.563	-0.389	0.644	0.588	-0.488	-0.516	-0.410	0.602
	0.004	**0.004**	**0.002**	**0.000**	**0.019**	**0.000**	**0.000**	**0.003**	**0.001**	**0.013**	**0.000**
Adj Stickiness	-0.886	-0.880	0.886	0.908	0.496	-0.796	-0.659	0.901	0.911	0.498	-0.895
	0.000	**0.000**	**0.000**	**0.000**	**0.002**	**0.000**	**0.000**	**0.000**	**0.000**	**0.002**	**0.000**
Adj Curdiness	0.907	0.894	-0.826	-0.899	-0.581	0.849	0.764	-0.877	-0.904	-0.680	0.931
	0.000	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
Adj Greasiness	-0.153	-0.091	0.190	0.248	-0.150	-0.105	-0.087	0.216	0.211	0.052	-0.093
	0.372	0.596	0.267	0.145	0.381	0.542	0.613	0.206	0.217	0.761	0.588

[3] The *p*-values in bold are significant at $p<0.05$

Table 29. Correlation coefficients[1] and probability values[2] for the pairwise correlation between the sensory scores and the rheological parameters from the compression testing

SENSORY ATTRIBUTE	RHEOLOGICAL PARAMETERS – COMPRESSION TEST					
	Young's modulus	Peak stress	Strain to peak stress	Work to peak stress	Work compression	Work decompr
Adj Fracturability	-0.016 [1] 0.928 [2]	0.018 0.915	0.150 0.382	0.135 0.433	-0.018 0.919	-0.153 0.373
Adj Firmness (compression)	0.952 **0.000** [3]	0.950 **0.000**	0.075 0.666	0.829 **0.000**	0.949 **0.000**	0.807 **0.000**
Adj Firmness (cutting)	0.890 **0.000**	0.876 **0.000**	-0.006 0.970	0.710 **0.000**	0.889 **0.000**	0.814 **0.000**
Rubberiness	0.547 **0.001**	0.579 **0.000**	0.378 **0.023**	0.633 **0.000**	0.581 **0.000**	0.569 **0.000**
Adj Stickiness	-0.892 **0.000**	-0.895 **0.000**	-0.062 0.718	-0.788 **0.000**	-0.879 **0.000**	-0.672 **0.000**
Adj Curdiness	0.929 **0.000**	0.941 **0.000**	0.173 0.312	0.855 **0.000**	0.946 **0.000**	0.871 **0.000**
Adj Greasiness	-0.190 0.266	-0.164 0.340	0.025 0.887	-0.083 0.631	-0.199 0.243	-0.319 0.058

[3] The *p*-values in bold are significant at $p<0.05$

Fracturability and greasiness showed poor correlation with all rheological parameters in compression (Table 29), mainly due to the fact that the variance in sensory response for these attributes (panel noise) is larger than the variance between cheeses. This is observed from the results in Table 9 (section 5.4), which showed that discrimination between experimental cheeses based on these two attributes was unsuccessful.

5.6.2.2. *Stepwise regression*

In order to ascertain which was the best predictor for each sensory attribute among those defined instrumental parameters (chemical and rheological), forward stepwise regression was performed (Hair *et al.*, 1998).

The purpose of using such a procedure was to find not only the best single predictor but also the simplest model, i.e., with the smallest number of terms possible, which can efficiently model individual sensory responses. Stepwise regression has been extensively used in food research for the generation of simple predictive models in a range of different applications. These include the prediction of carcass composition (Cisneros *et al.*, 1996; Fan *et al.*, 1992; Griffin *et al.*, 1999; Herring *et al.*, 1994; Johnson *et al.*, 1990b; Stanford *et al.*, 1995; Wishmeyer *et al.*, 1996) and of sensory characteristics of several dairy and non dairy products (Bevilacqua & Califano, 1992; Blankenship *et al.*, 1997; Fernandez-Garcia & Casp, 1998a,b; Imhof *et al.*, 1994; Najim & White, 1990; Sheen *et al.*, 1991), to name but a few.

Separate analyses were run for the chemical and for the rheological parameters. Determination of the simplest appropriate model was based on the yielded values of R-square, which tend to increase as more terms are included in the model. Evers & Withey (1989), when studying the use of image analysis of grains for the prediction of milling extraction rates of wheat, observed that addition of more than one shape factor to the predictive model increased the R-square values obtained. Similar observations were reported by Griffin *et al.* (1999) and Johnson *et al.* (1990a) in their studies.

The results of the stepwise regression analysis (with regression equations) for the chemical parameters can be found in Table 30 and those for the rheological parameters in Table 31. Consistent with the observations from the pairwise correlation (Tables 27 to 29), no models were obtained for the sensory attributes "fracturability" and "greasiness". The models obtained for "rubberiness" showed very poor fit, with R-squares values of 30.6% and 39.8% for the chemical and rheological parameters, respectively (Table 30 and 31), despite the significant statistical correlations shown in Tables 27-29.

The equations in Table 30 show that the variation in response for the attributes "firmness in compression" and "curdiness" can be modelled efficiently as a function of moisture content. Higher moisture content has a plasticising effect on the final texture of the cheeses and yields larger fat globules in the structure, making the cheeses weaker and less firm. This

plasticising effect also makes those experimental cheeses with higher moisture smoother, pastier and less curdy. Figures 45 and 46 show the relationship between moisture and firmness in compression and curdiness, respectively. "Stickiness", on the other hand, can be best modelled as a function of the fat content, as the increase in fat content of the cheeses promotes increased lubrication when they are handled in textural evaluation. Figure 47 graphically shows how "stickiness' relates to fat content of the experimental cheeses.

With regards to "firmness in cutting", the stepwise regression analysis showed that no single chemical parameter could model the variation in the sensory data with a satisfactory fit (R-square = 78.5%). The regression procedure then added a second term to the model, increasing the goodness of the fit from less than 80%, when only moisture was used, to 86.9% (Table 30). When moisture alone was used to model "firmness in cutting", the residuals of the regression analysis were not normal, which means the coefficients of that regression equation can not be assumed to be stable. Graphical representation of "firmness in cutting" as a function of moisture and fat content seems thus more appropriate and is found in Figure 48.

Table 30. Regression equations and adjusted R-square values for the best single chemical predictor and the simplest model (when appropriate) from the stepwise regression, for each sensory attribute

SENSORY ATTRIBUTE	REGRESSION EQUATION	R-SQUARE
Firmness compression	F(cp) = 42.75 – 0.671 moisture	92.4 %
Firmness cutting	F(ct) = 39.05 – 0.574 moisture	78.5 %
	F(ct) = 170.03 – 1.926 moisture – 3.14 fat	86.9 %
Stickiness	S = 21.30 – 0.998 fat	84.1 %
Curdiness	C = 44.27 – 0.639 moisture	89.6 %
Rubberiness	R = 1.164 + 0.194 fat	30.6 %

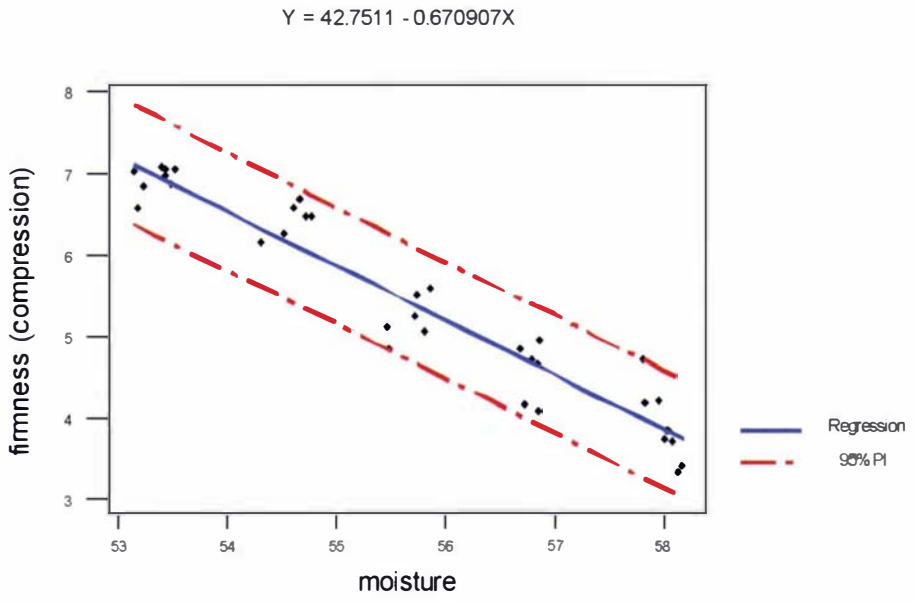


Figure 45. Sensory firmness in compression as a function of moisture content of the experimental cheeses (• raw data), with prediction interval

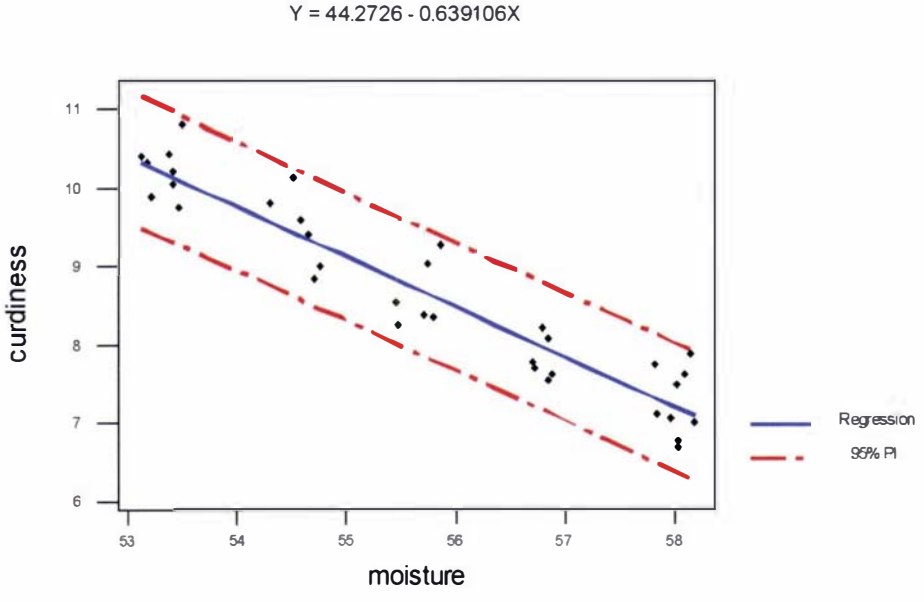


Figure 46. Sensory curdiness as a function of moisture content of the experimental cheeses (• raw data), with prediction interval

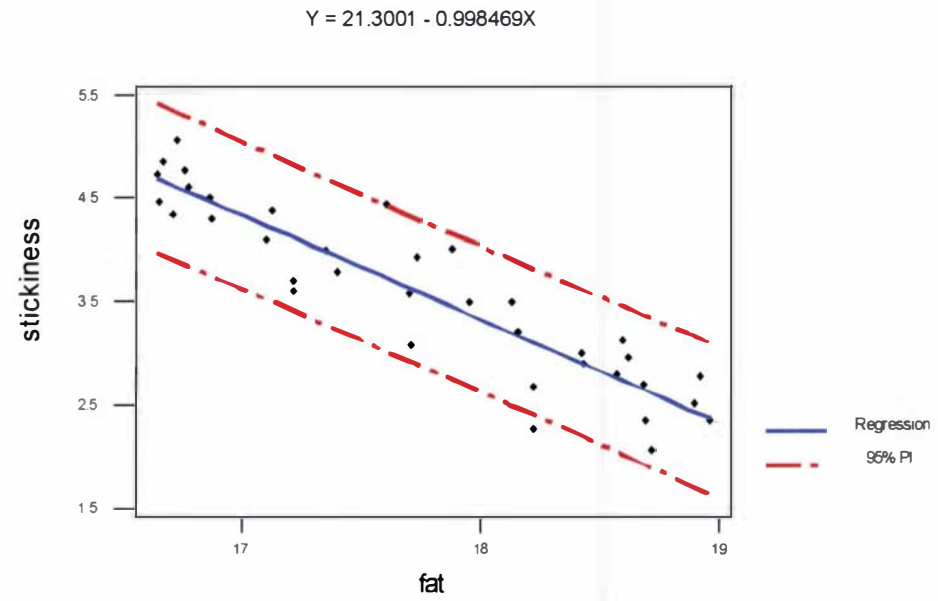


Figure 47. Sensory stickiness as a function of fat content of the experimental cheeses (• raw data), with prediction interval

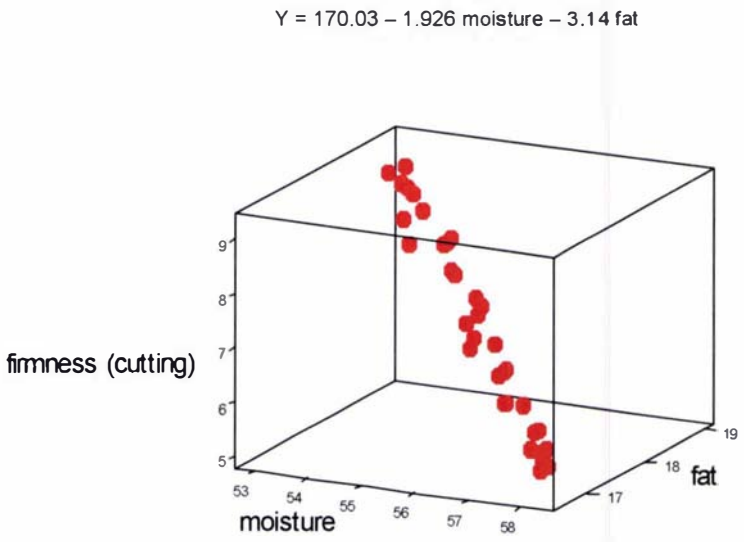


Figure 48. Sensory firmness in cutting as a function of moisture and fat content of the experimental cheeses (• raw data)

MacFie & Hedderley (1993) draw attention to the fact that the relations established using forward stepwise regression are often interpreted as causal, even though that is not always necessarily true. According to these authors, it is important to bear in mind that, by the mathematical principle used to derive the relations, other combinations of explanatory variables can potentially do just as well. Because of the interrelationship between the several compositional parameters of the cheeses in this study, in particular moisture and fat, the direct effect of each single predictor in the regression equations in Table 30 should not be assumed to be correct or exclude contribution of other causal factors.

Table 31. Regression equations and adjusted R-square values for the best single rheological predictor and the simplest model (when appropriate) from the stepwise regression, for each sensory attribute

SENSORY ATTRIBUTE	**REGRESSION EQUATION**	**R-SQUARE**
Firmness compression	F(cp) = 0.252 + 0.000027 Young	90.4 %
	F(cp) = 0.337 + 0.000015 Young + 0.000013 G'	94.1 %
Firmness cutting	F(ct) = 2.660 + 0.000023 Young	78.5 %
	F(ct) = 1.72 + 0.000015 Young + 0.253 P3 compliance – 0.188 P3 def – 48161 P2 compliance	85.7 %
Stickiness	S = 1.270 + 1.90 loge P2 def	87.8 %
	S = -17.5 + 2.82 loge P2 def + 1.06 loge P4 compliance	90.6 %
Curdiness	C = 3.23 + 0.000051 area_up	89.1 %
	C = 6.69 + 0.000031 area_up – 1.12 loge P2 def	90.7 %
Rubberiness	R = 4.15 + 0.00000002 P4 compliance	39.8 %

Similarly to the regression results using the chemical parameters, the regression equations for modelling the variation in sensory response using single rheological parameters (Table 31) showed this was possible for "firmness in compression, stickiness and curdiness". Firmness in cutting, similarly to its behaviour in relation to the chemical parameters, required a more comprehensive model (four terms) in order to yield good fit. Graphical

representation for this parameter is not available, due to the number of terms in the model, but Figures 49 to 51 show how 'firmness in compression, curdiness and stickiness", respectively, vary in relation to their best rheological predictors.

