Development of a Beverage Model to Test Appetite Control Food Ingredients

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Abstract

The present project is part of the broader 'Foods for Appetite Control' research programme of Plant & Food Research. The programme aims to deliver validated satiety effects (reduce appetite and provide more than four hours of satiety) in foods through phytochemicals and macro-nutrients. As it is necessary to validate the satiety effects through clinical trials, a beverage model was developed. It served as a 'vehicle' for incorporating phytochemicals (*e.g.* fruit extract) and macro-nutrients (*e.g.* viscous fibre – alginate) to deliver their satiety effects, which were validated by a satiety measurement trial.

The development work began with the characterization of viscous fibres. Based on the literature review, pectins and alginates appear to be more satiating than other viscous fibres. It is believed that gastric gelation can induce satiety, through the formation of a gel that has some strength (presumably in the stomach). Based on rheological measurements, Protanal® LF120 alginate and Grindsted® Pectin LA410 were selected for further evaluation in the beverage model. These viscous fibres met the criteria of providing viscosity to the beverage, showing sensitivity to acids and calcium ions resulting in gelation, and contributing to higher gel strength than others that were evaluated.

The beverage model was developed as a partial-meal replacer beverage, which is non-dairy, soy protein-based, fruit-flavoured (blueberry), 250 mL and of neutral pH (~7.2). The development work has established a base formulation and processing method for the beverage model and has successfully incorporated Protanal LF120 (0.25% and 0.5%) and fruit extract (0.2%). Due to its low viscosity and poor stability in UHT-processing even at high levels, Pectin LA410 was excluded from further evaluation. Incorporation of quercetin and isoquercetin into the beverage model was unsuccessful because of their insolubility in water and interactions with soy proteins.

A methodology for satiety measurement was established and a trial was carried out to validate the satiety effects (subjective appetite) of the fruit extract and Protanal LF120 in the beverage model. The trial used a preload (6 test beverages), within-subject (n = 12), repeated measures, completely balanced, crossover and randomized design. The satiety effect of Protanal LF120 was found to be dose-dependent; higher alginate level significantly increased the satiety effect of the beverage. Differences in mean appetite

ratings (P <0.05*)* between low and high alginate levels were 6.9%, 8.3%, 10.6%, 6.3% and 6.7% for hunger, fullness, satiety, desire to eat and prospective food consumption ratings, respectively.

On the other hand, the data did not reveal statistically significant results across all appetite scales (except for hunger, P = 0.015) between beverages with and without fruit extract. In addition, the interaction of alginate*fruit extract was not statistically significant, implying that the higher satiety effect of the high level alginate + fruit extract beverage could be purely due to the alginate. Further testing is warranted: (1) to incorporate higher levels of fruit extract in the beverage model to evaluate any dose-dependency, (2) to determine if an additive or synergetic satiety effect exists with a higher level of fruit extract and high alginate level in the beverage, and (3) to modify the current experimental design to increase power of the study to 80% by increasing the number of subjects.

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Chapter 1
Introduction

The rising prevalence of obesity around the world is having a substantial impact on public health. It is vital to understand how bodyweight and energy balance can be controlled (Benelam, 2009). There is increasing research on satiety, evaluation of whole foods and food ingredients that have satiety enhancing properties and development of food products or diets for controlling appetite and energy intake.

The present project is part of the broader 'Foods for Appetite Control' research programme of Plant & Food Research (PFR). The programme aims to discover and develop plant foods that could reduce appetite and provide more than four hours of satiety. Plant-derived phytochemicals and macro-nutrients are utilized as bioactives to target gut chemosensory mechanisms. This uses a combinatorial approach targeting multiple mechanisms at different levels of the gut to provide four hours of satiety: (1) bitter activation of duodenal cholecystokinin release, (2) carbohydrate activation of the ileal brake and (3) prebiotic activation of the colonic brake through fermentation of fibres (Sutton, 2012).

Other than *in-vitro* tests, *in-vivo* tests such as clinical trials are necessary to validate the satiety effects. For example, quercetin has shown positive satiety effects in *in-vitro* tests, so the next step is to validate the effects by incorporating quercetin in a food product and carry out clinical trials *i.e.* satiety measurement trial. Research using a consumer survey was previously conducted by PFR. The team has identified food product concepts that were perceived as acceptable satiety food models. One of the satiety food models is the breakfast drink (Huffman *et al.*, 2011). Thus, the current work is to develop a beverage model, which would serve as a 'vehicle' for incorporating phytochemicals (*e.g.* quercetin) and macro-nutrients (*e.g.* dietary fibres) to deliver their satiety effects, validated by satiety measurement trials.

The aim of the project is therefore to develop a base formulation and process for a beverage model that could affect satiety (subjective appetite) when consumed. In addition, the satiety beverage model should be one that is suitable for incorporation of potential phytochemicals and macro-nutrients that could reduce appetite and keep us feeling fuller for longer. The objectives of the project are as follows:

1. To formulate consumer-acceptable beverages with protein, fibre and/or phytochemical extracts, that could enhance satiety.
2. To determine the appropriate methodology and optimum parameters for processing of the beverage model.
3. To show proof of concept that phytochemical extracts could be incorporated into the beverage model.
4. To establish a methodology for satiety measurement and carry out a satiety measurement trial to validate the satiety effects of phytochemical extracts / fibres in beverages.
5. To evaluate the physical, chemical and sensorial properties of the beverages and correlate to satiety (subjective appetite).

Results and observations from the development work will provide a better understanding on the formulation of the beverage model, in relation to the use of potential phytochemicals and macro-nutrients for satiety effects. The beverage formulation and the satiety measurement protocol can be applied for future *in-vivo* testing of other phytochemicals and macro-nutrients. The research target is to deliver validated satiety effects of phytochemicals and macro-nutrients through whole foods or food products. This may translate into information/advice on weight management and lifestyle changes, may contribute to the production of specialized food products, may increase consumer choices for healthier foods, and may in turn contribute towards lowering levels of obesity and its associated health problems such as cardiovascular disease and type 2 diabetes.

Chapter 2
Literature Review

2.1 Introduction

A review of literature relating to appetite control was carried out with the following objectives:

1. To define satiation and satiety and their methods of measurement.
2. To review food ingredients that could enhance satiety, particularly dietary fibres, proteins and plant-based ingredients.
3. To determine the appropriate method for satiety measurement and suitable food ingredients for enhancing satiety of the model food.

2.2 Appetite control: Satiation and Satiety

2.2.1 Defining satiation and satiety

It has been a well-known fact that maintaining a healthy body weight and achieving energy balance is important for human survival. Controlling energy intake is vital to energy balance, and satiation and satiety are part of a complex appetite control system, which are involved in regulating food consumption or energy intake (Benelam, 2009).

Satiation is the process or feeling of fullness during a meal or eating episode which leads to the termination of eating. It may be accompanied by a feeling of satisfaction and is also known as intra-meal satiety. Satiety is the process or feeling of fullness after a meal that persists, inhibiting further eating and delaying hunger. It is also known as inter-meal satiety or post-ingestive satiety. Satiation and satiety therefore have a significant impact on energy balance, and enhancing them could facilitate the reduction of energy intake and the control of body weight (Benelam, 2009; Blundell et al., 2010).

Other than satiation and satiety, appetite and hunger are terms commonly used in the area of appetite control. Blundell and colleagues (2010) defined appetite as: (1) the entire field of food intake, selection, motivation and preference, and (2) the qualitative aspects of eating, sensory aspects or responsiveness to environmental stimulation, which can be compared to the homeostatic view based on eating responses such as physiological stimuli and energy deficit. They also defined hunger as the intervening

variable that indicates the drive to eat. It is not directly measurable but can be inferred from objective conditions. Another definition of hunger is the conscious sensation reflecting a mental urge to eat, and can be traced to changes in physical sensations in parts of the body (stomach, limbs or head) (Blundell et al., 2010).

2.2.2 Factors affecting satiation and satiety

Satiation and satiety can be affected by several factors; the 'Satiety Cascade' (Figure 1) presented by Blundell and colleagues (2010) provides a conceptual framework for examining the effects of foods from start of eating to late satiety. As shown in the figure, satiation and early satiety are initially affected by sensory factors such as the smell, taste and texture of the food or drink, and cognitive factors including expectations of the meal and associations with previous experiences (Benelam, 2009; Blundell et al., 2010). Once food or beverage reaches the stomach, post-ingestive factors such as distension of the stomach, sending of signals to the brain and initiation of satiation take place. Ghrelin, a peptide hormone mainly produced in the stomach associated with hunger and meal initiation, is thereby suppressed. As digestion occurs in the intestines, hormones such as cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1) and peptide YY (PYY) that promote satiation and satiety are released from the gut. Subsequently in the post-absorptive stage, nutrients are detected by receptors in various sites of the body and brain, conveying information about the nutrient status, which also affects satiety. In the longer term, satiety may also be affected by signals such as leptin, which provide information on the amount of fat stored in the body (Benelam, 2009; Blundell et al., 2010).

The 'Satiety Cascade' has demonstrated that the development of satiation and satiety involves several complex physiological mechanisms in the body. Modulation of satiation and satiety is affected by the sensory quality, physical structure, energy density and macronutrient composition of the foods or beverages we consumed (Blundell *et al.*, 2010). However, other than satiation and satiety, energy intake (what foods and how much we consume) is also affected by many other factors including the palatability of the food, variety of the meal, portion size provided, time of the day, level of physical activity, dietary restraint, prior knowledge and beliefs about test foods, and presence of other people (Benelam, 2009). Thus, when carrying out an appetite study, it is important to consider physiological, behavioural and environmental factors that influence satiation, satiety, eating behaviour and energy intake. Blundell and colleagues (2010) and Benelam (2009) have several important factors to consider when measuring satiation and satiety, which are summarized in Table 1 below.

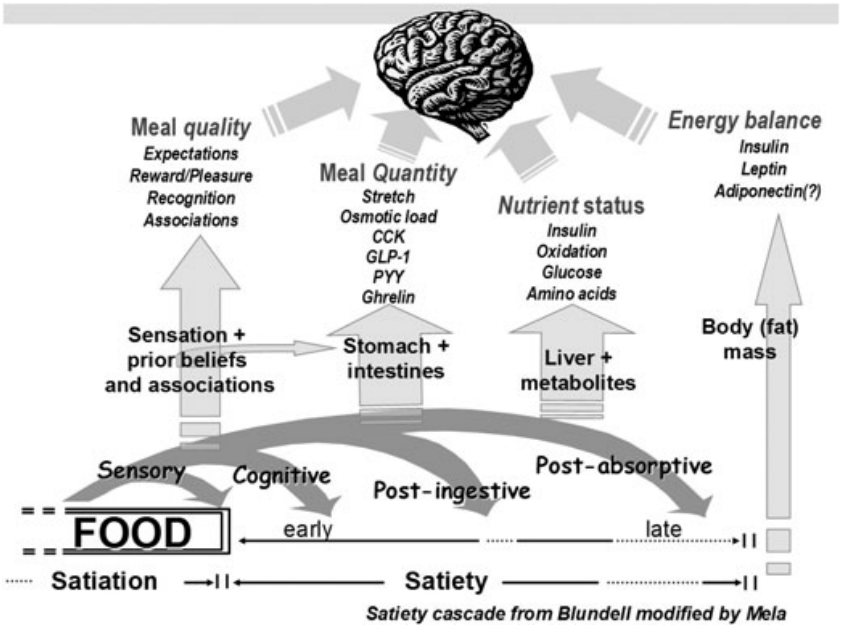


Figure 1 The 'Satiety Cascade' linking the timing and sequence of eating motivations and behaviours to associated cognitive and physiological processes (Source: Blundell *et al*, 2010)

Table 1 Factors affecting satiation and satiety (Adapted from Benelam, 2009; Blundell *et al.*, 2010)

Factor	Effect	Implication
Sensory – variety of the meal	'Fullness' and 'boredom with taste' are two major reasons to stop eating; consumption of a single food is more likely due to boredom of taste whereas composite dishes/meals is related to fullness and ending of the eating occasion. Sensory specific satiation – decline in reward value during consumption of a food due to repeated exposure to a particular sensory signal, also refers to boredom with taste of a particular food. It is responsible for the drive for variety and food choice.	Since satiation determines the meal size or amount of food intake, measurement of *ad libitum* (without restraint) food intake should consider sensory specific satiation – whether to restrict variety of foods eaten to stimulate satiation or to add variety of foods to stimulate food intake by avoiding sensory specific satiation.
Sensory – palatability of food / meal	Increasing the palatability of food (*e.g.* adding fat) increases appetite, meal size, meal duration and eating rate. The most palatable foods tend to be the least satiating and vice versa. Higher energy-dense foods tend to be more palatable than lower energy-dense foods.	It is important to control composition and energy density of test foods and to conduct pilot studies to test their palatability, ensuring that these test foods have similar 'liking' when studying the effect of particular food properties on satiation. In addition, the motivation/desire to eat or 'wanting' of foods should also be considered.

