EVALUATION OF FUNCTIONALITY OF COMMERCIAL RESISTANT STARCHES IN FOOD SYSTEMS

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TO MY MOTHER

ABSTRACT

The objective of this study was (i) to investigate the functional properties of commercial resistant starches in a fluid model food system, (ii) to determine the level of resistant starch that could replace the regular thickener without affecting the sensory properties of the system and (iii) to verify the claims made by manufacturers of resistant starches.

In order to evaluate four commercially available resistant starches, a chicken soup model food system was developed. The choice of food system was based on the ease of rheological measurement along with relatively easy method of preparation. A representative soup formulation was chosen which contained industrial starch, wheat flour and xanthan gum as thickening agents. A suitable experimental plan was developed using fractional factorial and central composite designs for evaluation of the soup model. The viscosity of the soup model was determined using Paar Physica rheometer and the sensory analysis was done using acceptance and simple difference testing.

The rheological properties, i.e. the consistency index (K) and flow behavior index (n), derived from the power law model, for the soup model were analyzed using response surface methodology, which enabled an evaluation of the functionality of the model and visualization of correlation between various factors (ingredients) and resistant starch. Results revealed that all resistant starches lacked any starch like functionality as none of them was able to replace the waxy maize starch functionality to any significant extent. Hence, it was necessary to allow for the replacement of waxy maize starch by increasing the amount of xanthan gum in the formulations. Thus, regression models, built to predict the optimum regions of response, were used in replacing waxy maize starch in soup with resistant starch by increasing the amount of xanthan gum.

Comparative sensory responses obtained from paired sample testing determined that the optimum level at which resistant starch could be added to soup model was only 20%. At higher levels (40% and 60%), a difference in

taste could be perceived.

The claims made by manufacturers regarding the thermal stability of resistant starches were validated and the *in vitro* assays showed no significant difference ($P>0.05$) in percent resistant starch (dry weight basis) level with the increase in holding time (5-20mins) at 95 °C while soup making.

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TABLE OF CONTENTS

CHAPTER 1

INTRODUCTION

Until recently, starch was thought of as a source of carbohydrate that was completely digested and assimilated in the small intestine. However, it is well known that there exists a small but variable starch fraction that is resistant to hydrolysis by enzymes of the pancreas. Passing through the small intestine, this fraction reaches the large bowel where it is fermented by the colonic microflora to a variable extent. This fraction is called resistant starch (RS) and on that account is defined as the sum of starch and the products of starch degradation not absorbed in the small intestine of a healthy human being (Niba *et al.*, 2002).

The main factors that influence rate and extent of starch digestion and absorption are both intrinsic and extrinsic. Processing conditions adopted and the resulting retrogradation steps may affect digestibility characteristics of starch-based foods. Also the duration and conditions of storage greatly influence the digestion of starch (Namratha *et al.*, 2002).

The rates of obesity continue to increase in most western countries despite the efforts made by health organization and state departments. For the last 20 years, reducing fat intake has been the primary focus of dietary prevention and treatment of obesity. Animal studies and human epidemiological studies have shown a relationship between dietary fat and body weight and a reduction in its intake can produce significant weight loss in obese subjects but weight regain often occurs (Brand-Miller *et al.*, 2002).

However, studies on the postprandial effects of carbohydrate dense, high-glycaemic index (GI) diets have helped to explain why low-fat diets have failed to inhibit weight gain in obese subjects. Rapid digestion and absorption accompanied by higher insulin response after high-GI foods dictates energy partitioning and satiety which favors expansion of fat stores over long term. Therefore, low GI diets are clearly justifiable for the prevention and treatment of

obesity in overweight subjects. Numerous studies have shown that food products high in resistant starches frequently result in low glycaemic and insulinaemic responses mainly due their resistance to digestion, which is the key determinant of the glycaemic index (Akerberg *et al.*, 1998; Brand-Miller *et al.*, 2002).

RS is a natural component of many foods but its quantity in our diets still needs to be increased. This can be achieved by careful selection of foods and changes in culinary practices. This phenomenon of the resistance of starch to human digestion needs to be exploited by the food industry to achieve high levels of RS in familiar food products such as breakfast cereals, breads, bars and biscuits. Such functional foods need to be manufactured in greater variety and with higher palatability than many of the familiar high fibre products currently available to consumers (Johnson and Gee, 1996).

New starch ingredients can be evaluated in food by using a model food system. This is a valuable tool to test ingredients on a small scale in a complex food system which can be extrapolated to real food products. Selected physical properties of starches have been used to study starch functionality using a food model system. Highly reproducible food models were used to evaluate functional and rheological properties of modified starches by Wischmann *et al.* (2002).

Following this line, the objective of this study was to investigate the functional properties of commercially available resistant starches in a fluid model food system. Moreover, it was the aim to determine the level of resistant starch that could replace the regular thickener without affecting the sensory properties of the system. Response surface methodology was used to collect rheological data on the food system which enabled us to describe the functionality of resistant starches and correlate it with their concentration. In this context, the claims made by manufacturers of resistant starches were also verified.

CHAPTER 2

LITERATURE REVIEW

2.1 Starch – chemistry, properties, various sources and uses in foods

Starch, a mixture of glucans, is the principal food reserve polysaccharide found mainly in the plant kingdom, where it may be utilized for growth. Out of the total calories consumed by humans worldwide, starch accounts for 70-80%. After cellulose, the most widely commercially utilized of all polysaccharides is starch (Greenwood, 1970).

All starches occur in nature as minute granules, each with its inherent characteristic size and shape irrespective of the source. The commercial starches are divided into three groups. The first group comprises of tuber (potato) and root (tapioca, arrowroot and sweet potato) and pith (sago) starches. Cereal starches (corn, rice, wheat and sorghum) make up the second group. Both these groups differ from each other in chemical composition and physical properties. Group three contains waxy starches (waxy maize, waxy sorghum and waxy rice), which are certain mutants of maize, rice and sorghum, cultivated for their special characteristics. Even though obtained from cereals, their physical properties are similar to those of root starches (Swinkels, 1985).

Starch is composed of a mixture of two polymers: an essentially linear polysaccharide called amylose and a highly branched polysaccharide called amylopectin. Amylogenin, a self-glucosylating initiator protein molecule, is attached to a single β-D- glucopyranosyl unit, from which the starch molecule grows. Adenosine diphosphate molecules donate α -D- glucopyranosyl producing a chain joined by 1→ 4 linkages. In addition to this, a branching enzyme is also active. In order to transfer a linear chain, the enzyme requires a linear chain of 40-50 units. The transferred portion becomes an α - 1→ 6 branch, whereupon

both non reducing ends continue elongation. This constitutes the amylopectin molecule. Some branches may occur as double helices of parallel chains (Whistler & BeMiller, 1997).

Amylopectin constitutes about 75% of most common starches. Some starches be entirely amylopectin and are called waxy starches. These are called so because, when the kernel is cut, the new surface appears vitreous or waxy but there is no wax present. Structures of amylopectin and amylose molecules are shown in Figure 2.1 (Whistler & BeMiller, 1997).

Amylose is essentially a linear chain of (1→ 4) linked α -D- glucopyranosyl units with an average molecular weight of about 10^6. Amylose takes a helical shape due to the axial equatorial position coupling of (1→ 4) linked α -D-glucopyranosyl units. This results in formation of more elastic films and fibers than formed by cellulose. Amylose molecules occur among the amylopectin molecules and can diffuse out of partially water-swollen granules (Whistler & BeMiller, 1997).

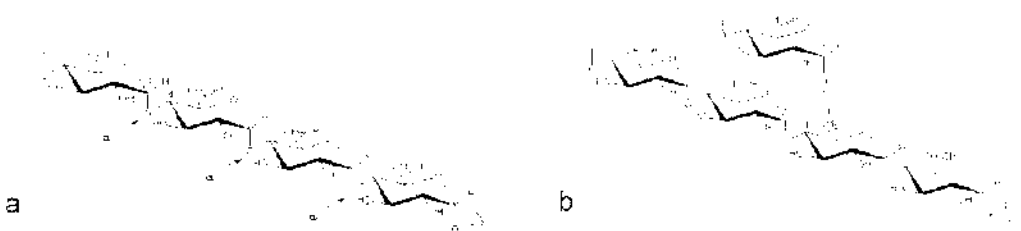


Figure 2.1: Representative partial structures of a) Amylose; b) Amylopectin (Pomeranz, 1991).

The quasi-crystalline nature of starch molecules in a granule is due to the radial ordered arrangement, as evident from the polarization cross (bifringence) seen using a polarizing microscope (Figure 2.2). The center of the cross is at the growth point, the hilum. Type-A X-ray pattern indicates parallel double helices separated by interstitial water, which is found in cereal starches. In tuber and root starches, a column of water molecules replaces one of the double helices, which produce Type-B X-ray pattern. The structure of the granule is formed by amylopectin molecules, arranged with their reducing ends towards the center of the granule (Whistler & BeMiller, 1997).

The unique particulate nature of starch and its properties such as high viscosity on gelatinization and its gelling or non-gelling characteristics, lead themselves to an array of uses. Their major applications in the food industry are as thickening agents (sauces, cream soups, pie fillings), as colloidal stabilizers (salad dressings), for moisture retention (cake toppings), as gel-forming agents (gum confections), binders (wafers, ice cream cones) and as coating and glazing agents (nut meats, candies) (Swinkels, 1985).

Figure 2.2: An image of wheat granule (A) using the first microscope by Van Leewenhoek, and an image of potato starch (B) viewed under polarised light (Source: Wang *et al.*, 1998).

In some instances, these properties are inherent in native starch, but occasionally, they can be augmented or introduced into the starch by (a) physical modifications, (b) non-degradative chemical modifications or (c) degradative modifications. Physical modifications are limited to variation in drying conditions. Starches react with a wide variety of chemical reagents to form ethers or esters which lead to an alteration of swelling properties along with associated properties of the starch molecule. Such starches comprise the non-degradative chemical modifications. The degradative modifications of starch lead to thin boiling starches, oxidized starches, dextrins and sugars and syrups (Pomeranz, 1991).

2.2 Gelatinization

Starch granules are insoluble in cold water due their semi-crystalline structure, stabilized by the hydrogen bonds, formed either directly via neighboring OH groups or indirectly by water bridges. Even though the forces of the hydrogen bonds are weak, they are present in large numbers and can confer considerable stability. However, the granules can imbibe water reversibly and swell slightly (10-15% in diameter) in cold water. On drying, they shrink back (Swinkels, 1985).

On progressively heating in water at high temperatures, the granules start to swell irreversibly and the characteristic polarization-cross starts to fade at the hilum. This loss of bifringence and the concurrent initiation of swelling is termed as gelatinization. Initiation of swelling takes place at the amorphous regions and starch molecules are hydrated by the disruption of weak bonds. The orderly radial arrangement is disturbed due to swelling leading to loss of bifringence. This is followed by more swelling and the disruption of hydrogen bonds in the crystalline region. Some amylose molecules may leach out increasing the viscosity of the surrounding phase. Because amylose is a relatively small, linear molecule, it can easily diffuse out of the granule (Swinkels, 1985).

Gelatinization occurs over a range of temperature with larger molecules gelatinizing first and the smaller ones later. However, this may depend on the granule type, starch-water ratio and the heterogeneities within the granule

population (Pomeranz, 1991). Amylose content also affects the temperature of gelatinization as high amylose varieties of corn have a higher temperature range compared to the low amylose ones. The gelatinization temperature range of starches derived from different sources is shown in Table 2.1. The granule finally ruptures and collapses on persistent heating and agitation at high temperatures yielding a viscous colloidal dispersion of swollen granule fragments, hydrated starch aggregates and dissolved molecules (Swinkels, 1985).

Gelatinization of granules occurs because the high temperature encourages vibration of the molecule, breaking the hydrogen bonds. More extensive hydration is produced when water molecules replace the broken hydrogen bonds. The starch molecules move freely and get sheathed in layers of water making it impossible for them to return to their original positions upon dehydration (Whistler & BeMiller, 1997).

However, the same end can be achieved at room temperature by the use of solvents such as liquid ammonia and dimethyl sulfoxide or mechanically, by milling (Blanshard, 1987). Gelatinization of starch can be examined by differential scanning calorimetry (DSC), which measures both temperature and enthalpy of gelatinization. The Brabender viscoamylograph is used to examine the cooking behavior of different starches. This instrument records the viscosity change when temperature is raised to 95°C, where it is held for a short time and then lowered (Whistler & BeMiller, 1997). The gelatinization of starch is affected by various factors. Concentration and degree of shear are the main ones. Proteins present in flour of cereal starches such as wheat interact due to the attraction of opposite charges. These form complexes during gelatinization. The interaction is low at alkaline pH levels, at which both starch and protein bear a negative charge, and high at acidic pH, at which both bear a positive charge.

Table 2.1: Physical and chemical properties of starch (From: Pomeranz, 1991).

Starch		Granule size (μm)		Amylose (%)	Swelling power (at 95°C)	Solubility at 95°C	Gelatinization range (°C)	Source	Taste
		Range	Average						
Barley		2-35[a]	20	22	-	-	56-62	Cereal	Low
Corn									
	Regular	5-25[b]	15	26	24	25	62-80	Cereal	Low
	Waxy	5-25	15	~1	64	23	63-74	Cereal	Low
	High amylose	-	15	Up to 80	6	12	85-87	Cereal	Low
Potato		15-100	33	22	1000	82	56-69	Tuber	Slight
Rice		3-8[c]	5	17	19	-	61-80	Cereal	Low
Rye		2-35[d]	-	23	-	-	57-70	Cereal	Low
Sago		20-60	25	27	97	-	60-74	Pith	Low
Sorghum		5-25[e]	15	26	22	48	52-64	Cereal	Low
Tapioca		5-35	20	17	71	48	52-64	Root	Fruity
Wheat		2-35[f]	15	25	21	41	53-72	Cereals	Low
Oats		2-10	-	27	-	-	56-62	Cereal	Low

[a] Large starch granules above 5; small starch granules below 5. Small starch granules gelatinize at 75-80°C
[b] Mainly 10-15.
[c] Some clusters 9-30; some as large as 40.
[d] 2-8; up to 35.
[e] Mainly 10-12.
[f] 2-5, 6-15, and above 15

Wheat proteins denature during the heat treatment and form complexes with starch molecules, preventing the escape of exudates and thus interfering with the decrease in viscosity. Gelatinization is also affected by the interactions of lipids with starch complexes. The availability of water for starch is affected by the presence of pentosan gums, which imbibe a lot of water. Wheat flour contains 2-3% of pentosan gums, one-fourth of which are water-soluble. They can absorb 9.2 times their weight of water in a starch-protein mixture (Pomeranz, 1991).

The extent of swelling, the swelling power, of granules is peculiar to particular starches. It can be calculated at a pasting temperature as the weight of sedimented swollen granules per gram dry starch. This indicates the nature of internal bonding. A relative ease in swelling indicates weak internal bonding and vice-versa (Swinkels, 1985).

The critical concentration value of starch is the weight of starch per 100 ml, on dry basis, required to produce a paste in which the swollen granules occupy the entire volume at 95°C. When the starch concentration is above the critical value, the swollen granules will form a continuous paste. Below this value there will be some free water left (Swinkels, 1985).

2.3 RETROGRADATION

The phenomenon in which some of the crystalline structure of the starch molecule, lost during gelatinization, is restored during storage is known as retrogradation. If a dilute starch solution is allowed to stand for a prolonged time, it gradually becomes cloudy and eventually deposits an insoluble white precipitate. Cooling leads to a rapid elastic gel formation in more concentrated systems. This may be accompanied by syneresis, which is the leakage of water from the gel along with hardening of the gel, increase in viscosity and turbidity development (Swinkels, 1985). The quality of food texture and other physical properties deteriorate making retrogradation an important factor in the inclusion of starch as an ingredient (Ishiguro *et al.*, 2000).

Amylose is predominantly responsible for the retrogradation processes. Dissolved amylose molecules can orient themselves in a parallel alignment, so that a large number of hydroxyl groups of one chain are in close proximity to those in the adjacent chains. This leads to formation of aggregates bound together by interchain hydrogen bonding of hydroxyl groups, which are insoluble in water (Swinkels, 1985).

A double helical association of 40-70 glucose units similar to structure to native granular starch may form (Ting-Jang *et al.*, 1997). In dilute solution, the aggregates of amylose chains precipitate whereas in concentrated dispersions, aggregated amylose molecules entrap fluid in the network forming a gel (Figure 2.3). To re-dissolve the retrograded amylose a temperature of 100-160°C is required (Swinkels, 1985).

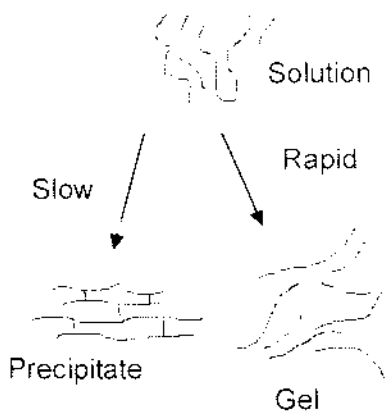


Figure 2.3: Schematic representation of retrogradation mechanism.

Studies of amylose dispersions indicate that amylose gels are composed of a network of double helical, semi-crystalline junction zones separated by amorphous regions. Ting-Jang *et al.* (1997) reported that retrograded amylose is a mixture of crystalline and amorphous regions and exhibits a B-type X- ray pattern. The crystalline regions are resistant to amylo-lytic and acidic hydrolysis. At elevated temperatures the double helix formation is slower and longer chains are required to maintain a stable double helical arrangement. Amylose with a degree of polymerization (DP) of 80-100 has the highest retrogradation tendency.

Amylopectin is much less prone to retrogradation than amylose due its highly branched structure. Therefore, amylopectin tends to be soluble and does not form gels under normal conditions and the double helices formed are shorter due to the restriction imposed by branching structures (Klucinec & Thompson, 1999). However, under freezing conditions and high starch concentration the molecules may undergo retrogradation. The amylopectin fraction in common starch gels has a moderating effect on the retrogradation of linear amylose fraction by slowing down the precipitation and diminishing the gel tendencies (Swinkels, 1985).

Normal retrogradation occurs generally on cooling and storage of starch pastes at a temperature of 70°C or below. High temperature retrogradation (75-95°C) is observed when corn starch is gelatinized at temperatures of 120-160°C. The precipitated particles are formed from inclusion complexes of cornstarch amylose with fatty acid, which occurs naturally in corn starch. Normal cereal starches contain 0.5 ~ 1.5% internal lipids (i.e., lipids extractable only by polar solvents such as water containing methanol, butanol, etc.), which remarkably influence the retrogradation of starch (Swinkels, 1985).

However, high temperature retrogradation does not occur when the corn starch is defatted. The dissociation of such complexes occurs at temperatures above 95°C, as the complex of amylose with fatty acids is not formed above 95°C (Swinkels, 1985). Lipids can indirectly affect the behavior of amylopectin towards water through complex formation with amylose, which may be associated with

amylopectin within a starch granule (Hibi *et al.*, 1990).

Retrogradation is a complex process and its rate depends on various factors, such as source of starch, starch concentration, cooking procedure, temperature, storage time, pH, cooling procedure, and the presence of other compounds (Jacobson *et al.*, 1997). Starches from different botanical sources retrograde differently. This is due to factors like the amylose and amylopectin ratio, the molecular weight of amylose and the chain length of amylopectin (Swinkels, 1985). Ishiguro *et al.* (2000) noticed rapid retrogradation in gels having small amylose molecules and long unit chain amylopectin. Sweet potato starch retrograded more slowly than tapioca starch due to its lower amylose content. Retrogradation is also affected by the chain length distribution of amylopectin as pea amylopectin retrogrades faster than cereal amylopectins, which have shorter average chain lengths.

Retrogradation, determined quantitatively by turbidometric analysis, in stored (4°C for 56 days) 2 % pastes prepared by atmospheric cooking under mild shear conditions of starches from various botanical sources revealed that retrogradation rates followed the order of wheat, common corn > rice, tapioca, potato >> waxy maize. Amylose in stored starch systems underwent very obvious changes whereas there was little change in amylopectin microstructure over the storage period. Solubilized amylose generally co-crystallized or precipitated with amylopectin and/or crystallized or precipitated onto the amylopectin-rich granule remnants. The latter interactions were weak indicating an interaction between amylose and amylopectin to form a network (Jacobson *et al.*, 1997). .

Lin *et al.* (2001) measured the degree of retrogradation of milled rice as the melting enthalpy (ΔH) of recrystallized starch for 30 days. The ΔH for rice varieties with high amylose content increased rapidly in the early stage and then reached a plateau. For varieties with intermediate amylose content, the ΔH did not change significantly for the first two days but rose as the storage time increased. For waxy varieties, the ΔH did not change for 9-12 days, and then started to increase. The ΔH for the latter two did not reach a plateau during the

30 days.

Fastest rates of retrogradation are observed at a pH of 5-7. The rate slows down below pH 2 and ceases above pH 10 (Swinkels, 1985). In dilute amylose solution, the rate of retrogradation, measured by decrease in amylose concentration, decreased with an increase in incubation temperature (Table 2.2). Different molecular sized amylose fractions have different retrogradation tendencies. Small sized amylose molecules such as potato amylose (DP 110) have a higher retrogradation tendency. Retrogradation temperature also affects the chain length of crystalline regions of retrograded amylose. The crystalline chain length increased as the incubation temperature of the retrograded amylose increased (Ting-Jang *et al.*, 1997).

Table 2.2: Total retrogradation of original potato amylose and retrograded amylose during incubation at different temperatures[1] (From Ting-Jang *et al.*, 1997).

Temperature (°C)	Total retrogradation (%)[2]
Original amylose	0
Retrograded amylose	
5	88.3 ± 4.5
15	70.8 ± 0.9
25	44.8 ± 3.2
35	14.2 ± 2.6
45	8.6 ± 0.4

[1] Means and standard deviation of duplicate samples for total retrogradation
[2] After 85 days incubation

The retrogradation of waxy starches is directly proportional to the mole fraction of branches with DP 14-24, and inversely proportional to the mole fraction of branches with DP 6-9 (Lin *et al.*, 2001). The polymeric form obtained during amylopectin recrystallization depends upon the storage conditions. Low water content and/or high temperature conditions favor the formation of the A polymorph while high water contents and /or low temperatures lead to polymorph

B form with intermediate conditions leading to mixed crystals (C type) (Farhat *et al.*, 2000).

A clear evidence of amylopectin retrogradation was observed in extruded potato starch stored at constant moisture conditions for 2 and 14 days at three different temperatures (22, 40, 60°C). The material stored at 22°C retrograded to B form while the one stored at 60°C gave a polymorph of type A. An intermediate of pure A and B polymorph was observed at a storage temperature of 40°C (Farhat *et al.*, 2000). Type-A polymorphs are known to have denser unit cells and contain very few bound water molecules as compared with polymorph B (Shamai *et al.*, 2003). The digestibility of the extruded material decreased on storage as a result of retrogradation depending on storage time and temperature (Table 2.3). The results suggest a higher resistance to digestion in polymorph A than polymorph B (Farhat *et al.*, 2000).

Table 2.3: PA[1] digestibility (% starch, 6 h incubation) of potato starch extruded at 35% water (w/w, wb) and stored at different temperatures. The digestibility of the freshly extruded material was 77.0% ± 0.9[2] (From Farhat *et al.*, 2001).