Even though the addition of terms to the fitting model in forward stepwise regression can improve the goodness of the fit, this regression procedure does not account for multicollinearity of the explanatory variables, i.e., it does not provide any information regarding the true independence of the parameters used (Hair *et al.*, 1998). Multicollinearity is related to parameters that inherently overlap in their influence on a dependent response (Myers, 1990). Its occurrence can lead to unstable coefficients in the regression analysis that might compromise the quality of prediction, even if additional parameters do not directly bias the model results. Multicollinearity problems in the use of multiple regression was detected and reported by Aparicio *et al.* (1992), Plotto *et al.* (1997), Togari *et al.* (1995) and Weigel *et al.* (1997) in their studies.

The Young's modulus, also called elastic modulus, and the storage modulus (G') are known to provide information about the strength of the tested materials, as discussed in section 5.5.5. These parameters have been reported as good discriminators of firmness between cheeses (Drake *et al.*, 1999; Wium & Qvist, 1997). It seems therefore reasonable that these parameters were the best predictors found for sensory firmness of the experimental cheeses during compression.

It can be seen in Figure 44 (section 5.5.5) that both moduli increase in the same direction, i.e., they are positively correlated. Increasing values of Young's modulus and G' are therefore associated with increased firmness in compression. The fact that these rheological parameters are highly correlated strongly suggests the occurrence of multicollinearity when both are used to model sensory "firmness is compression". In this case, the increase observed in the adjusted R-square value (90.4% to 94.1%) with the introduction of G' needs to be considered in relation to the instability of the coefficients produced for the regression equation. This, in turn, compromises the quality

of the prediction model. The best alternative, in this case, appears to be the use of one single predictor, which still provides a satisfactory quality of fit.

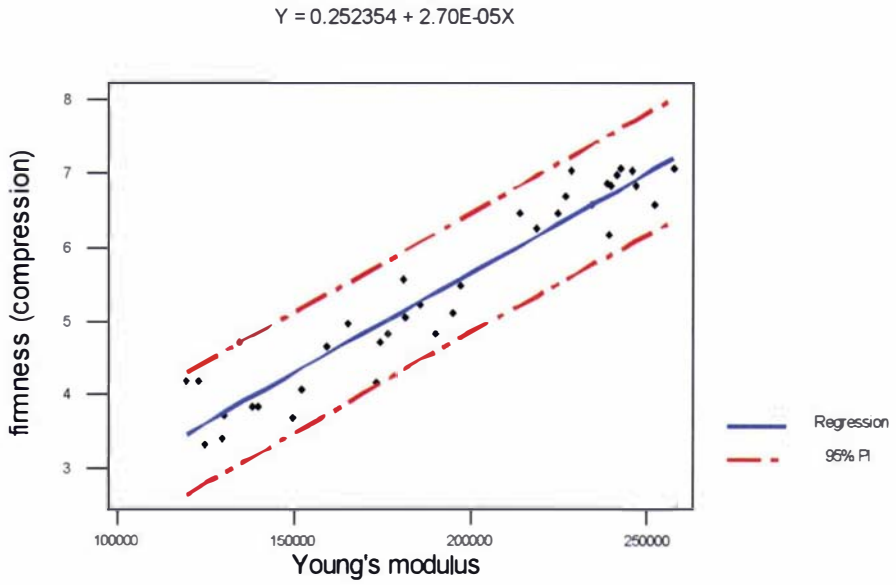


Figure 49. Sensory firmness in compression as a function of Young's modulus of the experimental cheeses (• raw data), with prediction interval

Curdiness is best modelled by the rheological parameter "area up", which is the work in compression. Large values of the area, which reflect the energy stored by the cheese samples during the compression stage, are related to increased curdiness. This observation could be linked to the effect of moisture on the experimental samples. Those cheeses with lower scores for curdiness were the ones with high moisture content. The plasticising effect of the water makes it easier for these experimental cheeses to flow, i.e., for planes of the product to slide over one another, which means less energy is stored during deformation. In other words, a bigger portion of the energy obtained in compression is dissipated through flow and permanent deformation for cheeses with lower curdiness scores.

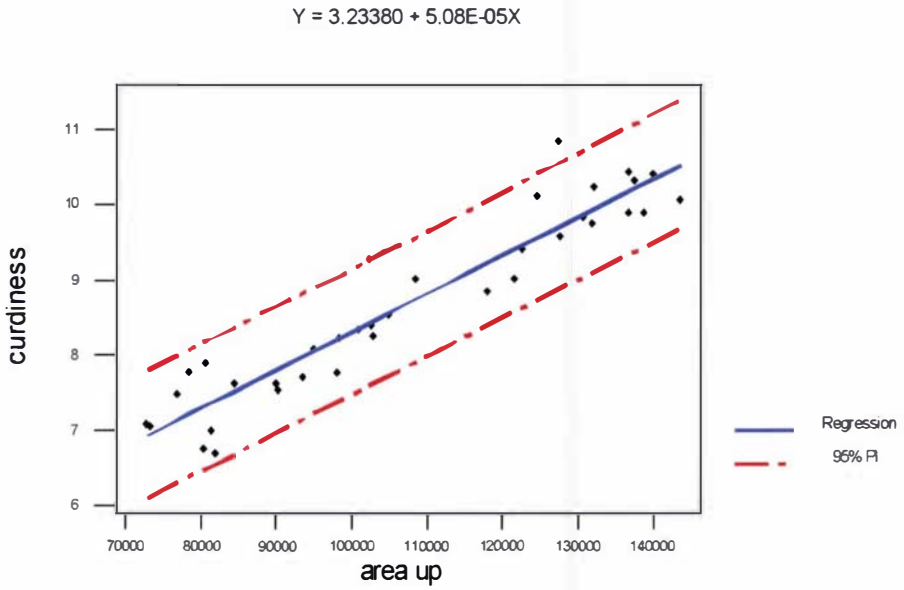


Figure 50. Sensory curdiness as a function of the area (of the compression curve) for the experimental cheeses (• raw data), with prediction interval

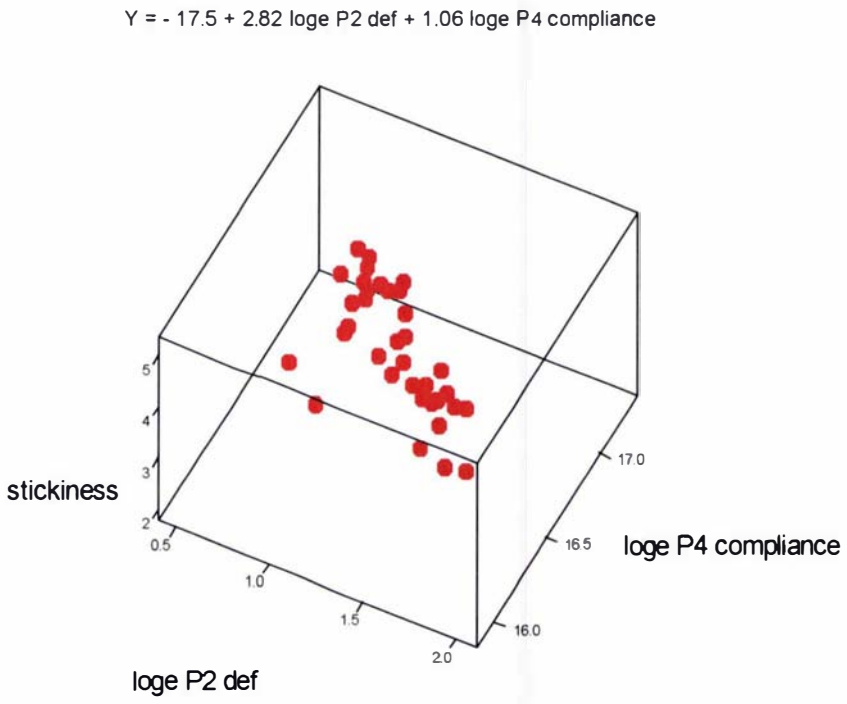


Figure 51. Sensory stickiness as a function log-transformed P2 in deformation and log-transformed P4 in compliance for the experimental cheeses (• raw data)

Even though "stickiness" can be modelled as a function of log-transformed P2 deformation alone, with an adjusted R-square of 87.8%, log-transformed P4 compliance was included in Figure 51 since both parameters can be obtained from the same rheological test and, used together, improve the R-square of the fit to 90.6%.

Also for the rheological parameters, the possibility of multicollinearity exists and, as previously discussed, is not accounted for by the stepwise regression. This regression procedure, however, is useful in generating simple models, when available, to fit the experimental data.

5.6.2.3. *Principal Component Analysis and Regression*

Principal Component Analysis (PCA) is one statistical technique that can handle the problem of multicollinearity. In fact, some degree of multicollinearity among the explanatory variables is desirable since, by the nature of the procedure, principal components are built to identify and combine variables that are not truly independent and also maximise the variance of experimental data that can be explained with the grouped variables. In other words, its objective is to find a way of condensing information contained in a number of original parameters into a smaller set of variables (principal components) with a minimum loss of information (Hair *et al.*, 1998; MacFie & Hedderley, 1993). It does not mean, however, that the correlation obtained between principal components and other parameters will be maximised as well.

An early example of the use of principal component analysis to study food texture was the work of Toda *et al.* (1971), in which the technique was used to clarify the relationship between different measurements. Since then, PCA has been often used in food research, commonly associated to principal component regression or partial least squares regression, to reduce the number of variables studied and facilitate the interpretation of results (Adams *et al.*, 1999; Garcia-Ruiz *et al.*, 1998; Molina *et al.*, 1999; Muir *et al.*, 1997; Rohm & Jaros, 1997; Ronn *et al.*, 1998; Skovgaard, 1995; Vaira *et al.*, 1999; Ward *et al.*, 1999).

The generation of principal components (PCs) for the chemical parameters in this study yielded three major principal component variables. The principal components and the coefficients for the terms that constitute each one are shown in Table 32. It is possible to observe that the first principal component, PC1 Chem, groups all chemical measurements in one single variable that can explain 94.2 % of the variance. PC2 Chem and PC3 Chem explain together around 5 % only of the variance in the experimental data and can be regarded an unimportant principal components relative to PC1 Chem.

Table 32. Principal components for the chemical parameters, with coefficients and proportion of variance explained

PARAMETERS	PC1 CHEM	PC2 CHEM	PC3 CHEM
Moisture	-0.368	0.565	0.092
Protein	0.383	-0.301	0.012
Fat	0.377	-0.421	0.189
pH day 1	-0.379	-0.329	-0.407
pH day 3	-0.378	-0.390	-0.377
pH day 7	-0.382	-0.272	0.093
pH day 14	-0.379	-0.280	0.800
Explained variance	94.2 %	4.5 %	0.6 %

It is clear, from the observation of the coefficients in PC1 Chem, that all terms have similar values, which indicates no one of these terms is truly independent from each other. Instead, these terms are all related and can be summarised as one single variable, PC1 Chem, with relatively equal importance for all terms. It is necessary, in a case like this, to be cautious when trying to make inferences about a predictive model that includes two or more of these chemical parameters as regressor factors, such as the one for the sensory attribute "firmness in cutting" presented in Table 30. Since the

two chemical variables (moisture and fat content) are not orthogonal or independent from each other, the coefficients obtained in the stepwise linear regression for the predictive model might not be as stable as expected. Because both variables are related, i.e., a change in moisture affects the fat content proportionally and vice-versa, the two effects are confounded. In turn, it becomes impossible to determine precisely which of these terms is more relevant to predicting "firmness in cutting" for the experimental cheeses. Thus, proper prediction of the sensory attribute can be poor, regardless of the quality of the fit of the model (86.9 %).

The use of PC1 Chem, as an example, to overcome the instability of the regression equation coefficients would be recommended, in theory, for the particular situation discussed above. However, it can be observed that the R-square value produced for the regression between "firmness in cutting" and PC1 Chem shown in Table 33 is not satisfactory, below 60%. This is because PC1 Chem, in addition to moisture and fat content, includes other parameters that do not correlate as well to "firmness in cutting". Hence, the use of stepwise regression, even with a single chemical predictor (R-square 78.5%, as opposed to 58.1%), seems more suitable than principal component regression for chemically modelling this sensory attribute.

The principal components and term coefficients for the rheological parameters were discussed previously in section 5.5.5 and the results displayed in Table 23. It was shown that the main rheological principal component combined all variables in PC1 Rheo to explain 77.1 % of the variance in the experimental data. Similarly to the chemical results, the following principal components (second and third) explained much less of the variance and can be regarded as unimportant relative to the first PC. The coefficients in PC1 Rheo (Table 23) were practically the same for all terms except "strain at peak stress", which indicates the latter is orthogonal to the combination of the other rheological parameters. This was also observed for Feta cheeses under uniaxial compression (Wium *et al.*, 1997)

Because "strain at peak stress" is an independent parameter on its own, it seems reasonable to proceed to remove this term from PC1 Rheo and add it as an independent term, in addition to PC1 Rheo, to the regression

procedure. This is done in order to try and improve the quality of the fit of the model yielded in the regression analysis (sensory scores versus PC1 Rheo). The results shown in Table 29, however, demonstrate the lack of correlation of the rheological parameter "strain at peak stress" with individual sensory attributes. Inclusion of "strain at peak stress" as an independent term showed no improvement to the regression R-square values and the quality of the fit. Hence, its addition to the regression model was not used.

The probability values for the correlation between individual sensory attributes and these principal components (PC1 Chem and PC1 Rheo) are presented in Table 33, together with the R-square values for the linear regression fitting. The plots for each individual sensory attribute, excluding fracturability and greasiness, against the principal components (raw data and prediction intervals) are presented in Figures 52 to 56.

Table 33. Probability values (*p*-values)[1] for the correlation of individual sensory attributes with the main chemical and rheological principal components and R-square values for the linear regression models

SENSORY ATTRIBUTE	PC1 Chem	PC1 Rheo	PC1 Chem regression R-square	PC1 Rheo regression R-square
Adj Fracturability	0.237 [1]	0.878 [1]	1.3 %	0.0 %
Adj Firmness (compression)	**0.000** [2]	**0.000**	79.1 %	**88.4 %** [3]
Adj Firmness (cutting)	**0.000**	**0.000**	58.1 %	74.4 %
Rubberiness	**0.001**	**0.000**	26.1 %	33.5 %
Adj Stickiness	**0.000**	**0.000**	79.6 %	79.8 %
Adj Curdiness	**0.000**	**0.000**	76.2 %	**90.0 %** [3]
Adj Greasiness	0.825	0.346	0.0 %	0.0 %

[2] *p*-values in bold are significant at $p<0.05$

[3] R-square values in bold indicate good fit of the linear model

The results in Table 33 show that both principal components (PC1 Chem and PC1 Rheo) are significantly correlated to sensory firmness

(compression and cutting), rubberiness, stickiness and curdiness, but no correlation was found for fracturability or greasiness. This is consistent with previous findings from the pairwise correlation shown in Tables 27 to 29, in which fracturability and greasiness were shown not to correlate with any individual chemical or rheological parameter.

For the significant correlations shown in Table 33, however, the *p*-values found are not an indication of good fit of the linear model in regression analysis. The R-square values highlight the fact that, despite the good correlation found between the individual sensory attributes and the principal components, the fit of the linear model was satisfactory, for prediction purposes, only for "firmness in compression" (88.4%) and "curdiness" (90.0%) in relation to the rheological principal component.