Table 1 *(Continued)*

Factor	Effect	Implication
Sensory – texture of food	Comparing *ad libitum* food intakes, we tend to consume higher amounts of more liquid foods than of more solid foods. This is related to the rate of eating, higher in liquids than in (semi-) solids.	It is important to match test foods for texture when studying the effect of particular food properties, unless texture variation is of interest.
Environmental (portion size) + Cognitive	In real life most meals/snacks are terminated through environmental factors/cues such as portion size. The amount of food we eat (or when to stop eating) can be influenced by visual cues concerning the emptiness of the plate/container containing the food. In most cases we finish our plate. Thus, greater portion size generally increase energy intake.	Subjects must be informed to eat till comfortably full during *ad libitum* food intake and they are not required to eat all the food provided.
Cognitive – learned response (expectations)	Learning mechanisms based on life-long food consumption determine our expectations about the satiating properties of foods and the amount of food we would eat for a meal. This affects how much we eat ad libitum in experimental situations. Sensory mediated satiation/satiety – signals that relate to learned satiety phase response issues; when tasting a food, people know instantly something about its satiety value based on previous experience. It is the sequence of sensory specific satiation that is linked to its metabolic consequences. The energy density of foods has a crucial role in our expectations of satiation. We tend to consume higher amount of low-energy dense foods than high-energy dense foods to feel satiated.	Subjects must be informed to eat till comfortably full during ad libitum food intake. It is important to match test foods for energy density when studying the effect of particular food properties.
Cognitive – knowledge about the time until the next meal	We tend to take into account future availability of food when deciding on current consumption; tendency to eat more when we know we have no access to food in the next few hours compared to having access to food in a short while.	It is important to inform subjects on the test procedure / schedule prior to start.
Cognitive – motivational state of subjects	This affects the amount of food that people eat, more when hungry and less when satiated.	It is important that subjects have a similar state of satiety when presenting them with an *ad libitum* meal.

Table 1 *(Continued)*

Factor	Effect	Implication
Sleep	Chronic lack of sleep has an impact on appetite hormones; decreased leptin and increased ghrelin levels result in stimulating hunger and increasing appetite.	Ensure subjects used in appetite study do not have sleep restrictions.
Physical exercise	Studies have found that an acute bout of physical exercise does not result in a compensatory increase in hunger and energy intakes, even when large energy deficits are induced. In contrast, studies that have induced negative energy balance by reducing energy intake resulted in increased appetite and energy intake. Intense physical activity appears to suppress hunger for short periods.	It is important to standardize physical activity/state of subjects prior to testing in order to ensure compatibility in glycogen stores.
Social situations	People generally consume more food and have longer meal duration when eating with others than when eating alone.	Depending on the variable of interest, the appetite study can either free-living or laboratory controlled.
Television viewing or other distractions	When distracted by television or other means, people tend to be less responsive to satiety signals and therefore consume more food.	Depending on the variable of interest, the appetite study can either free-living or laboratory controlled.

2.2.3 Measuring satiation and satiety

Satiation is measured through the measurement of *ad libitum* (freely; without restraint) food intake of particular test foods/meals (weight in grams or energy in kcal or kJ). Subjects are allowed to eat *ad libitum* and the quantity of food they consume to reach satiation is recorded, in comparison to a control food/meal. Satiety can be measured through self-reported measures of appetite, for example using visual analogue scales, which allow subjects to rate and record their feelings of hunger, fullness, satiety, desire to eat and prospective consumption. In addition, satiety can also be measured through the measurement of *ad libitum* food/energy intake (Benelam, 2009; Blundell *et al.*, 2010). Some of the principles and methods of measurements for satiety studies are outlined below.

2.2.3.1 Free-living versus laboratory studies

As mentioned earlier, there are several factors that could affect eating behaviour, satiation and satiety. Thus, satiety studies are usually performed in a controlled

laboratory setting under standardized conditions. Nevertheless, some satiety studies employ free-living subjects. Free-living studies theoretically have high external validity or naturalness while laboratory-based studies generally have high internal validity or precision. It is necessary to compromise on the requirements for internal and external validity; between precision and naturalness (Blundell *et al.*, 2010).

Free-living studies have high external validity, with results more applicable to the 'real world' and relevant to free-living populations. However, free-living studies have a number of methodological problems that limit their internal validity (precision). Errors in data collection are high, particularly measurements of self-reported dietary intakes are prone to bias, usually underreporting of energy intakes and misreporting of macronutrient consumption. Furthermore, there is a lack of control over the subjects' environment which may lead to difficulties interpreting results and making conclusions about the effects of the dietary manipulation of interest (Benelam, 2009; Blundell *et al.*, 2010).

Controlled laboratory studies have high internal validity because they offer the highest degree of sensitivity and control over environmental/external factors and the outcome measures of interest (Blundell *et al.*, 2010). Although the results of controlled laboratory studies are of high precision, extrapolating these results to free-living subjects, where conditions are of less rigorous control, may be met with undetermined relevancy. Nevertheless, the vast majority of satiety studies have been conducted in the laboratory under controlled conditions, as it is easier to obtain meaningful results than studies in uncontrolled conditions (Benelam, 2009).

2.2.3.2 Preload study design

The preload study design is often used in satiety studies to measure the effects of a particular variable or variables on the short-term regulation of food intake and appetite, typically carried out in part or all of a single day. The design involves first giving subjects a preload food or drink where the variable of interest is manipulated in order to monitor subsequent effect(s) *e.g.* satiety over a period of time, *ad libitum* food/energy intake (Benelam, 2009; Blundell *et al.*, 2010). In most cases when studying the effect of food properties or composition on satiation and satiety, it is necessary to vary one factor while holding other important factors constant (Blundell *et al.*, 2010). The preload test design is best conducted using (Benelam, 2009; Blundell *et al.*, 2010):

1. within-subject or repeated measures design (all control and test preloads will be given to an equal numbers of subjects within a test period),

2. double-blind conditions (neither experimenter nor subject aware of the identity of the foods presented),
3. control conditions (either by a non-preload or a placebo treatment), and
4. crossover procedure (subjects consume the control or test preload(s) on separate days/occasions).

Visual analogue scales (VAS), a type of self-reported measure of appetite, are widely used for subjects to rate and record their feelings of hunger, fullness, satiety, desire to eat and prospective consumption before and at pre-determined intervals after the preload (and test meal) is taken to monitor their changes in appetite. Table 2 shows the primary VAS for self-reported appetite in healthy adults, recommended by Blundell and colleagues (2010). At the end of the pre-determined time interval after the preload, test meals are given to the subjects and their food (energy) intake is measured, or they may self-report their own food intake. Depending on the study, subjects may be given another meal to test the effect of the preload over a longer time period or they may be asked to self-report their food intake for the rest of the day (Benelam, 2009; Blundell *et al.*, 2010). There are several issues to consider when using the preload study design, which include those listed in Table 3 below.

Table 2 Recommended primary scales for self-reported appetite in healthy adults, using line scales of 100 or 150 mm on paper or appropriate length for electronic capture systems (Adapted from Blundell *et al*, 2010)

Scale	Question	Anchors	
		Low	High
Hunger	How hungry are you?	Not at all	Extremely
Fullness	How full are you?	Not at all	As hungry as I have ever felt
Satiety	How satiated are you?	Not at all	Extremely
Desire	How strong is your desire to eat?	Very weak	Very strong
Prospective consumption (quantity)	How much do you think you could (or would want to) eat right now?	Nothing at all	A very large amount

Table 3 Issues and considerations when using preload study design (Adapted from Benelam, 2009; Blundell *et al.*, 2010)

Preload issue	Considerations
Matching the test and control preloads for taste, appearance and other sensory properties	This is to minimise/prevent the subjects from being able to differentiate the preloads. It is important to test palatability of the foods as it could affect food intake and satiety. However, this depends on the test variable of interest.
Standardization of preloads in terms of their energy content and density, macronutrient composition, physical state (solid vs. liquid) and weight or volume	Lack of standardization is one of the main reasons why preload studies often have highly variable outcomes.
Appropriateness of the preload (food) for that time of the day	This is important since eating is a function of the time of the day.
Deciding whether the test variable (outcome) should be the size of the test meal or the time taken before eating the test meal is required	The effect of the preload is measured as meal size (grams of food, energy intake) or time (minutes). Although both measures are suitable, meal size is more frequently used.
Time interval between preload and test meal	30 minutes or less is suitable to study the sensory, cognitive or gastrointestinal factors; whereas longer time (4 – 5 hours) interval is needed to measure post-absorptive effects on satiety. Differences in preload-test meal time interval can lead to quite different outcomes, thus it is necessary to justify the time interval selected in study protocols.
Sensitivity of test meal(s) to the test variable of the preload and the expected outcome (decreased or increased intake)	The composition of the test meal served after the preload can differ in the variety of foods offered – buffet style (various foods) or single course (one-dish meal). Buffet style test meal allows assessment of the effect of the preload variable on food choice and nutrient intakes, even if energy intake remains the same between two experimental conditions. However, this method does not represent the usual eating pattern of most people and the presence of a variety of foods is likely to delay satiation, stimulate appetite and enhance increased food intake. Single course test meal allows assessment of the effect of the preload variable on food and energy intakes rather than nutrient intake; thus it is used to assess short-term energy compensation, especially when no difference in food choice is expected from the preload variable.

2.2.3.3 Covert vs. overt experimental protocol

Satiety studies can be conducted either with covert or overt manipulations of diets or meals, depending on the intended outcomes. Other than the (preload and) test foods, subjects could have access to covertly manipulated experimental foods or have overt access to their usual, everyday foods. Studies that have used covertly manipulated foods/diets, ranging from a few days to a few weeks, are characterized by a general tendency to eat a constant quantity of food across treatments, brought about by learned associations between the weight and volume of familiar foods with the physiological consequences of ingesting those foods. In contrast, more immediate and complete compensation of energy was observed in studies following the overt experimental protocol, where cognitive and/or learning responses play an important role (Blundell *et al.*, 2010).

Although more studies favoured the covert experimental protocol, focus should also be given to the comparison of covert and overt manipulations using familiar foods, in order to differentiate whether feeding responses is attributed to the nutritional manipulation itself or to the covert or overt nature of experimental protocol (Blundell *et al.*, 2010).

2.2.3.4 Common models and designs of satiety studies

According to Blundell and colleagues (2010), there are several models and designs used for satiety studies and these are summarized as follows:

Acute food intake model: The effect(s) of a food or meal on satiation or satiety is studied through a single administration of the food on a single occasion. It is often assumed that the same effect would be observed on all future occasions if the food continued to be eaten on a daily basis.

Two-choice model (*e.g.* protein and carbohydrate) or **Three-choice model** (*e.g.* protein, carbohydrate and fat) model: Methods for measuring preferences for particular macronutrients and finding the determinants of food choice, achieved by restricting choices.

Simultaneous-choice model: Used for assessing food selection in the laboratory where the independent variable (*e.g.* macronutrient selection) is manipulated across multiple choices within one meal (*e.g.* high-protein vs. high-carbohydrate foods), generally considered a reliable measure of immediate energy intake and food selection.