	20 °C	40 °C	60 °C
2 days	45.8 ± 1.6	37.8 ± 0.3	32.9 ± 1.5
14 days	42.1 ± 0.3	35.4 ± 1.1	28.0 ± 1.7

[1]Porcine Pancreatic α amylase
[2]Results are means ± standard deviation.

The phosphorous in starch is mainly present in the form of phospholipids. Generally root starches contain low amounts of phosphorous compounds. Potato starch is the only commercial starch that has appreciable amounts of chemically bound phosphate ester groups (Swinkels, 1985). The effect of phosphate covalently linked to the starch is less known. Starch phosphorylation increases gel hydration and peak viscosity. However, potato starch with low bound phosphate shows a reduced peak viscosity, stronger gel, increased stickiness

and increased turbidity indicating increased retrogradation behavior. However, high amylose content, short chain amylopectin and high phosphate content suppress retrogradation (Thygesen *et al.*, 2003).

2.4 Rheological properties of starch dispersions

In most food products, starch is used as a texturing ingredient, and the texture depends on the concentration of the starch. Low levels are usually employed to impart thickness to the product whereas if it is the main component responsible for texture, the levels are relatively higher. Because of settling of the ungelatinized granules in rheological studies on starch dispersions of low concentrations, considerable heed should be exercised to ensure that the samples taken from the dispersion contain the same concentration of starch granules. Similar precautions are necessary in vertically oriented viscometer geometries like concentric cylinder and vertical capillary/ tube (Rao, 1999).

Starch dispersions, after gelatinization, have a continuous biopolymer matrix containing dispersed granule fragments along with swollen starch granules. Accordingly, rheology of gelatinized starch suspensions reflects the properties of the dispersed phase, the continuous phase, and the interactions between the components. The viscosity versus time profile is important for most food products containing starches (Rao, 1999).

Starch dispersions undergo crucial changes while being heat treated in heat exchangers. Significant changes in velocity profile occur resulting in pressure drops inside the process equipment. Shear rates ranging from 10 to 10^3 s^{-1} are encountered during most thermal processes carried out using plate heat exchangers or scraped surface heat exchangers. The flow regimes in heat exchangers depend heavily on the preparation procedure and the difference in viscosity of the product. Thus, for product development and process control strategies, modeling and rheological data for the starch dispersion are critical because of the effect on the process and the end product (Lagerrigue and Alvarez, 2000).

Extreme structural modifications take place during gelatinization, thermo-mechanical treatment and retrogradation of starches. These mainly result in the dramatic changes in apparent viscosity. During gelatinization, the starch granules swell irreversibly producing an increase in viscosity and an eventual leaching of amylose (linear component of starch granule). As soon as the granules start to rupture, due to shear, the viscosity starts to decease. The solubilized starch polymers and remaining starch granules re-associate in an ordered structure upon cooling during retrogradation (Lagerrigue and Alvarez, 2000).

Gelatinized starch dispersions exhibit a non-Newtonian, time-dependent and viscoelastic behavior. Their rheological properties revolve around the procedure adopted for gelatinization. Table 2.4 summarizes the rheological behavior of gelatinized starch dispersions found by different researchers for measurement conditions above and below 100°C. Minor variations in the procedure adopted for gelatinization were present along with differences in composition cause differences in viscosity (Lagerrigue and Alvarez, 2000).

Table 2.4: Modeling of rheological behavior of gelatinized starch suspensions: (a) T < 100 °C; (b) T > 100°C (From: Lagerrigue and Alvarez, 2001).

Authors	Starch type and concentration (w/w)	Viscometer	Gelatinization procedure	Measuring condition	Modelling
a) T < 100 °C					
Evans and Haisman (1979)	Corn, potato, tapioca and modified corn (0.5-10%)	Haake rotovisco RVI, Weissenberg Rheogonimeter R16 (cone and plate)	Heating at a fixed T in rotating flasks	T= 60°C; flow curves:0.0007-56 s^{-1} and 7-1142 s^{-1}	Power law model, Herschel Bulkley model
Bagley and Christianson (1982)	Wheat (7-25%)	Haake rotovisco coaxial cylinders (MV system cup, MV-II bob)	Corn industries research viscometer cooking T: 60-75°C cooking t : 15-75 min	T studied: 60°C and 23 °C; flow curves 1-1000 s^{-1}	Dilatancy if cooking 15- 45 min at 60 °C, master curve η/cQ function of cQ at a60 °C
Christianson and Bagley (1984)	Corn (8-24%)	Haake rotovisco coaxial cylinders (MV system cup, MV-II bob)	Corn industries research viscometer cooking T: 60-75°C cooking t : 15-75 min	T studied: 60°C and 23 °C; flow curves 3-500 s^{-1}	Herschel Bulkley model yield stress at 23°C is a function of cQ
Doublier et al. (1987)	Wheat and maize (3-10.5)	Rheomat-30 coaxial cylinders (A-system)	Double-walled round bottom vessel, stirring: 200 or750 rev. min^{-1} Cooking T: up to 96 °C, cooking t: up to 30 min; heating rate 1 °C min^{-1} or exp. rate	T=70°C up and down curves: (1) 0-6.6 s^{-1}, (2) 0-660 s^{-1}, (3) from 660 s^{-1} to 0 constant shear rate experiments,	Herschel Bulkley model K, n and σ_0 depends on the gelatinization procedure, thixotropy
Harrod (1989a,b,c)	Cross-linked and esterified potato (3-10%)	Rheomat-30 coaxial cylinders	Brabender viscograph scraped surface heat exchanger	T= 10-90°C up and sown step wise curves: 6-450 s^{-1}	Arrhenius law, power law, thixotropy
Lagarrigue et al. (1990)	Potato (3-6%)	Tube viscometer D=23mm; L=10.5m; q=0.05-0.5 kg s^{-1}	Steam-jacketed vessel cooking T: 90°C	T= 90°C; flow curves: 40-400 s^{-1}	Power law model, Bingham plastic model
Dolan and Steffe (1990)	Corn (5.5-7.3%) and bean (6%)	Brookfield RVTD mixer viscometer	Brookfield RVTD mixer viscometer cooking T: 85-90°C, cooking t: 2-25 min	T= 50-95 °C; impeller speed: 20-100 rpm	Shear -temperature –time model
Ramaswamy et al. (1995)	Cross-linked waxy maize (3-4%)	Hake RV20	Pregelatinized at 85-95 °C (2h) in a steam jacketed kettle thermal processing in an agitated retort (110-130°C; 10-20 rpm)	T= 25°C up and down curves :0-200 s^{-1}	Power law model, Herschel Bulkley model Casson model
Okechukwu and Rao (1995)	Corn (2.6%)	Carri-med CLS 100 cone-plate (6 cm, 2°C)	Isothermal heating in jacketed stainless steel vessel cooking T : 70-90 °C; cooking t:0-540 min	T= 20 °C; γ= 0.05-1200 s^{-1}	Power law model (20 -1200 s^{-1}): n function of the standard deviation in mean granule size, K and Casson yield stress increase with mean granule diameter, dilatant behaviour for cooking at 67 °C

Okechukwu and Rao (1996 a,c)	Cowpea (2.6 %)	Carri-med CLS 100 cone-plate (6 cm, 2°C)	Isothermal hating in jacketed stainless steel vessel cooking T: 67-86 C; cooking t: 0-2880 min	T= 20°C, γ= 0.05-1300 s^{-1}	Power law model; k increase exponentially with mean granule diameter, n decrease linearly with increasing extend of gelatinization dilatancy at early stage of gelatinization
Bhattacharya and Bhattacharya (1996)	Debranned maize flour (2-10%)	Rheotest –2 coaxial cylinders	Brabender viscograph	T= 60°C, flow curves: 3-1326 s^{-1}	Herschel Bulkley mode, Mizrahi-berk model, K, σ_0 increases with c, n decreases with c
Breton- Dollet (1996)	Maize and waxy maize (5%)	Carri-med CLS 100 cone-plate (6 cm, 4°C)	Autoclave cooking T: 100-136 C; cooking t: 30 min	T= 60°C, up an down curves: 0.1- 100 s^{-1}	Power law model, K function of cooking T, n increases with cooking T thixotropy depends on the kind of starch, c, cooking T
Nguyen et al. (1998)	Normal maize and waxy maize (6-7%)	Haake rotovisco RV 100 coaxial cylinders	Cooking T: 50-75°C (waxy) or 50-85°C (regular); heating rate: 2 C mn^{-1}	T = 25- 50°C, transient experiments at constant γ(90-225 s^{-1}), flow properties at steady state	Power law model: K (c,T) n is constant 0.48, structural kinetic model for thixotropy (which depends on γ, c, and T)
Xu and Raphaelides (1998)	Maize (7-30%)	TR-1 tube rheometer diameter: 2.05 mm; length: 30 mm	Gelatinization at 95 °C under continuous stirring	T=75-95 °C; 10-1000 s^{-1}	Power law model, n increase with cooking time
Nurul et al. (1999)	Sago (3-5.5%)	Contraves rheomat 115	Autoclave cooking T: 125°C cooking t: 30 min	T= 40-80 °C, γ = 13.64-704 s^{-1}	Power law model: exponential dependence with c, Arrhenius law
(b) T >100 °C					
Dial and Steffe	Waxy maize (1.82 – 2.72 %)	Tube viscometer D= 1.27 cm; L= 4.59 m	Gelatinization in a tubular heat exchanger	T studied: 121.1- 132.2-143.3°C; flow curves 10-150 s^{-1}	Power law model k depends on c and T, n increases with c and decreases with T dilatant behaviour
Abdelrahim et al. (1995)	Cross-linked waxy maize (3-6%)	Haake RV 20 equipped with a high T/high P attachment and a magnetic coupling coaxial cylinders	Gelatinization at 140 °C in an aseptic processing system	T studied: 60-140°C flow curves: 0-500 s^{-1}; constant γ experiment	Power law model, modelling of K and n as a function of C and T
Heydon et al. (1996)	Colflo 67 and C Flo (4-7%)	Tube viscometer D= 3.46-2.17 am; L= 2.85-3.2 m Q= 0.5-3.1 min^{-1}	Gelatinization in a scraped surface heat exchanger	T studied: 100-135°C; 10-70 s^{-1}	Arrhenius law, power law.

Predominantly, power law or Herschel- Berkley models are used to represent gelatinized starch dispersions in the shear rate range of 1-1500 s-1. However, other models such as Bingham plastic, Casson and Mirzrahi-Berk have been tested as well. These are illustrated below.

$\sigma = K \gamma^n$ (Power law Model)

$\sigma - \sigma_0 = K \gamma^n$ (Herschel-Bulkley Model)

$\sigma^{0.05} = K_{0c} + K_c (\gamma)^{0.05}$ (Casson Model)

Here, σ, σ_0, $\sigma^{0.05}$ are shear stress, yield stress and its square root, K is consistency index, γ is shear rate, n is flow behavior index,

Pre-gelatinization starch suspensions can show dilatant behavior. After gelatinization shear-thinning behavior is most often observed. The consistency index (K) and flow behavior index (n) obtained from the power law model depend upon the starch concentration and the processing temperature. Over the gelatinization temperature range, the consistency index (K) is also dependant on temperature and follows the Arrhenius law (Lagerrigue and Alvarez, 2000).

$K \alpha \exp (E_a/RT)$

Here, E_a is the activation energy in kJ mol^{-1}

Flow behavior index (n) has also been found to depend on concentration and temperature. A dependence of K and n on granule size has also been suggested (Lagerrigue and Alvarez, 2000).

2.5 Resistant Starch

2.5.1 History

Resistant starches were originally associated with raw starch with an X-ray diffraction pattern of Type B (raw banana and potato starch) as starch granules had been found in faeces of animals and human subjects. They were seen to decrease the energy value of starchy products. The medical and physiological interest in RS was boosted only after the identification of a specific fermentation profile (rich in butyrate) and the first studies on the effect of butyrate on cell proliferation and differentiation (Champ *et al.*, 2003).

The quantitative importance of the undigested fraction of starch was recognized, at first, by the laboratories of Cummings and Stephen (1983). Sandberg *et al.* (1981) studied the intestinal digestibility of ileostomy patients (patients without a colon) and made *in vivo* measurements. By doing this one could measure the RS content without administering invasive techniques. Besides this, intubation and perfusion techniques were developed for both small as well as the large intestine. The European Flair-Concerted Action Research Programme (EURESTA), which started in 1990, has since then instigated further research on resistant starch in Europe as well as in other parts of the world (Champ *et al.*, 2003).

2.5.2 Relationship with Glycaemic Index

An important number of studies in the past decade suggest a beneficial effect of low Glycaemic index (GI) diets in relation to insulin resistance syndrome. A low GI diet not only improves metabolic aftermaths of insulin resistance but also causes a reduction in insulin resistance *per se* (Bjorck *et al.*, 2003). Rapidly absorbed starch produces high blood glucose and insulin levels after meals. Free fatty acids thus released from the liver cause insulin resistance at the next meal, which is considered as a potential risk factor for the development of insulin resistance syndrome e.g. diabetes, atherosclerosis and obesity (Akerberg *at al.*, 1998).

However, food products rich in RS consistently give low GI and insulinaemic responses (Akenberg *et al.*, 1998). This is due to the indigestibility of RS in the small intestine, which prevents any contribution to postprandial glucose response. With addition of RS to meal there is no alteration in postprandial glucose. Propionic acid, a fermentation product of RS, has been shown to be involved in the metabolism as a moderator of hepatic glucose (Bjorck *et al.*, 2003).

Bjorck *et al.*, (2003) reported an improved insulin economy with a modified low GI diet compared to the usual high-GI one in women at risk to Type-2 diabetes. However, no correlation could be established between a low - GI diet and the postponement of Type-2 diabetes.

2.5.3 Resistant Starch as a Functional Food Ingredient

Uses of starch in the food industry are manifold, such as for bulking, functionality and texture and for nutritional quality as well. Therefore, its modification can easily be used in the field of disease prevention. In the UK, the daily intake of RS by an individual is estimated to be about 3g, which is quite low (Niba *et al.*, 2002). RS can be used in functional-food product development. By definition, functional foods are "foods similar in appearance to conventional foods that are consumed as a part of the normal diet and have demonstrated physiological benefits and/or reduce the risk of chronic disease beyond basic nutritional functions" (German *et al.*, 1999).

According to Niba *et al.* (2002), resistant starch can be exploited for the development of functional foods in following ways:

- Convert foods, with high RS, into more palatable and appealing forms;
- Increasing the level of RS in foods by various processing techniques;
- Developing processing techniques that ensure stabilization of resistant starch levels in foods.

Whole grains are considered as a rich source of RS and other fermentable carbohydrates as these escape digestion. Making these rich repositories of RS into palatable food material by appropriate processing techniques improves their usefulness for functional foods. Several methods have been suggested that can potentially enhance the RS content in foods such as high-pressure autoclaving (Escarpa *et al.*, 1996), enzymatic debranching (Shi *et al.*, 2003), partial degradation (Schmiedl *et al.*, 2000) etc.

Hitherto, most macronutrients including RS are difficult to quantify *in vivo*. In spite of that, there are a number of *in vitro* procedures, which provide an estimate of the physiologically available levels of RS (Englyst *et al.*, 1999). Therefore, physiologically relevant levels of resistant starch can be determined and applied to the production of functional food products.

2.5.4 Classification of Resistant Starch

Until recently, only three classes of RS were described in the literature but a fourth class has also been identified (Englyst *et al.* 1992, 1996). The four classes of RS are: physically inaccessible starch (RS1); RS granules (RS2); retrograded starch (RS3); chemically modified starch (RS4).

Physically inaccessible starch

RS1 is found in partly milled grains and seeds. Other main sources of RS1 are legumes such as beans or lentils where the starch is entrapped in a cellular matrix. These escape digestion due to the thickness of the cell walls. The preparation and cooking process is vital as these can disrupt the cell wall, lowering the resistance to digestion (Champ *et al.*, 2003).

RS granules

RS2 includes Type-B starches, such as raw potato and banana starch, which are known to be acutely resistant to enzymic hydrolysis when uncooked. Type-B refers to the X-ray diffraction pattern of the starch. Most raw starch in food gets gelatinized eventually leading to the disappearance of RS2. Banana is thought of as the main source of RS2 in the human diet, but the content in the

fruit depends on the degree of ripening (Champ *et al.*, 2003).

Retrograded starch

RS3 is present in most starchy foods after they have been hydrothermally treated. After gelatinisation, if the starch gels are allowed to cool, recrystallization occurs often forming double helices. Amylopectin, the branched fraction takes a longer time to retrograde than amylose, which is linear. Cooked and cooled potatoes have high RS3 mainly due to recrystalization. Retrogradation is partly reversible as reheating of starch reduces the RS3 content of the potato. Several cycles of heating and cooling, however, allow an increase in the RS3 levels. RS1, RS2 and/or RS3 can co-exist in the same food e.g. a meal of beans (Champ *et al.*, 2003).

Chemically modified starch

RS4, which has been recently established, includes starch ethers and esters as well as cross-bonded starches (Champ *et al.*, 2003).

2.5.5 Digestion and Fermentation of RS

By definition, RS is the fraction of starch that escapes digestion (Langkilde *et al.*, 1987). Therefore, it has been defined exclusively in terms of large bowel, as the rate of digestion in the small intestine is not relevant. Raw starch is digested poorly but cooking in presence of water enhances digestibility. While gelatinization increases digestibility, subsequent retrogradation increases resistance to digestion (Botham *et al.*, 1995). Another important factor affecting starch digestibility is the amylose: amylopectin ratio. Higher amylose content increases resistance to gelatinization and also makes the starch more susceptible to retrogradation (Topping *et al.*, 2003a).

Some physiological factors also influence the digestibility of starch that varies between individuals. Factors relating to gender, e.g. the female menstrual cycle, influence the RS uptake greatly with the uptake increasing in the mid-cycle. Also, particle size plays a crucial role. Large particle sizes have faster transit time and allow less access to digestive enzymes. Therefore, it is more

likely that people, who chew their food enough, would get less RS from a high RS diet as compared to the people who masticate sparingly. This is thought to be the biggest difference between resistant starch and non-starch polysaccharide. RS is a physiological outcome whereas the later is characterized by its chemical structure (Topping *et al.*, 2003b).

The digestion process does not end when food enters the large bowel. However, the main difference between the large and the small intestine is that the breakdown in the former is affected by bacterial enzymes rather than human ones. It is this bacterial system in the large bowel, which is responsible for metabolizing the undigested components in diet. RS is largely fermented, producing Short Chain Fatty Acids (SCFA) and bacterial cells. The molar quantity of SCFA produced depends on the accessibility of RS to the colonic microflora. Hitherto, there exists poor understanding of this complex bacterial system mainly due to limitations in technology as the present methods are labor intensive and consume enormous time. The net reaction of bacterial fermentation in adults is given below (Topping *et al.*, 2003a).

$$\text{Carbohydrates} + H_2O \rightarrow \text{Acetate} + \text{Propionate} + \text{Butyrate} + CO_2 + CH_4 + H^+ + \text{heat} + \text{more bacteria}$$

In humans, the principal SCFA are acetate, propionate and butyrate. They are useful metabolically and are readily absorbed from the large bowel lumen and used by the viscera for salt and water uptake (Henningsson *et al.*, 2002). The fermentability of RS is generally very high which makes them relatively poor laxatives. Thus, the effects of RS are mediated through their metabolic products rather than their physical presence so that SCFA become one of the key biomarkers for RS action. Fermentation is high in the proximal large bowel and so is the absorption of SCFA (Topping *et al.*, 2003a). However, as the faecal stream passes the fermentation slows down due to substrate depletion (Figure 2.4).

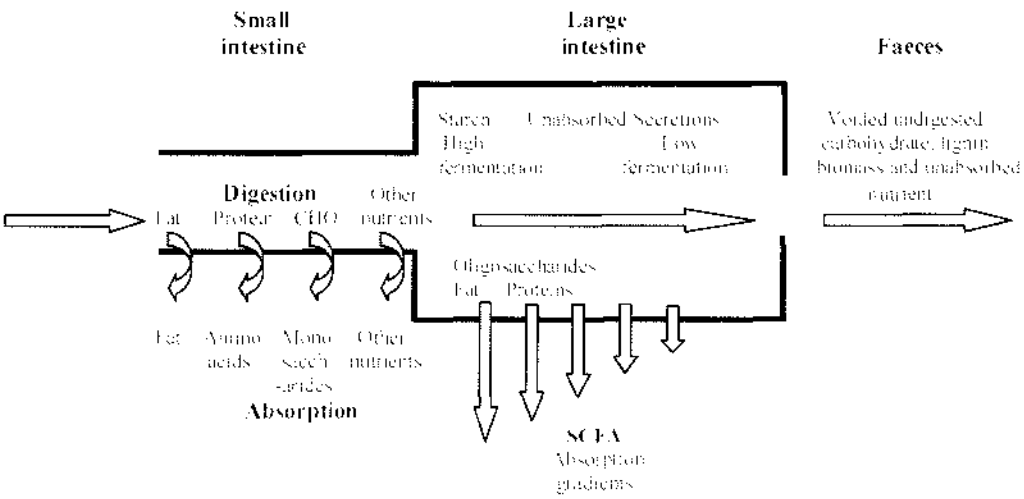


Figure 2.4: An overview of the transit of food through the small intestine and the large bowel (From Topping *et al.*, 2003a).

Of the principal SCFA, acetate does not have any specific actions in the large bowel and is largely transported to the liver for oxidative metabolism. RS fermentation by faecal bacteria leads preferentially to butyrate and propionate production (Brous *et al.*, 2002). Le Blay *et al.* (1999) reported that long-term intake of resistant starch for 2 to 6 months, derived from potato, increased butyrate production throughout the colon and also decreased the pH, effects which are considered beneficial. However, no key butyrate producing bacteria have been identified so far in human pilot studies (Schwiertz *et al.*, 2002).

Butyrate is thought to be pivotal for human colonic function as it contributes to the maturation of colonic epithelium. It has an anti proliferative effect on tumor cells and also promotes apoptosis (programmed cell death) *in vitro* in cancer cell lines (Brouns *et al.*, 1998). A study showed complete tumor regression by apoptosis probably induced by butyrate while testing the efficacy of sodium butyrate (NaB) and immune-factor interleukin 2 (IL- 2) against

experimental carcinomatosis induced in rats. Evidence based on these experimental observations suggests that butyrate plays a controlling role in colo-rectal cancer but such a link remains to be established in humans (Brouns *et al.*, 2002; Topping *et al.*, 2003b). However, Sakamoto *et al.* (1996) reported an increase in the butyrate production in rats with consumption of RS (raw potato starch) rich diet but this had no inhibiting effect on the dimethylhydrazine induced colonic carcinogenesis. Therefore, the literature on the effect of RS on colon cancer is contradictory (Champ *et al.*, 2003).

Many physiological attributes of butyrate are shared by propionate but at higher concentrations (Topping *et al.*, 2003a). Propionate also affects metabolism in peripheral tissue and its inhibitory action on hepatic cholesterol has also been proposed but not yet consistently shown *in vitro* (Henningsson *et al.*, 2002).

RS reduced body fat in rats and also increased fermentation in the lower GI tract, which is beneficial for colonic health (Hegsted *et al.*, 2003). However, this effect has not been confirmed in normolipidemic human subjects (Haralampu *et al.*, 2000).