The regression R-squares obtained for "firmness in cutting" and "stickiness" in relation to PC1 Rheo and for "firmness in compression, stickiness and curdiness" in relation to PC1 Chem, all between 70 and 80 %, suggest a reasonable fit of the linear model in each case. Values for the coefficient of determination (R-square) in this range is often considered good among sensory professionals due to the natural variance in sensory data. Caution is required, however, if the linear model is to be used for accurate prediction of sensory response.

"Rubberiness" of the experimental cheeses, despite having shown significant correlation with the principal components, could not be satisfactorily modelled by the linear regression due to the large variance in the data for this sensory attribute (Figure 54).

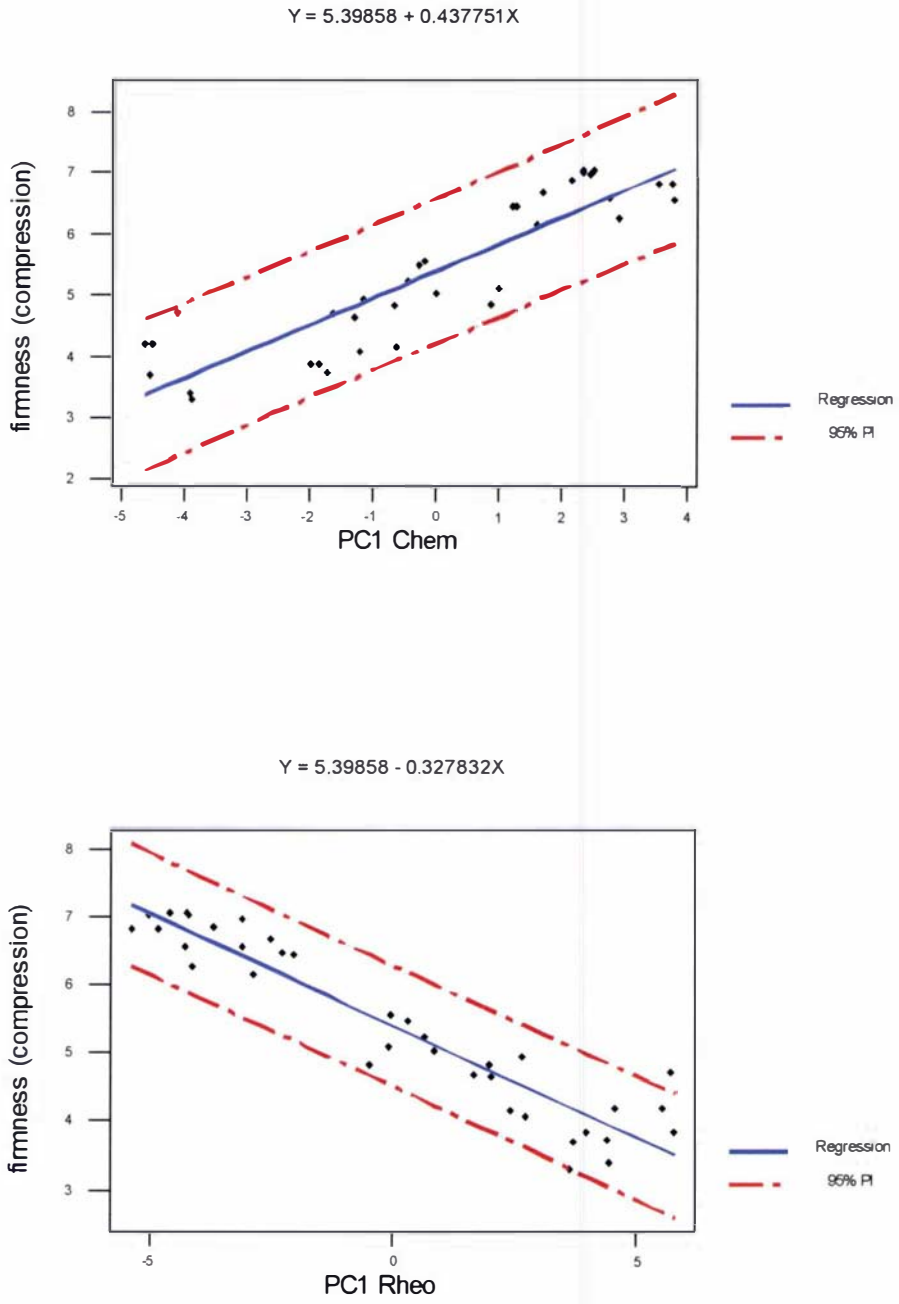


Figure 52. Sensory firmness (compression) as a function of the main chemical and rheological principal components (PCChem and PCRheo, respectively) for the experimental cheeses (• raw data), with prediction intervals

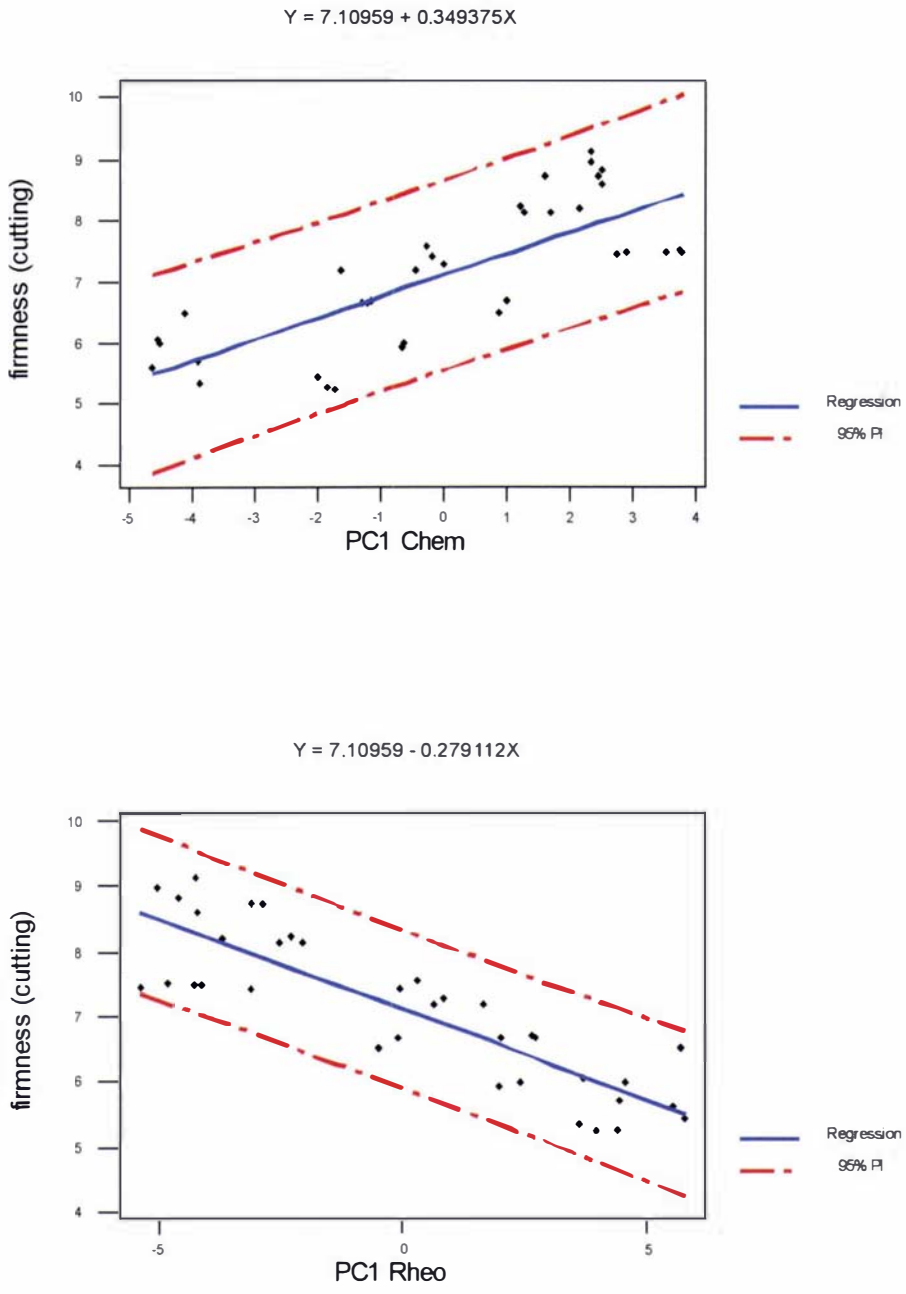


Figure 53. Sensory firmness (cutting) as a function of the main chemical and rheological principal components (PCChem and PCRheo, respectively) for the experimental cheeses (• raw data), with prediction intervals

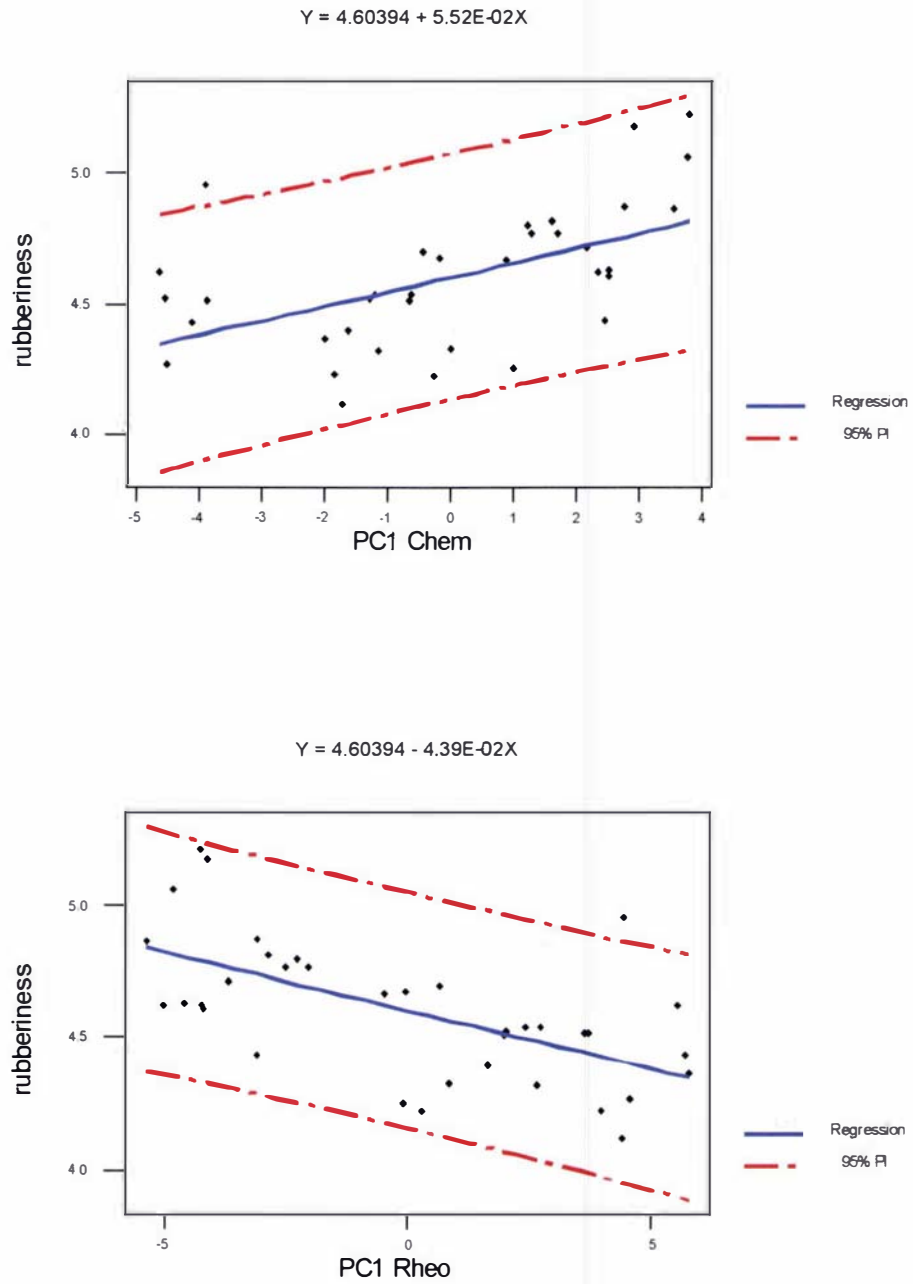


Figure 54. Sensory rubberiness as a function of the main chemical and rheological principal components (PCChem and PCRheo, respectively) for the experimental cheeses (• raw data), with prediction intervals

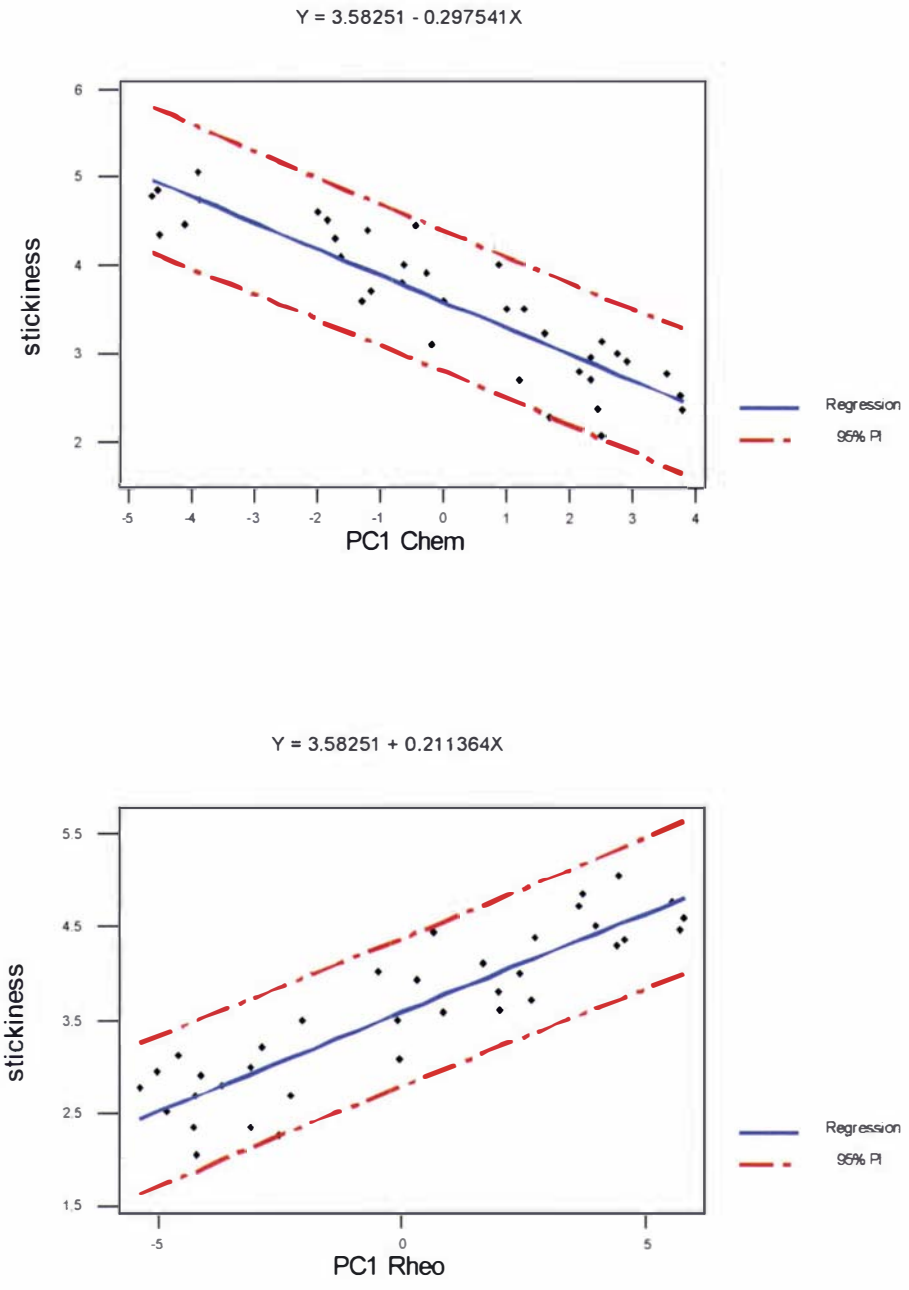


Figure 55. Sensory stickiness as a function of the main chemical and rheological principal components (PCChem and PCRheo, respectively) for the experimental cheeses (• raw data), with prediction intervals