Sequential-choice model: Also used for assessing food selection but the independent variable is manipulated across two or more meals, consumed on separate days.

Latin square design or Williams design: Experimental design that is completely balanced and randomized, balances the treatments over periods and subjects in such a way that is preceded by every other treatment an equal number of times. For possible carry-over effect, use the Williams design.

Within-subject, repeated measures design: In this design, each subject will receive all treatments once, and within a period, all treatments will be given to an equal number of subjects. For possible carry-over effect, the Luca's extra period design is used, to estimate both the direct effects (treatment effects) and extra effects (*e.g.* residual effect).

Between-subjects design: In this design, each subject will receive only one treatment, and the results from one treatment group are compared to that of the other treatment group(s).

Balanced incomplete block design: Used when more treatments are desired within a single experiment, each subject gets a subset of treatments, thus reducing the number of treatments per subject to a feasible level.

2.2.3.5 Types, reliability and validity of self-report scales in satiety studies

Self-report scales such as the visual analogue scale (VAS) were originally developed in the field of pain research and today they are commonly used in satiety research, which enable subjects to rate their feelings related to hunger, appetite and satiety. These scales are usually completed before and after consumption of the test food, and at regular time intervals (15-30 minutes, up to 1 hour), for 3 to 5 hours, or to the start of the next meal (Blundell *et al.*, 2010).

Types of scale

The most common self-report scale is the VAS (example in Table 2) that has unipolar unstructured line scales anchored by terms such as 'None' or 'Not at all' to 'Extremely' or 'As much as I have ever felt' in response to questions such as 'How hungry are you?' (Blundell *et al.*, 2010). The subjects will make a mark on each of the continuous line scale (100 or 150 mm on paper) for their rating of hunger, fullness, satiety, desire

to eat and prospective consumption. Measurements of length marked along each scale provide the quantitative data for mathematical and statistical analysis.

Similar in principle as VAS, category scales also require subjects to rate their feelings of hunger, satiety, etc in response to questions. However, instead of a continuous line, numbered categories (usually 1 to 9) are used. For example, 'How hungry are you?' 1 = not at all, 9 = extremely hunger or 1 = extremely hungry, 9 = extremely full (Benelam, 2009). Both line and category scales have the same theoretical problem that is the scored values do not necessarily reflect perceptual distances and do not have mathematical properties of true ratio scale *e.g.* distance between units 1 and 2 on the scale should not taken as equivalent to the distance between units 3 and 4, and a score of 50 mm along the line scale (of 100 mm) does not necessarily represent half the magnitude (Benelam, 2009; Blundell *et al.*, 2010).

The problem of line and category scales may have been overcome by The Satiety Labeled Intensity Magnitude (SLIM) scale (Figure 2) developed by Cardello and colleagues (2005). The SLIM scale was found to be more sensitive and reliable when compared to VAS, but it still requires further development before it can be widely used (Benelam, 2009; Blundell *et al.*, 2010). Nevertheless, self-report scales especially VAS remain one of the widely used tools in satiety studies, and often in conjunction with measures of food and energy intakes (Benelam, 2009).

Reliability

Under controlled laboratory conditions, use of VAS has been found to have variable repeat-reliability with individual subjects (*e.g.* single time points) but generally have good repeat-reliability with regard to group mean data (*e.g.* averaged values of ratings over several hours or area under the curve (AUC)) and comparisons of specific foods (*e.g.* composite scores), even over several months or years (Blundell *et al.*, 2010).

According to Blundell and colleagues (2010), 20–25 subjects are generally sufficient to capture a 10% difference in mean or AUC appetite ratings between foods, under good experimental conditions. A 10% difference or reduction (when compared to control) is a reasonable and realistic difference (Blundell *et al.*, 2010; Flint *et al.*, 2000). More subjects will be needed if there are more comparisons, more test foods, or for between-subjects design in a study (Blundell *et al.*, 2010).

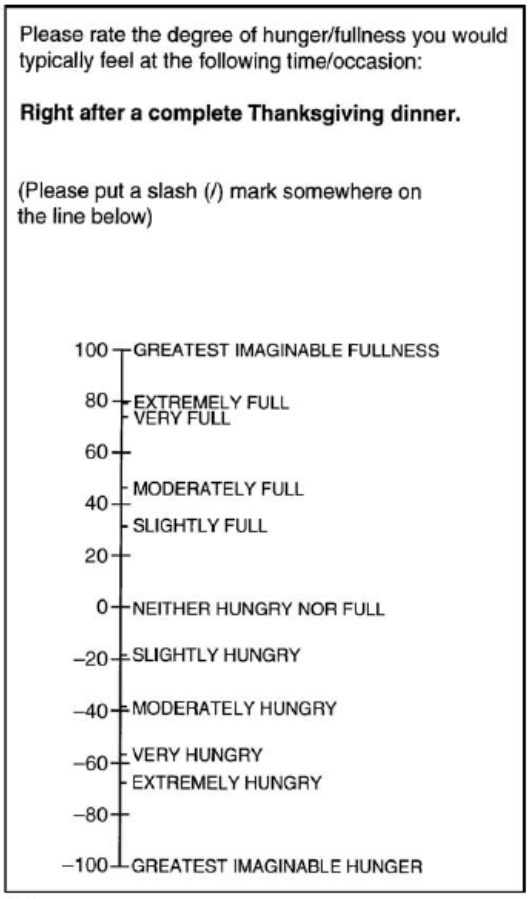


Figure 2 The SLIM scale (Source: Cardello *et al.*, 2005)

Validity

The validity of self-report scales is difficult to determine, as there is no objective measure of appetite for comparison (Flint *et al.*, 2000). Nevertheless, self-report scales are generally: (1) responsive to major characteristics of meals *e.g.* volume and energy load, (2) responses usually align in time and magnitude with corresponding postprandial physiological processes *e.g.* stomach filling and the movement and uptake of bulk and nutrients, and (3) variations in responses on such scales are 'normal' since there is no evidence that such variations are primarily reflecting or biased towards something other than the intent. Therefore, self-report scales have 'face validity' *i.e.* they faithfully show general agreement among people in certain experiences (of hunger, satiety, etc.) along a continuum, and allow for distinctions to be made within these (Blundell *et al.*, 2010). Methods to determine the validity of self-report satiety scores (data) is to correlate subsequent energy intake to: (1) the pre-meal satiety scores, (2) the change of satiety scores during the *ad libitum* meal (pre-post difference), and/or (3) the averaged values of satiety scores over several hours after consuming the test food (Flint *et al.*, 2000).

2.2.3.6 Confounders in satiety studies

The selection of human subjects for satiety studies is of high importance and would depend on the hypothesis or test variable of the study. There are some possible confounding factors to consider, including those of physiological (body mass index, age, gender) and behavioral (*e.g.* habitual diet, physical activity, etc) factors (Benelam, 2009; Blundell *et al.*, 2010), as summarized in Table 4.

2.2.3.7 Biomarkers of satiation and satiety

As noted from the 'Satiety Cascade', several gastrointestinal hormones or satiety-related peptides are involved in the regulation of appetite and food intake. Biochemical measures of blood plasma concentrations of CCK and GLP-1, as well as physical and chemical measures of stomach distension, could serve as useful biomarkers of satiation (meal termination). Studies have shown that higher concentrations of CCK and GLP-1 and greater stomach fullness correlate with lower subjective hunger ratings and lower food intake. On the other hand, measuring the plasma concentration of ghrelin and the rate of gastric emptying (the process by which food leaves the stomach and enters the duodenum) are excellent biomarkers of satiety (and meal initiation). Lower concentration of ghrelin and slower rate of gastric emptying correlate to higher subjective satiety ratings and longer time leading to the next meal (Benelam, 2009; de Graaf *et al.*, 2004).

Table 4 Confounders in satiety studies (Adapted from Benelam, 2009; Blundell *et al.*, 2010)

Confounders	**Key points**
Body mass index (obese or lean)	The energy requirements of obese people are generally higher than those of lean people, thus there would be differences in appetite ratings and energy intake. Some studies have suggested differences in some aspects of physiological appetite control (*e.g.* insulin levels, insulin sensitivity, genetics) between lean and obese people that could influence the results of satiety studies. When making claims about the effects of a food (product) in overweight or obese people, evidence from studies on these specific groups of people should be presented.
Age	The age of subjects may have an impact on sensory specific satiety. Sensitivity to sensory specific satiety seems to decline with age. For studies using buffet-style test meal, consideration on the age group of subjects is therefore important.

Table 4 *(Continued)*

Gender (men and/or women)	Women have lower energy requirements than men and are likely to eat less (lower energy intake). Women of childbearing age would have fluctuations in their energy intake within the menstrual cycle. These should be noted and controlled.
Habitual diet, alcohol consumption and physical activity	The study may exclude subjects who are significantly different from others in their habitual diet, alcohol consumption and physical activity. Subjects may be asked to refrain from alcohol consumption and physical activity one day before a study. They may also be asked to fast for several hours before the study. It may be necessary to control/standardize the diet of subjects who are not in energy balance prior to the study *e.g.* obese, restrained eaters.
Dietary restraint	This group of people has the tendency to avoid certain foods and restrict food intake in order to lose or maintain bodyweight. Dietary restraint may be associated with dieting behaviour such as binge eating, where control over eating is lost. Researchers commonly use questionnaires to access potential dietary restraint behaviour in subjects, such as the Dutch eating behaviour questionnaire (van Strien *et al.*, 1986) or the Three factor eating questionnaire (Stunkard & Messick, 1985). It is a standard practice to exclude subjects with a restraint score >13 (sometimes >11) from satiety studies.
Prior knowledge and beliefs about test foods	Some studies *e.g.* to test various formulations would require several visits to the laboratory by the same group of subjects, who can be accustomed to the experimental conditions and know what is expected of them, hence may affect their satiety responses. Subjects may still be able to detect differences between the control and test meals or preloads, thus it is important to match sensory properties of the test foods as closely as possible.

2.3 Food Ingredients for Enhancing Satiety

As mentioned earlier, modulation of satiation and satiety is affected by the sensory quality, physical structure, energy density and macronutrient composition of foods or beverages we consumed (Blundell *et al.*, 2010). Regarding the macronutrient composition of foods, there is evidence that proteins and specific fibres (viscous or gelling) have a greater impact on satiety than other macronutrients (Blundell *et al.*, 2010). Energy from proteins seems to have a larger effect on satiety than that from carbohydrates or fats (Benelam, 2009; Kleef *et al.*, 2011). Viscous fibres such as pectin and alginates appear to be more satiating than soluble fibres (Benelam, 2009; Slavin &

Green, 2007). The effects of proteins and/or fibre on satiety are heavily dependent on the types and amounts used and the background food matrix (Benelam, 2009; Blundell *et al.*, 2010).

The background food matrix refers to the energy density and physical structure of a food. Energy density is defined as the energy (kcal or kJ) per unit weight of a ready to eat food. Air, water and fibre are main constituents that can lower the energy density of a food (Kleef *et al.*, 2011). Generally, foods with high energy density tend to be more palatable but less satiating, whereas foods with low energy density are less palatable yet more satiating (Benelam, 2009; Kleef *et al.*, 2011). Studies found that water, when consumed as part of a food, has a greater effect on satiety than when it is consumed with a food (Kleef *et al.*, 2011). This is also related to the physical structure of a food; texture changes with varying water content *i.e.* solid, semi-solid or liquid. Studies have shown that the texture of foods influences satiety as well (Benelam, 2009; Kleef *et al.*, 2011). It has been suggested that solid foods or foods with a chewier, denser structure (*e.g.* apple) have a larger effect on satiety than liquid foods or drinks (*e.g.* apple juice) (Flood-Obbagy & Rolls, 2009; Kleef *et al.*, 2011). On the other hand, studies have shown that soups produced similar or larger effect on satiety when compared to energy-matched solid foods, thereby indicating the observed effects are not directly related to liquid or solid food structure (Benelam, 2009). Although findings are contradictory, the physical structure (texture) of foods remains an important factor on satiety. It is also noteworthy that the viscosity of liquids or semi-solids can affect the expected satiation (Hogenkamp *et al.*, 2011) and satiety (Benelam, 2009; Mattes & Rothacker, 2001). In addition, gel formation and viscosity imparted by viscous fibres can delay gastric emptying, prolong the absorption of nutrients and increase satiety (Benelam, 2009; El Khoury *et al.*, 2012; Mälkki & Virtanen, 2001; Wanders *et al.*, 2011). This section of the review focuses on fibres, hydrocolloids, proteins and novel ingredients for enhancing satiety.