The use of RS in probiotic compositions has been suggested as it promotes the growth of beneficial microorganisms such as Bifidobacterium (Haralampu *et al.*, 2000). Brown *et al.* (1997) reported an increase in the faecal concentration and excretion of Bifidobacterium longum after oral ingestion of RS as high amylose starch compared to those consuming conventional starch. In comparison to other Prebiotics, RS from high amylose starch yielded similar results in humans. However, pigs fed with rice baby food, with higher RS than conventional foods, did not show significant increase of ingested probiotics in faeces.

Ramakrishna *et al.* (2000) reported a major reduction in fluid loss and the halving of recovery time in children suffering from cholera induced diarrhoea after consuming RS (high amylose starch) along with the usual hydration therapy. This is due to greater fluid uptake as a result of increased SCFA production in the proximal colon.

2.5.6 Analysis of RS in Foods

The inclusion of RS in functional foods has led to the need for a valid analytical method for its appropriate quantification. Asp (1992) defined RS as 'the sum of starch and products of starch degradation that are not absorbed in the small intestine of healthy individuals. Thus, the analytical method should be applied to all the starch and α-dextrin present to quantify the RS content of foods. Besides, the ratification of the analytical method by a direct comparison of the data obtained *in vitro* with the true *in vivo* data from healthy subjects is important (Champ *et al.*, 2003).

A formalization of the physiological definition of RS seems to be particularly difficult for several reasons even though it would be conceptually most gratifying. Firstly, differences like the structural organization of the starch and/or the functional and physiological environment during the process of digestion exist between starch and RS. These factors are known to affect the digestive enzyme accessibility to the RS substrate. Others factors like the efficacy of chewing, the gastrointestinal transit time and the quantitative enzyme secretion also effect the starch digestion. All these factors may vary from one subject to another. These differences between digestible starch and RS are difficult to incorporate in an analytical method (Champ *et al.*, 2003)

Therefore, the *in vitro* method of choice has to provide results in contrast to the average response of a population of healthy individuals. Ideally, a large range of RS sources should be used to ratify the *in vitro* method (Champ *et al.*, 2003).

2.5.6.1 Current in vitro methods

The main step for the quantification of RS is to remove digestible starch from the sample by using α- amylase. To avoid a possible inhibition of the α-amylase by the products of the digestion (mainly maltose and maltotriose), some methods use amyloglucosidase. Proteolysis may precede amylolysis in order to reflect the action of pepsin and trypsin in the stomach secreted in the pancreatic juice along with α- amylase. RS is quantified after the digestion either from the

residue (isolated by ethanolic precipitation) or by calculating the difference between total starch and digestible starch (Champ *et al.*, 2003).

Björck *et al.* (1986) used the official AOAC method for estimation of dietary fibre to quantify total RS, as there was no regulatory definition of RS at that time. The residual dietary fibre was acquired after enzymatic solubilization (bacterial α-amylase {Termamyl} treatment at 95–100°C, according to Asp *et al.* (1983) and Prosky *et al.* (1988)). The amylase digestion was conducted at 95-100°C, which melted down some of the RS structures leading to an underestimation of the total RS. Therefore, to correct the method porcine pancreatin at 37°C was included (Haralampu, 2000).

The estimation of Rapidly Digestible Starch (RDS), Slowly Digestible Starch (SDS) and RS was first proposed by Englyst *et al.* (1992). The enzymatic digestion was accomplished at 40°C, which was close to that in the in vivo process, using protease, amylase and amyloglucosidase. A schematic of the method is given in Figure 2.5.

Berry's (1986) method was based on the Total dietary fibre (TDF) analysis methodology with remodelled scheme to improve the RS quantification. Goni *et al.* (1995) modified this method by including a proteolysis step before digestion, which was missing in Berry's (1986) method. In this method ethanol precipitation and acetone washing drying were also omitted from the Berry's (1986) method. Ethanol precipitation was thought of as time consuming and non-physiological and drying affected the RS values.

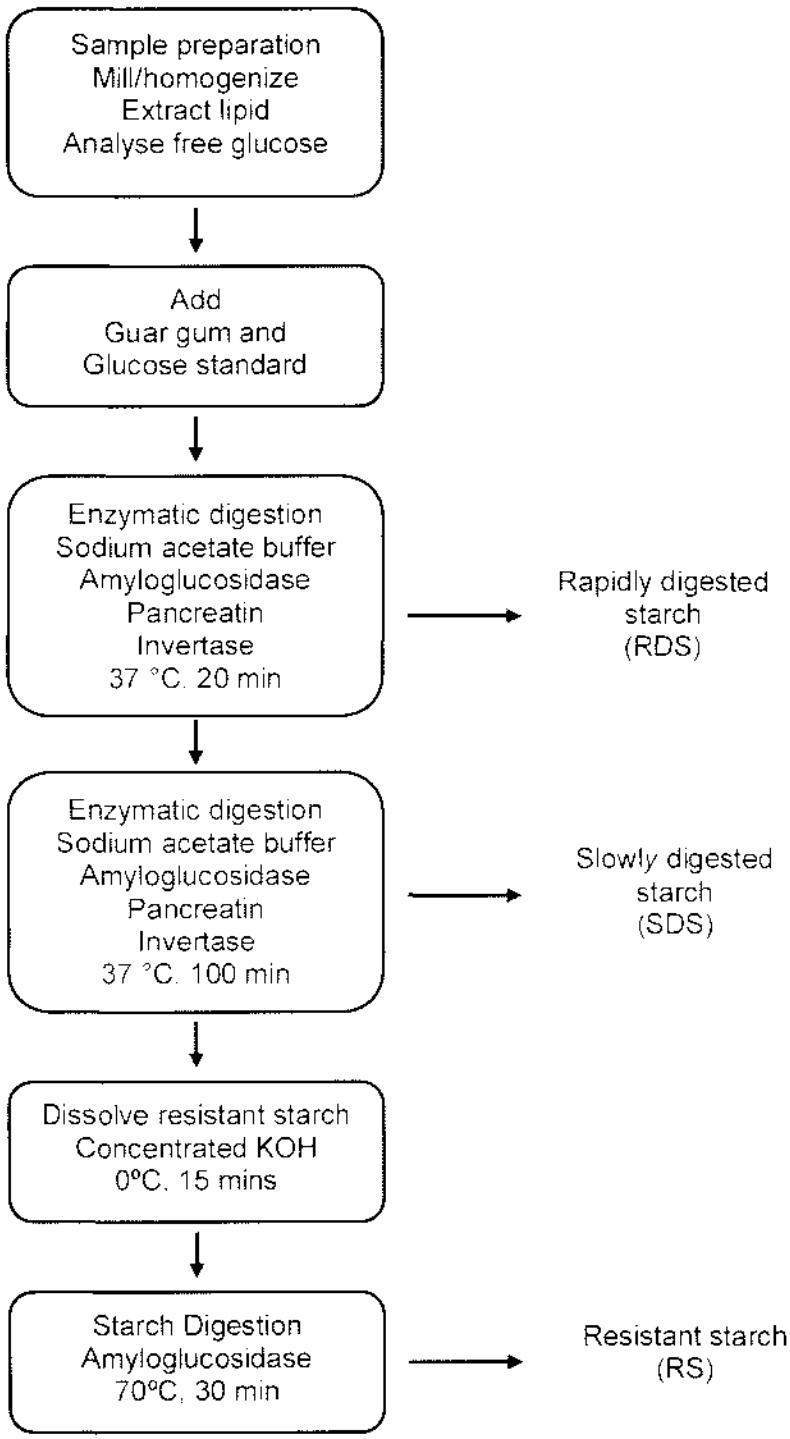


Figure 2.5: Englyst *et al.* (1992) method for resistant starch analysis (Haralampu, 2000).

Another method similar to the Berry (1986) method was proposed by Champ *et al.* (1999). The main modification to the Berry's (1986) method was the addition of amyloglucosidase. This method was derived from the one published earlier by the same author in 1992. A summary of the most recent methods is presented in Table 2.5. McCleary & Monaghan (2002) studied the strengths and weaknesses of the methods present in earlier literature (Englyst *et al.* 1992; Muir & O'Dea, 1992, 1993; Goñi *et al.* 1996; Åkerberg *et al.* 1998; Champ *et al.* 1999) and used the RS data derived from ileostomy model.

The following parameters were studied systematically:

- concentration of pancreatic α-amylase;
- need for pepsin pre-treatment;
- pH of incubation;
- importance of maltose inhibition of α-amylase;
- need for amyloglucosidase inclusion;
- effect of shaking and stirring on obtained RS values;
- problems in recovering and analysing the RS-containing pellets.

Table 2.5: Main *in vitro* methods to quantify resistant starch (RS) (Champ *et al.* 2003)

	Björck et al. (1986)	Englyst et al. (1992)	Muir & O'Dea (1992, 1993)	Goñi et al. (1996)	Akerberg et al. (1998)	Champ et al. (1999a)
Sample size	100 mg fibre residue (Asp et al. 1983 or Prosky et al. 1988(AOAC method)	0·8–4·0 g depending on the water and starch content of the sample	About 0·1 g carbohydrate basis	100 mg dry sample	1 g total starch basis	50 mg total starch basis
Sample pre-treatment		Minced (9 mm O holes)	Chewing	Dry samples, milled(o. 1 mm); fresh samples, homogenized	Chewing (15 X, 15 s)	Minced (9 mm O holes)
Protein hydrolysis	Pepsin pH 1·5, 1 h then pancreatin or bacterial protease pH 7·5 at 60 °C, both within DF analysis	Pancreatin (see 'starch hydrolysis')	Pepsin treatment (pH 2, 37 °C, 30 min)	Pepsin treatment (pH 1·5,40 °C, 60 min)	Pepsin treatment (pH 1·5, 37 °C, 30 min)	No protein hydrolysis
Digestible starch hydrolysis	Gelatinization step at100 °C + heat-resistant amylase then pancreatin or amyloglucosidase both within DF analysis	Pancreatin + amyloglucosidase + invertase + glass balls+ guar gum (pH 6·2, 37 °C). Time: 20 min =>RDS Samples collected ethanol (64·4 % in final concentration)	α-Amylase (Speedase PNA-8) + amyloglucosidase (pH 5·0, 37 °C, 15 h)	Pancreatic α-amylase(pH 6·9, 37 °C, 16 h)	Pancreatin + amyloglucosidase (pH 5·0, 40 °C, 16 h)	Pancreatic α-amylase+ amyloglucosidase (37 °C, 16 h)
Removal of starch hydrolysis products	Ethanolic precipitation filtration using celite as filter aid, within DF analysis	Ethanolic precipitation, centrifugation (10 min, 3000 g)	No ethanolic precipitation, centrifugation (10 min, 1200g)	No ethanolic precipitation, centrifugation (15 min, 3000g)	Precipitation,76 %EtOH, filtration centrifugation	Precipitation, 80 %EtOH
Dispersion of RS	Boiling (20 min) + 2 M-KOH (room temperature, 30 min)	No	Boiling (20 min)	2-M-KOH (room temperature, 30 min)	2-M-KOH (30 min	Boiling (20 min) + 2 M-KOH (0 °C, 30 min)
RS hydrolysis	Amyloglucosidase (pH 4·75, 60 °C, 30 min)	No	Amyloglucosidase	Amyloglucosidase (pH enzymic GOD-PAP4·75, 60 °C, 45 min)	Termamyl + Amyloglucosidase	amyloglucosidase (14 units ml; 70 °C, 30 min + 100 °C, 10 min)
Glucose determination	Enzymic, GOD-POD	Enzymic, GOD-PAP	Enzymic, GOD-PAP	Enzymic, GOD-PAP	Enzymic, GOD-POD	Enzymic, GOD-PAP
Validation	In vivo data obtained with antibiotic-treated rats	In vivo ileostomy data	In vivo ileostomy data	No	In vivo ileostomy data (mostly from literature)	In vivo ileostomy and intubation data
Specificity		RS = TS – (RDS+SDS)				

Consequentially, the predigestion step with pepsin was omitted. The refined procedure contained pancreatic α-amylase and amyloglucosidase acting together at pH 6·0 under defined shaking conditions followed by alcohol precipitation. RS was hydrolysed by amyloglucosidase after dissolution in 2 M-KOH and glucose was measured by using the GOD-POD reagent (glucose oxidase-peroxidase reagent; Megazyme International Ireland Ltd, Wicklow, Republic of Ireland). This method most resembles the Champ *et al.* (1999) method (Champ *et al.*, 2003). Various in vitro methods are compared with in vivo data using ileostomy method in Table 2.6 (Champ *et al.*, 2003).

Table 2.6: Comparison of RS (as percentage total starch) determined *in vivo* and *in vitro* (From Champ *et al.*, 2003).

Source of starch	In vitro RS					
	Englyst *et al.* (1992)	Faisant *et al.* (1995a)	Champ *et al.* (1999a)	Goñi *et al.* (1996)	McCleary & Monaghan (2002	In vivo RS
Potato starch, raw	66.5	83.0	77.7		77.0††	78·8†
HACS, raw	71.4	72.2	52.8		51.7††	50·3†
HACS, retrograded	30.5	36.4	29.6		42.0††	30·1†
Bean flakes	10.6	12.4	11.2		14.3††	9–10·9‡
Cornflakes	3.9	4.9	4.3		4.0††	3·1–5·0§
Beans	17.1		17.1		16.5††	16·5||
C*Actistar,						59·3†
Retrograded	63.0		57.0	57.0††	58.0††	78·8†

HACS, high-amylose maize starch.
†Ileostomy model; AM Langkilde, H Andersson and F Bouns (personal communication).
‡Ileostomy model; Schweizer *et al.* (1988).
§Ileostomy model; Muir & O'Dea (1993) and Englyst *et al.* (1992).
||Intubation technique; Noah et al. (1998).
¶Intubation technique; analysis by Dr Kettlitz, Cerestar Research and Development Centre, Vilvoorde, Belgium.
††Intubation technique; presented at AACC Meeting in Montreal 13–17 October 2002.

2.5.6.2 *Possibilities of validation in vivo*

At present, three prospective methods are available to obtain the *in vivo* values on the RS content of foods that are required for a ratification of the *in vitro* methods. These methods are discussed below.

Hydrogen breath test

One of the end products of carbohydrate fermentation is H_2. It is exclusively formed in the colon by bacterial fermentation, partly absorbed and cleared in a single passage of the lungs to be excreted in the expired air. The gas perfusion technique assumes that H_2 production is proportional to the rate of breath H_2 excretion. To quantify the mal-absorbed carbohydrate, a non-absorbable but quickly fermented oligosaccharide (lactulose) is used to 'calibrate' the subject. The area under the curve after the test meal is plotted together with the area under the curve after the intake of lactulose value, which gives the data. The amount of carbohydrate from the experimental meal can be calculated by knowing the amount of fermented lactulose. Rumessen (1992) proposed several ways of quantifying the data. Although this procedure was quantitative for oligosaccharides, it was only qualitative for insoluble or slowly fermented substrates (Champ *et al.*, 2003).

Ileostomy model

The ileostomy model can be performed over those subjects who have had a conventional ileostomy after colectomy. By minimising the bacterial degradation, direct and quantitative determination of small-bowel excretion can be obtained. The effluent is usually collected in a bag, which is changed every 2 hr during the day. The ileostomy bags are immediately deep-frozen on solid carbon dioxide. During the experimental period the subjects are given a plant polysaccharide-free diet with addition of the RS source under study (Champ *et al.*, 2003).

Intubation technique

Intubation technique uses a triple-lumen polyvinyl tube, which is passed through the gut with the help of a terminal inflatable bag containing Hg. The bag

is deflated when it reaches the caecum, which is confirmed fluoroscopically, and the subjects have to remain in a semi-supine position. The sample used in perfusion is drawn from 50mm above the ileocaecal junction in one lumen, and 250mm proximal to the aspiration port in the other. NaCl and polyethylene glycol 4000 are the main components of the perfusate used to mark the recovery so as to estimate water flow through the distal ileum. Throughout the experiment the tube is positioned in the same place and is confirmed fluoroscopically (Champ *et al.*, 2003). The advantages and shortcomings of the three methods for the in vivo validation of RS content are summarized in Table 2.7.

Ileostomy model v. intubation technique

Even though it is difficult to compare two methods, which use different type of meals as sample and have totally different data, one comparison has been made. The ileostomy model and the ileal intubation method have been compared with the same meal containing 16·3 g RS (30 g) from green banana. The ileostomates excreted 15·8 (SEM 0·4) of starch (i.e. RS) whereas healthy subjects showed 19·3 (SEM 0·7) g/d of ileal excretion. The explanation of the difference may be the underestimation of RS in ileostomates and/or the overestimation of RS when intubation techniques are used (Champ *et al.*, 2003).

Table 2.7: Advantages and shortcomings of the studies performed with human subjects (Champ *et al.* 2003).

	Advantages	Shortcomings
H2 breath test*		
	Simple and non-invasive	Semi-quantitative
	Healthy subjects	Strict standardization necessary
		Large intra- and inter-individual variation in H_2 excretion
Ileostomy model†		
	Direct collection of the ileal effluent (i.e. quantitative)	Cannot be considered as healthy
		Physiological adaptation
	Easy to perform	Water and electrolytes absorption
		Bacterial overgrowth
		Transit time (different from normal)
Intubations of healthy subjects	Healthy subjects	Disturbance of the normal physiology by the long triple lumen tube
	Direct collection of the ileal effluent	Quantification of the flow rate using a liquid phase marker
		Risk of selectivity of the tube in case of heterogeneous food
		Expensive and long

* Determination of the increase in H2 in the breath after the consumption of malabsorbed carbohydrates.
† Patients who have had a colectomy for ulcerative colitis.
‡ Collection of the ileal content in healthy subjects after intubation using a constant perfusion technique of solution containing an unabsorbable marker.

The microbial population is different in a normal distal ileum with 10^5–10^6 bacteria/g as compared to that of the terminal ileum in ileostomy subjects which is 10^7–10^8 bacteria/g. However, these differences might sound small when compared with a population of 10^{12}/g found in the caecum. Changing the bag every 2 h and deep-freezing it prevents the substantial bacterial degradation of NSP and RS.

Thus, the model gives a slight underestimated amount of carbohydrate recovered, especially of easily fermented carbohydrates such as oligosaccharides. In contrast, when intubation is used, the tube is believed to decrease intestinal efficiency because of the decrease in the oro–ileal transit time. This is believed to be the cause of overestimation of RS.

Of the three techniques, the intubation technique might be the only one, which can be exploited directly. The main disadvantage is the presence of the tube along the small intestine and its possible control on the passage of food. Using ileostomy, the direct quantification of excretion can be achieved with minimal bacterial degradation but there is a slight underestimation of the RS content. The hydrogen breath test will be more acceptable if the calibration could be improved (Champ *et al.*, 2003).

2.5.7 Production of Resistant Starch

Present day eating habits tend to result in diets which are very high in digestible carbohydrate leading to obesity. This could be ameliorated by increasing the level of RS in the diet, with the added benefit of increasing the production of butyrate in the large bowel. The most common RS in diet, out of the classified four categories, is retrograded starch RS3 as it is formed during food processing (Escarpa *et al.*, 1996). This is also the most extensively studied type. The class and extent of derivatization that may be legally permitted restricts the use of RS4 in the food industry on a large scale.

Inside the native granule, starch is tightly packed in a radial pattern, relatively dehydrated. Due to the compact nature of the native starch granule the

accessibility of digestive enzymes is limited (Haralampu, 2000). Banana, which is predominantly consumed raw, is the chief source of RS2 (Resistant starch granules) in the human diet (Champ *et al.*, 2003). Heating in excess of water disrupts the native semi-crystalline granule rendering it accessible to digestive enzymes.

However, if the gelatinized starch is allowed to cool, recrystallization of polymers occurs forming microcrystalline filaments, often as double helices. These are created from amylose chains and upon further retrogradation these double helices form hexagonal units. When this happens, a partial crystalline structure is formed, which is responsible for their resistance to the degradation by digestive enzymes (Haralampu, 2000). A scheme of the process is shown in Figure 2.6.

An elementary method for production of RS3 is by controlled hydrothermal treatment. Pomeranz and Sievert (1990) reported an increase of up to 20 –35 % in the RS3 content of high amylose cornstarch (Hylon VII) by repeated cycles of autoclaving and subsequent cooling. It was quite evident from the literature that the factors that affected the yield of RS were amylose/amylopectin ratio, along with the temperature and sample/water volume ratio (Escarpa *et al.*, 1996). Table 2.8 shows how the yield of RS3 is related to the amylose content in the source.

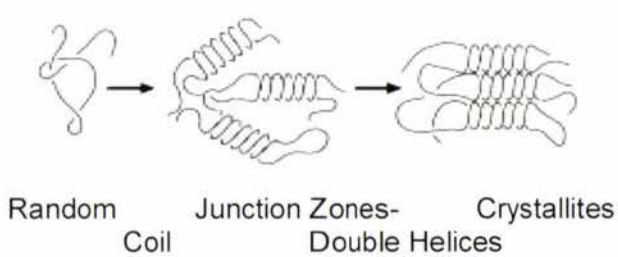


Figure 2.6: Schematic of amylose retrogradation (From Haralampu, 2000).

Table 2.8: RS yields in autoclaved and retrograded amylase / amylopectin mixtures (From Escarpa *et al.*, 1996).

Amylose/Amylopectin (%, dm)	RSa (%, dm)
100/0	36.45 ± 2.31
40/60	19.07 ± 0.40
75/25	28.06 ± 1.46
25/75[b]	18.16 ± 0.23
50/50	21.48 ± 0.41
15/85	8.97 ± 0.29
0/100	7.61 ± 0.38

a Values are average of three gelatinization treatments in HCHPA.
b Potato starch.

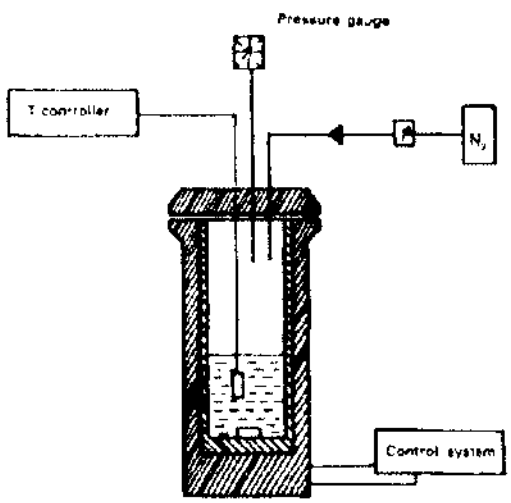


Figure 2.7: Scheme of high pressure autoclave (Escarpa *et al.*, 1996).

Table 2.9: comparison of RS yields by different authors (From Escarpa *et al.*, 1996).

Standard	RS (%)	RS (%)
0% Amylose	2.8[a]	7.6[b]
100% Amylose	31.0[a]	36.4[b]
Potato starch	4.4[c]	18.2[b]

[a] Values of Berry (1986), [b] Values of Escarpa *et al.*(1994), [c] values of Sievert & Pomeranz (1989)

The Hydrothermal Treatment used by Escarpa *et al.* (1996) improved on previous gelatinization studies by using a heat controller within the high-pressure autoclave, shown in Figure 2.7, so as to standardize the internal conditions. Higher yields of RS3 were reported as compared to the studies conducted earlier (Table 2.9).

Skrabanja & Kreft (1998) and Huth *et al.* (2000) showed that debranching and/or lintnerization were much more effective procedures for the molecular weight reduction of starch than other nonenzymatic hydrothermal treatments, e.g., autoclaving or extrusion cooking (Lehmann *et al.*, 2003). The fine structures of branched molecules markedly influence the rate and extent retrogradation of amylopectin (Botham *et al.*, 1995).