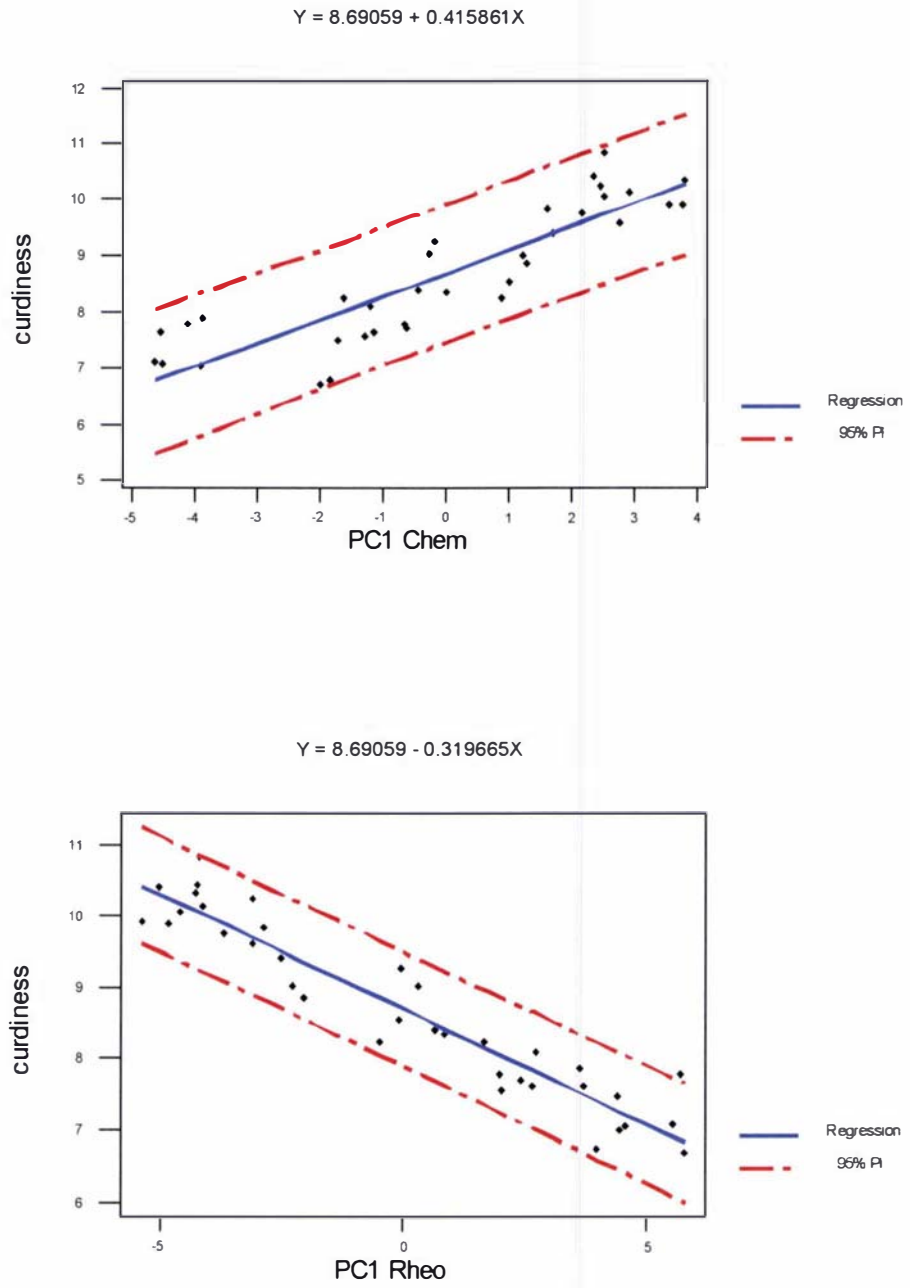


Figure 56. Sensory curdiness as a function of the main chemical and rheological principal components (PCChem and PCRheo, respectively) for the experimental cheeses (• raw data), with prediction intervals

Chemical and rheological parameters were subjected together to principal component analysis in order to assess if any improvement in the correlation R-squares occurred. This led to a main principal component, PC1 Rheo+Chem, that was able to explain 79.8% of the variance in the experimental data and that included all parameters but "strain at peak stress". Linear regression of this principal component (PC1 Rheo+Chem) with individual sensory attributes showed no improvement in R-square values when compared to the ones reported in Table 33. The exception was "stickiness", with a new adjusted R-square of 81.9% (data not shown). Because the increase in the quality of the fit was not large and restricted to one sensory attribute, the use of the expanded principal component (Rheo + Chem) is not further discussed.

Principal component analysis was also performed on the sensory attributes and the coefficients obtained for each term in PC1 Sens, PC2 Sens and PC3 Sens are shown in Table 34. Both PC1 and PC2 Sens, in this case, are of some importance in explaining the variability in the experimental data, even though PC1 Sens by itself explains more than 50% of the variance. PC3 Sens explains less than 10% of the variance and can be regarded as unimportant for regression and prediction purposes.

Table 34. Principal components for the sensory parameters, with coefficients and proportion of variance explained

ATTRIBUTES	PC1 SENS	PC2 SENS	PC3 SENS
Adj Fracturability	-0.098	-0.683	0.114
Adj Firmness (compression)	0.477	-0.103	0.156
Adj Firmness (cutting)	0.472	0.130	0.176
Rubberiness	0.284	-0.232	-0.922
Adj Stickiness	-0.446	0.208	-0.239
Adj Curdiness	0.473	-0.090	0.074
Adj Greasiness	-0.192	-0.633	0.137
Explained variance	58.6 %	27.1 %	9.5 %

The comparison of the coefficients in Table 34 show that PC1 Sens is composed mainly by the attributes "firmness in compression, firmness in cutting, stickiness and curdiness", confirming the results for sensory pairwise correlation, and PC2 Sens by "fracturability and greasiness". "Rubberiness", on the other hand, constitutes the main factor in PC3 Sens.

With regards to PC1 Sens and PC1 Rheo, the regression analysis produced an adjusted R-square of 88.2%, indicating good fit of the model. The relationship between these two principal components is presented in Figure 57, with the regression equation.

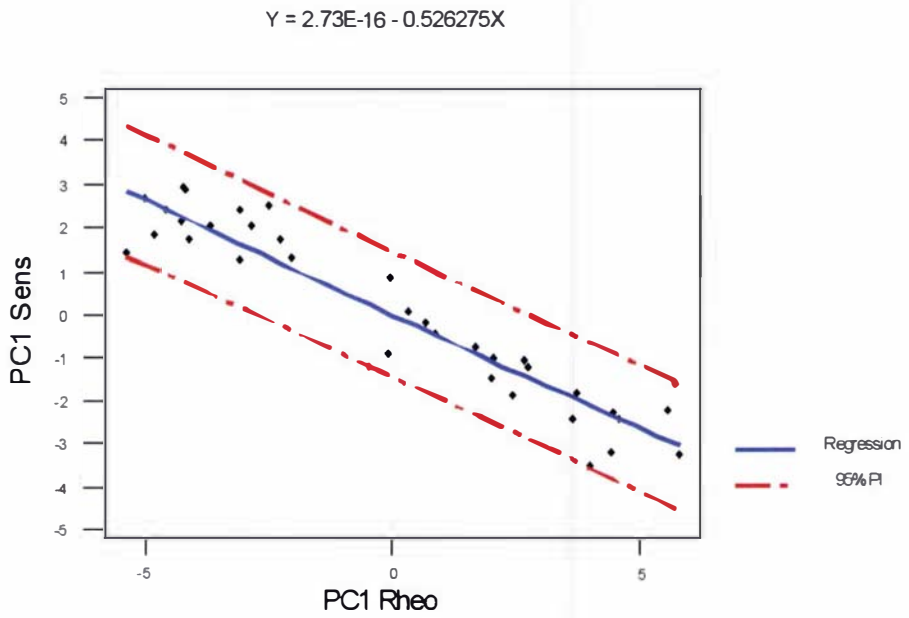


Figure 57. Sensory PC1 as a function of the main rheological principal component (PC1 Rheo) for the experimental (• raw data), with prediction intervals

The regression analysis of the two main sensory principal components with PC1 Chem showed, in both cases, inadequate fit of the model, with R-square values of 71.9% and 13.0% for PC1 Sens and PC2 Sens, respectively (regression coefficients not shown). Similarly, the R-square of the regression

analysis of PC2 Sens and PC1 Rheo (2.2%) indicates poor fit of the linear model. Even though the R-square value obtained for PC1 Sens x PC1 Chem is above 70%, which is at times considered satisfactory among sensory professionals, the residuals of the regression analysis were found not to be normally distributed, which enhances the lack of predictive power of this model.

It is important to note that even though a good fit of the linear model was obtained for both principal components (Figure 57), with a reasonably narrow prediction interval, the practical usefulness of such correlation is not great. This is because it becomes difficult, in a prediction application, to know which sensory attribute is being predicted and how well it is being predicted. For that purpose, it seems more reasonable to consider the correlation between individual sensory attributes and the chemical and instrumental combined variables (principal components).

5.6.2.4. *Canonical correlation*

The fact that the first principal components in this study (PC1 Chem/Rheo) explained most of the variance in the data set does not necessarily mean that the correlation of such principal components with specific, individual sensory attributes is maximised. The same is valid for the correlation with the sensory principal components (PC1 Sens and PC2 Sens). In order to achieve that, the technique of canonical correlation was used, as its main objective is to maximise the correlation between two sets of multiple random variables, regardless of the amount of variance explained by each set (Dijksterhuis, 1995; Hair *et al.*, 1998).

According to MacFie & Hedderley (1993), canonical correlation analysis is unique in that it makes no assumptions about dependencies of one set on another. However, because the direction of maximum correlation not necessarily explains large proportions of the variance in either set, the method is not as frequently used in food studies as partial least squares (PLS)

regression. Examples of the application of canonical correlation analysis in food research are found in Dever *et al.* (1995), Keteleer *et al.* (1993), Plotto et al. (1997), Rouzaud & Martinez-Anaya (1997), Vainionpaa *et al.* (2000) and van Lil *et al.* (1995). Partial least squares regression, commonly used in sensory-instrumental correlation studies (Drake *et al.*, 1999b; Hough *et al.*, 1996; Martens *et al.*, 2000; Meullenet & Gross, 1999; Wium & Qvist, 1998), was not used in this study for being the least restrictive of the various multivariate extensions of the multiple linear regression model (StatSoft, Inc., 1999).

Because of its principle of maximising correlation, the rheological canonical variables (CV) in this study that best correlate with individual sensory attributes and with the sensory canonical variable are not necessarily the same, i.e., they do not necessarily include the same terms in them. Table 35 shows the terms for each of the rheological canonical variables that best relate to individual sensory attribute, as well as the amount of variance explained and correlation R-square values.

Standardised canonical coefficients for the relevant terms in each canonical variable are presented (Table 35). Rheological parameters without coefficients were not included in the canonical variable due to the lack of good correlation between the individual parameter and the respective CV. It is possible to observe that the canonical correlation technique was not able to produce good correlation between sensory fracturability (R-square 18.5%) and greasiness (R-square 11.7%) and the respective rheological canonical variables. This is consistent with the findings of the previous statistical procedures presented and discussed here, which also indicated the lack of good correlation between these sensory attributes and rheological or chemical parameters.

Despite the lack of correlation for sensory fracturability, the inclusion of those rheological parameters obtained in the compression tests in the canonical variable for this sensory attribute was expected, as compression tests are reported in the literature to be associated with the fracture properties of a food material. The parameters from the compression tests were not, however, suitable for the prediction of the other sensory attributes.

Table 35. Rheological canonical variables for each individual sensory attribute, correlation R-square values and respective amount of variance explained with the CVs

	Sensory attributes (individual) [1]						
Rheo variables	**FRACT**	**FIRM (CP)**	**FIRM (CT)**	**RUBBER**	**STICK**	**CURD**	**GREASE**
G'		0.635	2.123		0.075	1.165	
G"		-0.291	-1.889		-0.065	-0.772	
P1 compliance		-0.103	0.290		0.273	0.079	-0.425
P2 compliance		-0.537	-0.547	-1.277	0.371	-0.334	1.737
P3 compliance	0.816						
P4 compliance		0.012	-0.051	0.697	0.490	-0.185	
Recovery				0.350		0.109	
P1 deformation		-0.312	-0.665		0.055	0.170	0.226
P2 deformation		0.542	0.190	1.705	0.092	-0.016	-0.835
P3 deformation							
P4 deformation		0.265	0.048	0.092	-0.710	0.605	
Young's mod	3.194						-3.957
Peak stress	0.294						2.954
Strain peak	-6.959						
Work peak	-4.858			0.319			
Work compr							0.517
Work decompr	14.616						
S proportion[2]	100.0 %	100.0 %	100.0 %	100.0 %	100.0 %	100.0 %	100.0 %
R proportion[3]	16.3 %	88.7 %	81.3 %	62.6 %	87.1 %	83.5 %	41.5 %
Corr R-square[4]	18.5 %	94.8 %	86.5 %	55.1 %	89.6 %	91.4 %	11.7 %

[1] sensory attributes are fracturability (FRACT), firmness in compression (FIRM CP), firmness in cutting (FIRM CT), rubberiness (RUBBER), stickiness (STICK), curdiness (CURD) and greasiness (GREASE)

[2] standardised variance of the individual sensory attributes explained by their own canonical variables

[3] standardised variance of the reduced set of rheological parameters explained by their own canonical variables

[4] adjusted canonical correlation R-square values

Even though the adjusted correlation R-square obtained for sensory rubberiness was larger (55.1%), the value indicates an unsatisfactory fit of the regression model and the inability to predict this specific sensory attribute with the set of rheological results that constitute the canonical variable.

Figures 58 to 61 graphically show the good fit of the regression model between the sensory attributes "firmness in compression, firmness in cutting, stickiness and curdiness" and the canonical variables. The latter, as seen in Table 35, are composed basically of the same rheological parameters and show that the sensory attributes can be efficiently modelled and predicted by the results of a frequency sweep and a creep test, without the need for compression tests to be performed.