2.3.1 Fibres and hydrocolloids

Dietary fibres, like proteins, fats and carbohydrates, vitamins and minerals, constitute an essential, healthful part of our daily diet. As defined by Food Standards Australia New Zealand, ‘dietary fibre’ refers to ‘the fraction of the edible part of plants or their extracts, or synthetic analogues that: (1) are resistant to the digestion and absorption in the small intestine, usually with complete or partial fermentation in the large intestine and (2) promote beneficial physiological effects (laxation, reduction in blood cholesterol and/or modulation of blood glucose). It included polysaccharides, oligosaccharides

(degree of polymerization >2) and lignins (FSANZ, 2001). In addition, fibre-rich diets are considered to bring about five main beneficial physiological effects (Lunn & Buttriss, 2007):

1. improvements in gastrointestinal health,
2. improvements in glucose tolerance and insulin response,
3. reduction of hyperlipidaemia (excess blood lipids), hypertension and other coronary heart diseases risk factors,
4. reduction in the risk of developing some cancers, and
5. increased satiety and hence some degree of weight management.

Many studies have been conducted to investigate the effects of dietary fibres on satiety. Epidemiological studies have shown that a higher intake of dietary fibre is associated with smaller waist circumference and lower body weight, and controlled intervention studies have shown that dietary fibre intake may reduce subjective appetite, energy intake and body weight (Kleef *et al.*, 2011; Slavin & Green, 2007; Wanders *et al.*, 2011). Generally, studies that used high doses of fibre (*e.g.* 10g or more in one dose or 30g over the course of a day) had more positive results (Benelam, 2009; Perrigue *et al.*, 2010). In New Zealand and Australia, dietary recommendations for adults on fibre intake per day are 25g as 'adequate intake', 28g as 'suggested dietary targets' and 25–30g as 'New Zealand dietary goals' (Department of Health, 1991; NHMRC/MOH, 2006).

The suggested mechanisms by which dietary fibres affect appetite, energy intake and body weight include (Slavin & Green, 2007; Wanders *et al.*, 2011):

- dietary fibre reduce the energy density of foods (displaces calories and nutrients), which may directly lead to reduced energy intake and indirectly reduced appetite,
- fibre-rich foods generally take longer to chew, which may increase sensory specific satiety and reduce the meal size,
- dietary fibres may decrease intestinal passage rates, leading to a more gradual nutrient absorption and prolonged feelings of satiety,
- dietary fibres may decrease energy absorption by lowering the bioavailability of fatty acids and proteins, and
- dietary fibres can be fermented in the colon, which increases the concentration of short chain fatty acids, which may enhance satiety via various mechanisms.

There are many types of dietary fibres and it is understood that different fibres would have different or varying effect on satiety. The effect that a particular type of fibre has on satiety depends on its physicochemical properties when consumed and its physiological effects in the gastrointestinal tract (Benelam, 2009; Wanders *et al.*, 2011). In their review, Wanders and colleagues (2011) have grouped dietary fibres based on their chemical structure (*e.g.* glucose polymers with alpha linkages, polymers mainly consisting of mannose) or unique origin (*e.g.* marine polysaccharides, chitosan). In addition, the fibres were classified based on their physicochemical properties as being: (1) more fermentable (whether the fibres are fermented by anaerobic bacteria in the colon), (2) more soluble, and/or (3) more viscous (whether the fibres impart viscosity or are gel-forming) than others. An adaptation of Wanders and colleagues (2011)'s grouping of fibres is shown in Table 5.

The systematic review by Wanders and colleagues (2011) revealed that for appetite, acute energy intake, long-term energy intake and body weight, there were clear differences in effect rates depending on the physicochemical properties of dietary fibres. Key findings summarized from the review include (Wanders *et al.*, 2011):

- Fibre groups with the largest proportion (%) of appetite-reducing effects were pectins (100%), pectin-rich fibres (100%), glucans (62%), mannans (50%) and marine polysaccharides (50%).
- Averaged over a 4-hour time interval, more viscous fibres reduced appetite more often than those less viscous fibres, by 7.4% and 1.3%, respectively, at a mean fibre dose of 8.1g. Similar but less pronounced effects were found for more soluble fibres compared to less soluble fibres. As for more- and less-fermentable fibres, the effect rate was similar.
- Across fibre groups, reduction in appetite ratings was 0.18% per gram increase in fibre intake. As for viscous fibres, this reduction was the greatest (0.41%).
- Fibres provided as liquids reduced appetite by 6.6%, over 4 h, compared to 3.1% when fibres were provided as solids. The hydration rate of dietary fibres may differ depending on the food matrix, which might also explain differences in physiological effects within the group of viscous fibres.
- Fibre types with the highest effect rate for acute energy intake were β-glucan-rich fibres (100%), resistant starch (100%), dextrins (100%) and pectins (100%).

Table 5 Grouping of fibres and their assumed physicochemical properties (Adapted from Wanders *et al*, 2011)

	Grouping based on chemical structure and/or unique origin		Assumed physicochemical properties			
			More fermentable	More soluble	More viscous	Modification
Isolated fibres	Glucans (glucose polymers with β-linkages)	Cellulose				✓
		Methylcellulose	✓			
		Ethyl hydroxyl ethyl cellulose		✓	✓	
		β-glucans	✓	✓	✓	
	Resistant starch (glucose polymers with α-1,4 linkages)	Type 2	✓			
		Type 3	✓			
	Dextrins (glucose polymers with other α-linkages)	Dextrins	✓	✓		
		Polydextrose	✓	✓		
		Reuteran	✓	✓		
		α-cyclodextrin	✓	✓		
	Mannans (mainly mannose)	Guar gum	✓	✓	✓	✓
		Locust bean gum	✓	✓	✓	
		Fenugreek gum	✓	✓	✓	
		Konjac glucomannan	✓	✓	✓	
	Fructans (mainly fructose)	Inulin	✓	✓		
		Fructo-oligosaccharides	✓	✓		
	Xylans (mainly xylose)	Arabinoxylan	✓			
		Xylo-oligosaccharides	✓	✓		
	Pectins (mainly galacturonic acid)	Pectin	✓	✓	✓	✓
	Chitosan	Chitosan			✓	
	Marine polysaccharides	Alginates		✓	✓	
		Carrageenan		✓	✓	
		Agar		✓	✓	
Complex fibres	Pectin-rich	Sugar beet fibre		✓	✓	
		Lupin kernel fibre		✓	✓	
	Arabinoxylan-rich	Psyllium fibre		✓	✓	
		Wheat fibre				
		Rye fibre				
		Corn fibre				
	β-glucan-rich	Oat fibre		✓	✓	
		Barley fibre		✓	✓	

- Fibre types with the greatest number of comparisons showing a reduction in long-term energy intake were arabinoxylan-rich fibres (88%), mannans (83%), fructans (80%) and resistant starch (67%).
- Fibre types with the highest effect rate for long-term body weight reduction were dextrins (100%), marine polysaccharides (100%), chitosan (86%), fructans (67%) and arabinoxylans (67%).

Based on the review by Wanders and colleagues (2011) and other literature (Benelam, 2009; Cho *et al.*, 2009; El Khoury *et al.*, 2012; Kleef *et al.*, 2011; Slavin & Green, 2007), it is assumed that viscous fibres are more effective in promoting satiation, increasing satiety and reducing short-term energy intake. Most of these viscous fibres such as pectins, mannans (*e.g.* guar gum, locust bean gum) and marine polysaccharides (*e.g.* alginate, carrageenan) are hydrocolloids, which are also commonly used food ingredients or additives for their thickening, gelling and/or stabilizing functionality. Viscous fibres typically have good or complete solubility in water. The soluble portion of viscous fibres was initially believed to be the contributing factor for effects of satiety. However, many of the more recent trials using soluble fibres that are not viscous (*e.g.* inulin, fructooligosaccharides, polydextrose) found no effect on satiety or hunger, even when large amounts of these soluble fibres were fed. Therefore, other than soluble fibre, insoluble fibre and viscosity have as much impact on satiety (Slavin & Green, 2007).

Several mechanisms of actions of viscous fibres on satiety effects have been proposed. Firstly, solutions of viscous fibre impart high viscosity, which may increase exposure time in the oral cavity and induce sensory specific satiety (Slavin & Green, 2007; Wanders *et al.*, 2011; Zijlstra *et al.*, 2007). It was found that reductions in hunger were greater and more prolonged with the thicker shake (16000 cps) than the thin shake (600 cps) (Mattes & Rothacker, 2001). The *ad libitum* intake of milk-based products differing only in viscosity (liquid, semi-liquid and semi-solid) increased with decreasing viscosity (Zijlstra *et al.*, 2007). Secondly, viscous fibres have high water holding capacity and thereby increase stomach distension which may trigger afferent vagal signals of fullness (Wanders *et al.*, 2011). Thirdly, viscous fibres may delay gastric emptying, thereby prolonging the absorption of nutrients and extend the time available to stimulate pre- and post-absorptive mechanisms of satiety (Benelam, 2009; Lunn & Buttriss, 2007; Wanders *et al.*, 2011). Brownlee (2011) listed seven studies reporting consistent results that inclusion of viscous fibres, particularly pectins in liquid test meals can delay gastric emptying (Brownlee, 2011). Last but not least, the

increased viscosity of digesta in the small intestine can lead to prolonged presence of nutrients which in turn stimulates the release of appetite-regulating hormones (*e.g.* CCK, GLP-1, PYY) (Wanders *et al.*, 2011).

Among all dietary fibres, pectins, alginates and β-glucans appeared to be more promising for enhancing satiety. These ingredients are suitable for beverages and can be sourced locally. Several studies involving these viscous fibres in liquid test meals or beverages and their effects on satiety, are reviewed and presented in Table 6.

2.3.1.1 Pectins

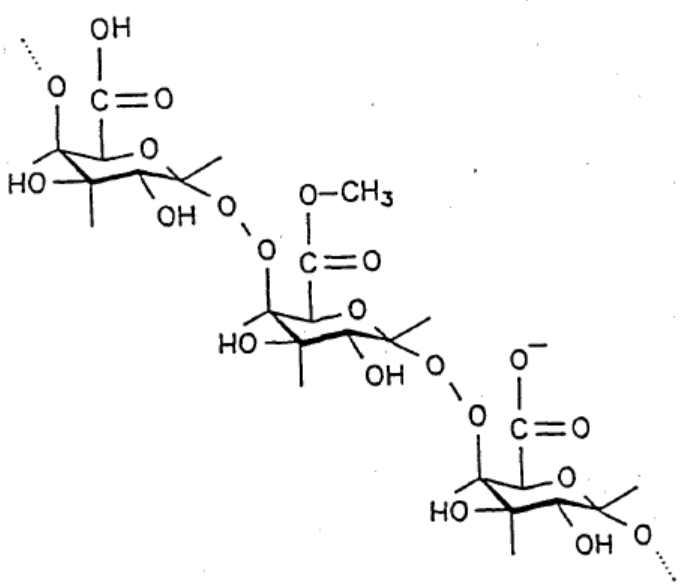


Figure 3 Chemical structure of pectin (a repeating segment of the molecule) (Source: Thakur *et al.,* 1997)

Pectins are high molecular weight (~50,000–150,000 Da) hetero-polysaccharides containing galacturonic acid units (at least 65% by weight) and a range of neutral sugars including rhamnose, galactose and arabinose (Figure 3). The chemical structure of pectin is shown in Figure 3. Pectins are present in all plant primary cell walls and are commercially isolated from citrus peels (by-product from citrus juice and oil extraction) and apple pomace (dried residue from apple juice extraction). The most common method of pectin isolation is to use an organic solvent (methanol, ethanol or isopropanol) in which pectin is insoluble (EndreB & Christensen, 2009). After separating pectin from as much alcohol as possible, it is dried and ground into a fine powder. The resulting pectin have high methoxyl (HM) content *i.e.* its degree of esterification (DE) above 50% (normally 67–73% and up to 80%) and it will yield a

rapidly-setting gel under traditional jam-making (acidic and high soluble solids) conditions (EndreB & Christensen, 2009). On the other hand, low methoxyl (LM) pectins, with DE below 50% can be produced by de-esterification of HM pectins *i.e.* hydrolyzed under acidic or alkaline conditions or by methylesterases. LM pectins will form gel in the presence of calcium ions, under suitable pH (3.0 to above 5.0) and soluble solids (10 – 80%). Another type of pectins is the amidated pectins, which are produced by reaction of HM pectin with ammonia. Thus, the galacturonic acid group in pectins may be free, as methyl esters or as acid amides (EndreB & Christensen, 2009).