Besides being slow, the retrogradation of amylopectin is reversed by heating at 70°C. Debranching and /or lintnerization aims at releasing linear, recrystallizable polymers chains by debranching the amylopectin, which results in an effective retrogradation in fairly quick time (Lehmann *et al.*, 2003; Kettlitz *et al.*, 2000). Schmiedl *et al.* (2000) illustrated that the formation of RS from gels containing 30% poly-1,4 -α-D-glucans (linear chains with low molecular weight) was very rapid as 50-65% resistant structures were achieved after two hours of retrogradation. With the same gel concentration and a retrogradation temperature of 4 - 25°C, up to 94% of high α -amylase resistant starch can be obtained.

The retrogradation of debranched maltodextrin generates a much higher RS3 content. Maltodextrins are starch hydrolysis products with a wide distribution of oligomeric and polymeric α-D-glucans with a DP ≤ 20. To obtain linear, low molecular weight, recrystallizable polymer chains, maltodextrin was partially debranched. An RS3 content of up to 65 % could be achieved if the recrystallization was carried out at 25°C on a 30% (w/w) maltodextrin gel previously debranched by isoamylase (Schmiedl *et al.*, 2000).

High concentrations of oligosaccharides retarded RS3 formation and their reduction in the gel markedly accelerated the formation of RS3 structures up to 56% within 24 h. Large amounts of optimally long linear polymer chains (poly-1,4 -α-D-glucans) were produced using amylosucrase. Figure 2.8 shows a chromatogram of poly α-D-glucans preparation obtained by in vitro synthesis with amylosucrase according to the method described by Buttcher *et al.* (1997). A relatively high concentration of polymers with DP 10 – 20 can be seen from a range distribution between DP 10 and 35 (Schmiedl *et al.*, 2000).

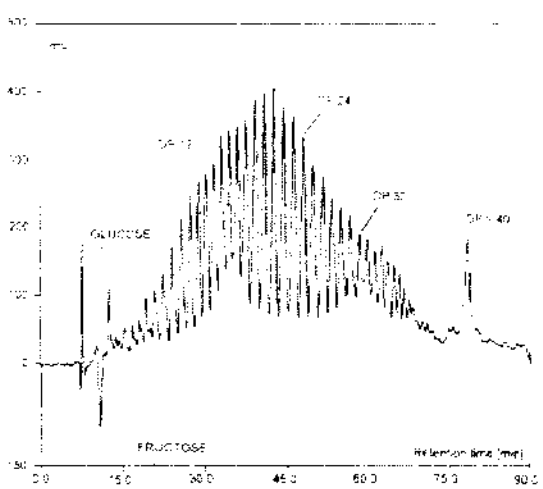


Figure 2.8: Chromatogram showing poly α-D-glucans preparation containing polymers ranging between DP10and 35 (From Schmeidl *et al.*, 2000).

Interestingly, there was no separate step for retrogradation in the technique outlined by Kettlitz *et al.* (2000). The maltodextrin solution (45%) was cooled to 50 °C and incubated for 48 hrs at a pH of 4 after the addition of isoamylase (0.1% starch d.b.). Enzymatic debranching and retrogradation occurred at the same time during the incubation, which made the process faster and cheaper, according to the author.

Lehmann *et al.* (2003) used lintnerization in addition to debranching for reduction of molecular weight. It was accomplished by stirring of starch for 1 to 7 days after adding a fivefold volume 7.5% HCL. Later, the samples were freeze-dried after being washed and centrifuged. Retrogradation after lintnerization up to 7 days led to a low yield of approximately 20% probably due to insufficient reduction in molecular weight. However, acid hydrolysis followed by enzymatic debranching led to an increase in RS3 content in pea starch. Table 2.10 shows a total of 51% RS3 yield after one day which could probably be explained by the higher susceptibility of α-1,6 linkage to acid hydrolysis than α-1,4 linkages.

According to Brumovsky & Thompson (2001), the native starch granule consists of a metastable state, packing the semi crystalline material structure inside the granule quite efficiently. However, the resistance to digestive enzymes is reduced or lost completely, on heating the granules in water. The heat stability of granular starch can be effectively increased by hydrothermal treatment. Annealing (ANN) and Heat Moisture Treatment (HMT) are two types of hydrothermal treatments that have been used to modify the starch granule, physiochemically, without wrecking its structure. ANN relates to the treatment given to starch at a moisture level of more than 40% whereas the treatment given at lower than 40% moisture is known as HMT. Annealing is achieved by incubating the sample at a gel concentration of 30% (w/w) at specified temperatures (50-70°C) above the glass transition temperature but below the gelatinization temperature. Granular RS yield was higher with ANN followed by Partial Acid Hydrolysis than ANN alone (Brumovsky & Thompson, 2001).

Table 2.10: Resistant starch content of native enzymatically debranched and lintnerized pea starch products analyzed after different retrogradation conditions (From Lehmann *et al.*, 2003).

Sample	Retrogradation conditions		Yield of RS (%)
	Storage temperature (°C)	Starch concentration in gel (%)	
Native starch	-	-	21.4 a ± 0.7
Debranched starch	4	10	37.3 b ± 1.6
	4	20	42.4 c ± 0.1
	25	10	38.0 b ± 0.9
	25	20	42.1 c ± 1.3
Acid hydrolyzed (1 day) and Debranched starch	25	10	51.3 d ± 0.9
	25	20	37.5 b ± 0.9
Acid hydrolyzed (7 days) and Debranched starch	25	10	17.2 a ± 0.4
	25	20	19.7 a ± 1.5

Data are means ± SD; n = 3, Numbers in the column followed by a letters in common were not significantly different ($p > 0.05$).

Table 2.11: Resistant starch content of a 1 day lintnerized, enzymatically debranched, and retrograded (24 h at 25 °C, 20% w/w starch concentration in gel) products after 10 min of tempering at 93 °C and a further tempering at 93 °C for different storage times (From Lehmann *et al.*, 2003).

Sample	Storage time (hr)	Yield of resistant starch (%)
Starting substance	-	57.3 a ± 0.7
	1	46.2 b ± 0.7
	8	46.9 b ± 0.6
	24	74.4 c ± 2.9

Data are means ± SD; n = 3; numbers in the column followed by a letter in common were not significantly different ($p > 0.05$).

Lehmann *et al.* (2003) tempered (annealed) the lintnerized, enzymatically debranched and retrograded product with the highest RS3 yield to investigate the increase, if any, in polymer interaction. The retrograded product was stored for 24 hrs at 93 °C, which eventually generated 74% RS3, even higher than the commercially available products (Table 2.11). This was probably due to the perfection of crystalline regions and recrystallization of imperfect crystals. The product formed from a higher starch concentration in gel increased the gelatinization temperature indicating a higher thermal stability. Schmiedl *et al.* (2000) reported a rise in thermal stability of RS3 product with an increase in recrystallization temperature.

The technique described by Haralampu *et al.* (1998) to produce RS2 related to the heating of starch granule under conditions sufficient to make them swell but preventing them from rupturing. The product obtained from this method had a Total Dietary Fibre (TDF) of 20% to about 50% by weight. Interestingly, the product displayed heat stability in the range of 90°C to 150 °C rendering it quite resistant to normal cooking temperatures in most food processes.

2.5.8 Thermal analysis of resistant starches using differential scanning calorimeter (DSC)

DSC is the most widely used instrument to study the thermal behavior of starches because of its high sensitivity to of weak transitions and its high resolution (Liu *et al.*, 1991). Generally, DSC is used to determine the specific heat, heat of fusion, and heat of reaction or heat of polymerization of materials and involves heating or cooling a sample and a reference over a temperature range, under such conditions that the two are always maintained at identical temperatures. The additional heat required to maintain the sample at the same temperature as the reference is measured, and is a function of chemical or physical changes, which are talking place. Basically, the difference between the independent supply of power to the sample and reference is recorded against the programmed temperature (Brown, 2001). A schematic is shown in the Figure 2.9.

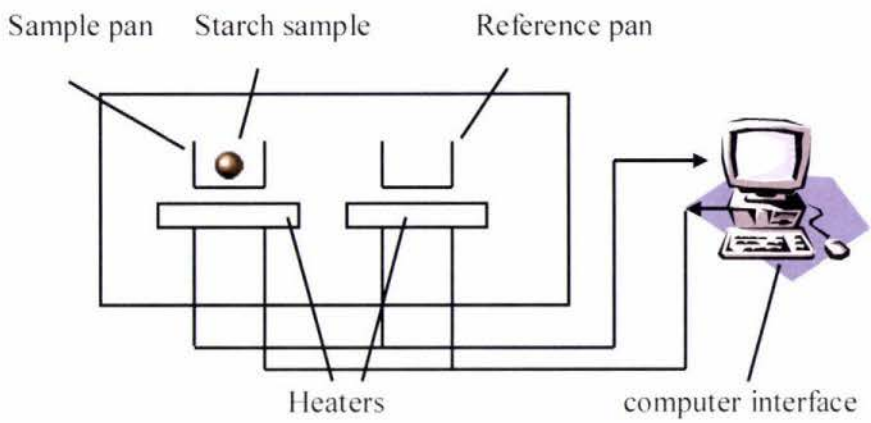


Figure 2.9: Diagram of Differential scanning calorimeter (DSC) with a computer interface to monitor and regulate heat flow.

The first reported application of the DSC to starches was the measurement of heat of gelatinization in 1971 (Biliaderis, 1990). Since then it has been widely used to study the thermal behavior of starches including gelatinization, glass transition temperature and crystallization. However, due to different measuring conditions and the complexity of starch structure, the results are often controversial. Many physiochemical changes can take place during the thermal analysis of starches including gelatinization, melting, glass transition, crystallization, molecular degradation, volume expansion and water migration which makes it a complex process (Yu and Christie, 2001).

Typical thermal profiles of starches containing more than 50% moisture reflect two kinds of thermal phenomena. The first involves the rearrangement of starch crystals and the second one connotes the disorganization of amylose-lipid complexes. The melting of the starch crystallites follows swelling of the amorphous region of the starch granule by the water in the system. If the water content is limited the melting of the crystallites is spread over a wider

temperature range and can sometimes give rise to a second peak. The melting temperature thus depends upon the moisture content (Lui *et al.*, 2005). Overall, the structural reorganization of starches and their thermal behavior is water content dependent. Also, there are reported variations in thermal profiles due to heating rates (Biliaderis, 1990).

To avoid sloping and bending of DSC curves, baseline subtraction has been widely used particularly when the starch sample contains water. It has also been noticed that shaken samples or starch granules packed tightly using an ultrasonic bath gave better defined peaks than unshaken ones. Because the thermal enthalpies of starches are quite low, the mass of sample used for DSC is generally high. This leads to a compromise between resolution and sensitivity. It is generally recommended to have a sample mass of 5-20mg to obtain well-defined endotherms (Yu and Christie, 2001).

2.5.9 Applications of RS

These days most Americans seem to view bread as the "kiss of death". That's because millions of people in US, as well as in many other countries have adopted low-carbohydrate diets or the so called "Carb Craze" that push proteins and cut carb-laden foods such as pasta, potatoes and bread to a minimum.

The survey by Morgan Stanley analysts, US estimated that 13 percent of the U.S. population was on the Atkins, South Beach, or other low-carb diet in January, 2003. Participation has since tailed off to 11 percent.

Caloric control is paramount to combat obesity. Depending on the RS product used, resistant starches contribute 1.6-2.5 kcal/gm versus 4 kcal/gm for rapidly digested starches. In addition to this, RS has numerous potential health benefits and functional properties. The physiological benefits of RS, which are similar to those of fibre, include increased faecal bulk and the production of butyrate, both of which have been shown to promote good colonic health. Because it is not absorbed in the small intestine, the products, which contain RS, don't raise blood glucose levels as other carbohydrate sources do.

Products like, "NiteBite", "Gluc-O-Bar", and "ExtendBar" snack bars all contain RS2. These foods are specifically formulated for people suffering from type- 2 diabetes. The "Choice" bar which contains RS3 called CrystaLean, manufactured by Opta food ingredients based in Bedford, US is another example of a medical food.

Quite a few leading food ingredients companies are now manufacturing RS. The leading supplier of resistant starch to Australia and New Zealand is the National Starch Company. HI-MAIZE and NOVELOSE, which are RS2 and RS3 respectively, have made a considerable impact on the food industry of Australia and New Zealand. A number of products with HI MAIZE as an ingredient are being marketed in Australia and New Zealand (Appendix 1).

Another product called Cerestar ACTISTAR, manufactured by the Cargill Company, USA, is another functional food ingredient rich in RS. It is claimed to have 53% of RS in the end product. It is promoted as being an appropriate ingredient for incorporation in breads biscuits/ cookies, muesli, milk drinks etc.

The MGP Ingredients Inc. began marketing its wheat based RS in July, 2003. FIBERSYM, as it is called now, is capable of delivering 70-80% of total dietary fibre. The company refers to it as an "invisible fibre" because of its ease in blending. The product can be incorporated in a variety of bakery products and snack foods as well.

Using functional ingredients like resistant starch is a step forward to a balanced approach to eating and improving diets. Balancing nutritional needs and taste preferences is the challenge for food scientists and for food manufacturers as well.

2.6 Conclusions

The versatility of starch as a food ingredient is incomparable to any other ingredient in terms of application in the food industry. Designed by nature as a plant energy reserve, this polymeric carbohydrate is only second to cellulose in abundance. Modification of starches has resulted in numerous highly functional derivatives. The choice of starch for a certain food product depends upon a range of features. These include sensory properties of final product, manufacturing process, shelf life requirement and other ingredients in the food product.

Gelatinization of starch is critical to building the structure and texture of most food products. Starches upon cooking are high in viscosity and imparts desirable body to a variety of foods. The amount of thickness of the final product depends of the concentration of starch, amount on water and the shear applied during processing. The type of starch applied depends on the textural requirements of the final product. In baked goods, where limited amount water is present, it is desirable to have starches, which bind with water early on whereas in liquid foods such as soups and gravies quick thickening starches are used.

By definition, resistant starch is the fraction of starch that escapes digestion. RS is classified in to four classes i.e., physically inaccessible starch (RS1); resistant starch granules (RS2); retrograded starch (RS3); chemically modified starch (RS4).

Raw starch is digested poorly but cooking in presence of water enhances digestibility. While gelatinization increases digestibility, subsequent retrogradation increases resistance to digestion. Another important factor affecting starch digestibility is the amylose: amylopectin ratio. Higher amylose content increases resistance to gelatinization and also makes the starch more susceptible to retrogradation

Earlier, RS was quantified using the official AOAC method for estimation of dietary fiber. Berry (1986) modified the AOAC method, which gave better

estimates of RS. Since then, many researchers over the past decade have tried to devise an *in vitro* method to precisely quantify RS in foods without total success. However, McCleary & Monaghan (2002) refined the procedure of quantifying RS by studying the strengths and weaknesses of the methods in earlier literature and is expected to become generally accepted method.

At present, three prospective methods are available to obtain the *in vivo* values on the RS content of foods that are required to ratify the *in vitro* methods. These are (1) hydrogen breath test, (2) intubation technique and (3) ileostomy model. Of the three techniques, the intubation technique might be the only one, which can be exploited directly.

An elementary method for the production of RS3 is by controlled hydrothermal treatment e.g. autoclaving. It has been showed that debranching and/or lintnerization were much more effective procedures for the molecular weight reduction of starch than other nonenzymatic hydrothermal treatments, e.g., autoclaving or extrusion cooking. Debranching and /or lintnerization aims at releasing linear, recrystallizable polymers chains by debranching the amylopectin, which results in an effective retrogradation in fairly quick time.

RS2 can be produced by heating of starch granule under conditions sufficient to make them swell but preventing them from rupturing. Annealing (ANN) and Heat Moisture Treatment (HMT) have been successfully used to modify the starch granule, physiochemically, without wrecking its structure in order to produce boiling stable RS2.

With increasing awareness and health consciousness among consumers, the demand of RS has grown rapidly. Quite a few leading food ingredients companies are now manufacturing resistant starch as a standard product. Most of these products fall under the solid food category, like breads, pasta products, fruit bars etc. In most of these products RS has been included as an addition ingredient to the existing formulation rather than substituting a filler ingredient.

However, replacement of starch with RS in food systems has not been studied. There is a need to test the substitution of resistant starch for the other

thickeners in a fluid food system. Such a study will help in understanding the functional behavior of RS in real foods with respect to rheological and sensory characteristics. The results for the study can be used in evaluating RS in small-scale food model, which can be extrapolated later to real food products.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

The RS samples were, a commercial resistant maize starch type II with 52% TDF (Hi-Maize 1043, National starch and Chemical NZ Ltd., Green Mount, Auckland, New Zealand.), another Type II commercial resistant maize starch with 22% TDF (Hi-Maize 958, National starch and Chemical NZ Ltd., Green Mount, Auckland, New Zealand.), a commercial Tapioca RS Type III with 54% RS (Cerestar Actistar, Cargill food company, Cedar Rapids, Iowa, United states), and a Type III commercial RS with 30% TDF (Novelose 330, National starch and Chemical NZ Ltd., green mount, Auckland, New Zealand.). For standard formulations commercial waxy maize starch (Novation 2700, National starch and Chemical NZ Ltd., green mount, Auckland, New Zealand.) was used.

Other ingredients (salt, sugar, pepper, garlic powder) were purchased from a local supermarket and onion powder was supplied by Stonemill (G.S. Hall and company, Auckland, New Zealand). Dairy Products Supply, Emmen (Holland) and Chr. Hanson Pty. Ltd., Bayswater (Victoria, Australia) supplied chicken flavor and spray dried vegetable fat respectively. Archer Daniels Midland Company, Decatur, USA, provided xanthan gum NF/FCC, and skim milk powder was provided by NZMP, New Zealand. All chemicals used in the analysis were of analytical grade obtained from either Sigma Chemical Co. (St. Louis, MO) or Megazyme international Ltd. (Wicklow, Ireland).

3.2 Approach to development of Model Food system for functionality testing of resistant starch

In order to be able to evaluate RS with new functional properties under realistic conditions of use, a soup model was developed. The choice of food system was based on the ease of rheological and sensory measurement along with a straightforward method of preparation. The model also presented an opportunity to verify thermal stability of RS.

The approach utilized has been

1. Various formulations for the manufacture of particular food system of interest were reviewed.

2. The simplest combination representative of the majority of formulations was selected. Multiple use of a single additive was avoided wherever possible.

3. The laboratory process of making the food system on small scale was then investigated.

4. For the evaluation of the system, quantifiable food characteristics were selected.

5. A suitable experimental design was developed thereafter. Generally fractional factorial or central composite design is most applicable for a number of ingredients at multiple compositional levels.

6. The formulation that provided for differentiation of RS by standard functionality testing was chosen.

Four representative cream of chicken soup formulations were the basis of initiating the study. These formulations were sourced either from text books or from the Internet (Appendix 2). The formulations included both industrial starch and wheat flour as thickening agents. A modified formulation based on these four basic formulations was selected (Appendix 3).

3.3 Method of Soup Preparation

The soup was prepared in laboratory by slurring the dry mixture of ingredients with water at 50°C and rapidly heating in a rotary evaporator (Rotavapor-R, Nicholas Watson Victor Ltd) to a temperature of 95°C by placing it in a boiling water bath. The heating was done without the application of vacuum by using just the rotating mechanism of the apparatus. As soon as the temperature was reached, the slurry was held for 20min in a controlled temperature water bath at 95°C (Evans and Haisman, 1979). A schematic of preparation of soup is given in Figure 3.1.

3.4 Experimental design.

3.4.1 The 2^k factorial design

Five factors in manufacturing process for soup preparation were investigated in a 2^{5-1} design with the objective of screening the ingredients which had profound effect on the viscosity of soup. The five factors were starch; skim milk powder, spray-dried fat, xanthan gum and wheat flour. The construction of 2^{5-1} design is shown in Table 3.1. The design was constructed by writing down the basic matrix having 16 runs (a 2^4 design in A, B, C and D), selecting ABCDE as generators. And then setting the levels of the factor E = ABCD (Montgomery, 2001: Minitab Release 14, 2004). Figure 3.2 gives a pictoral representation of the design.

The designing relation of the design was I = ABCDE. Consequently every main effect was aliased with a four-factor interaction (e.g. l_A = A +BCDE), and every two-factor interaction was aliased with a three-factor interaction (e.g. l_{AC} = AC + CDE) (Minitab Release 14, 2004). Thus, the design was of resolution V, it was expected that the 2^{5-1} design would provide appropriate information concerning the main effects and two-factor interaction.

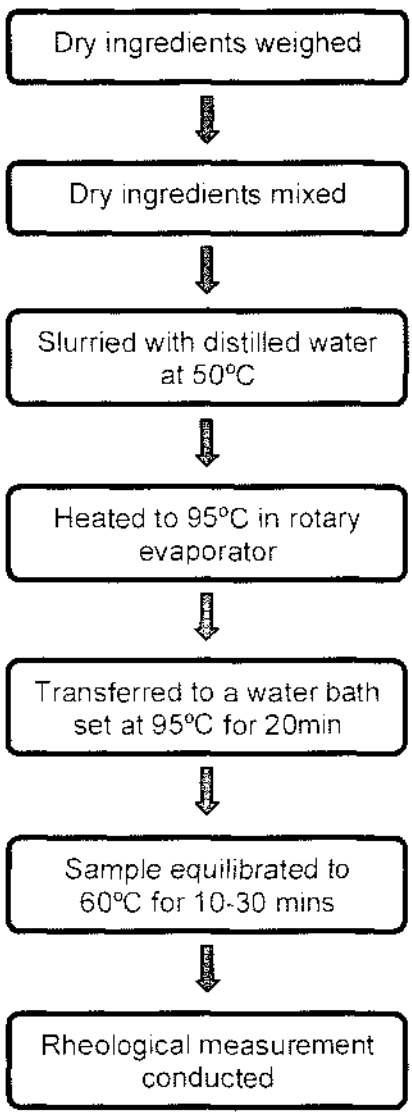


Figure 3.1: A schematic exhibiting a step-by-step method of soup preparation.

Table 3.1: A 2^{5-1} design for the experiment.

Run Order	A	B	C	D	E = ABCD	Treatment combination
1	-	-	-	-	+	e
2	+	-	-	-	-	a
3	-	+	-	-	-	b
4	+	+	-	-	+	abe
5	-	-	+	-	-	c
6	+	-	+	-	+	ace
7	-	+	+	-	+	bce
8	+	+	+	-	-	abc
9	-	-	-	+	-	d
10	+	-	-	+	+	ade
11	-	+	-	+	+	bde
12	-	-	+	+	-	abd
13	+	-	+	+	+	cde
14	+	-	+	+	-	acd
15	-	+	+	+	-	bcd
16	+	+	+	+	+	abcde

Generated by Minitab Inc. (2004)

3.4.2 Response surface methodology

To investigate the effects of two main factors and to optimize the response, response surface methodology was put to use. It was decided that the region of exploration for fitting the first order model was starch (3, 4) and wheat flour (0.5, 1.5). The design used to collect this data was a 2^2 factorial augmented by five center points. Replicates at the center were used to estimate the experimental error and to allow for checking the adequacy of the first order model. The design was centered about the current conditions for the process. It simply augmented design points of a 2^2 design with design center points at (0, 0, ...,0). Figure 3.2 contains design points for a 2^2 with center points (Montgomery, 2001; Minitab Release 14, 2004).