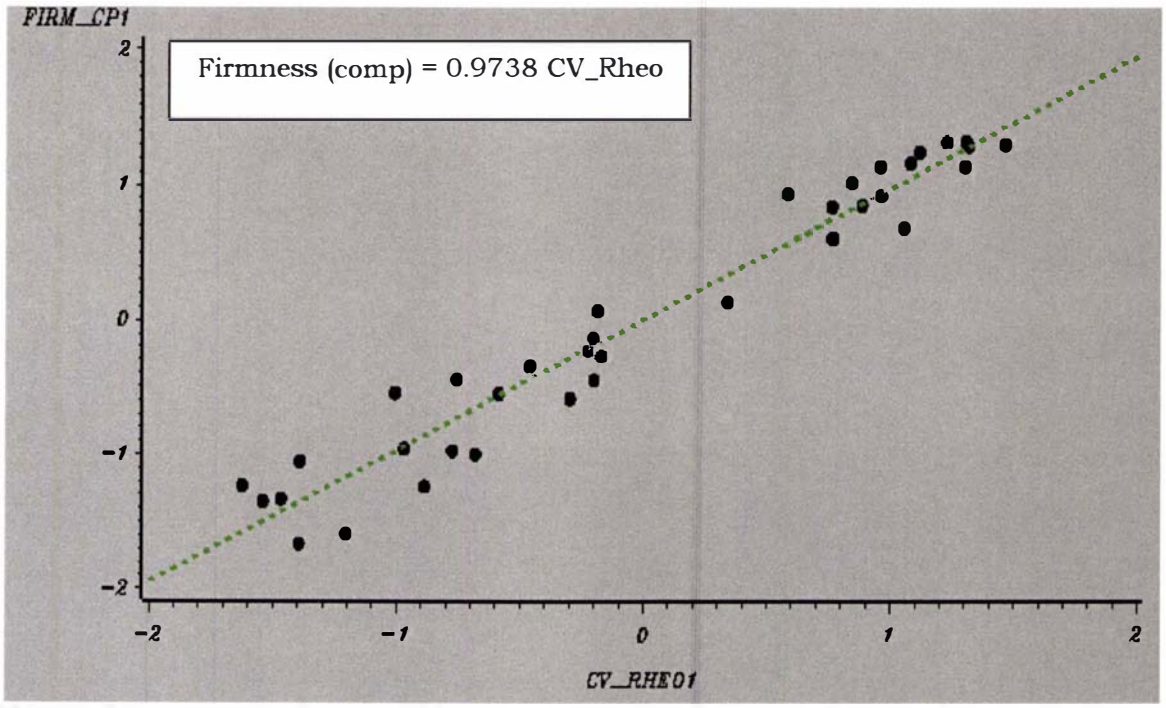


Figure 58. Canonical correlation between firmness in compression and the reduced set of rheological parameters (raw data and regression line)

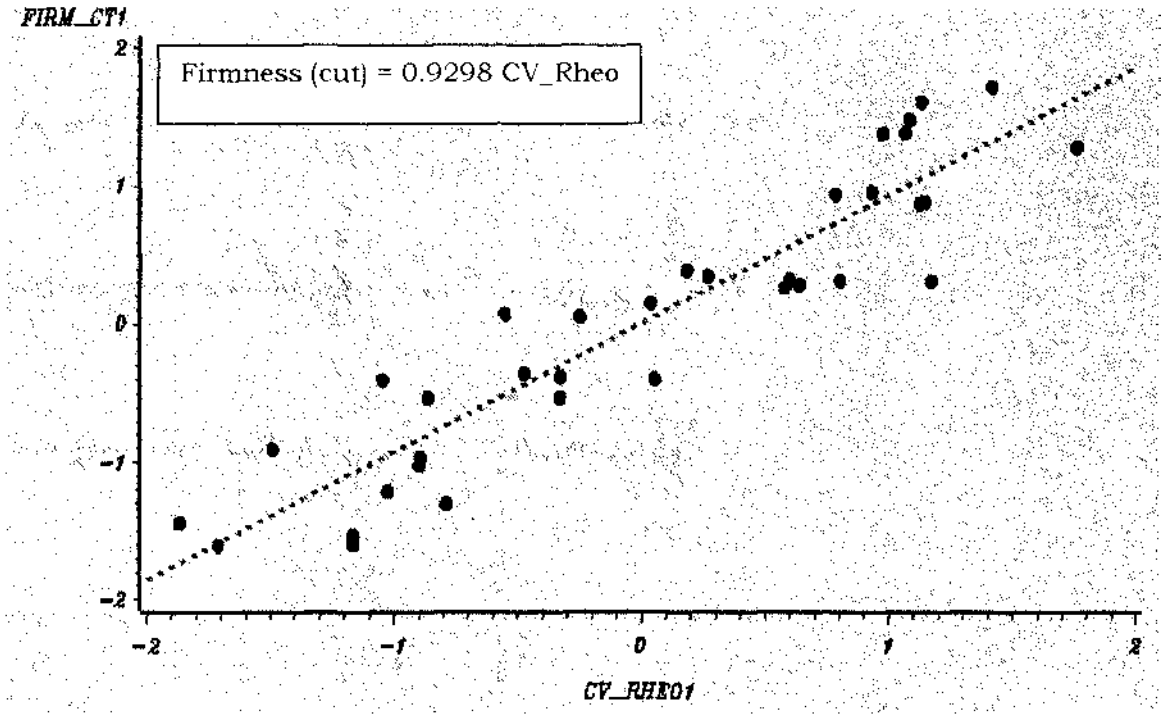


Figure 59. Canonical correlation between firmness in cutting and the reduced set of rheological parameters (raw data and regression line)

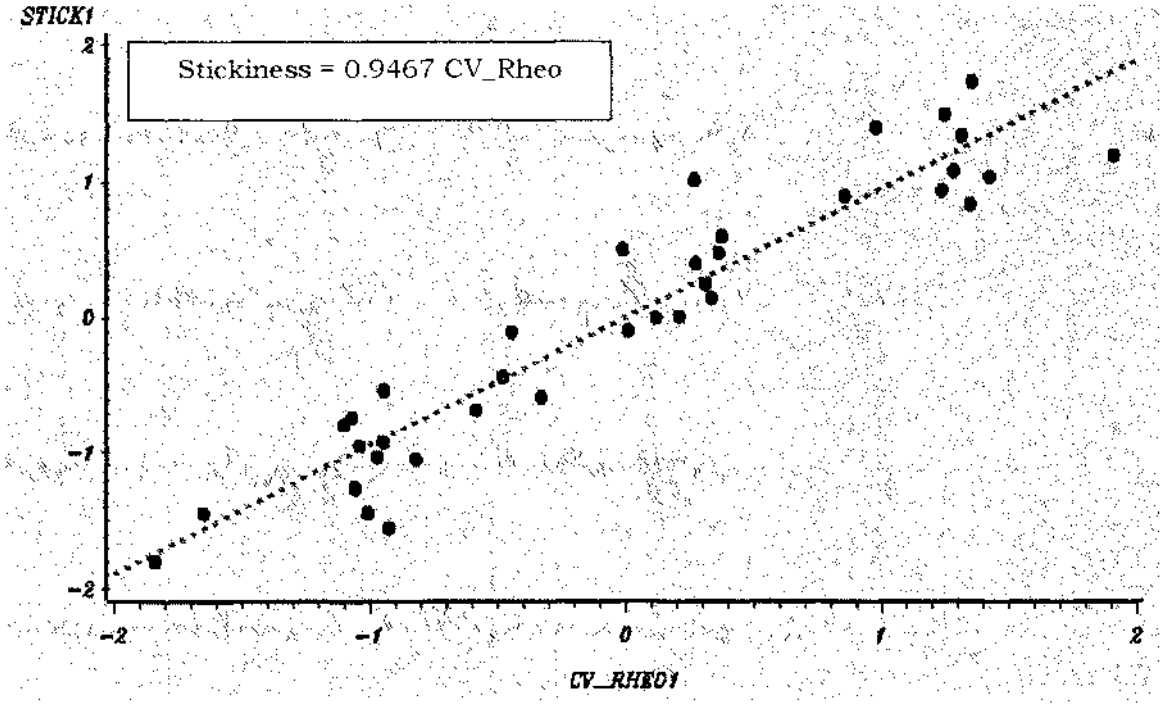


Figure 60. Canonical correlation between stickiness and the reduced set of rheological parameters (raw data and regression line)

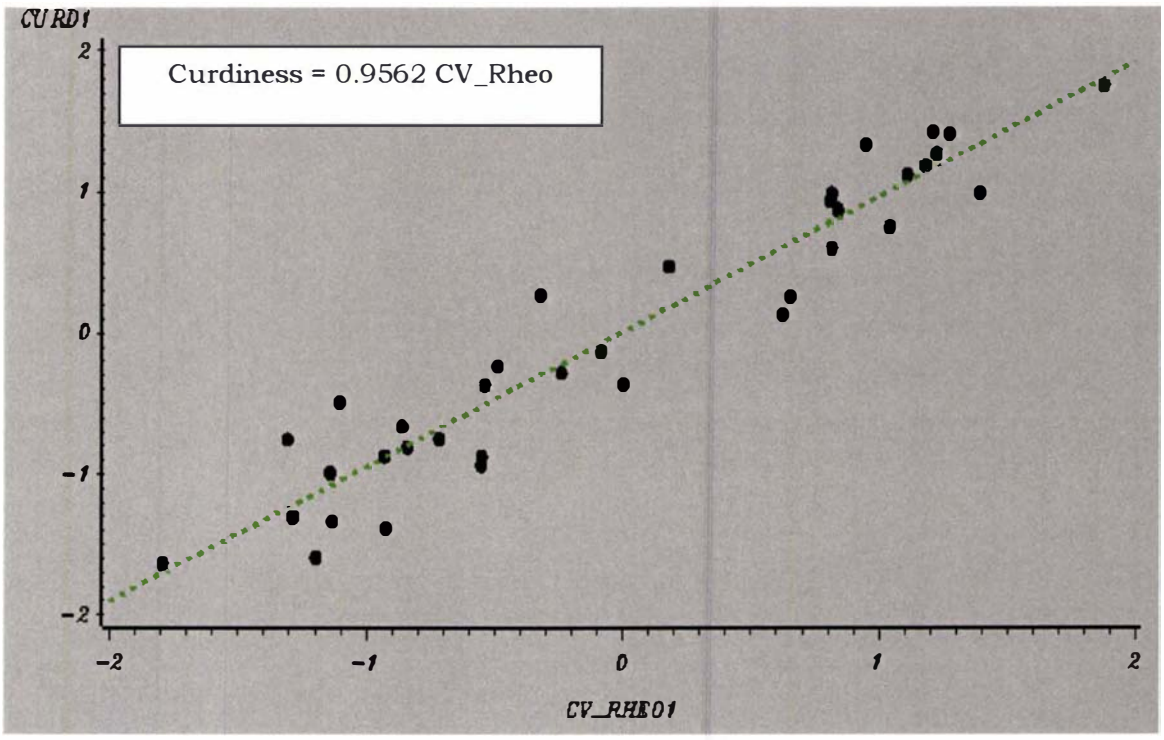


Figure 61. Canonical correlation between curdiness and the reduced set of rheological parameters (raw data and regression line)

The R-squares values obtained in the canonical correlation (Table 35) show an improvement of the sensory x instrumental correlation for individual sensory attributes when compared to the results of the principal component analysis (Table 33). It is also interesting, however, to consider the correlation between a sensory canonical variable and a rheological canonical variable. This is done so that the group of sensory attributes that best discriminates between the experimental cheeses is regressed against the group of rheological parameters that provides the best prediction model for the former variable (canonical).

The grouping of the sensory attributes in a canonical variable was done by combining "firmness in compression, firmness in cutting, stickiness and curdiness". These were the sensory attributes that explained most of the variance in the sensory experimental data. Fracturability, rubberiness and greasiness showed poor correlation coefficients in relation to the canonical

variable and, when included in the grouping, did not improve the quality of the fit of the linear regression model. Maximum correlation was achieved through regression of the sensory variable against a rheological canonical variable composed by G' and G" (frequency sweep) plus the creep test parameters P1, P2 and P4 (both in compliance and deformation) and creep recovery. Significant values from the canonical correlation procedure (the correlation coefficients of the several terms involved and quality of the fit) are shown in Table 36 and the correlation plot in Figure 62.

It is observed that all correlation coefficients are quite high and indicate the terms selected to be combined in the canonical variables were appropriate to maximise the variance explained in the experimental results. Likewise, the rheological canonical variable was shown to explain 87.6 % of the variance in the reduced set of sensory scores, i.e., the sensory canonical variable (Table 36).

Figure 62 graphically reinforces the very satisfactory fit of the regression model between the two canonical variables, which resulted in a correlation coefficient of 0.9791 and an adjusted R-square of 95.85 % (Table 36). This indicates that the parameters obtained from the frequency sweep and creep compliance tests provide a good predictive model, within the range of textures considered in the study, for a sensory compound attribute named "sensory canonical variable". The latter, in turn, represents a combination of four individual sensory attributes that, as shown before in this study (principal component analysis, Table 34) are not truly independent from each other.

Table 36. Correlation coefficients for the terms of each canonical variable against their own and the opposite CVs, amount of variance explained and correlation R-square value

	CV_SENSORY	**CV_RHEOLOGY**
FIRMNESS (compression)	0.9889	0.9682
FIRMNESS (cutting)	0.9172	0.8980
STICKINESS	-0.9555	-0.9355
CURDINESS	0.9608	0.9407
G'	0.9460	0.9662
G"	0.9345	0.9545
P1 compliance	-0.8989	-0.9181
P2 compliance	-0.9402	-0.9604
P4 compliance	0.8627	0.8812
RECOVERY	0.6978	0.7127
P1 deformation	-0.9412	-0.9613
P2 deformation	-0.9327	-0.9526
P4 deformation	0.9412	0.9613

Reduced S proportion	91.38 % [1]	87.60 % [2]
Reduced R proportion	81.48 % [4]	85.00 % [3]
Correlation R-square [5]	95.85 %	

[1] standardised variance of the reduced set of sensory attributes explained by their own canonical variable

[2] standardised variance of the reduced set of sensory attributes explained by the opposite canonical variable

[3] standardised variance of the reduced set of rheological parameters explained by their own canonical variable

[4] standardised variance of the reduced set of rheological parameters explained by the opposite canonical variable

[5] adjusted canonical correlation R-square value

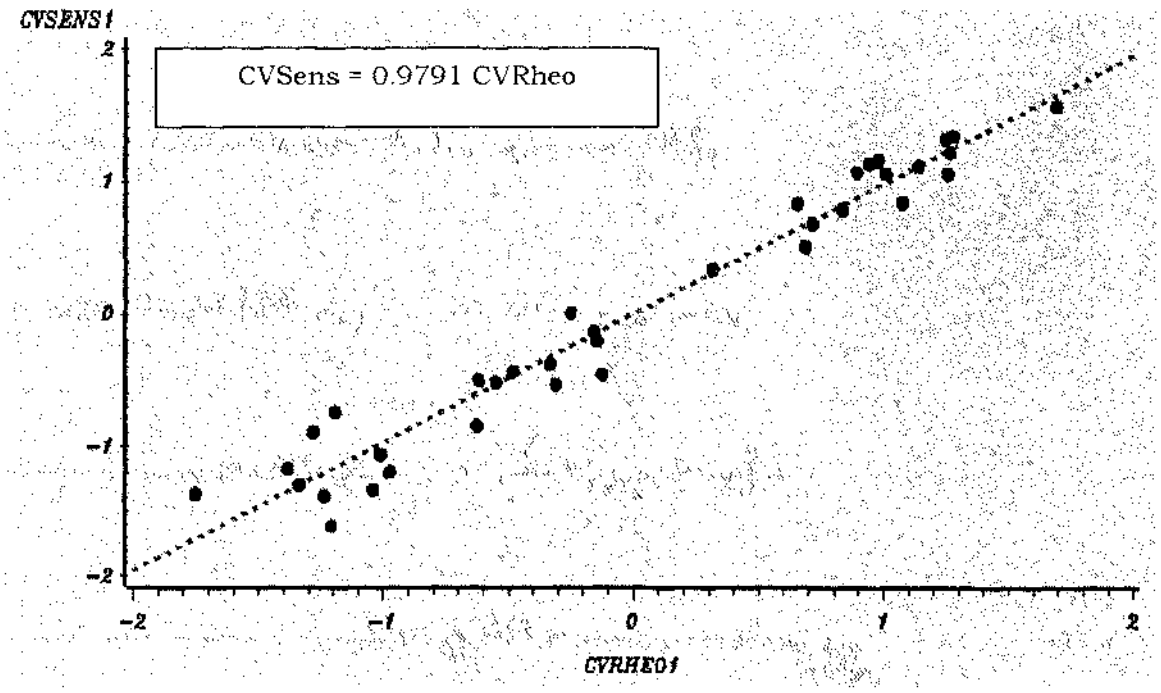


Figure 62. Canonical correlation between a reduced set of sensory attributes (sensory canonical variable) and the reduced set of rheological parameters (rheological canonical variable)(raw data and regression line)

It is important to emphasise that the predictive model can not be extrapolated, without reservations, for analogue processed cheese textures outside the range studied in the course of this research. Any attempt to expand the predictive ability of the model would require confirmation through experimental work.