Pectins are commonly used in many foods including jams, fruit preparations for baked and dairy products, fruit jellies, yogurt, yogurt drinks, acidified milk drinks, desserts and juices. As shown in Table 6, pectins used in satiety studies have been dosed into orange juice, apple juice, flavoured beverages and an egg-sandwich meal with water. Effective dose of pectin fibre ranges from 4.8g to 30g; about 1.1%, 1.9% and 2.2% w/v showing increased satiety and/or decreased acute energy intakes, and about 1.0% w/v and 7.5% w/w showing delayed gastric emptying. The type of pectin fibres used in these studies was not clearly stated, but there was mention of using low viscosity pectins to minimize differences in viscosity, texture and appearance between preloads with and without the pectin addition.

Processing of food products containing pectin generally requires heating, which can affect the physico-chemical and functional properties of pectins (Thakur *et al.*, 1997). In acidic solutions, at low temperatures, de-esterification of pectin molecules occurs more rapidly, whereas at high temperatures, depolymerization is predominant. In alkaline solutions, saponification of the methyl ester groups occurs more rapidly at low temperatures, while depolymerization is predominant at high temperatures (Thakur *et al.*, 1997). Thermal degradation of pectin molecules in alkaline solution is due to β-elimination cleavage of the glycosidic linkage, which occurs at glycosidic bonds adjacent to an esterified carboxyl group. The rate of degradation (pH 6.1, 100°C) is affected by the DE of the pectin; higher rate of degradation with higher DE. The nature and quantity of ions and salts present in the system can also affect the heat degradation of pectin. For example, divalent cations caused more depolymerization than monovalent cations during the heating of pectin (Thakur *et al.*, 1997).

2.3.1.2 Alginates

Commercial production of alginates utilizes marine brown seaweeds (*Phaeophyceae*, mostly *Laminaria*), which contain up to 40% alginates dry weight. Sodium alginate is

the most common form of alginates; other soluble forms include potassium alginate, ammonium alginate and propylene glycol alginate. Alginates are linear binary copolymers of β-(1→ 4)-linked D-mannuronic acid (M) and α-(1→ 4)-linked L-guluronic acid (G) residues (Draget, 2009). Depending on the source, alginates have different M/G ratios and block distributions *e.g.* MMMM, GGGG and MGMGMG blocks, and hence variations in their gel properties. The chemical structure of alginates is shown in Figure 4. Typically, high G alginates are highly sensitive to calcium ions and produce strong brittle gels with good heat stability but are prone to syneresis on freeze-thaw. High M alginates produce weaker, more elastic gels with good freeze-thaw stability. Owing to its high water holding capacity, gelling and emulsification properties, alginates are used in many restructured meat-, surimi- and vegetable-based foods *e.g.* meat patties, sausages, pet food chunks, crabsticks, onion rings and stuffed olives. Other foods using alginates include pie fillings, low fat spreads, dressings, pâté, imitation caviar and acidic beverages (Draget, 2009). Similar to pectins, thermal processing of alginate-containing foods such as heating (high temperature), autoclaving and sterilization often cause polymer breakdown, depolymerisation, loss of viscosity and possibly reduced gel strength (Draget, 2009).

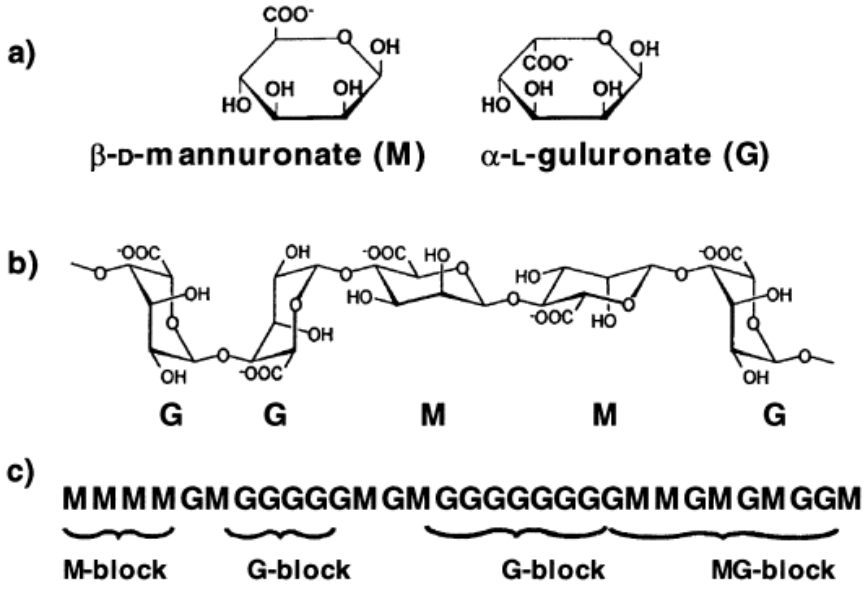


Figure 4 Chemical structure of alginate (a) monomers, (b) chain conformation, and (c) block distribution (Source: Draget *et al.*, 2005)

The satiety studies shown in Table 6 indicate that effective doses of alginates were between 0.8% to 3% w/v in test beverages or in water. These studies specify the use of high G alginates and either those of high molecular weight or strong gelling type. In a study by Paxman *et al* (2007) alginate was used in combination with pectin, probably for synergistic effects. HM pectins form gels only with high sugar solids levels and low

pH, but when they are used with alginates, gel formation is possible at low solids and below pH 3.8 the gel is reversible (Thakur *et al.*, 1997). The preload was a 2-part beverage; Part 1 beverage contained alginate-pectin (0.4% and 1.2% w/v) and Part 2 beverage contained calcium, which when consumed consecutively forms a stable, fibrous gel in the stomach. They concluded that the consumption of the 2 part, calcium-gelled alginate-pectin beverage twice per day reduced energy intake and overweight and obese women (Paxman *et al.*, 2008). In other studies, sodium alginate was used in combination with konjac glucomannan and xanthan gum in the specialty blend PGX® from InovoBiologic. Effective doses of PGX® were 1.2% w/v in beverages and 15g in 3 meals a day, showing increased satiety and/or decreased acute energy intakes.

2.3.1.3 β-glucans

Beta-glucans (β-glucans) are hydrocolloid polysaccharides found in cereal grains, particularly higher concentrations are found in oats and barley. The primary structure of mixed-linkage β-glucans is a linear chain of glucopyranosyl monomers linked by a mixture of single β-(1→3) linkages and consecutive β-(1→4) linkages (Figure 5) (Gómez *et al.*, 1997). The mixed-linkage of cereal β-glucans is resistant to the digestion and absorption in the small intestine, but undergoes fermentation in the large intestine. As a good source of dietary fibre, oats, barley and isolated highly concentrated β-glucan ingredients are used by food manufacturers for fibre enrichment in many foods (Stevenson & Inglett, 2009). Cereal β-glucans have high solubility in water at ambient or higher temperature, forming pseudoplastic viscous solutions at concentrations above 2 g/L. The viscosity is dependent on the concentration of dissolved β-glucan, molecular weight and the methods used in its extractability (Mälkki & Virtanen, 2001).

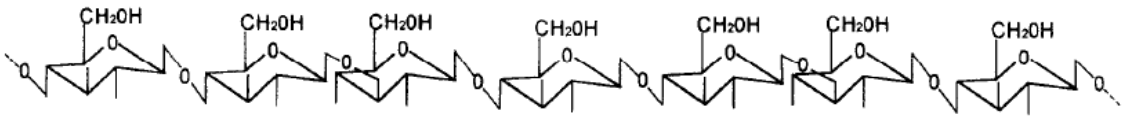


Figure 5 General structure of cereal β-glucans (Source: Gómez *et al*., 1997)

Processing conditions such as high or low temperatures, acidic or alkaline pH, high pressure, mechanical shear or presence of enzymes can alter their structure, molecular weight, physical properties and physiological effects. For example, dry or wet milling and hydrothermal treatments could improve extractability by reducing the particle size and/or by opening physical barriers for water penetration, whereas frozen storage

could reduce extractability as molecular weight is reduced by freeze-thaw cycles. Ideally, the molecular weight of β-glucan should not be reduced unless viscosity and gelation properties and physiological function remained unchanged (Mälkki & Virtanen, 2001; Stevenson & Inglett, 2009).

Diets rich in β-glucans are known to have many health benefits; these includes lowering blood cholesterol levels, reducing risk of coronary heart diseases, reducing diabetic symptoms, lowering blood pressure and cancer prevention (Stevenson & Inglett, 2009). Furthermore, oat β-glucans have been shown to delay gastric emptying, prolong the absorption of nutrients, affect motility in the small bowel and prolong postprandial satiety (Mälkki & Virtanen, 2001; Stevenson & Inglett, 2009). As shown in Table 6, cereal β-glucans used in satiety studies were oat bran concentrate, oat fibre (Nutrareal GI-trim®) and barley β-glucan (Glucagel™) with effective dose of 3.4%, 2.6% and 1.2% w/v in test beverages, respectively.

Several satiety studies were also conducted using β-glucan ingredients in solid and semi-solid foods, but both positive and negative effects on satiety were reported (El Khoury *et al.*, 2012). Test foods used in studies that had shown higher satiety ratings and lower acute food intake than the control foods included the 3% β-glucan-enriched bread using Glucagel™ (Vitaglione *et al.*, 2009) and the extruded breakfast cereals containing 5.45g β-glucan/45g serve using OatWell™ (Beck *et al.*, 2009). Other test foods that had shown increased satiety but no difference in subsequent energy intake were biscuit snack containing 5.2% barley beta-glucan (Vitaglione *et al.*, 2010) and hot cereal (56g) and snack mix (30g) with 9g β-glucan from whole-grain high fibre barley, Sustagrain® (Schroeder *et al.*, 2009). The test foods used in studies with no significant effect on satiety, subsequent food intake and/or gastric emptying compared to control foods include, wholemeal rye bread containing 5.4g β-glucan/169g serve, using oat β-glucan concentrate (Juntunen *et al.*, 2002), meal-replacement bar containing 1.2g β-glucan/57g serve, using Sustagrain® (Peters *et al.*, 2009) and muesli (mostly oat bran flakes, OatWell™) containing 4g β-glucan/26.5g serve (Hlebowicz *et al.*, 2008). These inconsistent results could be due to several factors that affect the efficacy of β-glucan on satiety, such as dose, molecular weight, solubility, viscosity and carrier food. Almost all studies that reported no significant effect on satiety used solid or semi-solid foods as carrier foods. Solids foods generally are more satiating than liquids, thus when a solid test food is compared to a solid control food, the larger satiating effect of solid food *per se* may mask the satiating potential of β-glucan (El Khoury *et al.*, 2012).

Table 6 Studies investigating the effects of viscous fibres on satiety, with a focus on pectin, alginate and β-glucan in beverages or liquid test meals. Abbreviations: Visual analogue scales (VAS), *ad libitum* (AB).