A similar model was also used to investigate the response region of xanthan (0.15, 0.55) and starch (2, 6) augmented by fives center points. The design consisted of four runs at the corner of the square, plus four at the center of this square, plus four axial runs (Minitab Release 14, 2004).

In coded variables the corners of the square are (x1, x2) = (-1, -1), (1, -1), (-1, 1), (1, 1); the center points are at (x1, x2) = (0, 0); and the axial points are at (x1, x2) = (-1.414, 0), (1.414, 0), (0, -1.414), (0, 1.414). A central composite design for the response region for starch and xanthan is shown in Figure 3.3.

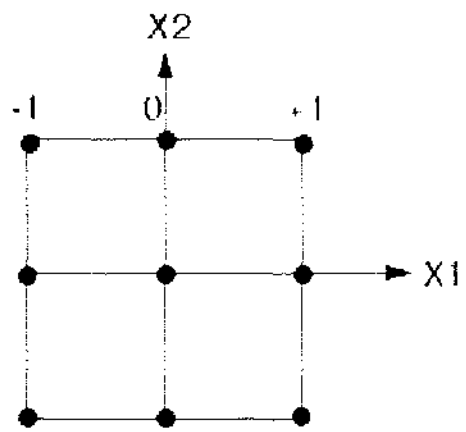


Figure 3.2: Treatment combination in the 2^2 design showing design points (•) at high (+), centre (0) and low (-) levels.

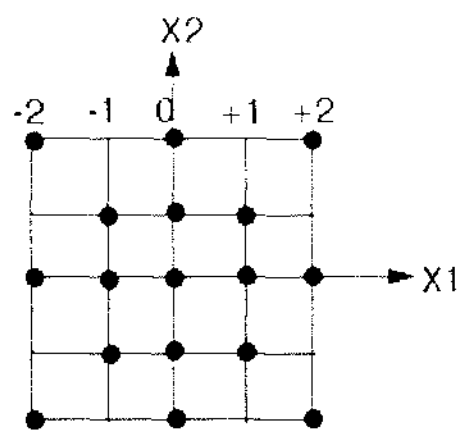


Figure 3.3: Treatment combination in a Central composite design (CCD) for starch and xanthan with four axial points in addition to the normal 2^2-design.

3.5 Viscosity measurement of model food system (soup)

Controlled shear rate measurement of viscosities as a function of shear rate was performed using a Paar Physica Rheometer (Rheolab MC1, Ashland, Virginia, United states; Figure 3.4). The soup samples, which were equilibrated at 60 °C in a water bath for 15-20 mins, were poured into the cup (MS-Z3, 17 mL, Shear rate factor 1.291, shear stress factor 1.1418; Figure 3.4) to the indicated level and were loaded in to the temperature-controlled jacket of the rheometer for 2 min before measurements were taken over the shear rate range of 10 -1000 s^{-1}. The viscosity, the shear rate and the shear stress were measured. After each measurement the cup and the spindle were washed and dried. Two replicate measurements per sample were conducted and the average value of the measurements was used in the statistical analysis.

The apparent viscosity-shear rate data and the general log η_a – log γ curve was used to characterize soup samples. The plots became linear when plotted on double logarithmic coordinates, and all flow curves were approximated well by power law model given below.

$$\sigma/\gamma = K\gamma^{(n-1)} \quad (1)$$

$$\eta_a = K\gamma^{(n-1)} \quad (2)$$

Here,

K is consistency index (Pa s^n), n is flow behavior index, σ is shear stress (Pa), γ is shear rate (s^{-1}) and η_a = Viscosity (Pa s^{-1})

Figure 3.4: Paar Physica Rheolab MC1 Rheometer (Top) and Z3 cup and spindle (Bottom) used for rheological measurements.

From log η_a versus log γ plot, the resulting straight line intercept was log K and slope was n. Due to the large number of points, linear regression of log η_a versus log γ provided statistically accurate values of K and n. All measurements were conducted at 60°, which corresponds to the temperature at which soups are generally consumed.

3.6 Sensory evaluation

3.5.1 Acceptance test

The test was used to determine whether the experimental soups enjoyed sufficient acceptance to the panelists against the standard soup formulation. The test samples were three different chicken soups with varying amounts of wheat flour (0%, 0.75% and 1.5%), which were compared with a standard recipe of chicken soup. The standard formulation resembles packet chicken soups available in the market. The questions were structured using least possible jargon and were framed in a way relevant to pivotal sensory characteristics of soups (appearance, feel in the mouth, creamy feeling, overall flavor, chicken flavor, sharpness, and liking). A total of eight questions per sample were designed, out of which four were based on 9 point attribute scoring, three on 7 point attribute scoring and one was an open ended question asking comments on the sample. A sample questionnaire is attached in Appendix 4.

An hour prior to the commencement of the test, all prepared soup samples were poured into sampler cups and marked with three digit codes accordingly. The codes were taken from the random order digit table. These were then stored at 60-65°C temperatures in a hot air oven until the test was executed. The data was analyzed applying analysis of variance (ANOVA) and Tukey's "honestly significantly different" (HSD) test using Minitab.

3.6.2 Simple difference test

The same/different test was used to determine whether a sensory difference existed between four soup samples with varying amount of xanthan and starch as well as resistant starch in them. This test was used because the

samples had a strong lingering flavor and could have been mentally confusing for the panelists. The information on the possible product difference was obtained comparing responses obtained from different pairs (A/B and B/A) with those obtained from matched pairs (A/A and B/B). The presentation of matched pairs enabled the evaluation of the magnitude of "placebo" effect of simply asking the difference question (Meilgaard *et al.*, 1991).

Each subject was presented with two samples, asking whether the samples were the same or different. Thirty test panelists received two pairs of samples, one matched and one unmatched. A typical worksheet for the test is attached in Appendix 5. As the simple difference test was chosen because of the complexity of stimuli, no more than one pair of sample should have been given. But, because of the need to calculate the placebo effect and to handle four different samples each panelist was presented with two pairs but a record of the subject's test score was kept.

The McNemar's test was used to calculate the significance of the results is shown in Table 3.2. The probability of no difference was observed at the value of chi^2 for one degree of freedom. The chi^2 analysis is used most often to compare the placebo effect with the treatment effect.

Table 3.2: Analysis of the McNemar procedure (Meilgaard *et al.*, 1991).

		Subjects received A/B or B/A and responded	
		Same	Different
Subjects received A/A or B/B and responded	Same	S	T
	Different	U	V

McNemar's test chi $^2 = (|U-T|-1)^2/(U+T)$

3.7 Resistant starch assay

The characterization of the *in vitro* rate and extent of starch was performed on lyophilized soup samples according to the AOAC method 2002.02 (AACC method 32 – 40). The method allowed the measurement of resistant starch, solubilized starch and total starch content of samples without any artificial mechanical disintegration or additional treatment.

Sample preparation

In order to convert soup samples in to an easily quantifiable form for RS analysis, they were stored at –35°C for 1 hours and then lyophilised using a freeze dryer (Cuddon freeze dryer, Blenhiem, New Zealand) for 48hr at 30°C. This also minimized the damage caused to the sample by conventional drying. These freeze-dried samples were then analyzed for RS.

Enzyme solution I

Amyloglucosidase (AMG, Megazyme, Ireland) solution -12 mL, 3300 U/mL in 50% glycerol.

Enzyme solution II

Dilute amyloglucosidase solution – 300 U/mL. 2 mL of concentrated AMG (Megazyme, Ireland) solution was diluted to 22 mL with 0.1 M sodium maleate buffer (pH 6.0).

Enzyme solution III

Pancreatic α-amylase (10 mg/mL, Megazyme, Ireland) plus AMG (3 U/mL) – was prepared immediately before use. 1 gram of pancreatic α - amylase was dispersed in 100 mL of sodium maleate buffer and stirred for 5 min after which, 1.0 mL of AMG (300 U/mL) was added and mixed well. This was then centrifuged at > 1500 g for 10 min, and the supernatant was decanted carefully. This solution was recommended to be used on the day of preparation.

Other used materials

Sodium maleate buffer (0.1 M, pH 6.0) - 23.2 g of maleic acid was diluted

in 1600 mL of distilled water and the pH was adjusted to 6.0 with 4M (160 g/L) sodium hydroxide. 0.6 g of calcium chloride and 0.4 g of sodium azide were added thereafter and the volume was adjusted to 2 L.

Sodium acetate buffer (1.2 M, pH 3.8) - 69.6 mL of glacial acetic acid was added to 800 mL of distilled water and adjusted to pH 3.8 using 4 M sodium hydroxide. The volume was later adjusted to 1 L with distilled water.

Sodium acetate buffer (100 mM, pH 4.5) - 5.8 mL of glacial acetic acid was added to 900 mL of distilled water and adjusted to pH 4.5 using 4 M sodium hydroxide. The volume was later adjusted to 1 L with distilled water.

Potassium Hydroxide (2 M) - 112.2 g KOH was added to 900 mL of deionised water and dissolved by stirring over a magnetic stirrer. The volume was adjusted to 1 L.

Aqueous IMS – About 50% v/v. 500 mL of ethanol (95% or 99%) or industrial methylated spirits (IMS; denatured ethanol; ~ 95% ethanol plus 5% methanol) was diluted to 1 L with H2O.

Glucose oxidase peroxidase aminoantipyrine reagent (GOPOD) - GOPOD was prepared by diluting 50 mL of buffer concentrate to 1.0 L with distilled water. Part of this diluted buffer was used to dissolve the entire contents of the vial containing freeze-dried glucose oxidase peroxidase aminoantipyrine mixture. The contents of the vial were quantitatively transferred to 1 L volumetric flask containing diluted buffer.

Other used equipment

Shaking water bath, vortex mixer, bench centrifuge (capable of holding 4 X mm centrifuge tubes), spectrophotometer (capable of operating at 510 nm).

Samples were incubated in a shaking water bath (Julabo SW-20) with pancreatic α–amylase and amyloglucosidase (AMG) for 16 hr at 37°C, aligned in the direction of motion, during which time non-resistant starch was solubilized and hydrolyzed to glucose by the addition of the two enzymes (Figure 3.5).

The reaction was terminated by the addition of ethanol or industrial methylated spirit (IMS, denatured ethanol) and the RS is recovered as a pellet on centrifugation. This is then washed twice by suspension in aqueous IMS of ethanol (50% v/v), followed by centrifugation. Free liquid is dissolved in 2M KOH by vigorously stirring over an ice water bath over a magnetic stirrer.

The solution was neutralized with acetate buffer and the starch was quantitatively hydrolyzed to glucose with AMG. Glucose was measured with glucose oxidase/peroxidase reagent (GOPOD), and this was the measure of the RS content of the sample. By combining the supernatant of the washings by IMS, non-resistant starch was quantified as well. A schematic of the method is shown in Figure 3.6.

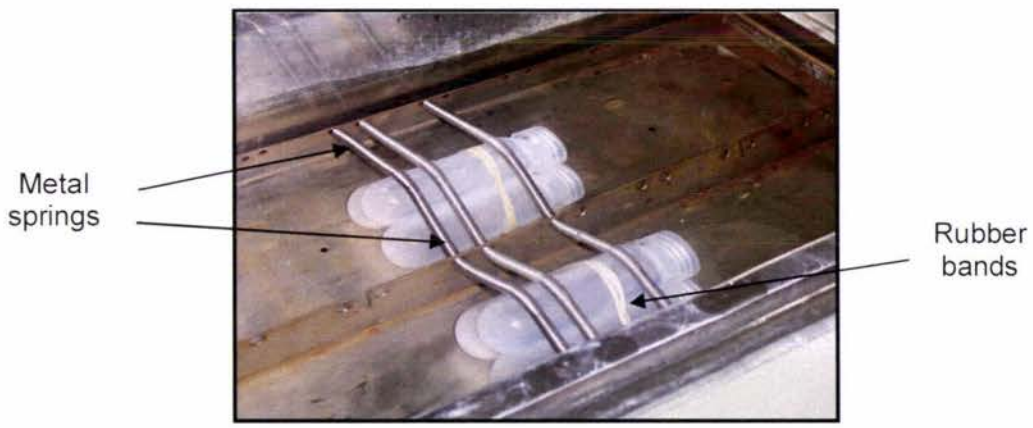


Figure 3.5: Attachment of culture tubes to the shaking tray in a shaking water bath. The metal springs hold the tubes tightly and the rubber band prevent them from slipping loose from the metal springs.

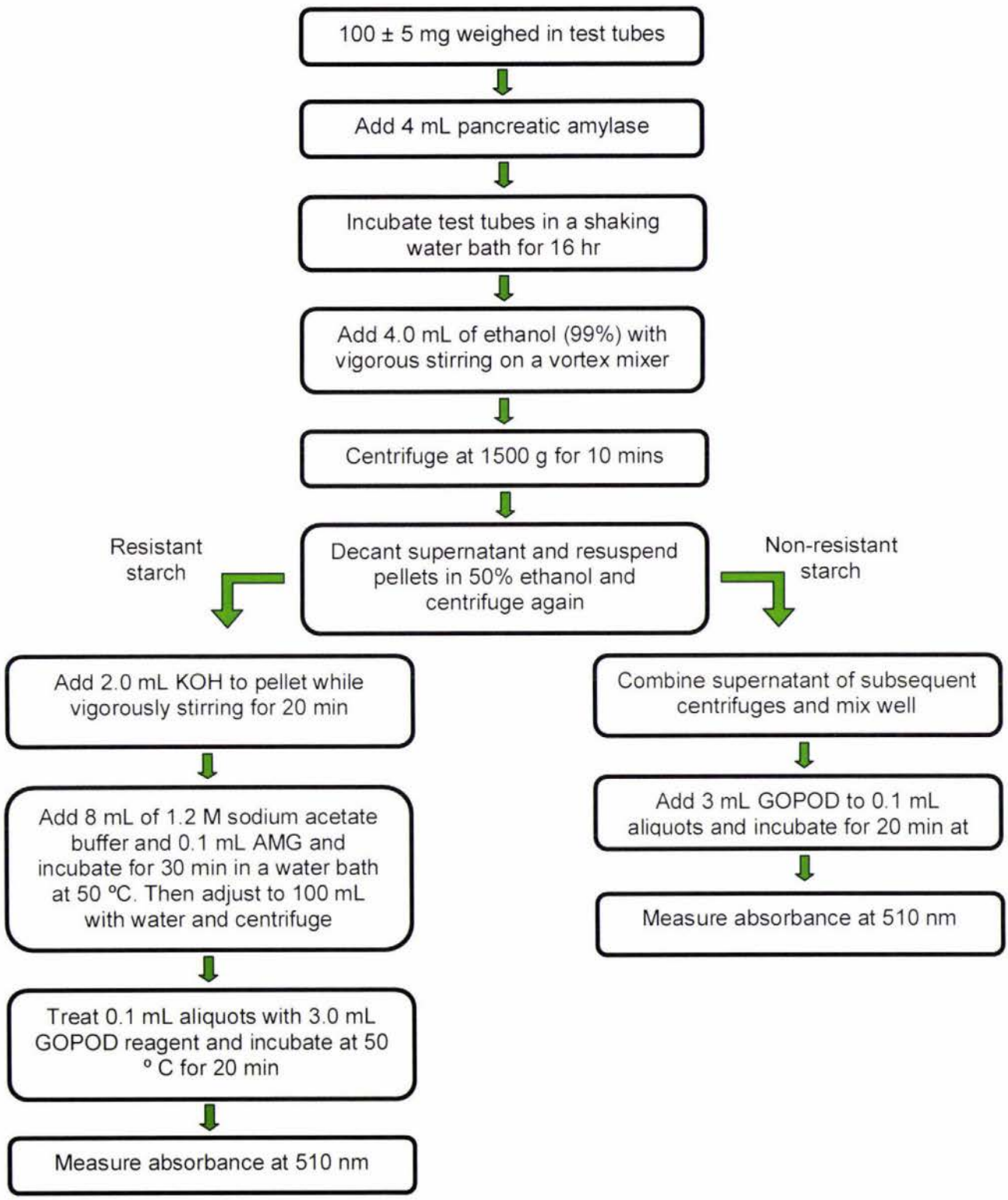


Figure 3.6: A schematic of the measurement of resistant starch using AOAC official method 2002.02.

3.8 DSC thermal analysis

The heat stability of products containing RS was determined using a differential scanning calorimetry (DSC 7, Perkin-Elmer, Norwalk, CT) equipped with a thermal analysis data station, Pyris windows (Perkin-Elmer)as shown in Figure 3.7. Empty stainless steel pans (Perkin-Elmer No. 03190218) were accurately weighed along with the lids on a Mettler Toledo weighing balance and 10-15 mg (dry weight) of resistant starch sample was weighed into them with an equal amount of Milli-Q water (water purified by the treatment with a Milli-Q apparatus, Millipore corp. Bedford, MA), which was added directly with a 25µL syringe (Yu *et al.*, 2000).

Figure 3.7: A Pyris DSC 7 Differential Scanning Calorimeter attached to a computer interface.

The contents of the pan were stirred gently with a steel needle and hermetically sealed. These were then kept in an ultrasound bath for half an hour to improve the thermal transition of sample by packing the particles more tightly. The pans were then stored at room temperature for 24h to ensure equilibration of starch samples and water. The pans were reweighed before scanning to check weight loss. Analysis was performed on the samples over a temperature range of 20°C to 170°C at a heating rate of 4°C/min (Shamai *et al.*, 2003).

The melting behavior of high purity Indium metal was used for the calibration of DSC (onset temperature; T_o, 158.88 °C; peak temperature, T_p, 159.68 °C; conversion enthalpy, ΔH , 20.7 mJ/mg). Experiment was replicated twice. The correction of baseline was done using the endotherm of pure water to avoid slope and bend of curves in DSC measurement. A sealed empty pan was used as a reference. The onset temperature (T_o), peak temperature (T_p), completion temperature (T_c) and melting enthalpy (ΔH) were obtained and recorded as thermograms (Shamai *et al.*, 2003; Yu *et al.*, 2000).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Introduction – scheme of research

Initially, evaluation was done to determine the relative significance of each constituent on the properties of the model food system (MFS). For this screening, ingredients which did not alter MFS functionality were eliminated from the formulation. Five factors, which were thought to have a significant effect on the functionality of MFS, were investigated in a 2^{5-1} fractional factorial design in order to simplify the system. These were waxy maize starch, wheat flour, skim milk powder, spray dried fat and xanthan gum.

The aim was to explore critical factors and the extent to which the model food system was affected by the change in the levels of ingredients. The viscosity of the different formulations was measured with the Paar Physica rheometer.

After the factors which had large effect on the model food system were identified, a central composite design was used to determine the region of response in which rheological characteristics of the model food system were optimized.

Three thickeners were present in the soup formulation used for food system testing out of which wheat flour and waxy maize starch had similar effects. Wheat flour gives cloudy, slightly cohesive and soft cooked pastes. It is used a great deal in soup mixes because of its bland flavor and high viscosity (Binsted and Devey, 1970). The screening experiment showed that the level of wheat flour in the formulation significantly affected the consistency index (k, Pa s^n). In planning to eliminate wheat flour from the formulation, it had to be verified that it did not have a profound effect on the sensory profile of the soup. In situations where it is required to determine the effective status of the product, an acceptance test is most often used. Therefore, soup samples with varying levels

of wheat flour were tested by untrained panelists against a standard formulation using an acceptance scale.

Keeping in mind the main objective, which was to replace waxy maize starch with RS, the functionality of all RS was investigated. Interestingly, RS lacked any "starch like" functionality at low concentrations. Thus the loss of thickness in soup due to replacement of waxy maize starch had to be filled in by xanthan gum.

It is well known that the addition of plant gums such as xanthan lowers the gelling temperature and increases the viscosity of starch gels. They also improve taste and texture, as well as mechanical properties of products obtained from starch as a thickener. A small amount of xanthan can increase the viscosity of starch gel remarkably. The amount of increase in viscosity and effects on taste depend on the origin of starch and the type of gum and also the presence of other ingredients (Sikora *et al.*, 2003). A second order response surface model was used to determine the required region of response.

The gelatinization temperature and melting enthalpies of RS were determined by differential scanning calorimetry. This was done to validate the claims made by manufacturers regarding their stability during processing. Also, the effects of processing time and temperature on the levels of RS in soup formulations were studied using the AOAC official method (2002.02) for RS analysis.

The most process stable RS was used in a final soup formulation to replace waxy maize starch at various levels. Once again, sensory testing was used to determine the optimum level at which the RS could replace waxy maize starch without being distinguishable by taste.

4.2 Screening

Screening is usually performed in the early stages of a research when it is likely that many factors that are initially considered have little or no effect on the response. This stage gives an opportunity to identify important factors so that

they can be investigated more thoroughly in later experiments (Montgomery, 2001, Moen *et al.*, 1991).

4.2.1 Experimental design

A fractional factorial (2^{5-1}_{V}), shown in Table 4.1 with consistency index (K, Pa s^n) as response, was used to identify important factors in the model food system. A separate estimate for all factors was not possible, thus some of the effects were confounded (Montgomery, 2001). The factors were labeled alphabetically (skim milk powder, waxy maize starch, wheat flour, spray dried fat and xanthan as A, B, C, D and E, respectively).

4.2.2 Results and discussion

Table 4.2 contains the levels, estimated effects and the model regression coefficients of the 15 effect estimates for this experiment. The absolute value of the effects determined the strength of the effects, the higher the value the greater the effect on the response (Montgomery, 2001). The sign of the effects indicated the level, high or low, resulting in a higher response

The effect estimates of waxy maize starch (WMS), wheat flour (WF) and xanthan gum (XG) were comparatively higher than the other factors and interaction effects. These factors had positive signs indicating that all the significant effects had greater response at higher levels. Also, for each factor there was a coefficient, which helped in constructing a linear equation representing the relationship between the factors and the responses (Myers and Montgomery, 1995; Mead, 1988).

Table 4.1: Fractional factorial design (2^{5-1}_{V}) for the screening experiment.

StdOrder[1]	RunOrder[2]	A (%SMP[3])	B (%NWMS[4])	C (%WF[5])	D (%SPRAY DRIED FAT)	E (%XANTHAN)	k[6] (CONSISTENCY INDEX)
6	1	1.5	4	0	3	0.15	7.8180
8	2	1.5	4	1	3	0.00	5.5880
3	3	2.5	3	1	2	0.15	8.8195
4	4	1.5	4	1	2	0.15	14.4120
1	5	2.5	3	0	2	0.00	0.4901
2	6	1.5	4	0	2	0.00	2.9431
7	7	2.5	3	1	3	0.00	2.7078
5	8	2.5	3	0	3	0.15	4.4640
16	9	2.5	4	1	3	0.15	14.2190
10	10	2.5	4	0	2	0.15	9.0324
15	11	1.5	3	1	3	0.15	9.8498
12	12	2.5	4	1	2	0.00	6.5862
14	13	2.5	4	0	3	0.00	3.0341
13	14	1.5	3	0	3	0.00	0.8289
11	15	1.5	3	1	2	0.00	3.3225
9	16	1.5	3	0	2	0.15	5.7926

[1]Standard order (StdOrder) is the order designed by Minitab
[2]RunOrder is the order in which the experiment is conducted
[3]Skim milk powder (SMP)
[4]Waxy maize starch (NWMS
[5]Wheat flour (WF)
[6]Obtained using power law model for shear thickening fluids

Table 4.2: Levels, effects estimates and regression coefficients for the screening experiment

Variable	Name	-1 Level %	+ 1 Level %
A	Skim milk powder	1.5	2.5
B	Starch	3	4
C	Wheat flour	0	1
D	Spray dried fat	3	2
E	Xanthan gum	0.00	0.15

Variable	Regression coefficient	Estimated effects
Constant	6.2443	
A	-0.0751	0.1502
B	1.7098	3.4197
C	1.9439	3.8877
D	-0.1806	0.3611
E	3.0567	6.1133
AC	-0.0299	0.0597
AD	0.1176	0.2353
AE	-0.0921	0.1842
BC	0.3034	0.6067
BD	-0.1088	0.2176
BE	0.3596	0.7192
CD	0.0836	0.1672
CE	0.5803	0.1606
DE	-0.0327	0.0653

The normal probability plot of the effect estimates for the screening experiment is presented in Figure 4.1. This plot was useful in comparing the relative magnitude of main effects and interaction effects. Significant effects were larger and farther from the straight line than non-significant ones at an α-level of 0.05 (Minitab Inc., 2004). The main effects of waxy maize starch (WMS), wheat flour (WF), xanthan gum (XG) and interactions between XG and WF were large.