Similarly to the correlation between sensory and rheological principal components, the practical usefulness of the correlation between sensory and rheological canonical variables is not great, for the same reason previously discussed. Thus, use of the canonical variables to predict textural sensory response based on correlation results for individual attributes is more appropriate.

Rheological and chemical parameters were combined and a new canonical correlation analysis was done to assess the improvement in the correlation coefficients of determination (R-square values) for individual

sensory attributes. Compositional analysis and measurement of pH are common practices in the processed cheese industry and could be used, together with rheological testing, to predict individual sensory textural responses with enhanced degree of accuracy. The results of the expanded canonical correlation analysis are presented in Table 37 and include the canonical variables, the standardised canonical coefficients, the amount of variance explained and the R-square values for the seven sensory attributes.

It can be observed in Table 37 that the inclusion of chemical parameters in the canonical correlation analysis improved the quality of the fit for all sensory attributes, as seen by the R-square values, in comparison to the analysis with the rheological parameters only (Table 35). This increase in the quality of the fit of the predictive models was obtained to the detriment of the amount of variance explained by the rheological + chemical canonical variables. The latter, as seen in Table 37, decreased for all sensory attributes.

For the attributes "fracturability" and "greasiness", the amount of variance explained by the respective canonical variables once the chemical and the rheological parameters were considered together decreased to values below 10%. In such cases, the considerable increase in the values of R-square for fracturability and greasiness, from 18.5% and 11.7% (Table 35) to 72.9% and 64.9% (Table 37), respectively, can not be assumed to be completely accurate and could have occurred by chance. Hence, it is possible to infer from the results that these two sensory attributes can not be satisfactorily predicted using a combination of rheological and chemical parameters, which confirms the findings from the previous canonical correlation analysis and also the principal component analysis.

For "rubberiness", the quality of the fit of the predictive model using rheological and chemical parameters increased from 55.1% (Table 35) to 76.8% (Table 37), which is still not good to guarantee accurate prediction of the sensory attribute. The decrease in the percentage of variance explained by the canonical variable for this attribute was also significant, even though the amount was still above 30%. In addition to that, it is possible to observe that 20 different parameters were required for the increase in the quality of the fit, making the use of the model as a control tool at the industry not practical.

Table 37. Canonical variables (rheological + chemical parameters) for each individual sensory attribute, correlation R-square values and respective amount of variance explained with the CVs

Rheo + Chem variables	**Sensory attributes (individual)** [1]						
	FRACT	**FIRM (CP)**	**FIRM (CT)**	**RUBBER**	**STICK**	**CURD**	**GREASE**
G'		0.622	2.053	-2.509	-1.117	0.968	
G"		-0.443	-1.925	1.804	1.187	-0.804	
P1 compliance		-0.029	0.338	-0.008	0.267	0.182	-0.660
P2 compliance		-0.410	-0.448	-0.654	0.310	-0.172	-0.061
P3 compliance	-0.337			-2.307			
P4 compliance		0.026	-0.002	1.445	0.316	-0.174	
Recovery				1.298		0.089	
P1 deformation		-0.292	-0.687	-2.368	0.222	0.235	0.131
P2 deformation		0.488	0.121	2.499	0.126	-0.111	1.289
P3 deformation				2.647			
P4 deformation		0.107	0.005	-3.253	-0.275	0.459	
Young's mod	3.939						-0.124
Peak stress	-0.598						0.838
Strain peak	1.120			-9.778			
Work peak	-0.843			0.947			
Work compr							-1.440
Work decompr	7.312			12.798			
Moisture	0.622			0.612			
Protein	0.182						-0.398
Fat	-0.306	0.522	0.203	2.253	0.211	0.737	
pH day 1	-4.309			-3.323			
pH day 3	-0.264	0.172		-0.581	0.898	0.322	-1.593
pH day 7	-0.672	0.191	0.545	1.370	-0.826	0.217	
pH day 14	2.936	-0.290	-0.484	0.687	0.393	-0.365	
S proportion[2]	100.0 %	100.0 %	100.0 %	100.0 %	100.0 %	100.0 %	100.0 %
R+C proportion[3]	6.9 %	82.7 %	75.8 %	32.6 %	81.9 %	79.0 %	8.2 %
Corr R-square[4]	72.9 %	96.3 %	87.3 %	76.8 %	93.7 %	93.8 %	64.9 %

[1] sensory attributes are fracturability (FRACT), firmness in compression (FIRM CP), firmness in cutting (FIRM CT), rubberiness (RUBBER), stickiness (STICK), curdiness (CURD) and greasiness (GREASE)

[2] standardised variance of the individual sensory attributes explained by their own canonical variables

[3] standardised variance of the reduced set of rheological parameters explained by their own canonical variables

[4] adjusted canonical correlation R-square values

Unlike the three attributes discussed above, firmness (in compression and in cutting), stickiness and curdiness showed a more subtle increase in the quality of the fit of the model when the chemical parameters were used in combination with the rheological ones. A small decrease in the amount of variance explained was observed, but not to an extent that compromises the quality of the predictive models for these attributes. In view of the subtle, small changes obtained for these four sensory attributes, use of the expanded set of parameters (Table 37) or the restricted one (Table 35) for predictive purposes is considered to be equivalent and is left to the discretion of those pursuing a textural prediction.

Figures 63 to 69 provide an illustration of the line of best fit and linear regression equation for the correlation between individual sensory attributes and the canonical variables comprising rheological and chemical parameters. All sensory attributes were presented rather than just the ones for which satisfactory correlation was found.

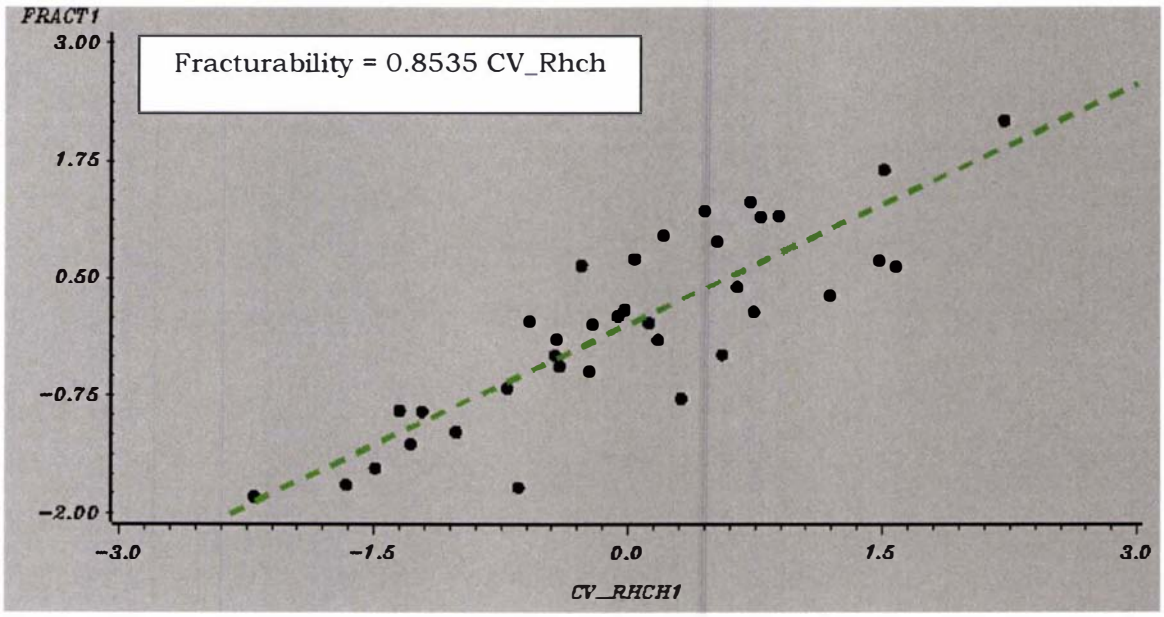


Figure 63. Canonical correlation between fracturability and the reduced set of rheological + chemical parameters (raw data and regression line)

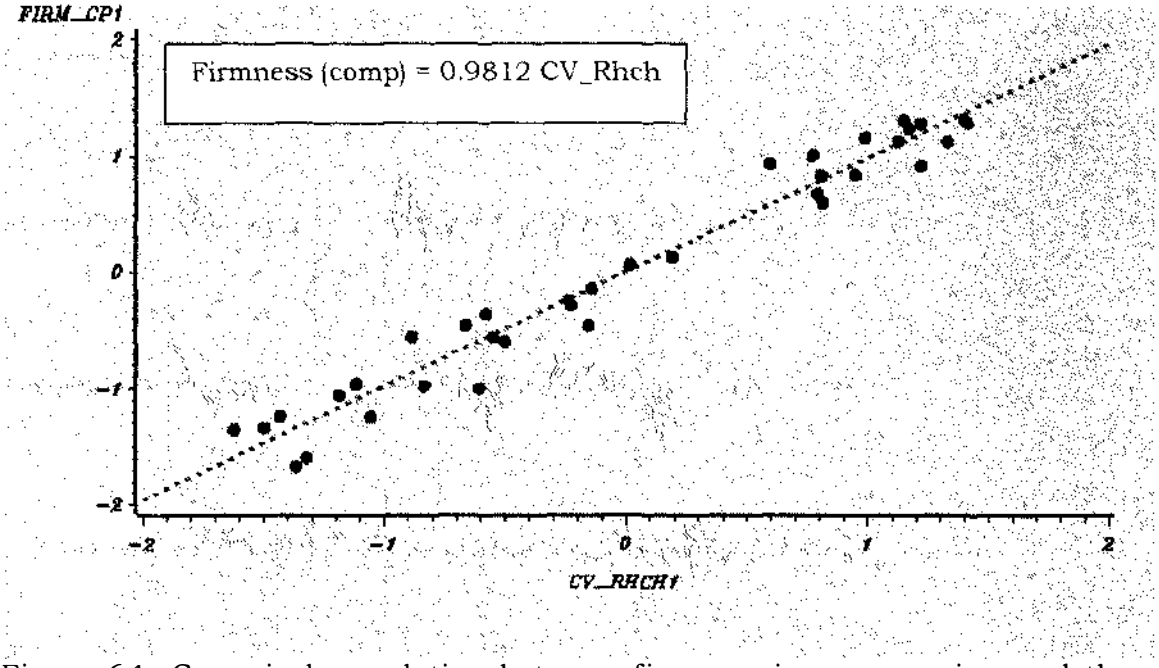


Figure 64. Canonical correlation between firmness in compression and the reduced set of rheological + chemical parameters (raw data and regression line)

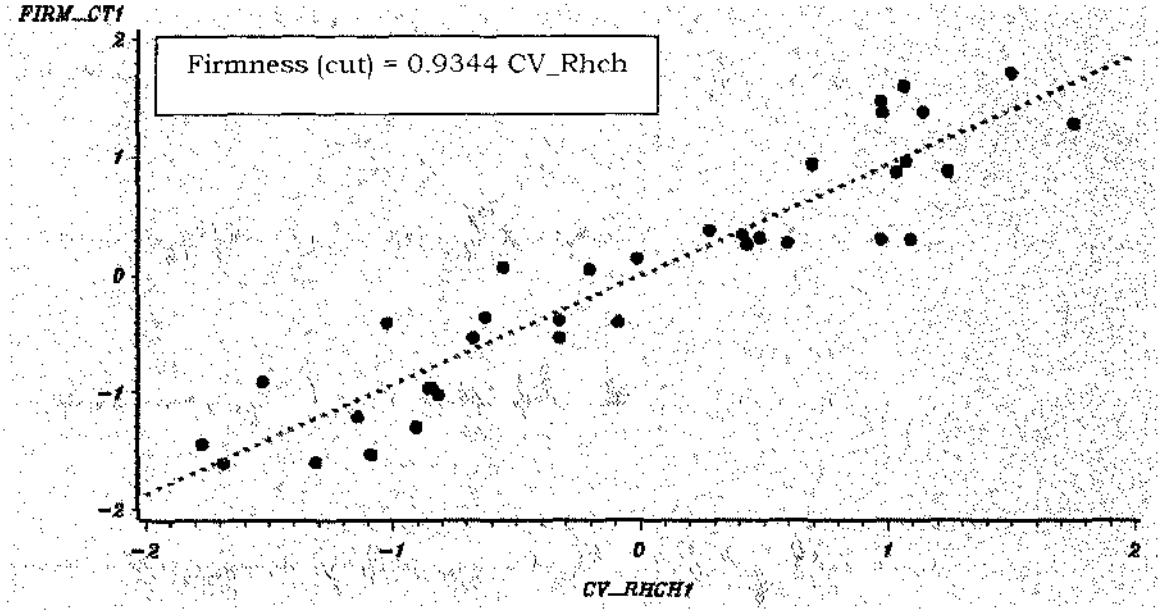


Figure 65. Canonical correlation between firmness in cutting and the reduced set of rheological + chemical parameters (raw data and regression line)

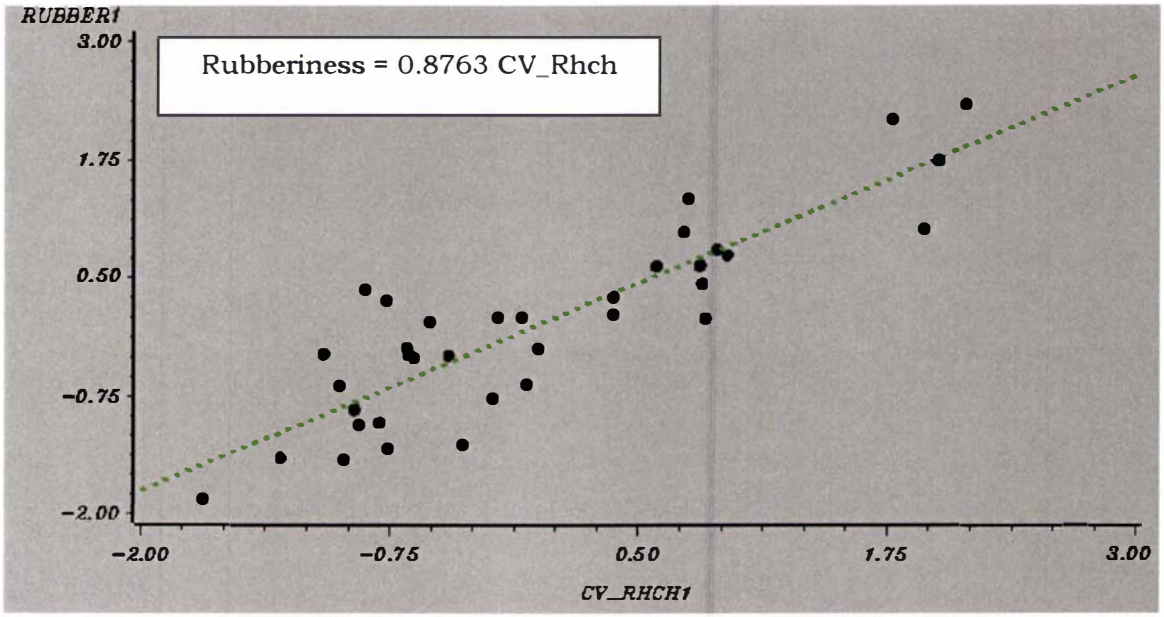


Figure 66. Canonical correlation between rubberiness and the reduced set of rheological + chemical parameters (raw data and regression line)