Fibre(s)	Fibre doses	Pre-load	Test meal	Design	Measurement	Results	Reference
Pectin	5, 10, 15 or 20g pectin	448 ml orange juice	0.473 L ice-cream	49 males and 25 females, US Army employees within normal weight limits	Appetite rating (VAS) before and at 0, 1, 2, 3 and 4 hours after orange juice and at 0, 30 and 60 minutes after ice cream	Pectin in doses as small as 5 g mixed with orange juice increases satiety.	Tiwary *et al.*, 1997 (abstract)
Pectin, 100% apple, low viscosity, Herbstreith & Fox	0 or 4.8g fibre	266g of apple (325 ml), applesauce (258 ml), apple juice with fibre (254 ml), apple juice without fibre (254 ml), control (no pre-load) Taken 3 hours after standard AB breakfast and 15 minutes before AB lunch	AB breakfast: bagels and yogurt AB lunch: 612g cheese tortellini, 280g tomato sauce, 1 L drinking water (2°C) - energy density of 2.2 kcal/g	5 sessions, 30 males and 28 females, 19-45 years old, BMI 18-40 kg/m^2, within subjects	Appetite rating (VAS) before and after pre-load and after lunch Food / energy intake	Comparing apple juice with and without fibre – adding pectin at naturally occurring levels of fibre to apple juice did not enhance satiety significantly, but decreased acute energy intake.	Flood-Obbagy & Rolls, 2009
Low viscosity, soluble apple pectin fibre	0 or 8g pectin fibre	355 ml, low-calorie (8 kcal) beverages, lemonade flavour and pink colour Taken at 2 time intervals between preload and test lunch (90 or 15 min)	Standard breakfast: Cheerios and milk AB lunch:24 bagel bites, mini pizzas (4 flavours), 591 ml water - 1215±50 kcal	4 sessions, 19 males and 22 females, 20-40 years old, BMI 18-29.9 kg/m^2, within subjects, Latin Square design	Appetite rating (VAS) at 15 min interval from before breakfast to 30 min after lunch Food / energy intake	Consumption of beverage with 8g pectin fibre reduced energy intakes at the next meal. Timing of preload consumption is important.	Perrigue *et al.*, 2010

Table 6 *(Continued)*

Fibre(s)	Fibre doses	Pre-load	Test meal	Design	Measurement	Results	Reference
Pectin and methylcellulose	15g pectin or 15g methylcellulose (control)	-	100g, 250 kcal egg-sandwich meal and 100 ml water	2 sessions, 9 obese subjects (1 male, 8 females), 24–53 years old, mean BMI 45.1 kg/m^2	Appetite rating (VAS), gastric emptying, CCK and pancreatic polypeptide measurements	Pectin increased satiety and delayed gastric emptying time, more than methylcellulose.	Di Lorenzo *et al.*, 1988
Agar and pectin	No fibre, 2g agar fibre or 5.2g pectin fibre	-	500 ml, 450 kcal ready-made nutritive drink	3 sessions, 10 healthy males, 21-33 years old, BMI 19.4-23.9 kg/m^3, within subjects	Gastric emptying and postprandial glycaemic response	Agar- and pectin-supplemented meals delay gastric emptying but have no post-prandial glucose response, compared to the control meal.	Sanaka *et al.*, 2007
Alginate-pectin fibre 1:1 Manugel LBA and GHB alginates (ISP); 15:85 blend of USP-L220 pectin (CP Kelco)	0, 1 or 2.8g fibre	Part 1: 237 ml fruit-flavoured aqueous solution, sweetened with sucralose, with 1g or 2.8g fibre or 0g fibre (control); Part 2: 118 ml fruit-flavoured beverage also sweetened with sucralose, with 500 mg calcium or no calcium (control). Subjects consumed both beverages (Part 1 followed by Part 2 (within 3 minutes for each) once before breakfast and once mid afternoon	Standard breakfast: choice of bagels or cereal and yogurt, milk, juice, coffee or tea. AB lunch and dinner including sliced meats, bread, cheeses, vegetables and fruits.	3 sessions, 29 overweight and obese women, 20-40 years old, BMI 27.5-34.4 kg/m^2, within subjects	Appetite rating (VAS) before and after each meal and every hour between meals Food / energy intake	Significant reduction in food intake at dinner for both formulations with alginate-pectin fibre, compared to control.	Pelkman *et al.*, 2007

Table 6 *(Continued)*

Fibre(s)	Fibre doses	Pre-load	Test meal	Design	Measurement	Results	Reference
Alginate, high guluronate (65-75%) and high molecular weight (150-195 kDa), Protanal, FMC	1.5g sodium alginate (1.35g fibre)	100 ml, 27 kcal vanilla-flavoured, viscous beverage according to patent W02007039294, contained 1.5g sodium alginate, 0.7g calcium carbonate, 2.8g glucono-delta-lactone, 0.5g sodium bicarbonate, 0.05g malic acid, 0.24g vanilla flavour and 7g fructose. Control: 100ml Slim Fast Simply Vanilla Milk Shake Powder (Unilever, UK), contains 2g fibre and 66 kcal Taken 30 minutes before either breakfast or evening meal for 7 days	Free-living	Two 7-day sessions, 68 free-living adult subjects (30 males and 38 females), BMI 18.5-32.8 kg/m^2	7-day estimated measures food dairies	Daily preprandial ingestion of the alginate beverage produced a significant 135 kcal (7%) reduction in mean daily energy intake.	Paxman *et al.*, 2008
Alginate, high guluronate (65-75%) and high molecular weight (150-195 kDa), Protanal, FMC	1.5g sodium alginate (1.35g fibre)	100 ml sodium alginate satiety beverage according to patent W02007039294 or 100 ml control beverage Taken 3 hours after breakfast and 30 minutes before lunch	Breakfast: 60g Crunchy Nut Cornflakes with 160g skimmed milk and 200g orange juice. AB lunch: penne pasta in tomato sauce	2 sessions, healthy subjects (1 male and 10 females), mean age 21.7 years, mean BMI 23.3 kg/m^2	Appetite rating (VAS) before, during and 2 h after meal	No effect of the sodium alginate satiety beverage upon test meal food intake, but it significantly reduced the onset of hunger following that meal compared to control.	Dettmar *et al.*, 2011

Table 6 *(Continued)*

Fibre(s)	Fibre doses	Pre-load	Test meal	Design	Measurement	Results	Reference
Alginate, high guluronate (70%), strongly gelling and fully hydrated, Manugel DMB, FMC	0, 0.6 or 0.8% alginate	-	Breakfast: 325ml, 190 kcal low viscosity meal replacement shake (Optima milk chocolate RTD, Slim.Fast, Unilever), containing (1) whey protein isolate that is low in freely available calcium (in replacement of skim milk powder and caseinate) and (2) added calcium (0.26% w/w $Ca_3(PO)_4$) that is insoluble at product pH of 7, but soluble at gastric pH.	3 sessions, 23 subjects, 36-60 years old, BMI 21.7-30.3 kg/m^2, balanced treatment order, random allocation, double-blind, and 3-way crossover design	Appetite rating (VAS) for 5 hours post-consumption Product viscosity and strength of gel formed under stimulated gastric conditions	Significant hunger reduction with the 0.6% and 0.8% alginate drinks, clear dose-response observed; greater effects with the 0.8% alginate drink. Acceptable product viscosity of <0.5 Pa.s at 10/s, gastric gel strength was 1.8 N and 3.8 N for the 0.6% and 0.8% alginate drinks, respectively.	Peters *et al.*, 2011
Alginates, weak- and strong-gelling on exposure to acid Guar gum, viscosity unaffected by acid	Control, 1% Manucol DM (~40% G), Manugel DMB (~70% G) or Vidogum GH200	-	325 ml sweetened, milk-based meal replacer beverage, 921 kJ/325 ml per serving (3g fat, 10g protein, 35g sugar)	4 sessions, 12 subjects (3 males and 9 females), mean age of 24 years, BMI 22 kg/m^2	Intragastric gelling, gastric emptying, meal dilution and satiety (VAS)	Gastric emptying was similar for all 4 meals. The sense of fullness at the same gastric volume was significantly greater for all 3 viscous meals than for the control. Compared with the control meal, the strong-gelling alginate and guar meals increased fullness at 115 min, and the strong-gelling alginate decreased hunger by the 115-min and 240-min time points.	Hoad *et al.*, 2004

Table 6 *(Continued)*

Fibre(s)	Fibre doses	Pre-load	Test meal	Design	Measurement	Results	Reference
Alginates – M:G ratios of 0.8, 1.3 and 2.5	1.5% (~4.95g) M:G 2.5 alginate or 3.0% (~9.9 g) M:G 0.8 alginate	300ml water containing the alginate First preload taken 30 minutes before standard breakfast Second preload taken 3 hours after breakfast and 30 minutes before AB lunch	Breakfast: yogurt, bread and cheese (2 MJ) AB lunch: pizza with tomato, cheese and ham	8 subjects (4 males, 4 females), 20–45 years old, BMI 22.5±2.8 kg/m^2	Mannuronic : guluronic (M:G) acids ratio determination, rheology, water retention capacity (WRC) measurements, appetite rating at 0, 15, 30, 45, 60, 90, 120, 150, 180, 210 and 240 minutes after consuming the first preload, AB food/energy intake and report occurrence and severity of gastrointestinal symptoms	M:G 0.8 alginate (high in guluronic acid) solutions exhibited stronger acid-gel formation and higher WRC than the M:G 2.5 alginate (high in mannuronic acid). Appetite rating for fullness was higher and prospective food intake was lower after consumption of preloads with M:G 0.8 alginate than that with M:G 2.5 alginate.	Georg Jensen *et al.*, 2012

Table 6 *(Continued)*

Fibre(s)	Fibre doses	Pre-load	Test meal	Design	Measurement	Results	Reference
Novel viscous polysaccharide (NPV) – PGX® contains xanthan, glucomannan and sodium alginate, high viscosity Glucomannan (GLM), moderate viscosity Cellulose (CE), low viscosity	5.1g in preload drinks (~1.2% fibre)	56g powdered formula (8.3g sugar, 5.1g fibre, 19.8g protein, 6.9g fat, 226 kcal) in 350 ml water Plus 350 ml water after consuming the preload drink Taken 90 minutes before AB lunch	AB lunch: Vegetarian pizzas	3 sessions, 31 subjects (6 males and 25 females), mean age of 16.1±0.6 years, BMI 22.2±3.7 kg/m^2	*In vitro* viscosity (Brookfield) of the drinks measured every 15 minutes for up to 90 minutes Appetite rating, gastrointestinal symptoms, food intake at AB lunch and 24-h food intake	Final viscosity of NPV, GLM and CE at 30/s was 700, 410 and 10 P, respectively. AB lunch intake was significantly lower after consumption of preload drink NVP, compared to GLM and CE. No difference in appetite ratings, gastrointestinal symptoms and 24-h food intake between the treatments.	Vuksan *et al.*, 2009
PolyGlycopleX (PGX) contains konjac (glucomannan), sodium alginate and xanthan gum, 87.4% fibre, of which 81.1% is soluble	15g PGX or placebo (rice flour)	-	3-day, 1000 kcal per day diet, breakfast, lunch and dinner meals provided, sprinkle each meal with 5g PGX or rice flour, and 500 ml water, all within 20 minutes. Additional 500 ml water allowed in-between meals but no water 30 minutes before completing premeal VAS	2 sessions, 3-day structured, low-calorie diet, 45 overweight and obese females, 38±9 years old, BMI 29.9±2.8 kg/m^2 Only 35 women completed the study.	Appetite rating (VAS) before, in-between and after each meal.	Adding 5g PGX to meals at the start of the low-calorie diet helps manage appetite by increasing satiety and decreasing prospective food consumption, this effect was only significant on day 3.	Kacinik *et al.*, 2011