Because the design was confounded, these effects actually were B + ACDE, C + ABDE, E + ABCD and CE + ABD (Minitab Inc., 2004). However, because it seemed plausible that three factor and higher interactions were negligible, it could be concluded that only WMS, WF, XG and WF*XG interaction were important effects.

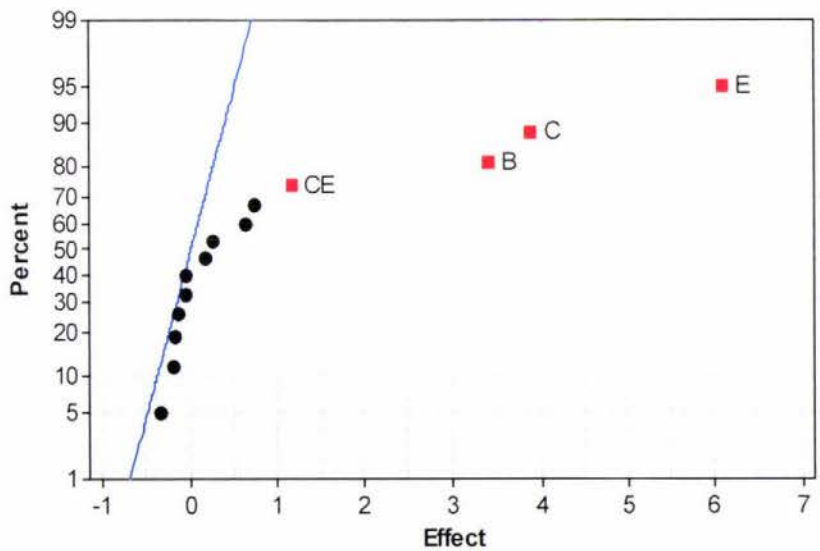


Figure 4.1: Normal probability plot of effects for screening having consistency index (k, Pa s^n) as response with effect types being non significant (•) and significant (■) and factors being NWMS (B), WF (C), XG (E) and WF * XG interaction (CE).

To confirm these results, a main effects plot (Figure 4.2) was used, which compared the overall grand mean of the factors in the form of a reference line with the main effects. A main effect would only be present when the change in mean response across the level of factors was significant (Minitab Inc., 2004). It was clear from Figure 4.2 that skim milk powder and spray-dried fat were insignificant ($P>0.05$) because the effect line was aligned horizontally to the overall mean reference line for these factors whereas it was non-parallel for NWMS, WF and XG.

Table 4.3 summarizes the analysis of variance for this experiment. The model sum of squares is $SS_{Model} = SS_B + SS_C + SS_E + SS_{CE} = 268.79$, and this accounts for 96.61 (R^2 value) percent of the total variability in the consistency index. The three factors viz. NWMS, WF and XG had large positive effects.

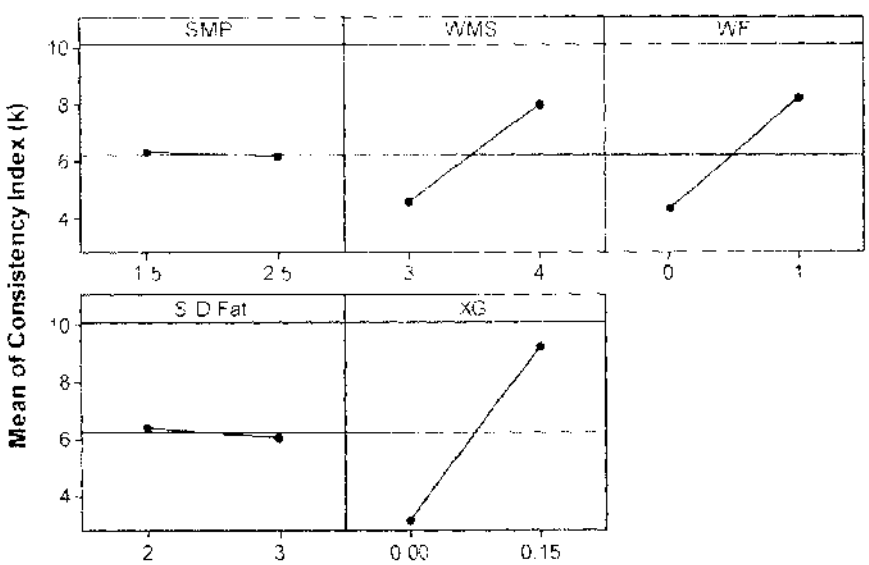


Figure 4.2: Main effects plot of data means for screening trial with consistency index (k, Pa s^n) as response.

Table 4.3: Analysis of variance for screening experiments.

Source of variation	Sum of squares	Degrees of freedom	Mean square	F_O	P- Value
Main effects	256.725	3	85.5751	140.9	
2-way interaction	5.388	1	5.3882	8.87	0.000
Residual error	6.681	11	0.6073		0.013
Lack of fit	3.763	3	1.2542	3.44	0.072
Pure error	2.918	8	0.3648		
Total	268.794	15			

[1]F factor or interaction
[2]P probability of a factor having an effect on the response

The fitted model was examined to ensure that it provided an adequate approximation to the true system and also to verify that none of the least square regression assumptions were violated. Proceeding with the exploration and optimization of a fitted response surface was likely to give a misleading result unless the model was a good fit (Myers and Montgomery, 1995).

Residual analysis plays an important role in judging model adequacy. Constructing a normal probability plot of the residuals can make a check of normality assumption (Myers and Montgomery, 1995). The Anderson-Darling normality test was used to determine whether the data was randomly selected from a normal population. The P-value (0.409) from the test was larger than α = 0.05. Also, a roughly straight line was present, which rejected the possibility of non-normality, outliers, skewness and unidentified variables (Meyer and Kruger, 2001).

Figure 4.3 plots the residuals versus the predicted response. This plot is useful in checking for any non-constant variation, which is exhibited by a funnel shaped scattering of the residuals (Minitab Inc., 2004). Here, the residuals were randomly scattered around zero suggesting that the variance of the original observations is constant for all values of the response.

If two or more observations on the response are obtained at the same

setting of regression variables, a test for the lack of fit can be conducted. The replicate points are used to obtain a model-independent estimate of variance, which means that the model will be satisfactory as a prediction equation. The test procedure may be easily introduced into the analysis of variance conducted for the significance of regression (Minitab Inc., 2004, Lorenzen and Anderson, 1993). The analysis of variance Table 4.3 clearly showed that the lack of fit for the main effects was non significant at a 95% confidence interval.

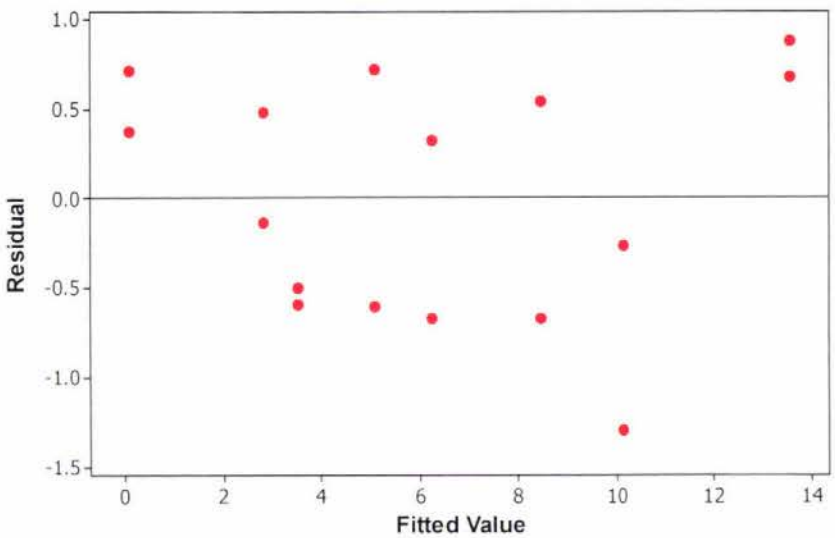


Figure 4.3: Plot for the residuals versus the predicted response with consistency index (k, •) as response.

4.2.3 Conclusions

The 2^{5-1}_{V} design will collapse into two replicates of a 2^3 design in any three of the original five factors. Figure 4.4 is a cube plot in the factors WF, WMS and XG with the average yields superimposed on the eight corners. It is clear from inspection of the cube plot that the highest yields are achieved with all factors at high level. Factors A and D have little effect on the consistency index (k. Pa s^n) of soup and could be eliminated from the model for further investigation in order to achieve research objectives.

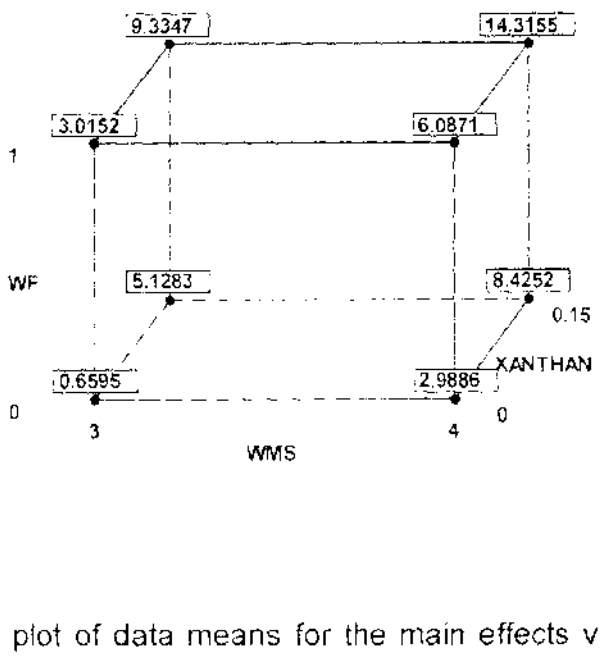


Figure 4.4: Cube plot of data means for the main effects viz. NWMS, WF and xanthan with consistency index as response at all corners of the cube corresponding to the respective levels of the effects.

4.3 A second-order model to predict the effect of WMS and WF on model food system

In order to be able to evaluate RS with a meaningful model food system under realistic conditions of use, further simplification of soup model was required. The screening experiment involved five factors, and isolated WMS, XG and WF as the three main variables.

WMS and WF are both starch thickeners and have similar functions in the formulations. The objective of the research was to test the effect of substituting RS for the other thickeners on the properties of the soup. It would be simpler if there was only one starch thickener to consider, so the contribution of WF to the measured and sensory thickness of the soup was necessary. A central composite design (CCD) was constructed with WMS and WF, with XG as constant (fixed level) factor, to model their behavior in actual system. The objective was to obtain a regression equation, which could be used to reduce the percentage of WF in the system, if not completely take it out.

4.3.1 Experimental design

A second-order model would provide adequate information to help keep the consistency of the soup in the vicinity of the optimum even if the levels of the factors were changed within the elected range (Montgomery). In addition to a general first order model, four axial points were added at (x1, x2) = (-1.414, 0), (1.414, 0), (0, -1.414), (0, 1.414) (Myers and Montgomery, 1995).

If the experimental runs are not simultaneous, blocking is necessary (Montgomery, 2001; Minitab Inc., 2004). As the experiment was conducted on two separate days, blocking was incorporated into the design. Also the design was augmented with enough points to fit a quadratic model (Montgomery, 2004). The complete experiment design is shown in Table 4.4 with consistency index (K, Pa s^n) as the response.

Table 4.4: A central composite design (CCD) for starch and wheat flour

Std Order[1]	Run Order[2]	Pt Type[3]	Blocks	A (%Starch)	B (%Wheat flour)	Consistency index (k)
11	1	-1	2	3.50000	1.70711	9.6670
12	2	0	2	3.50000	1.00000	6.8688
13	3	0	2	3.50000	1.00000	7.4034
9	4	-1	2	4.20711	1.00000	11.1330
8	5	-1	2	2.79289	1.00000	4.7800
10	6	-1	2	3.50000	0.29289	4.3080
14	7	0	2	3.50000	1.00000	6.8663
5	8	0	1	3.50000	1.00000	6.8912
6	9	0	1	3.50000	1.00000	7.0226
4	10	1	1	4.00000	1.50000	10.9180
2	11	1	1	4.00000	0.50000	8.0348
7	12	0	1	3.50000	1.00000	8.1192
3	13	1	1	3.00000	1.50000	7.3054
1	14	1	1	3.00000	0.50000	4.7625

[1]Standard order (StdOrder) is the order designed by Minitab
[2]RunOrder is the order in which the experiment is conducted
[3]Point type (pt type) is the type of point viz. high (1), low (-1) or centre (0)

4.3.2 Results and discussion

The experiment design had five replicate runs; on that account, the residual sum of squares was partitioned into pure error and lack of fit components (Myers and Montgomery, 1995; Lorenzen and Anderson, 1993). Table 4.5 shows the analysis of variance for this design. The P-value for lack of fit test was large (p= 0.274), implying that the quadratic model was adequate. Accordingly, the residual mean square with 8 degrees of freedom was used for the remaining analysis. The F-test for the significance of regression showed zero P-value for linear terms but was large for the square and the interaction terms, concluding that only the linear terms contributed to the model significantly. T - tests on individual variable is shown in Table 4.6.

The T values for the square and quadratic terms were small enough to indicate that they were non-significant variables for the model, resulting in a reduced quadratic model for the experiment. The regression equation for the model is given below.

$$K = -9.70 + 3.97\,WMS + 3.25\,WF$$

The consistency index response surface and contour plot, respectively, for the fitted model are shown in Figure 4.5 (a) and (b). The response surface here fits the quadratic model best. It can be seen in the plot that similar trends are produced for both independent variables at all three levels.

Diagnostic statistics for the model are presented in Figure 4.6 (a) in the form of a normal probability plot. The Anderson-Darling test for non-normality exhibited a high P-value (0.205) confirming normality and the absence of any outliers (Meyer and Kruger, 2001). The plot for residual versus predicted response in Figure 4.6 (b) showed no unusual scattering of the fitted values. A positive slope was exhibited for NWMS as the levels of WF increased from low to high, which is similar to the slope for WF with an increase in NWMS content.

Table 4.5: Analysis of variance table for the experiment.

Source	Degrees of freedom	Sum of squares	Mean square	F[1]	P[2]
Regression	5	54.1885	10.8377	34.34	0.000
Linear	2	52.6206	26.3103	83.37	0.000
Square	2	1.5390	0.7695	2.44	0.149
Interaction	1	0.0290	0.0290	0.09	0.770
Residual error	8	2.5247	0.3156		
Lack of fit	3	1.2907	0.4302	1.74	0.274
Pure error	5	1.2390	0.2468		
Total	13				

[1]F factor or interaction
[2]P probability of a factor having an effect on the response

Table 4.6: Tabulated T-test results for individual variables for the model.

Source	Coefficient	SE Coefficient	T[1]	P[2]
Constant	7.19525	0.2293	31.373	0.000
Starch	1.98367	0.1986	9.987	0.000
Wheat flour	1.62561	0.1986	8.185	0.000
Starch-Starch	0.45142	0.2067	2.184	0.061
Wheat Flour-Wheat Flour	0.03308	0.2067	0.160	0.877
Starch-Wheat flour	0.08507	0.2809	0.303	0.770

[1]T factor or interaction
[2]P probability of a factor having an effect on the response

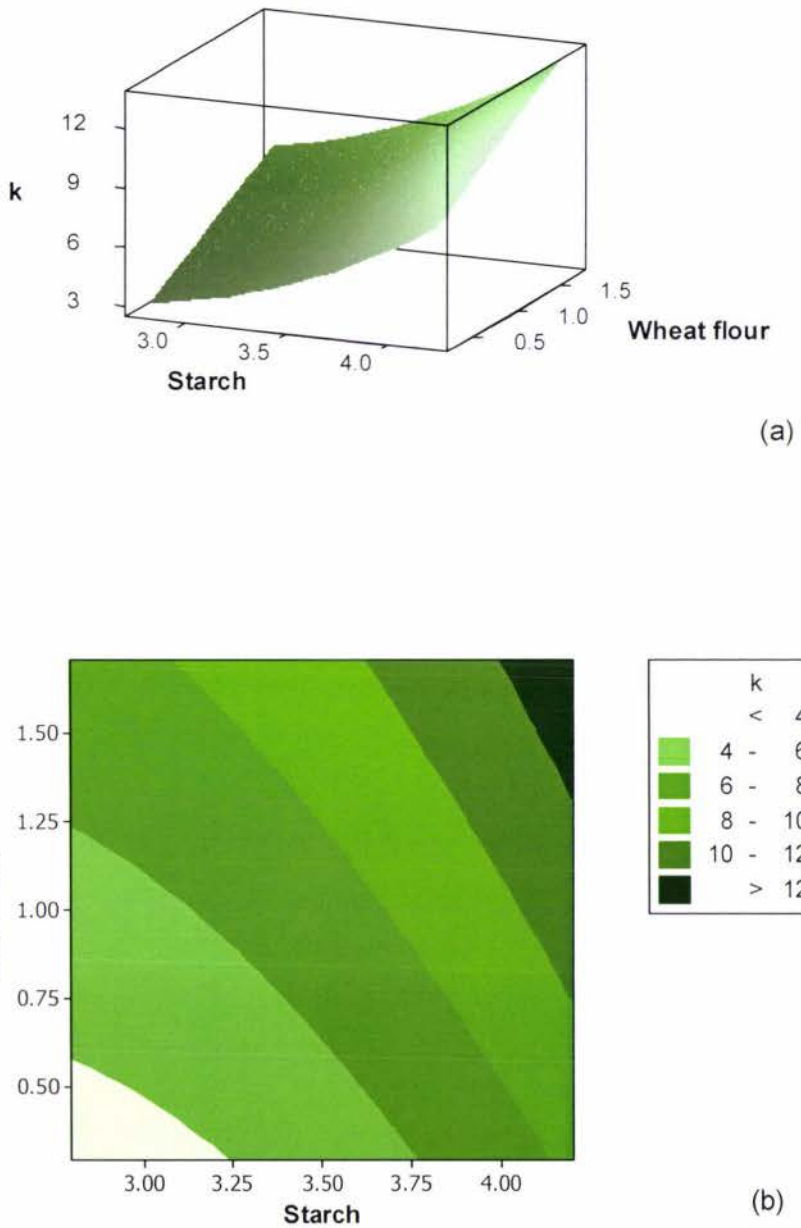


Figure 4.5: Response surface plot (a) for WF and NWMS along with the contour plot (b) of the same with consistency index (K, •) as response.

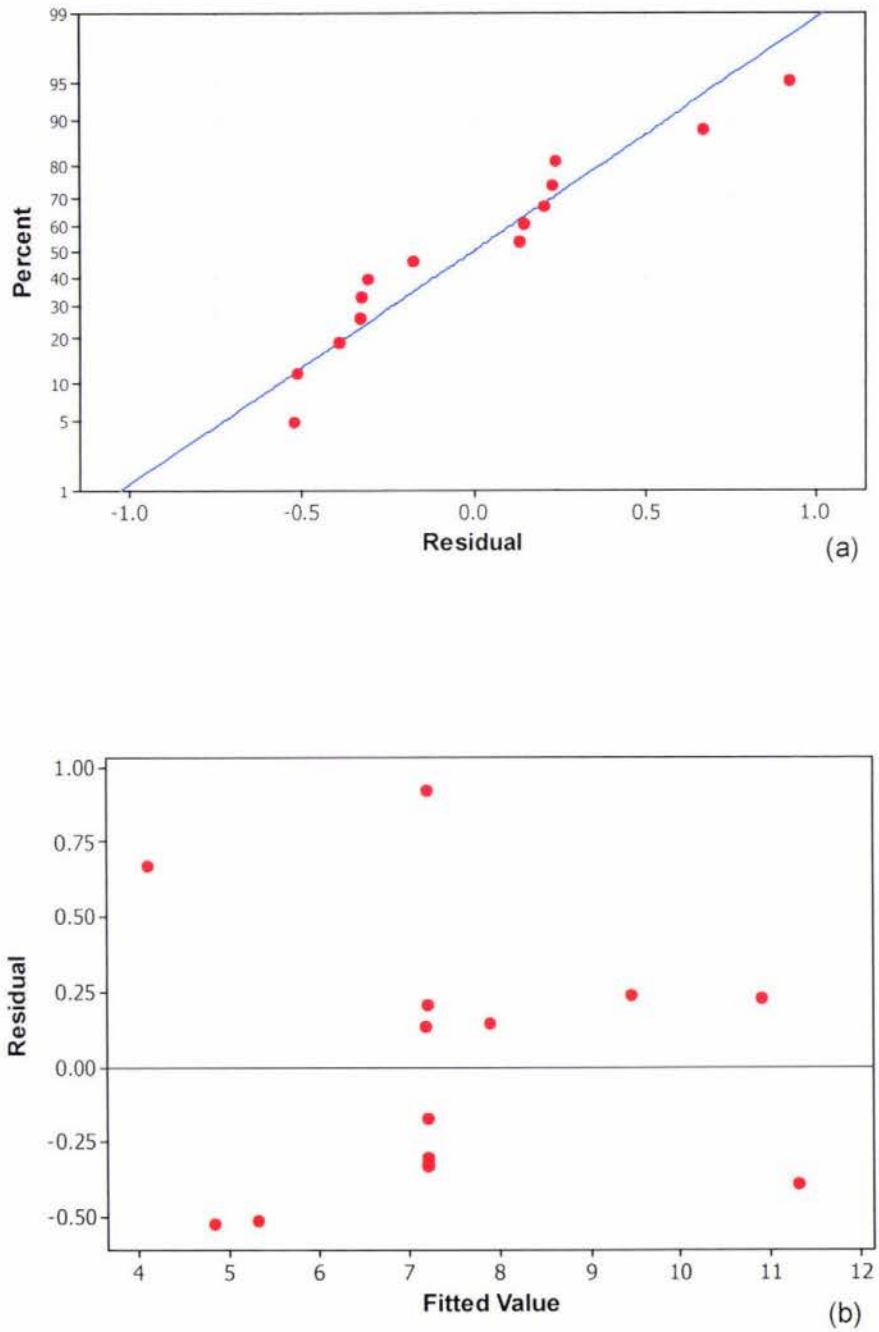


Figure 4.6: The normal probability Plot for residuals (a) and the plot for the residuals versus predicted response (b) with consistency index (K, •) as a response.