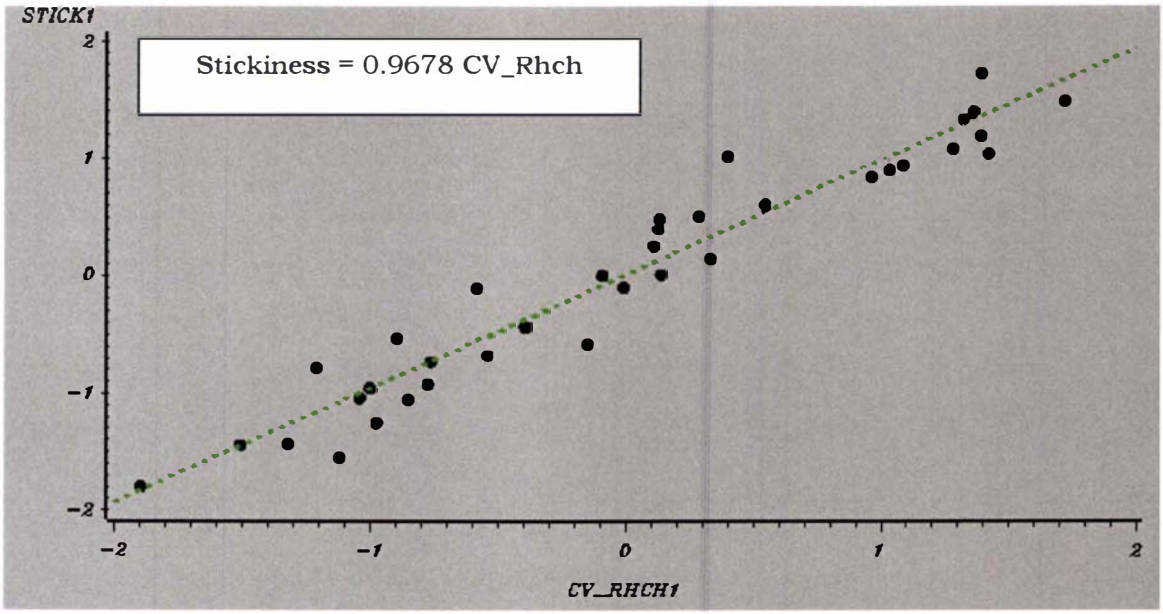


Figure 67. Canonical correlation between stickiness and the reduced set of rheological + chemical parameters (raw data and regression line)

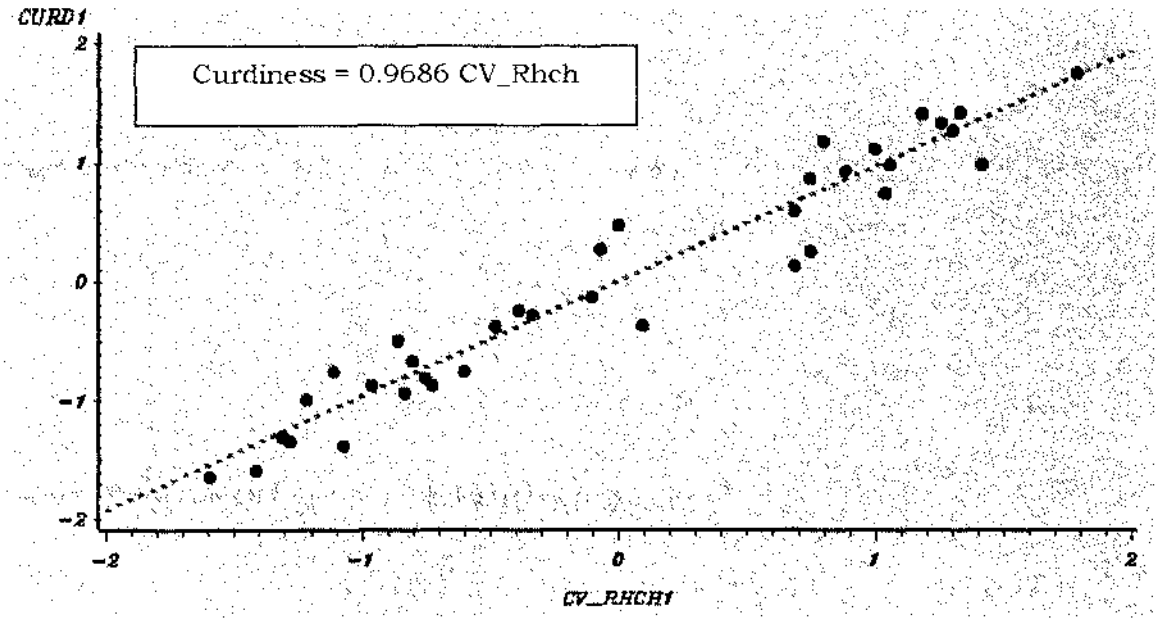


Figure 68. Canonical correlation between curdiness and the reduced set of rheological + chemical parameters (raw data and regression line)

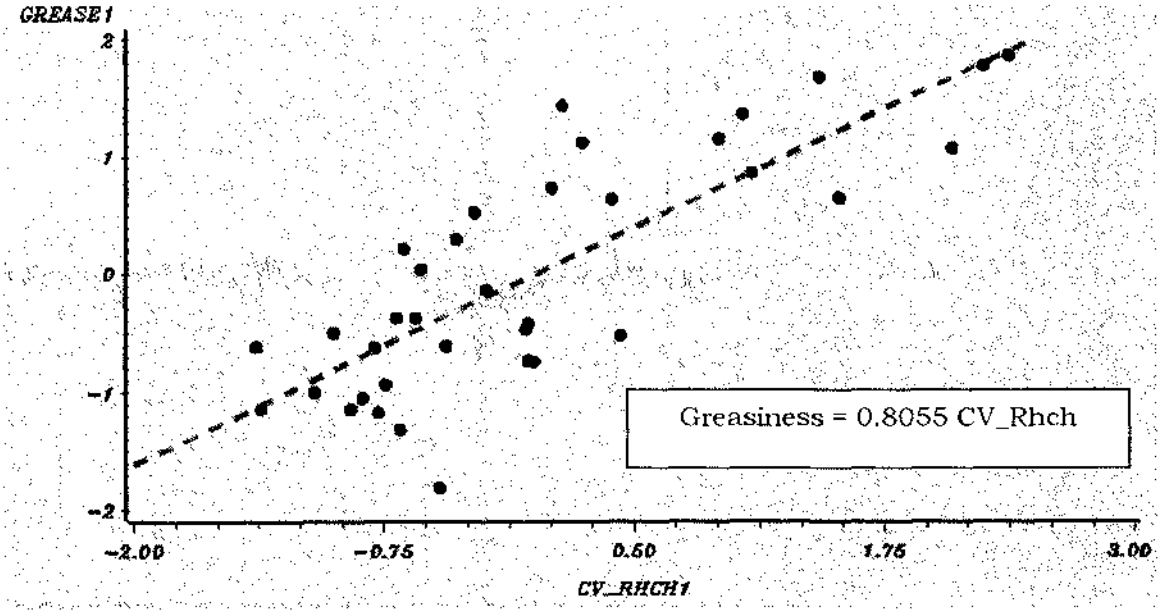


Figure 69. Canonical correlation between greasiness and the reduced set of rheological + chemical parameters (raw data and regression line)

It is worth noting that the spread of the raw data in Figures 63, 66 and 69 (fracturability, rubberiness and greasiness, respectively) makes the regression lines (line of best fit) questionable regarding their precision. In the case of the sensory attributes "firmness (compression and cutting), stickiness and curdiness", the raw data are seen to be much tighter around the regression lines, improving the quality of the predictive model.

"Firmness in compression, stickiness and curdiness" (Figures 64, 67 and 68, respectively) are the sensory attributes best predicted by instrumental and chemical measurements. "Firmness in cutting" data (Figure 65) appear to display larger deviation from the regression line, which in turn causes the prediction interval for this attribute to be slightly wider than the intervals for "firmness in compression, stickiness and curdiness". In spite of this fact, "firmness in cutting" can also be satisfactorily modelled with the reduced set of rheological and chemical parameters.

Because of the lack of practical usefulness of the correlation data, the canonical correlation analysis between a sensory canonical variable (including the four attributes adequately modelled when individually considered) and a rheological + chemical canonical variable is not presented in this discussion.

In conclusion, stepwise regression and canonical correlation were useful and powerful statistical tools for generating predictive models for some individual sensory textural attributes in the cheese analogues studied. While stepwise regression provides simple models for such predictions, canonical correlation analysis used a complete set of rheological and chemical results to yield models with maximum predictive ability. Principal component analysis, while eliminating the problem of multicollinearity in the fitting models, did not perform well in improving their correlation coefficients. Pairwise correlation showed that significant correlation exists, in general, between the microstructural data and the sensory, chemical and rheological ones. Use of the microstructural information in regression analysis and prediction of sensory attributes, however, has yet to be verified.

6.0. CONCLUSIONS

The results obtained in this experimental work showed that it is possible to satisfactorily model and predict a number of sensory textural attributes of the processed cheese analogues studied by using compositional data, fundamental rheological parameters and microstructural information. Stepwise regression analysis and canonical correlation were useful techniques for that purpose. This type of correlation study has not been reported before within one cheese type.

In the course of the present experimental work, the results obtained and reported here made it possible to conclude that:

1. Moisture content played a more important role than mixing speed in yielding textural differences between the experimental cheeses, as assessed by microstructural, sensory and rheological evaluation techniques and response surface regression analysis. The effect of mixing speed on the sensory, rheological and microstructural parameters was seldom found to be significant. For those parameters for which the effect of mixing speed was significant, the coefficients were much lower than the ones obtained for moisture content.

2. The effect of changes in mixing speed on the texture of the experimental cheese analogues used in the study could not be detected due to the narrow range of speeds investigated.

3. Fat globule size and distribution, rather than total fat content, was also a determinant factor in the textural characteristics of the different experimental cheeses. Increased moisture content caused the cheeses to be less viscous and to experience lower shear stress, which resulted in larger fat globules and a less uniform protein matrix. Reduction in the fat globule size increased the number of globules in the matrix, consequently increasing the occurrence of interactions between proteins and between proteins and fat. This increased cheese firmness.

4. Variation in pH between the different experimental cheeses was not large and is unlikely to have contributed much to the textural differences.

5. Confocal laser scanning microscopy was a powerful and easy to use technique for the structural study of the experimental cheeses. Image analysis provided quantitative information about the protein matrix and fat globules that correlated well to most of the textural properties evaluated by sensory and rheological techniques, as assessed through pairwise correlation analysis.

6. Sensory characterisation of the textural attributes of the experimental cheeses included seven descriptors – fracturability, firmness (in compression and in cutting), rubberiness, stickiness, curdiness and greasiness – used in hand evaluation. Fracturability, rubberiness and greasiness were not suitable attributes to differentiate between the experimental cheeses. Firmness in compression and cutting, stickiness and curdiness were found to be significantly different between the several cheeses studied. Cheeses with higher moisture content were less firm, less curdy and stickier than cheeses with lower moisture content. Cheeses with intermediate moisture content showed intermediate values for those attributes. Testing temperature affected all the sensory scores. These had to be adjusted for the effect of temperature prior to statistical analysis.

7. The three different tests used in the rheological evaluation of the experimental cheeses – frequency sweep (small deformation, short times), creep compliance (small deformation, long times) and compression (large deformation) – yielded consistent, reproducible results that allowed, in most of the cases, significant differentiation between the experimental cheeses. Cheeses with higher moisture content were less elastic and less firm than cheeses with lower moisture content, showing lower values of G' and G", higher creep compliance values and lower values for those parameters from the compression to fracture. Testing temperature appears not to have affected the rheological results to any significant extent when tests were carried on at 5-8°C.

8. Pairwise correlation showed that microstructural information correlated highly with sensory, rheological and chemical characteristics of the different cheeses across the experimental blocks. Pairwise correlation between the sensory attributes and chemical parameters and between the sensory attributes and rheological parameters showed no significant correlation for fracturability and greasiness. Firmness in compression and cutting, stickiness, curdiness and rubberiness correlated with all chemical and rheological parameters except "strain to the peak stress", from the compression tests, which did not correlate to any of the sensory attributes.

9. Stepwise regression analysis failed to find good predictive models for the sensory attributes "fracturability, rubberiness and greasiness". Simple models with satisfactory fit were obtained for "firmness in compression and cutting, stickiness and curdiness", with firmness in cutting being the least precise of the models. Firmness in cutting could not be modelled adequately with a single chemical or rheological predictor and required additional terms in the model for satisfactory fit of the predictive models. Firmness in compression, stickiness and curdiness were satisfactorily modelled with either single chemical or single rheological predictors.

10. Sensory attributes, with the exception of fracturability and greasiness, correlated significantly with the main principal components (PC1) for the chemical and rheological parameters. No sensory attribute could be satisfactorily modelled with the chemical PC1. Firmness in compression and curdiness could be satisfactorily modelled with the rheological PC1, but not the other sensory attributes. Principal component analysis, despite handling the problem of multicollinearity in multiple regression, was not an effective method for sensory versus instrumental texture correlations.

11. Canonical correlation proved to be a useful tool for correlation between sensory and instrumental (rheological only or rheological + chemical) results by maximising the correlation coefficients and the amount of variance explained by the canonical variables. No satisfactory models were found for fracturability, rubberiness and greasiness. Models with satisfactory fit were obtained for "firmness in compression and cutting, stickiness and curdiness", with firmness in compression being the most

precise of the predictive models. Parameters obtained in the compression tests were not necessary for the generation of good predictive models.

In conclusion, the success in finding good sensory versus instrumental correlation provides an important tool for the dairy industry for use in product development and product and process control. Direct application of the predictive techniques for a particular product would require verification of the models for that specific product.

7.0. SCOPE FOR FUTURE WORK

The results of this experimental work showed it is possible to model some hand evaluated sensory textural attributes using instrumental (chemical and physical) techniques. The accuracy of the models found makes them useful for the prediction of those sensory attributes and, as such, valuable tools for product development and process control. Microstructural information was shown to significantly correlate to several sensory, chemical and rheological parameters, but its inclusion in the predictive models was not possible. Further work to substantiate the findings reported here and to expand the number of sensory attributes possible to be instrumentally modelled is of interest.

Some of the areas where further experimental work could be done include:

1. Development of a more appropriate methodology for microstructural evaluation of the model processed cheese analogues by confocal laser scanning microscopy in which both the protein matrix and the fat are successfully stained. This would be followed by a methodology for the generation of consistent quantitative data from image analysis to be used in multivariate correlation analysis with sensory and rheological characteristics of the products.

2. Studies on the application of the instrumental techniques used in this research to predict sensory textural attributes in commercial processed cheese products (block and slice type products).

3. Determination of perceived sensory attributes and appropriate instrumental tests as well as development of techniques for sensory-instrumental correlation in soft type cheese products such as cheese spreads.

4. Studies on sensory textural attributes evaluated in the mouth and subsequent development of instrumental techniques that allow satisfactory modelling of the perceived texture during the complex process of mastication.

5. Studies on the physico-chemical nature of the interactions between the components of the model cheese analogues to better understand the chemistry of structure formation and efficiently control the functional properties of the investigated food materials.

6. Investigation into other microscopy techniques, namely electron microscopy, that could potentially maximise the amount of structural information of the food systems used and enhance the predictive accuracy of the models for individual sensory attributes.