Table 6 *(Continued)*

Fibre(s)	Fibre doses	Pre-load	Test meal	Design	Measurement	Results	Reference
Oat bran concentrate (34g fibre/100g, 1:1 insoluble and soluble fibre)	10.2g fibre	Two 300ml, 1250 kJ beverages containing wild berry flavoured juice, aspartame, acesulfame K, blackcurrant juice concentrate, sucrose, oat bran; one of the beverages was treated with β-glucanase to yield low viscosity of <250 mPas, while the other beverage was at >3000 mPas. Plus 200ml water Taken 180 minutes before the AB lunch	AB lunch: vegetable soup, oat bread, rye bread, margarine, cheese, tomato slices, cucumber slices, noncaloric juice and water	3 sessions (incl. 1 training), 20 subjects (4 males, 16 females), mean age of 22.6 years, BMI 21.6 kg/m^2	Appetite rating (VAS) and blood tests (ghrelin, CCK, GLP-1, PYY, insulin and glucose) at 0, 15, 30, 45, 60, 90, 120 and 180 minutes after consuming the preload, gastric emptying and 24-h food intake	The low viscosity oat bran beverage induced a greater postprandial increase in satiety, and plasma glucose, insulin, CCK, GLP-1 and PYY, and a greater decrease in postprandial ghrelin than the high viscosity oat bran beverage. Gastric emptying as measured by paracetamol absorption was faster with the low viscosity beverage.	Juvonen *et al.*, 2009

Table 6 *(Continued)*

Fibre(s)	Fibre doses	Pre-load	Test meal	Design	Measurement	Results	Reference
Oat fibre (Nutrareal GI-trim®, β-glucan 17g/100g), wheat bran and guar gum	0, 2.4, 7.8 or 10.5g fibre	-	400 ml, 1000 kcal beverages containing juices (blackcurrant, strawberry), sugar, maltodextrin, water and with/without fibre – 0g (control beverage), 7.8g (guar gum beverage), 10.5g (oat β-glucan / wheat bran beverages) 1000 kcal, 2.4g fibre in wheat bread	6 sessions (incl. 1 training with rye bread), 19 subjects, 18–30 years old, BMI 23.2 kg/m^2	Appetite rating (10-unit graphic intensity scales) at 0, 20, 40, 60, 90 and 120 minutes after intake, viscosity (rheometer) and Satiety Index	Viscosity of beverages control, wheat bran, oat β-glucan and guar gum at 50/s and room temperature was 1.7, 2.6, 33.5 and 1740 mPas, respectively. Higher satiety and lower desire to eat was perceived for the guar gum beverage, compared to control. Oat β-glucan beverage was perceived with increased satiety, fullness and decreased desire to eat.	Lyly *et al.*, 2009
Oat fibre, Nutrareal GI-trim®, β-glucan 17g/100g	2 energy levels – 700 and 1400 kJ 700 kJ – 0, 5, 10g fibre 1400 kJ – 0, 10g fibre 700 kJ – 10g fibre, reduced viscosity of beverage by enzyme	-	300g beverages containing juice concentrates (berry-flavoured, blackcurrant), Canderel sweetener, citric acid, water and with/without the oat fibre 61g, 700 kJ white wheat bread (control)	8 sessions (incl. 1 training with oat beverage, 2.5g fibre), 29 subjects (11 males, 18 females), 19–39 years old, BMI 23.2 kg/m^2	Viscosity (rheometer) measurements at 50/s, 20°C Appetite rating (10-unit scale) before and at 0, 20, 40, 60, 90, 120, 150 and 180 minutes after intake, time taken to consume each beverage and viscosity (rheometer) measurements	Addition of the oat fibre rich in β-glucan and high viscosity of beverages enhance post-meal satiety. This effect was not related to the amount of ingested fibre or energy.	Lyly *et al.*, 2009

Table 6 *(Continued)*

Fibre(s)	Fibre doses	Pre-load	Test meal	Design	Measurement	Results	Reference
Barley β-glucan, Glucagel™ (≥75% barley β-glucan, DKSH Italy)	0g fibre, 3g barley β-glucan fibre or 2.5 fibre from fruits (mainly pectin)	250ml beverages: Fruit-based beverage, tropical fruit-flavoured, 2.5g fibre from fruits mainly pectins, 34.3g sugars, 623 kJ β-glucan-enriched beverage, tropical fruit-flavoured, 3g β-glucan, 34.5g sugars, 617.1 kJ Control beverage, tropical fruit-flavoured beverage, 0g fibre, 37.3g sugars, 624.2 kJ Taken with breakfast within 15 minutes.	Breakfast: 2245.6 kJ, 4 slices of toasted bread and preload AB lunch: bread, pasta with tomato sauce, pasta with zucchini, beef meat with tomato, fish, green salad, chips and apples	2 trials, 3 sessions each, 14 subjects (8 males, 6 females), 24–39 years old, BMI 20.2–24.6 kg/m^2	Sensory evaluation (VAS) of the beverages Appetite ratings (VAS) at -15, 0, 15, 30, 60, 120 and 180 minutes after consuming the breakfast and preload, AB food/energy intakes Gastrointestinal hormones and glucose metabolism biomarkers	Viscosity of the Fruit-based, β-glucan-enriched and control beverage at 50/s and 20°C was 90, 55 and 1.2 mPa.s, respectively. Fruit-based and β-glucan-enriched beverages increased fullness / satiety and suppressed ghrelin response over 3 h post-breakfast. Only the β-glucan-enriched beverage significantly reduced energy intake at lunch and over the rest of the day, and increased pancreatic polypeptide (PP) response.	Barone Lumaga *et al.*, 2012

2.3.2 Proteins

2.3.2.1 High protein diets

Many satiety studies have demonstrated that under most conditions, protein is more satiating than the isoenergetic (equivalent quantities of energy) ingestion of carbohydrate or fat. This implies that an increase in dietary protein, by reduction of other macronutrients, may promote satiety and reduce subsequent energy intakes and hence facilitate body weight loss (Benelam, 2009; Halton & Hu, 2004; Kleef *et al.*, 2011; Paddon-Jones *et al.*, 2008; Westerterp-Plantenga *et al.*, 2009). Diets containing at least 25–30% energy from proteins are considered higher or high protein diets, since the recommended protein level for adults is 10–15% according to World Health Organization (Westerterp-Plantenga *et al.*, 2009) or 15–25% as the 'Acceptable Macronutrient Distribution Ranges' (AMDRs) in New Zealand and Australia (NHMRC/MOH, 2006). However, the US dietary reference intake or the AMDR for proteins is 10–35% of total energy intake (National Research Council, 2005); the upper acceptable limit of 35% energy intake from proteins is considered 'extremely high' for a typical diet. Dietary protein intakes are also recommended based on body weight and vary according to age and physiological conditions. For adults, recommendations by the Nutrition Task Force and ESFA Panel are 0.8–1.6g and 0.83g protein/kg of body weight/day, respectively (Department of Health, 1991; European Food Safety Authority, 2012). The ESFA Panel considered a protein intake of up to twice their recommended amount *i.e.* 1.66g protein/kg body weight as safe (European Food Safety Authority, 2012).

In the review by Halton and Hu (2004), a number of short-term satiety studies using high protein preloads ranging from 29% to 100% energy from proteins and test period of 1 to 24 hours, were listed. Out of 14 studies, 11 found that the higher protein preloads significantly increased subjective satiety, and 8 out of 15 studies found that higher protein preloads reduced subsequent energy intakes more than the control (Benelam, 2009; Halton & Hu, 2004). It is noted that these studies with positive effects were through high protein foods or meals containing at least 2-fold to about 6-fold greater protein load, a condition unlikely to represent the normal dietary intake for most individuals (Paddon-Jones *et al.*, 2008). Studies that found no differences between high protein preloads and controls were probably due to factors such as palatability, food form (solid or liquid foods), energy density, fibre content and glycemic index. It seems that the closer the test methodology was to real life situations (real foods vs. liquids, free living vs. whole body calorimeter, sense of taste unaltered), the more likely

it was for protein to exert a significant difference in subsequent energy intake (Halton & Hu, 2004). Some longer term studies have shown that higher protein diets may increase body weight loss and fat loss, possibly because of the increased satiety and reduced energy intakes under *ad libitum* condition (Benelam, 2009; Paddon-Jones *et al.*, 2008). Halton and Hu (2004) also reviewed 15 studies on the effect of high protein diets on weight loss. Seven of the studies found significant decrease in total body weight with the higher protein diets, of which 5 studies were those that allowed *ad libitum* food intakes and were of a longer duration (6 months or longer) (Benelam, 2009; Halton & Hu, 2004). The 8 studies that found no significant difference in weight loss were mostly of a relatively short duration (10 weeks or less) and small sample size (6–35 subjects). A wide variety of macronutrient ratios were seen in these 15 studies, some alter protein relative to carbohydrate content of the diet and hold fat constant while some alter protein relative to fat and hold carbohydrate constant (Halton & Hu, 2004). Therefore, it is difficult to ascertain the effect of protein and satiety on weight loss with high protein diets.

For many years, high protein diets have been promoted for weight loss and have become very popular. Examples include the Atkins and the Protein Power diets (with about 35% energy from proteins, over 50% from fat and 8% or less from carbohydrate), and the Sugar Busters and the Zone diets (with about 28% energy from protein, 40% from carbohydrate and 32% from fat) (Anderson & Moore, 2004). Some studies may have shown that both the Atkins and Protein Power diets contributed to weight loss and reduced risk factors for cardiovascular diseases in obese subjects, but the role of protein or the effect of protein intake in these diets remains unclear (Anderson & Moore, 2004). This is because some of the studies did not achieve protein intakes recommended by either the Atkins diet or the Protein Power diet. Otherwise, one study suggested that the greater weight loss with the low carbohydrate diet was due to lower energy intakes, with no mention of protein as a possible factor. It was the same for another study with no report on the effect of protein intake or effect of the low carbohydrate diet on satiety (Anderson & Moore, 2004).

2.3.2.2 Different sources of protein

Some studies have suggested that different sources of protein affect satiety and subsequent energy intake differently. Based on reviews, these include: (1) higher satiating effects of proteins from fish, than beef and chicken, (2) liquid preloads with whey or soy proteins suppressed subsequent energy intake, but not with egg albumin; and whey protein was more effective than soy protein (Benelam, 2009), (3) larger

satiety effect was observed after a whey preload than a casein preload, (4) higher satiety ratings and lower energy intake at lunch after an alpha-lactalbumin or gelatin breakfast, compared with after a casein, soy or whey breakfast, and (5) higher satiety after consumption of pea protein hydrolysate or whey protein, than milk protein or combination of whey protein and pea protein hydrolysate (Westerterp-Plantenga *et al.*, 2009). On the other hand, there were studies that did not show any significant difference between different sources of protein on satiety and energy intake. A possible cause is that when different proteins are consumed at very high levels, satiety was very high and therefore differences in the satiating effects were no longer observed (Westerterp-Plantenga *et al.*, 2009). Another reason could be that the satiating effect of a specific protein may be masked by the concurrent ingestion of a mixture of proteins and other macronutrients in a normal mixed diet (Paddon-Jones *et al.*, 2008).

2.3.2.3 Mechanisms of action

The satiety effect of proteins is primarily due to increased thermogenesis, which is the increase in energy expenditure above the baseline following consumption (Kleef *et al.*, 2011; Westerterp-Plantenga *et al.*, 2009). The thermic effect of proteins is 20–35% of the energy consumed and 5–15% of the energy consumed for carbohydrates. As for fats, it could be the same or lower than carbohydrates (Halton & Hu, 2004). More recently cited by Westerterp-Plantenga and colleagues (2009), the thermic effects of food or diet-induced energy expenditure (DEE) are: 0–3% for fat, 5–10% for carbohydrate, 20–30% for protein and 10–30% for alcohol. The higher thermic effect of protein can be explained by the fact the body has no storage capacity for protein and thus it has to be metabolically processed immediately. Increased thermogenesis is probably contributed by digestion rate, high ATP costs of protein and peptide bond synthesis, high cost of urea production and gluconeogenesis (Halton & Hu, 2004; Paddon-Jones *et al.*, 2008; Westerterp-Plantenga *et al.*, 2009). Consumption of protein foods leads to increased thermogenesis or elevated energy expenditure, which implies oxygen consumption and body temperature increase, leading to feeling deprived of oxygen and thus promoting satiety (Paddon-Jones *et al.*, 2008; Westerterp-Plantenga *et al.*, 2009). The thermic effect of proteins from different sources did not differ significantly. Mikkelsen and collegues (2000) found that ingestion of animal (pork) protein resulted in a 2% higher energy expenditure than ingestion of plant-based (soy) protein, while Tan and colleagues (2010) reported no significant differences between meat-, dairy- and soy-based meals for effects on energy expenditure.