4.3.3 Conclusions

The contour plot suggested that the levels of both waxy maize starch and wheat flour could be varied significantly in the range k value of 8-10, which is the optimum target consistency for the MFS. It was concluded the quadratic model was adequate in predicting the response region of the experimentation and the regression equation could be used for sensory testing in the next stage.

4.4 The effect of wheat flour on sensory perception of model soup system

The quadratic model for wheat flour provided the regression equation, which was used to make soup samples with decreasing levels of wheat flour in them without affecting the overall consistency (K. Pa s^n). To make up for the decrease in viscosity contributed by wheat flour, the content of waxy maize starch was increased. The test also provided the opportunity to test the standard soup recipe for general acceptability by consumers.

4.4.1 Test objective

To measure the acceptability of two soup samples with varying amounts of wheat flour along with a standard soup formulation.

4.4.2 Test design

An acceptance test was used to determine the effective status of the soup samples. Questions were framed which were relevant to the pivotal sensory characteristics of soups. They were structured in easy language with least possible jargon. A total of eight questions per sample were designed out of which four were based on 9 point acceptance scale and three on 7 point scale. One question was open ended asking for comments on the sample. A copy of the questionnaire is attached in the Appendix 4.

Three formulations viz. a standard formulation with 1.5% wheat flour, a formulation with 0.75% wheat flour and a formulation with no wheat flour, were tested.

4.4.3 Results and discussion

To determine whether there was difference among samples to a significant level, general linear model of analysis of variance was applied. The probability of significant difference (α=0.05) in samples in relation to each characteristic are listed in Table 4.7.

The P-values for all sensory parameters viz., overall appearance, creaminess, mouth feel, overall flavor, chicken flavor, sharpness and overall liking were not significant , at α = 0.05.

4.4.4 Conclusions

Wheat flour had no significant effect on the overall sensory profile of the soup samples.

Table 4.7: Probability of significant difference among soup sample for each scrutinized sensory character.

Overall appearance[1,3]	DF	SS	MS	F- Value	Probability
Sample	2	5.056	2.528	1.26	0.297
Error	33	66.167	2.005		
Total	35	71.222			

Mouth feel [1,3]	DF	SS	MS	F- Value	Probability
Sample	2	5.056	2.528	1.83	0.176
Error	33	45.500	1.379		
Total	35	50.556			

Creaminess[2,3]	DF	SS	MS	F- Value	Probability
Sample	2	3.389	1.694	1.37	0.269
Error	33	40.917	1.240		
Total	35	44.306			

Overall flavour[1,3]	DF	SS	MS	F- Value	Probability
Sample	2	1.056	0.528	0.14	0.869
Error	33	123.5	3.742		
Total	35	124.556			

Chicken flavour[2,3]	DF	SS	MS	F- Value	Probability
Sample	2	0.222	0.111	0.08	0.926
Error	33	47.667	1.444		
Total	35	47.889			

Sharpness[2,3]	DF	SS	MS	F- Value	Probability
Sample	2	1.056	0.528	0.33	0.723
Error	33	53.250	1.614		
Total	35	54.306			

Overall liking[1,3]	DF	SS	MS	F- Value	Probability
Sample	2	1.056	0.528	0.14	0.873
Error	33	127.250	3.856		
Total	35	128.306			

[1] Responses input on a 9 pt hedonic scale where 1 = dislike extremely, 9 = like extremely.
[2] Responses input on a 7 pt hedonic scale where 1 = dislike extremely, 7 = like extremely.
[3] 12 panelists evaluated the soup samples.

4.5 Functionality of RS

4.5.1 Results and discussion

Initially the functionality of four RS was investigated. RS suspension, at concentration of 5% and 10%, were prepared by the standard procedure used for making samples, and thier viscosity was measured by Paar Physica rheometer. Figure 4.7 shows the plot for the consistency indices of the various RS suspensions at different concentrations. It was evident that RS lacks any "starch like" functionality at lower concentrations. Only Novelose and Hi-maize 958 had slightly higher values of consistency index (K) at higher concentrations.

4.5.2 Conclusions

None of the RS samples were able to replace the WMS functionality to any significant extent. Hence it was necessary to allow for the replacement of waxy maize starch by increasing the amount of XG in the formulations.

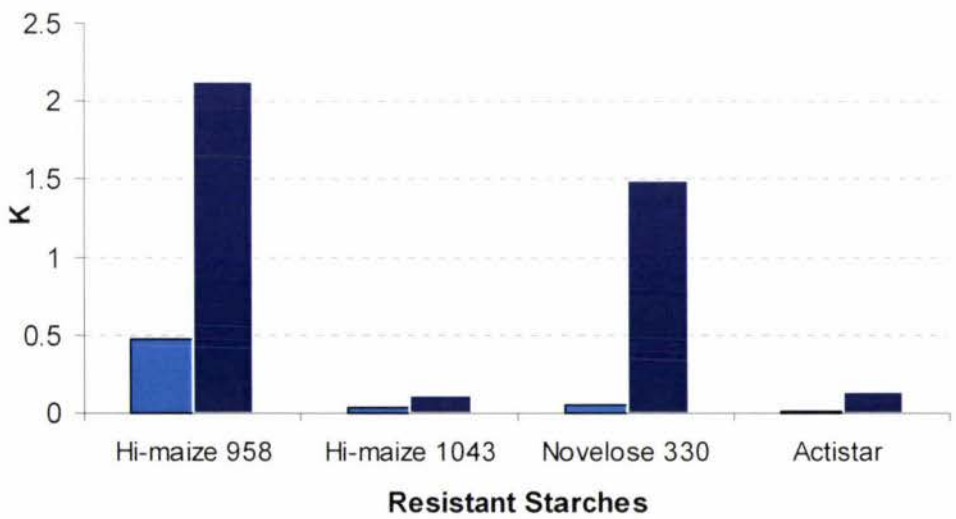


Figure 4.7: Bar chart showing the consistency indices (k, Pa sn) of four resistant suspensions at 5% (■) and 10% (■).

4.6 A second-order regression model to predict the effect of waxy maize starch and xanthan gum on model food system

Waxy maize starch and xanthan gum caused the two main effects in the model food system, which required thorough investigation. *This will primarily be useful in fitting a regression model, the equation of which could help optimize consistency index (k, Pa s^n) of the MFS because changes made in the levels of both factors. The observed synergistic increase in the viscosity by the addition of XG to starch showed a second order model would be a better fit.

4.6.1 Experimental design

A central composite design was constructed, similar to WF and NWMS model, with four axial points added at (x1, x2) = (-1.414, 0), (1.414, 0), (0, -1.414), (0, 1.414). Construction of the central composite design is shown in Table 4.8. The levels of the factors were determined based on the results from previous experiments, with wider exploration of the region of interest.

4.6.2 Results and discussion

The analysis of variance output is shown in Table 4.9. It was observed that the P-value for the interaction effects was insignificant, which meant that there was no higher order interaction between the two factors at the given levels. The table also showed small P-value for square terms but because the square terms of NWMS and XG were not important in the experiment, they were ignored.

The normal probability plot shown in Figure 4.8 exhibited right skewness suggesting abnormality in the model. Because the appearance of a histogram can change depending on the choice of number of intervals to group the data, the test of lack of fit was used to assess whether the residuals were normal or not (Minitab Inc., 2004).

* It was anticipated that this would be used primarily to fit a regression model

Table 4.8: Central composite design (CCD) for WMS and XG.

StdOrder[1]	RunOrder[2]	PtType[3]	NWMS	XG	Consistency index (k)	Corrected k
8	1	-1	3.50000	0.632843	11.1480	3.33886
3	2	1	2.00000	0.550000	8.3699	2.89308
7	3	-1	3.50000	0.067157	3.4500	1.85742
12	4	0	3.50000	0.350000	8.4733	2.91089
5	5	-1	1.37868	0.350000	3.8574	1.96403
4	6	1	5.00000	0.550000	17.4840	4.18139
9	7	0	3.50000	0.350000	7.5976	2.75637
6	8	-1	5.62132	0.350000	18.7180	4.32643
1	9	1	2.00000	0.150000	2.0182	1.42063
13	10	0	3.50000	0.350000	7.5225	2.74272
11	11	0	3.50000	0.350000	7.6774	2.77081
10	12	0	3.50000	0.350000	8.6630	2.94330
2	13	1	5.00000	0.150000	8.1703	2.85837

[1]Standard order (StdOrder) is the order designed by Minitab
[2]RunOrder is the order in which the experiment is conducted
[3]Point type (pt type) is the type of point viz. high (1), low (-1) or centre (0)

Table 4.9: Regression coefficients and analysis of variance for NWMS and XG model.

Term	Coefficient	SE coefficient	T	P
Constant	7.9868	0.4871	16.397	0.000
Starch	4.5353	0.3851	11.778	0.000
Xanthan	3.3190	0.3851	8.619	0.000
Starch-starch	1.5798	0.4129	3.826	0.006
Xanthan-xanthan	0.4146	0.4129	1.004	0.349
Starch- xanthan	0.7405	0.5446	1.360	0.216

Source	Degrees of freedom	Sum of squares	Mean square	F	P
Regression	5	274.957	54.991	46.36	0.000
Linear	2	252.677	136.338	106.50	0.000
Square	2	20.087	10.044	8.47	0.014
Interaction	1	2.193	2.193	1.85	0.216
Residual error	7	8.304	1.186		
Lack of fit	3	7.147	2.382	8.24	0.035
Pure error	4	1.157	0.289		
Total	12	283.261			

[1]F/ T factor or interaction
[2]P probability of a factor having an effect on the response

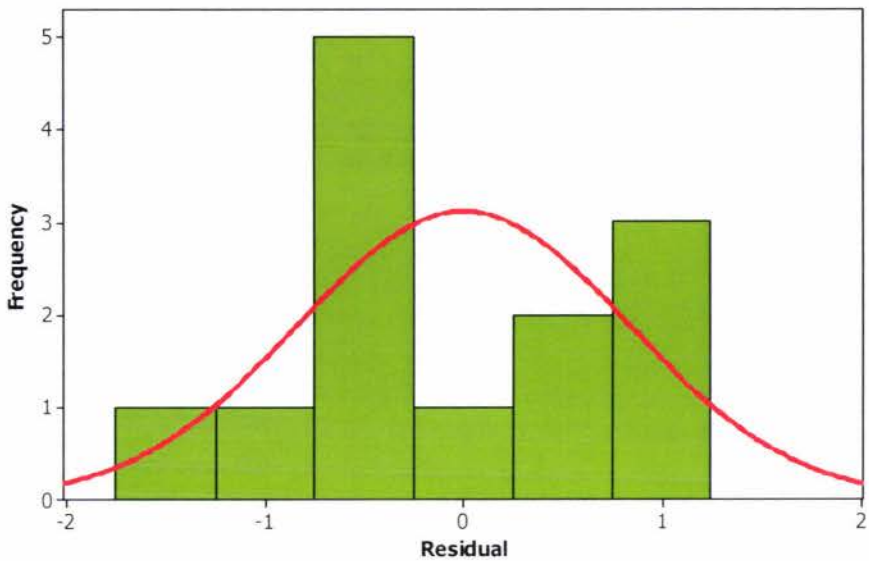


Figure 4.8: Histogram showing the distribution of the residuals for all observation having consistency index (K, Pas^{n}) as response (Green bars) and normal distribution (Red line).

Analysis of variance showed the probability of lack of fit of the model was very high (P= 0.035) and it had to be accepted that the model did not adequately describe the data. When lack of fit is detected, it implies that the predictive knowledge of the model is not statistically correct. This will cause the bending and twisting of response surface in a more complex manner than that which can be easily described by the model (Minitab, Inc., 2004; Lorenzen and Anderson, 1993). This also means that some of the important terms may have been excluded from the model or the regression equation may not explain well the presence of several large residuals. In order to correct the lack of fit the data was transformed. Box-Cox transformations can be useful in eliminating non-normality and improve fit (Draper and Smith, 1996).

The Box Cox curve (Appendix 6) gave the best value (0.5) for

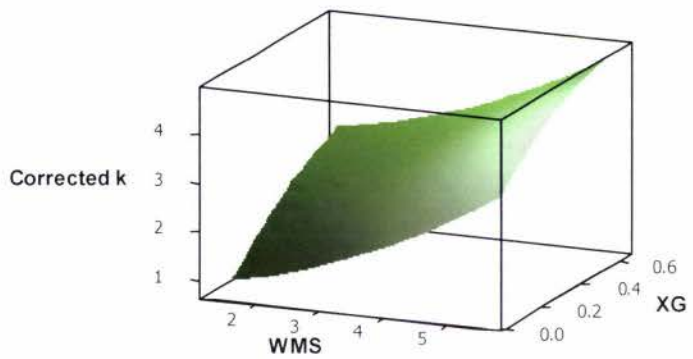
transformation which suggested that a square root transformation would be ideal. Analysis of variance for the transformed data showed large P value for lack of fit. No quadratic effect existed between model variables, as the P value for the quadratic terms is large as well.

4.6.3 Conclusions

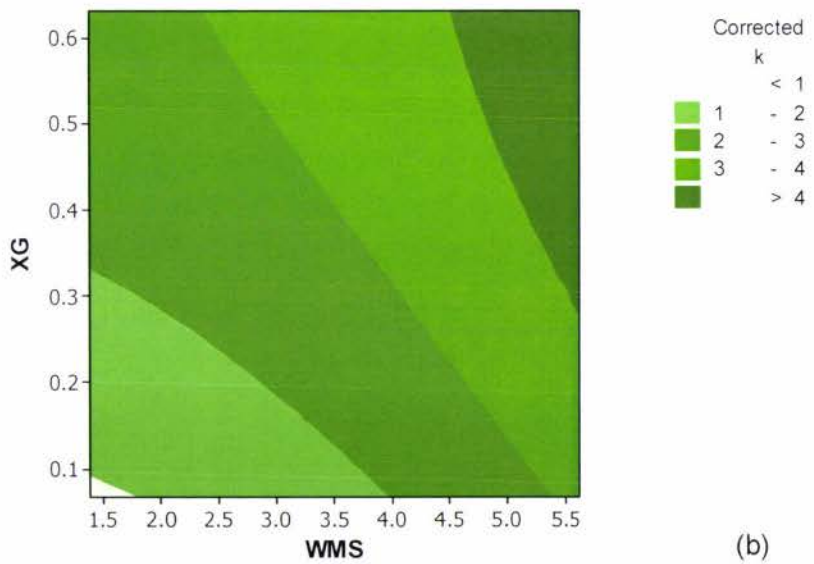
The transformed model adequately fits the data and the regression equation for the model is given below.

$$\sqrt{K} = 0.004 + 0.506 * WMS + 3.06 * XG$$

The response surface plot and contour plot with the transformed data of consistency index as response are shown in Figure 4.9 (a) and (b). The highest value of consistency index was observed at high levels of both variables.



(a)



(b)

Figure 4.9: Response surface plot (a) for WF and NWMS along with the contour plot (b) of the same with consistency index (K, •) as response.

4.7 Thermal behavior of RS

4.7.1 Experimental design

Differential scanning calorimeter was used to measure the gelatinization temperature of RS on their own and in soup formulations within a temperature range 20 to 150° C and heating rate of 4° C/min.

4.7.2 Results and discussion

Figure 4.10 shows the thermograms of four different RS as determined by DSC studied at water content of approximately 50%. Due to limiting moisture content of the samples typical broadening on both sides of melting endotherm was observed. The position and the appearance of this broadening varied and affected the temperature range of melting. Hi-Maize 1043 was the only exception showing two distinct peaks.

The transition temperatures and corresponding enthalpies are shown in Table 4.10 All starches showed characteristic gelatinization curves with endothermic transition at ranges of T_m = 98-113°C, 120-124 °C, 116-120 °C and 97-110 °C for Actistar, Novelose 330, Hi-maize 1043 and Hi maize 958 respectively.

Table 4.10: DSC results for four RS.

Sample	DSC parameters				
	Onset (°C)	Peak (°C)		Completion (°C)	ΔH (J/g)
		Claimed	Observed		
Actistar	98.8	110	102.8	113.8	2.784
Novelose 330	120.4	121.5	121.1	123.9	2.275
Hi-maize 1043	116	114	119.6	119.8	1.663
Hi maize 958	97.3	99.5	99.7	109	1.783

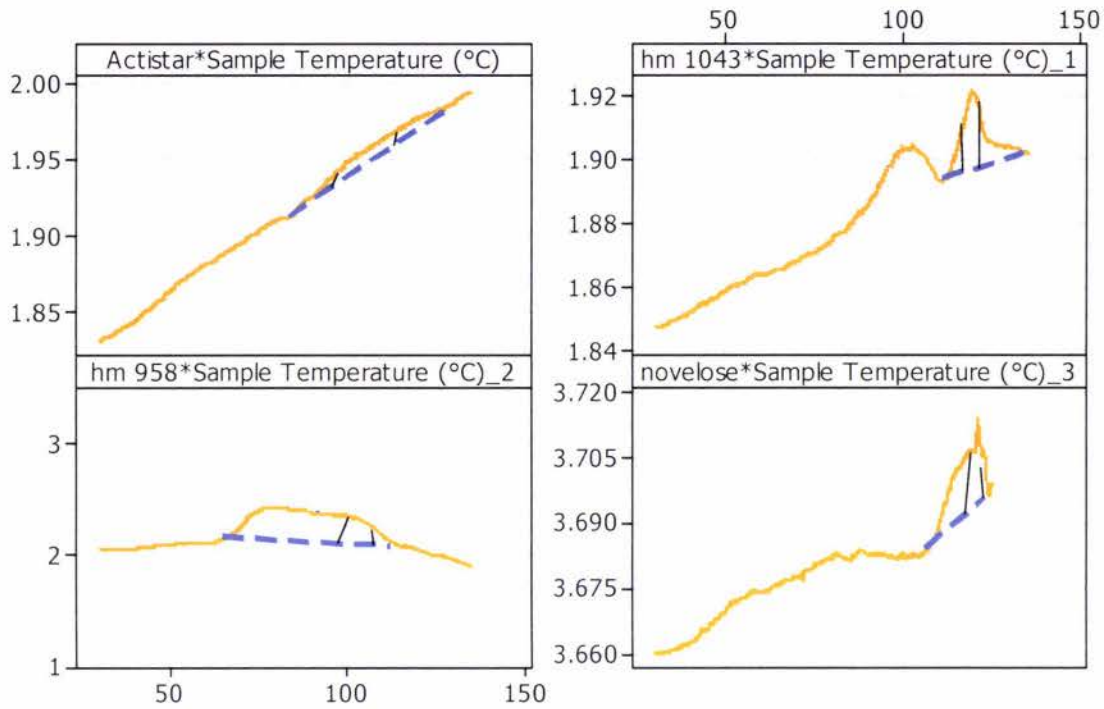


Figure 4.10: Thermograms of RS samples analysed using DSC.

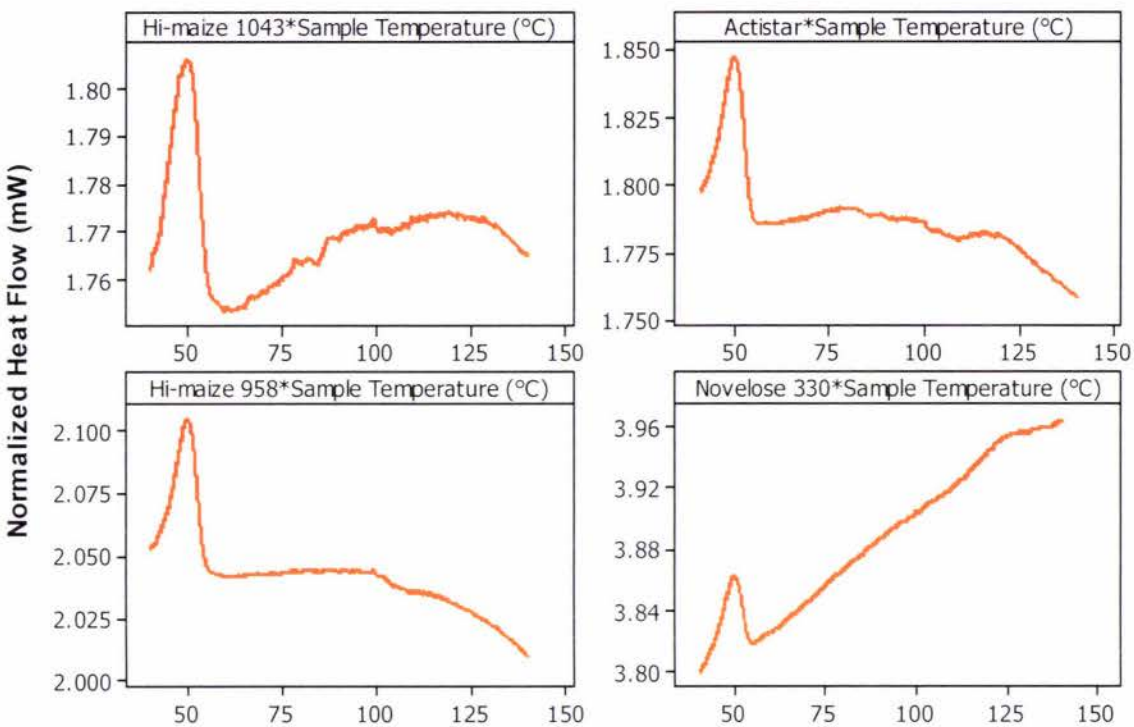


Figure 4.11: Thermograms of soup samples with RS analysed using DSC

The melting enthalpy is a measure of overall crystallinity of RS i.e. the quality and quantity of starch crystals. Pure crystalline A-spherulites exhibit a melting enthalpy of approximately 35 J/g, which is very similar to that of B-spherulites (Freddikson, 1971). For RS, the resistance to degradative enzymes is due to the formation of partial crystallites, which is reflected in the enthalpies. The enthalpies were between 1.663 and 2.74 J/g.

The melting of crystallites only occurs if there is excess of water present in the system. However, if the water is insufficient for the process to be completed, the remaining crystals melt at a higher temperature in accordance with the theory of polymer-diluent interaction. The temperature of the second melting depends on the thermal stability of the remaining crystals and the amount of water available (thermal analysis of foods).

The DSC thermograms of soup samples containing different RS are shown in Figure 4.11. The endothermic transition in all curves fell in the range of 42 – 55 °C, which relates to the gelatinization of WMS. No other peak was noticed in the curves predominantly because of very low mass (dwb) of RS added, which was below detection level

4.7.3 Conclusions

The peak melting temperatures were 102.8 °C, 121.1 °C, 119.587 °C and 99.7 °C for Actistar, Novelose 330, Hi-maize 1043 and Hi maize 958, respectively, which are in agreement with data provided by the manufacturers. The Hi-maize starches (RS 2) showed lower enthalpy values than Actistar and Novelose 330 (RS 3), which confirmed the higher order of crystallinity in the later group. The melting peaks for RS in soup samples couldn't be detected.

4.8 The effect of holding time on %RS content of model food system

4.8.1 Experimental design

The AOAC official method (2002.02) was used for RS analysis described by McCleary and Monaghan. The moisture content of all starch samples and soup samples was determined by oven drying according to AOAC method 925.10 (Appendix 7). Soup samples containing various RS at 60% substitution level of waxy maize starch were subjected to different holding times (5, 10, 15 and 20 mins) at 95°C and the %RS level analyzed after the treatment.