8.0. REFERENCES

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9.0. APPENDIX

The research results presented and discussed in this thesis were obtained after extensive preliminary work to define the materials to be used, conditions for manufacture and techniques for textural evaluation. Even though some preliminary trials are mentioned in the discussion of results, specific information was not provided there. More detailed information is presented in this section.

1. Development and definition of equipment to be used for the model systems

Two formulations for model processed cheese analogues were provided by the New Zealand Dairy Research Institute (NZDRI) as a starting point for this study. The formulations varied in their ingredients and consistency of the finished products (hard and soft types). Only the hard type formulation was chosen for the initial work regarding equipment definition and cheese production. Development of the formulation at pilot plant scale was directly related to the development/determination of the most appropriate piece of equipment for cheese manufacture. A piece of equipment was initially designed by Dr Osvaldo Campanella and built at the Institute's workshop to be used for the production of the model systems.

The designed stainless steel kettle (capacity 25 kg) was jacketed for indirect heating using hot water and mounted on a frame without the possibility of tilting for discharge. Mixing blades were welded to a vertical shaft connected to a torque-meter in an attempt to measure the changes in torque during mixing of the raw ingredients and formation of the emulsified molten cheese mass. It was assumed that the torque would increase from the beginning of mixing and reach a maximum once emulsification was complete (creaming). Mixing beyond the point of critical emulsification would be possible to be detected by means of a decrease in the torque (loss of stability) from the maximum torque value. This phenomenon was to be used for determination of the maximum mixing time in manufacture.

Failure to achieve proper emulsification of the cheeses (insufficient temperature and engine power for mixing) and practical difficulties in the use of such equipment called for a new machine to be tested. The chosen piece of equipment was a Brabender Farinograph borrowed from the NZDRI and adapted with a load cell to measure the changes in torque during product manufacture. The capacity of the equipment was small, with a maximum batch size of 400 g. Due to the design of the equipment, leakage of the liquid ingredients occurred once they were added to the cooking compartment and the losses could not be measured. Cleaning after cheese manufacture was not practical, also because of the design. Torque measurements proved to be very noisy due to a very sensitive load cell. The Farinograph was also ruled out as an appropriate piece of equipment for this research work due to its practical limitations.

Equipment definition followed with pilot plant trials at Pastoral Foods Ltd. (Eltham, New Zealand) using a Kustner cooker. This piece of equipment had good batch size capacity (15 kg) and proved to work well with the formulation in use, yielding homogeneous products adequately emulsified under visual examination. Different amounts of emulsifying salts in the formulation were also investigated in the search for a more stable product for study. However, because the lease and transportation of the Kustner cooker to Massey University was not agreed upon by Pastoral Foods Ltd., its further use in the experimental work was abandoned.

Finally, a pilot plant scale Blentech cooker (capacity 12 kg) belonging to the NZDRI was returned to the Institute from a previous lease and subsequently loaned to Massey University for use in this research work. The equipment is a miniature version of the Blentech cooker normally used at the NZDRI pilot plant and proved to be efficient in producing well emulsified, homogeneous cheese analogues. The idea of torque measurement as a manufacture control tool was completely abandoned, since such measurements were not possible to be made with the Blentech cooker.

This process of determining the appropriate piece of equipment for use in the manufacture of the cheese analogues took 15 months, from December 1996 to March 1998.

2. Determination of the range of textures for the correlation studies – processing parameters

Several different processing variables were tested using the formulation provided by the NZDRI to assess the extent to which the textural characteristics of the model systems could be widened. Extent of pre-mixing, mixing time, mixing temperature, mixing speed and use of rework are examples of processing parameters that were investigated. None of them, used alone, appeared to provide a wide enough range of textures for correlation study. Combinations of the processing parameters mentioned were also investigated, without much success.

Following recommendations from a technician at the NZDRI, Mr Robbie Buwalda, the moisture content of the formulation was altered to try and expand the textural characteristics of the cheese samples. Mixing speed was chosen as a complementary factor to expand the textural range in the experimental cheeses. Further investigative work was carried on to guarantee the consistent manufacture of homogeneous products, properly emulsified and able to be evaluated using the different techniques for textural assessment (sensory, rheological and microstructural evaluations).

A range of textures was found to be satisfactorily produced by varying moisture content and mixing speed of the model systems, while cooking for a period of 8 minutes at the maximum temperature of 86°C.

3. Determination of the occurrence of slip in a creep compliance test using the stress controlled Rheometrics rheometer (stress 2000 Pa, gaps 2 and 4 mm, base formulation – experimental cheese 6)

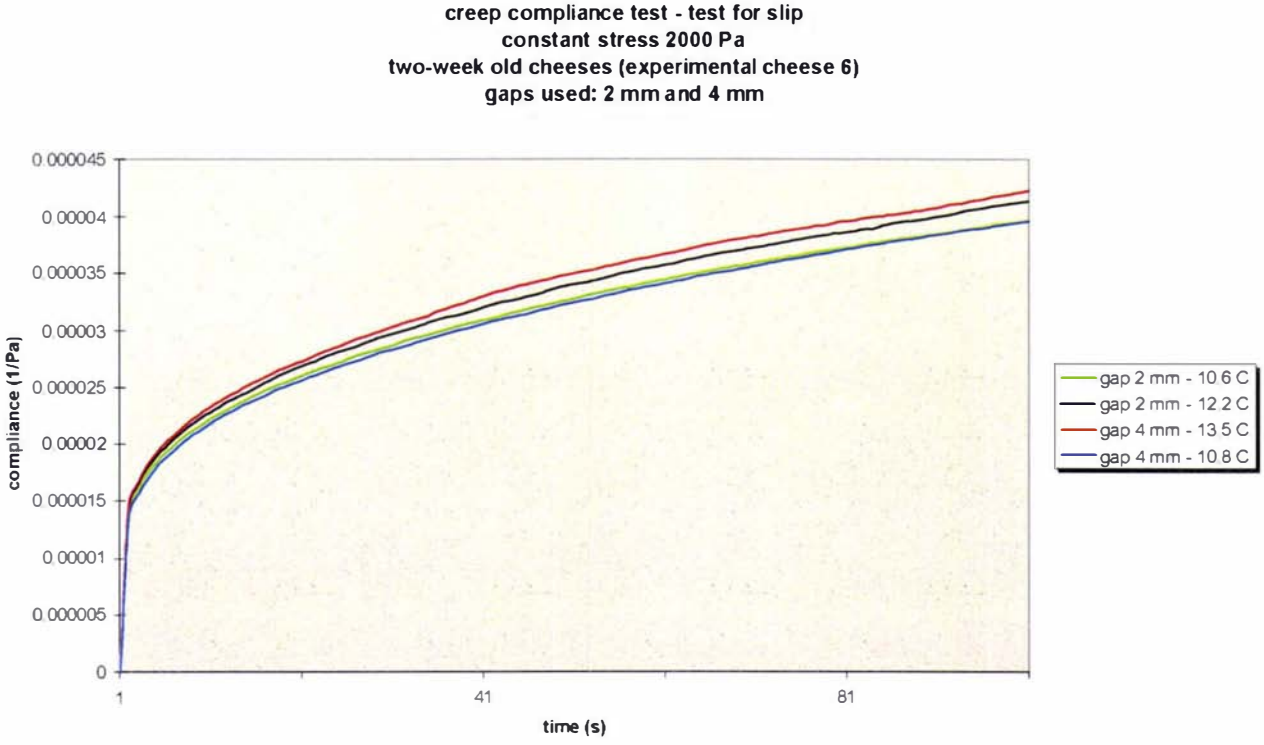

Figure 70. Creep compliance curves (constant stress of 2000 Pa) of experimental cheese 6 using different gaps (2 and 4 mm) for assessment of slippage

Exact control of temperature was not possible, so the temperature of each sample after the creep test was measured with a thermocouple. The test was run with the plates covered with sandpaper to avoid or reduce slip. The Peltier element was set to the temperature of 10°C.

The curves presented are the average of 4 runs. Temperature values reported are also average values over the 4 runs. It can be seen that the creep curves for the samples with gap 2 mm/10.6°C and 4 mm/10.8°C practically superimpose, as expected when slippage is controlled. Use of sandpaper to avoid slippage was adopted for the subsequent phases of the experimental work.

4. Determination of the linear viscoelastic region

This was done using a strain sweep test. Experimental cheese 6 (base formulation from NZDRI) was used, with a frequency of 1 Hz and temperature of 25°C.

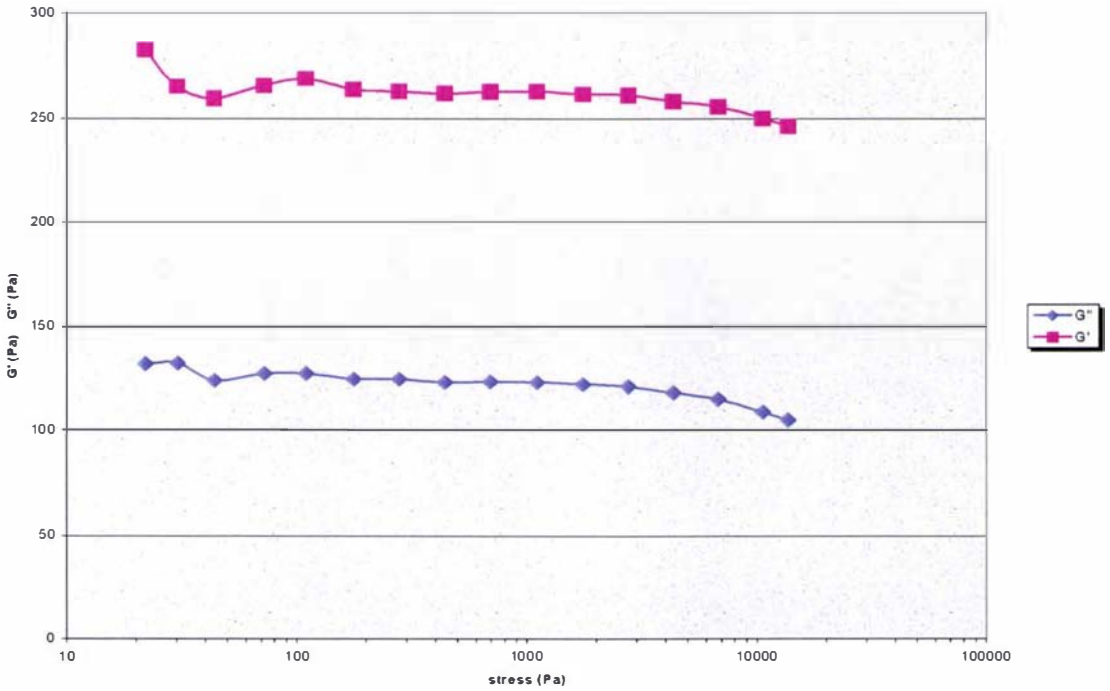

Figure 71. Strain sweep (frequency 1 Hz) for experimental cheese 6, at 25°C, for assessment of linear viscoelastic region

The curves of storage and loss moduli (G' and G", respectively) as a function of stress show that stresses between 20 and 3000 Pa fall within the linear viscoelastic region. These values are well within those reported by Ma *et al.* (1996) for the linear viscoelastic region of Cheddar cheeses.

As shown by Subramanian & Gunasekaran (1997), the linear viscoelastic range decreases, in general, with increasing temperature because cheeses behavemore like viscoelastic solids at lower temperatures. Hence, it is expected the linear viscoelastic range for the cheeses in this study will be slightly wider for tests performed at cold temperatures.

5. Determination of the effect of testing temperature on the rheological results

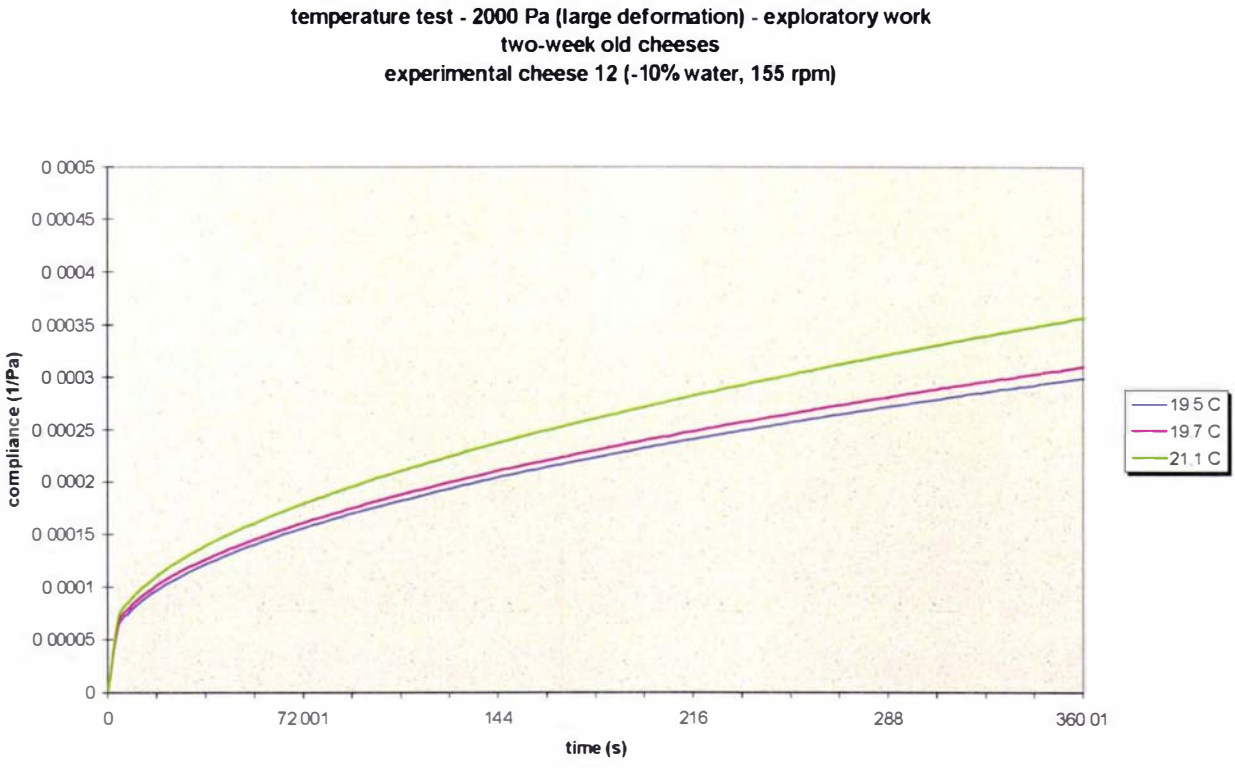

Figure 72. Creep compliance curves (constant stress 2000 Pa) for experimental cheese 12 using different sample temperatures

These tests were conducted at room temperature (around 21°C), when the fat is going through a phase change, from solid to liquid. Results from experimental cheese 1, with the highest moisture content, could not be reported due to the problems in running the test with those samples. The samples were handled with difficulty, as they were very sticky and easy to break.

Variations of less than 1°C in the cheese sample temperature caused the rheological response to vary slightly, around 3-8%. The significance of these differences was not verified through statistical analysis; however, measurements lay in general within the acceptable variation due to the precision of the instrument. Variation larger than 1°C, on the other hand, caused differences to be larger than 10%.

The rheological properties of the cheeses in the study are expected not to change much at lower temperatures, such as those used in refrigeration. Temperature values around 4°C to 8°C are far from the "fat melt transition" range, which is around 20°C to 25°C. Hence, a decision was made to run the rheological tests at temperatures close to refrigeration temperature, also for the ease of sample preparation and for more appropriate correlation to the sensory results, also obtained under colder temperatures than 21°C.