4.8.2 Results and discussion

Actistar obtained from tapioca was found to have 48.5% RS, whereas Novelose 330 had 43.2%RS. Shin *et al.* (2003) reported an RS content of 39.9% in Novelose 330 using AOAC total dietary fiber method. Hi maize 1043, which was a New Zealand equivalent of Hi maize 260, had a RS % of 50.3.

As RS was included in the dietary fiber definition along with non-starch polysaccharides, the data provided by manufacturers have been described in terms of %TDF. Hi- Maize 958 is a new equivalent of Hi-maize 240 (57.9%, shin *et al., 2003*) but with a relatively lesser amount of RS (26.3%) as compared to the standard product.

No significant difference ($P > 0.05$) for %RS (dwb) of the four soup samples, held for different times, was observed. Figure 4.12 shows the plot for holding time versus %RS content of model soups. The comparison of means and standard deviations for soup samples based on average %RS (dwb) at different holding times is shown in Table 4.11. The standard deviation of all soups was quite small except for Novelose 330 due an outlying value at holding time of 10 minutes, which may have been due to an experimental error. The observed %RS (dwb) values of only two of RS were relative to the added levels. The analysis of variance output by Minitab is attached in Appendix 8 along with the analysis sheets for RS assays.

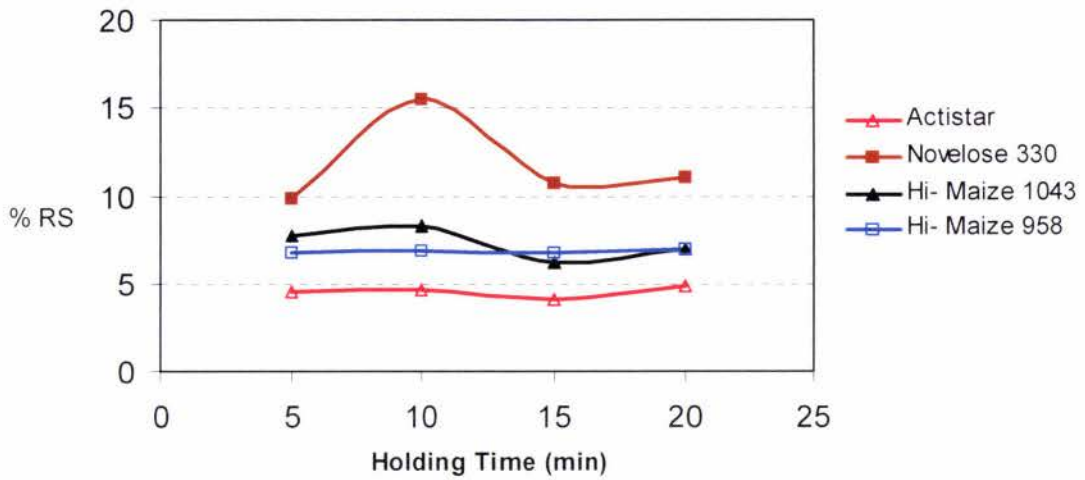


Figure 4.12: Scatter plot for % RS (dry weight basis) of soup samples held at different times at 95° C during processing.

Table 4.11: comparison of means and standard deviations of % RS in soups.

RS	%RS in Raw material	%RS added in soup samples	%RS observed in soup sample	
			Mean	Stdev.
Actistar	52	12.9	4.4	0.34
Novelose 330	40	10.1	11.7	2.54
Hi-maize 958	25	6.3	6.8	0.14
Hi–maize 1043	50	13.1	7.3	0.9

4.8.3 Conclusions

RS values determined by AOAC official method (2002.02) showed that all RS are process tolerant and holding time has no effect on their content in the final product. However, the comparative values of added and observed %RS (dwb) in soup samples were different. The discrepancy could have been due to the minute levels of added RS in soup samples and complexities in the method of sample preparation.

4.9 Replacing NWMS by RS in soup model

Novelose 330 was added to the final soup as it exhibited the highest %RS (dwb) level in the *in vitro* tests. The viscosity test (section 4.4) showed that it also had partial functionality but at the level of addition this was not expected to be significant. As claimed by manufacturers, Novelose 330 had a bland taste upon addition to food products. But due to the increase in amount of XG to rebuild the lost viscosity caused by inclusion of RS, which was non functional, a comparison had to be made.

4.9.1 Test format

The same/different test was used to determine whether a sensory difference existed between soup with no RS and ones with part of NWMS replaced with RS. This attempt would help establish the level of RS that could be incorporated without affecting the organoleptic properties of the soup.

The soups were formulated using the regression equation obtained by modeling NWMS and xanthan by a central composite design in the previous section. Four soup samples were used with increasing level of NWMS substitution viz. 0%, 20%, 40% and 60%. A total of 60 responses, 30 matched and 30 unmatched were collected from 30 subjects. Each subject evaluated both matched and unmatched in a single session. The score sheet and worksheet are attached in the Appendix 9.

Because each panelist evaluated both matched and unmatched pairs, the standard chi ^{2} - test would have been inappropriate; therefore a McNemar test

was used instead, which used both responses for each panelist.

4.9.2 Results and discussion

The results of the same/difference test are shown in Table 4.12. The test statistic for McNemar test took the placebo effect into account and the results were evaluated on the basis of correct responses for both unmatched and matched pair (Meilgaard *et al.*, 1991). The McNemar two-tailed P-value for sample with 20% 40% and 60% substitution of WMS with RS was calculated as 0.1824, 1.778 and 1.778. The P-value for the sample with 20% substitution was not significant at α=0.05. However, for the samples at 40% and 60% substitution the P-values suggest that samples were significantly different (α=0.05).

4.9.3 Conclusions

The replacement of waxy maize starch with RS can be differentiated by taste at substitution levels of 40% and 60%. However, at 20% replacement level the soup samples taste the same.

Table 4.12: Responses for both matched and unmatched pair for McNemar's test for all three-soup samples (20%, 40% and 60% substituted with RS).

		Subjects received A[δ]/B or B[Ψ]/A and responded*	
		Same	Different
Subjects received A/A or B/B and responded[#]	Same	S = 1	T = 7
	Different	U = 2	V = 0

		Subjects received A/C[λ] or C/A and responded	
		Same	Different
Subjects received A/A or C/C and responded	Same	S = 0	T = 7
	Different	U = 0	V = 3

		Subjects received A/D[ρ] or D/A and responded	
		Same	Different
Subjects received A/A or D/D and responded	Same	S = 1	T = 7
	Different	U = 0	V = 2

*McNemar test $x^2 = (|U - T| - 1)^2/(U - T)$
[δ] soup with no RS
[Ψ] soup with 20% of NWMS replaced by RS
[λ] soup with 40% of NWMS replaced by RS
[ρ] soup with 60% of NWMS replaced by RS
[#] 30 panelists evaluated the soup samples.

CHAPTER 5

OVERALL CONCLUSIONS AND RECOMMENDATIONS FOR FURTHER WORK

A RS based model food system has been developed and its rheological and sensory properties characterized. The rheological data was evaluated using response surface methodology, which enabled an evaluation of the functionality with correlation to the concentration of RS. The results from the rheological data were used to develop regression models for determining optimum region of response.

Interestingly, all RS lacked 'starch like' functionality, thus could not replace normal starch in soup formulation alone. The region of response of the main ingredients was investigated by fitting a quadratic model, the equation of which helped optimize the response while increasing the amount of other thickener.

The thermal stability and process tolerance of various RS samples, tested using DSC and *in vitro* analysis, were in agreement with the claims made by manufacturers. The peak melting temperatures of all RS were at least or more than 100 °C, which can be classified as boiling stable. This was confirmed by in vitro RS analysis as holding time (at boiling temperature) had no effect on the content of RS in final product.

The sensory test to investigate the optimum amount of RS that could replace waxy maize starch in the soup formulation showed that only 20% of replacement was achievable without a difference being identified between the soup with RS and standard soup formulations by taste.

This study has shown that RS is a potential food ingredient which can be incorporated into fluid food products only to certain extent. These findings have commercial potential and may result in packet soup mixes with lower glycaemic response. It is recommended that further work be carried out on improving the

sensory properties of model soup system so that the levels of RS addition may be increased. Other thickeners may also be tested which may impart a better mouthfeel and mask the sandy texture of RS.

Commercially available RS are commonly derived from major cereal and tuber crops such as maize, potato, wheat and rice. It is well known that the formation of RS is highly dependent on the botanical source. There is a need for testing other raw materials for RS production such as barley which is the fourth major cereal crop in the world and is most commonly used in malting, brewing and in animal feeds. Barley is known to have a high content of dietary fibre and also high proportions of soluble fibre especially β-glucan. Another alternative source of obtaining RS with better physiochemical and functional characteristics is banana. Also, starches derived from legumes can be tested as their rate of digestion is considered to be lower than cereal starches.

An important problem lies in determining the amount of RS in a given diet. A collaborative inter-laboratory study reported that the procedure involving incubation of samples with pancreatin at 37°C for 16 h showed higher RS yields than other methods for almost all samples. However, the true value of RS of food can only be derived from that found in the contents of the terminal ileum. To date two methods, ileostomy and intubation, have been used for *in vivo* measurement. However, these methods have certain physiological problems. In the former method, intestinal modification can alter results which may depend on individuals. Moreover, the possibility of break down of carbohydrate by fermentation by the microflora inhabiting the terminal ileum may exist. The later measurement method may drift from accuracy because of the presence of tube leading to slower gastric emptying but shortening intestinal transit time. Therefore, a standard measurement procedure needs to be developed by modification of the existing techniques which can predict true amount of RS in foodstuffs.

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APPENDIX

A1: Products with Hi-maize (RS) as an ingredient marketed in Australia and New Zealand

- **Buttercup Australia** Wonder white bread
- **Quality Bakers NZ** Fibre white bread
- **Uncle Toby's Australia** Health-Wise cereals and soft break muesli bars
- **Heinz Watties Australasia** Kidz finger fruit snacks and pasta
- **Lanes biscuits ltd.**
    - Premium high fibre crisp bread
    - Animal bites savory biscuits
- **Greens** Simpsons fruit snacks and Rug rats
- **Sanitarium** Up and Go liquid breakfast
- **Paul's Ltd.** Vaalia breakfast yoghurt
- **Select foods** Hi-maize ingredients in 400g packs for cooking
- **Sigma pharmaceuticals pty ltd** Nucolox
- **Mead Johnson Nutritionals** Sustagen hospital with fibre
- **Freedom foods** Gluten-free cookies, pasta and vegetable burgers
- **Griffins (NZ)** Vita wine biscuits

Source: http://www.foodstarch.com/productlist_au_nz
Dated: 17/06/04

A2: Basic soup recipes sourced to formulate cream of chicken soup for the research

Formulation 1: Cream of chicken soup

Chicken stock	15 gals.
Milk	11.5 gals
Wheat flour	16 lb.
Butter	9 lb.
Salt	2.5 lb.
Hydrolyzed vegetable protein	1 oz.
Monosodium glutamate	10 oz.
Onion powder	10 oz.
Sugar	6 oz.
Celery	4 oz.
Ground white pepper	1 oz.

Source: Binsted and Devey (1970) (for reference see bibliography section of the report)
Units are gallons (gals.), pounds (lb.) and ounces (oz.)

Formulation 2: Cream of chicken soup

Water	76.055%
Chicken broth	5%
Maltodextrin	2.9%
Chicken soup base	5.2%
Modified food starch	3.2%
Precooked chicken cubes	2%
Barley betafiber	1.2%
Whey protein conc.	1%
Soybean oil	1%
Mono- and diglycerides	0.5%
Flavor	0.8%
Spices and seasoning	0.97%
Microcrystalline cellulose	0.15%
Color	0.025%

Source: http://www.cargilhft.com/industry_products_beta_formulation.html#soups
Dated: 20/09/05

Formulation 3: Cream of chicken soup

Vegetable oil	1 tbs
Chicken breasts without skin	1 ½ lb
Diced celery	¾ cup
All-purpose flour	½ cup
Chicken broth	1 ½ cups
Water	1 cup
Mushrooms	½ lb.
Onion powder	1 ts
Thyme leaves	½ ts
Ground white pepper	¼ ts
Milk	¼ cup

Source: http://www.recipesource.com/text/soup/recipe1919.txt
Dated: 20/09/05
Units are tablespoons (tbs.), pounds (lb) and teaspoons (ts.)

Formulation 4: Cream of chicken soup

Milk	4 cups
All-purpose flour	2 tbs.
Vegetable oil	2 tbs
White sugar	2 tbs
Chicken meat	2 cups
Salt	½ ts
Black pepper	½ ts
Garlic powder	½ ts

Source: http://soups.allrecipes.com/92/creamofchickensoup.asp
Dated: 20/09/05
Units are tablespoons (tbs.) and teaspoons (ts.)

A3: Final soup formulation used in present research

Final formulation: Cream of Chicken soup	
Waxy maize Starch	5%
Skim milk powder	2.5%
Spray dried fat	2%
Chicken flavour	0.5%
Xanthan	0.15%
Onion powder	0.1%
Citric acid	0.15%
Garlic powder	0.1%
Salt	0.9%
Sugar	0.1%
Pepper	0.1%

A4: A sample questionnaire for acceptance test

Massey University
Te Kunenga ki Pürehuroa

Consumer Sensory Evaluation of Chicken Soup

Researcher(s) Introduction

Researcher's Name:	Amit Taneja	Supervisor's Name:	Prof. Harjinder Singh Dr. Derek Haisman
Contact Details:	06 350 5799 extn 2535	Contact Details:	Extn 5579

You are invited to take part in a consumer sensory evaluation, to evaluate 2 pairs of chicken soup. Your participation in this activity will take approximately 10 minutes. We are selecting people for this exercise who meet the following criterion:

Are consumers of chicken soup?

The foods you will taste contain milk and milk derivatives that can be harmful or cause allergic reactions to certain group of people. You are requested not to participate if you may be adversely affected by milk and milk derivatives

The information collected in this study will be used in a thesis in partial fulfilment of the Masters of Technology in Food Technology. No data linked to an individual's identity will be collected.

If you have any questions about this work, please contact one of the people indicated above.

Consumer Sensory Evaluation of Chicken Soup

CONSENT FORM

THIS CONSENT FORM WILL BE HELD FOR 12 MONTHS FROM DATE OF SIGNING
(For minors aged 8-15 consent form is to be signed by parent or guardian)

I have read and understood the Information Sheet and have had the details of the study explained to me. My questions have been answered to my satisfaction, and I understand that I may ask further questions at any time.

I agree to voluntarily participate in this study under the conditions set out in the Information Sheet.

I understand that I have the right to withdraw from the study at any time and to decline to answer any particular questions.

I have advised and discussed with the researcher, any potentially relevant cultural, religious or ethical beliefs that may prevent me from consuming the Foods under consideration.

Participant's Signature: ..... **Date:** .....

Full Name - printed .....

Please answer the following questions:

Age: Please tick

☐ 15-19 ☐ 20-24 ☐ 25-29 ☐ 30+

Gender: Please tick

☐ Male ☐ Female

How often do you have a soup?

☐ More than once a week ☐ Once a week ☐ Once a fortnight ☐ Once a month ☐ Once a year ☐ Never

These questions relate to sample #932/553/797

Please **look** at sample #932/553/797

1. How do you like the overall appearance of sample #932/553/797?

☐	☐	☐	☐	☐	☐	☐	☐	☐
Dislike Extremely	Dislike very much	Dislike moderately	Dislike slightly	Niether like nor dislike	Like slightly	Like moderately	Like very much	Like Extremely

Please eat sample #932/553/797

2. How do you like the overall feel in your mouth of sample #932/553/797?

☐	☐	☐	☐	☐	☐	☐	☐	☐
Dislike Extremely	Dislike very much	Dislike moderately	Dislike slightly	Niether like nor dislike	Like slightly	Like moderately	Like very much	Like Extremely

3. What do you think about of the creamy feeling of sample #932/553/797?

☐	☐	☐	☐	☐	☐	☐
Not creamy enough			Just right			Too creamy

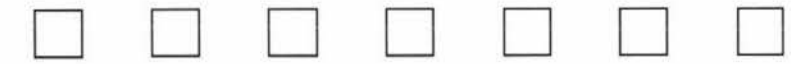
4. How do you like the overall flavor of sample #932/553/797?

☐	☐	☐	☐	☐	☐	☐	☐	☐
Dislike Extremely	Dislike very much	Dislike moderately	Dislike slightly	Niether like nor dislike	Like slightly	Like moderately	Like very much	Like Extremely

5. What do you think of the chicken flavor of sample #932/553/797?

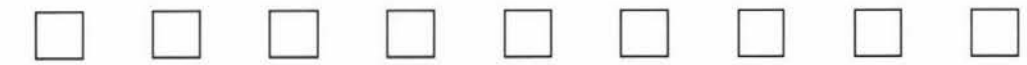
☐	☐	☐	☐	☐	☐	☐
Too intense			Just right			Too mild

6. What do you think of the sharp in taste of sample #932/553/797?



Too little Sharpness | Just right | Too much sharpness

7. Taking everything into consideration, how do you like the sample #932/553/797?



Dislike Extremely | Dislike very much | Dislike moderately | Dislike slightly | Niether like nor dislike | Like slightly | Like moderately | Like very much | Like Extremely

8. Do you have any comments about the soup?

Please take a sip of water and eat a piece of cracker before continuing.

Please rank the 3 samples in order of preference, 1 = most liked to 3 = least liked.
Sample numbers # 932, 553 and 797.

1._____

2._____

3._____

Thank you for your time.

A5: A typical worksheet for simple difference test

Worksheet

Test code: Date:

Type of sample: **XXXXXXXXXXX**

Type of test: Same/difference test

Sample identification:	***code***
5-117-36 (old process)	36
5-117-39 (new process)	39

Code serving containers with 3-digit random numbers and divide into lots, one lot to receive sample 36, the other sample 39.

When preparing panellists trays, place samples from left to right in the following order.

Panellist Code	Sample Order
1-15	36-36
16-30	36-39
31-45	39-36
46-60	39-39

Source: Meilgaard *et al.* (1991)

A6: Box- Cox transformation plot and the lambda table for the second order model of xanthan and waxy maize starch

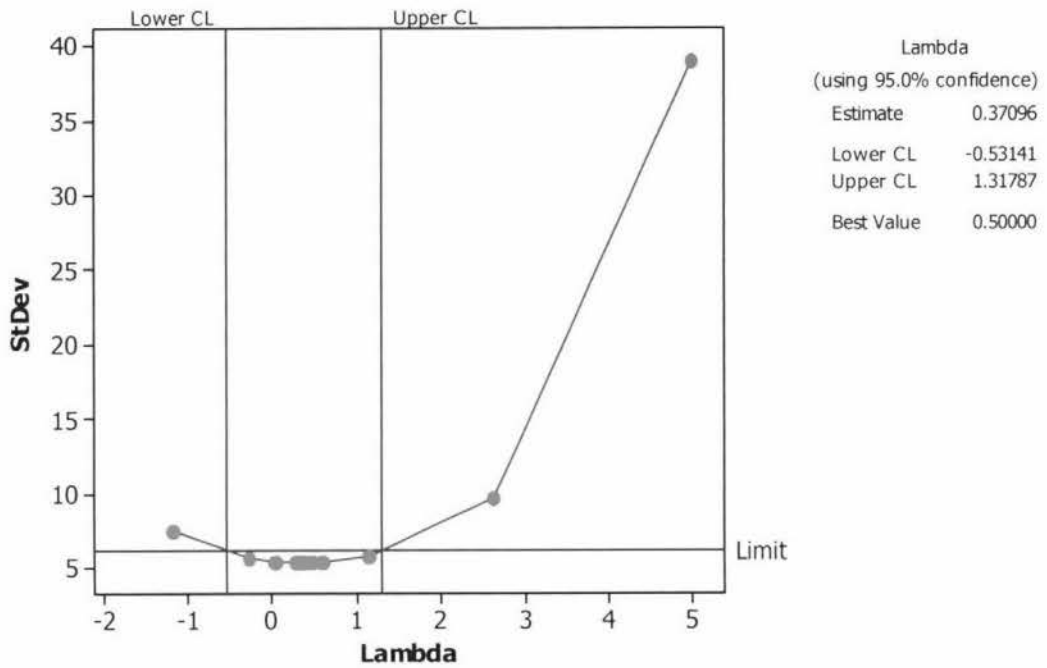


Figure A1: Box Cox plot for the second order model for xanthan and waxy maize starch to eliminate non-normality.

A7: Moisture content determination (AOAC Official method 925.10)

- Set the Vacuum Oven temperature at 98 - 100° C for wheat flour.
- Disposable aluminium pans are pre-dried at 100° C for 15hr in an Air Oven or at 100° C for 3hr in a Vacuum Oven and cooled in a desiccator.
- Weigh pre-dried disposable aluminium pans.
- To each pre-dried pan add 2-3g of ground beef or wheat flour (weighed to the nearest 0.1mg) and distribute uniformly.
- Place the samples in a desiccator.
- Once the above temperature is reached in the Vacuum Oven place the samples and create a partial vacuum with a pressure equivalent < 50mm of Hg.
- Dry samples to a constant weight 5hr for ground beef and wheat flour.
- Remove samples after drying and place in a desiccator to cool (15-30 min).
- Weigh the sample after cooling.

A8: The analysis of variance for %RS versus holding time for model soups

Source	Degree of freedom	Adj. sums of squares	Adj. mean squares	F	P
Soup models	3	112.37	37.46	23.02	0.000
Holding time	3	2.53	2.53	1.55	0.267
Error	9	14.65	1.63		
Total	15				

Generated by Minitab inc. (2004)
[1]T factor or interaction
[2]P probability of a factor having an effect on the response

A9: Worksheet and score sheet for same/different test

Worksheet

Date: 11/Jan/05 Test code: s/d-soup

Type of samples: Chicken soup with varying amounts of starch and xanthan
Type of test: same/different test

Sample identification:			Code
Xanthan 0.15%	&	5g starch (0%)	A
Xanthan 0.32%	&	4g starch (20%)	B
Xanthan 0.4%	&	3g starch (40%)	C
Xanthan 0.54%	&	2g starch (60%)	D

Code serving containers with three digits random numbers and divide them into four lots.

When preparing panelists trays, place samples left to right in the following orders (one matched and one unmatched pair).

Panellist no	Sample order
1	AB, AA
2	AC, CC
3	AD, DD
4	BA, BB
5	CA, AA
6	DA, AA
7	AB, AA
8	AC, CC
9	AD, DD
10	BA, BB
11	CA, AA
12	DA, AA
13	AB, AA
14	AC, CC
15	AD, DD
16	BA, BB
17	CA, AA
18	DA, AA

Aim of the test is to find out whether difference exists between sample A & B, A & C and A & D, which vary in content of starch and xanthan.

Each panellist will receive two pairs of samples, one matched and one unmatched. Six panellists will judge the similarity/ difference of each sample. At a confidence interval of 95%, five out of six panellists have to correctly judge the difference/similarity for the samples to be significantly different (alpha 0.05) in taste. The results may be analysed separately for sample A & B, A & C and A & D using McNemar test.

Score-sheet

Same/Different test

Test no:

Taster's name: _____

Type of sample: **Cream of Chicken soup**

Instructions

Please taste samples (in pairs) from left to right,
Please determine if the samples (in pairs) are identical or different,
Mark your responses below.

Please take a sip of water and eat a piece of cracker before moving from one pair to the next.

Note that some of the sets consist of two identical samples

Sample XXX

☐ **Identical** ☐ **Different**

Sample XXX

☐ **Identical** ☐ **Different**

Comments:

_____

_____