Another possible mechanism of protein on satiety is the aminostatic hypothesis by Mellinkoff, who suggested in 1956 that an elevated concentration of blood or plasma amino acids that cannot be channeled into protein synthesis, serves as a satiety signal to stop hunger, regulates food intake and decreases energy intake (Halton & Hu, 2004; Westerterp-Plantenga *et al.*, 2009).

2.3.2.4 Controversy on the safety of high protein diets

The safety of high protein diets with regard to kidney function has been controversial. There is evidence showing high protein diets may have a negative effect on renal or kidney function (Kleef *et al.*, 2011); may promote renal damage via excretion of nitrogenous waste products generated from protein metabolism, thereby increasing glomerular pressure and hyperfiltration (Westerterp-Plantenga *et al.*, 2009); may increase the risk of kidney stones, uric acid stones and calcium stones (Halton & Hu, 2004); and may have blood pressure-raising effects caused by acidifying amino acids during maintenance of acid-base homeostasis through excretion of the excess acid load by the kidneys (Westerterp-Plantenga *et al.*, 2009). It has been shown that in subjects with established renal diseases, limiting protein to the recommended level may slow progression of the disease (Halton & Hu, 2004). However, there is insufficient evidence to show that high protein diets pose a serious risk to kidney function in healthy individuals. In subjects without renal disease, changes in dietary protein intake caused adaptive alterations in renal size and function without adverse effects. The changes in renal function, induced by high protein intakes, are normal adaptive mechanism. One study has shown that long-term daily protein intakes under 2.8g/kg body weight have no negative effects on renal function in athletes (Westerterp-Plantenga *et al.*, 2009). Nevertheless, it is suggested that the more susceptible groups particularly those with existing renal diseases and/or diabetes, should use high protein diets with extra caution (Halton & Hu, 2004; Westerterp-Plantenga *et al.*, 2009).

Other than kidney function, concerns relating high protein diets to osteoporosis and cardiovascular disease (CVD) have been raised. However, there is insufficient evidence to show that high protein diets have negative effects on calcium balance and/or increase the risk of CVD (Anderson & Moore, 2004; Westerterp-Plantenga *et al.*, 2009). On the contrary, positive effects with higher protein diets as well as negative effects with low protein diets have been observed in studies. These include: (1) higher protein intake (24% of energy vs. 15% of energy) was associated with a decreased risk of CVD during a 14 years follow-up; both animal and plant protein contributed to the lower risk, (2) very low levels of animal protein intake were associated with an

increased risk of hemorrhagic stroke, (3) inverse relationship between dietary protein intake and blood pressure were found in both men and women (Halton & Hu, 2004), (4) relatively high protein intake is associated with increased bone mineral mass and reduced incidence of osteoporosis, and (5) protein/nitrogen intake during weight loss apparently have a positive effect on calcium balance and consequent preservation of bone mineral content (Westerterp-Plantenga *et al.*, 2009).

2.3.3 Plant- and lipid-based ingredients

A number of plant- and lipid-based ingredients have claims for their efficacy for satiety, weight loss, weight management and/or anti-obesity. Some of these ingredients do have the potential to produce significant effects on metabolic targets such as satiety, thermogenesis, fat oxidation and/or fat or glucose absorption blocking (Hursel & Westerterp-Plantenga, 2010; Tucci, 2010), while some others do not. Functional ingredients that have shown some research evidence such as increased satiety, decreased subsequent energy intake and/or greater body weight loss include conjugated linoleic acid, diglycerides, medium-chain triglycerides, PinnoThin™ Korean pine nut oil and Olibra® novel fat emulsion, green tea, oolong tea, Svetol® green coffee extract, caffeine and capsaicin. Several naturally-occurring phenolic compounds such as epigallocatechin gallate, quercetin, kaempferol and luteolin may also help in obesity prevention (Hsu & Yen, 2008; Sergent *et al.*, 2012). Other ingredients such as *Garcina cambogia* extract (hydroxycitric acid), *Citrus aurantium* (bitter orange), Hoodia *gordonii* and *Caralluma fimbriata* extracts are lacking in evidence for what they have been claimed for. The source, composition or active constituents, effects and possible mechanism of these ingredients are summarized in Table 7 below.

Table 7 Potential ingredients for satiety enhancement and/or weight management

Ingredient	Source	Composition or active constituents	Effect(s)	Possible mechanism	Reference
Conjugated linoleic acid (CLA) Tonalin® CLA (Cognis GmbH)	Dairy products, animal-based products (*e.g.* beef) and safflower oil	Isomers of linoleic acid: *trans*-10, *cis*-12 and *cis*-9, *trans*-11 octadecadienoic acid	CLA appears to reduce body fat; it affects body composition rather than body weight *e.g.* 1 year supplementation of CLA led to a 9% decrease in body fat in overweight subjects.	CLA decreases body fat mass through four possible actions: (1) decreasing the amount of fat that is stored after eating (decreases lipoprotein lipase), (2) increasing the rate of lipolysis and rate of fat burning in the mitochondria (carnitine palmitoyltransferase and β-oxidation), (3) reducing proliferation and differentiation of preadipocytes to mature adipocytes, and (4) decreasing the total number of fat cells (apoptosis).	Cai, 2009; Kovacs & Mela, 2006
Diglycerides (DG)	Various oils (<1% to ~10% DG) and commercially available DG-rich oils (~80% DG) from soybeans	DG: fatty acids esterified in the 1,2- or 1,3-positions	Compared to triglycerides (TG), consumption of DG or DG-rich oil was shown to (1) have lower postprandial triglyceridemia, (2) result in greater reduction in body weight and abdominal fat, (3) reduce appetite scores and (4) enhance fat oxidation.	Dietary TG are hydrolyzed by lipase to form 1,2-DG and further hydrolyzed to 2-monoglyceride (MG). The 2-MG and their hydrolyzed fatty acids are absorbed and rapidly re-esterified to TG in the small intestinal epithelial cell, and appear as chylomicrons in postprandial plasma. As for DG, those of the 1,3 conformation are catabolised to 2 free fatty acids and a glycerol molecule, which cannot be reformed into chylomicrons due to absence 2-MG. These molecules travel in circulation and are diverted into the liver, where they are mostly oxidized. A rise in fat oxidation may act to give suppressed appetite.	Kovacs & Mela, 2006; St-Onge, 2005

Table 7 *(Continued)*

Ingredient	Source	Composition or active constituents	Effect(s)	Possible mechanism	Reference
Medium-chain triglycerides (MCT)	Naturally high in coconut and palm oils. Commercially produced MCT-oil by lipid fractionation comprises >90% C8 and C10 fatty acids	MCT with fatty acids having a chain length of 6–12 carbon atoms	Compared to long-chain triglycerides, consumption of MCT in meals was shown to (1) have greater postprandial energy expenditure and fat oxidation, (2) increase satiety and (3) decrease subsequent energy intakes. However, relatively high doses of MCT (>10g per day) are needed, which may affect product palatability and quality and may have adverse effects on plasma lipids, cardiovascular and gastrointestinal function.	MCT are hydrolyzed by lipases into medium-chain fatty acids (MFCA), which are absorbed directly into the portal circulation and transported to the liver for rapid oxidation. MFCA bypass peripheral tissues such as adipose tissue, which makes them less susceptible to the actions of hormone-sensitive lipase and to the deposition into tissue stores.	Kovacs & Mela, 2006; St-Onge, 2005
Korean pine nut oil PinnoThin™ triglyceride (TG) or free fatty acid (FFA)	Natural pressing of Korean pine nuts (*P. koraiensis)*	Triglycerides and >92% poly- and mono-unsaturated fatty acids including pinoleic acid (C18:3), linoleic acid (C18:2) and oleic acid (C18:1); contains 15% pinoleic acid	PinnoThin™ TG and FFA forms have shown to increase the release of satiety hormones CCK and GLP-1, suppress appetite, increase satiety (VAS) and reduce subsequent energy intake.	Mainly through a peripheral mechanism, release of satiety hormones.	Scott, 2009; Tucci, 2010
Novel fat emulsion Olibra® / Fabuless™	Plant-based	Mixture of fractionated palm oil (40%) and fractioned oat oil (2.5%) in water	Olibra® may increase satiety through its physico-chemical properties, suppress appetite, reduce subsequent energy intake and could be beneficial for weight maintenance.	Ileal brake mechanism; ileal brake initiates a feedback loop that inhibits upper gut motility (to slow gastric emptying and intestinal transit) in response to nutrients in the distal small intestine, which cause satiety signals.	Hursel & Westerterp-Plantenga, 2009a; Tucci, 2010

Table 7 *(Continued)*

Ingredient	Source	Composition or active constituents	Effect(s)	Possible mechanism	Reference
Oolong tea (OT), green tea (GT) and green tea extract (GTE)	Leaves of *Camellia sinensis* L. species of the Theaceae family	Catechin polyphenols: epicatechin, epicatechin gallate, epigallocatechin, epigallocatechin gallate and caffeine	OT and GT (with adequate amounts of caffeine and catechins) have shown to increase diet-induced thermogenesis, enhance energy expenditure and fat oxidation over 24 hours. Several long-term studies reported that consumption of OT, GT and GTE resulted in decreased body weight, body fat and waist circumference; dose-dependent effect was noted.	Thermogenesis and fat oxidation; inhibition of 2 enzymes that help regulate the sympathetic nerve system. Tea catechins inhibit catechol-*O*-methyltransferase, which degrades noradrenalin, and caffeine inhibits phosphodiesterase, which degrades intracellular cAMP to AMP. Inhibition of these enzymes resulted in sympathetic nerve system that is constantly activated, thereby leading to an increase in energy expenditure and fat oxidation.	Hursel & Westerterp-Plantenga, 2009b; Kovacs & Mela, 2006; Tucci, 2010
Green coffee bean extract Svetol® (Naturex)	Green, unroasted coffee beans of the Robusta variety (*Coffea canephora robusta* Pierre)	Rich in chlorogenic acid (27%) unlike roasted coffee beans, source of caffeic and quinic acids and low in caffeine (<2%)	Reduction in body fat and body weight, related to a reduction in the absorption of glucose.	Blocking of α-amylase resulted in reduction of glucose absorption which would eventually lead to an increase in the uptake of fat reserves due to the reduced availability of glucose as an energy source.	Naturex Group, 2008; Tucci, 2010
Caffeine	Plants such as coffee, tea, cola nuts, cocoa beans, mate and guarana	Caffeine	Short-term thermogenic effects (increased energy expenditure) of caffeine (100–600 mg) have been reported in lean, obese and post-obese subjects, dose-dependent and may be different in caffeine users and non-users. Caffeine seems to stimulate lipolysis and fat oxidation but requires further investigation.	Caffeine acts through inhibition of phosphodiesterase, which degrades intracellular cAMP to AMP, and through adenosine antagonism, which prolongs noradrenalin release.	Hursel & Westerterp-Plantenga, 2009b; Kovacs & Mela, 2006; Tucci, 2010

Table 7 *(Continued)*

Ingredient	Source	Composition or active constituents	Effect(s)	Possible mechanism	Reference
Capsaicin	Hot, sweet, and bell peppers (*Capsicum)*, chillies, ginger, mustard CH-19 Sweet pepper	Capsaicin Capsiate in CH19 Sweet (similar structure as capsaicin but no pungency)	Short-term studies have shown that consumption of capsaicin-containing foods increased diet-induced thermogenesis, increased fat and carbohydrate oxidation, increase satiety, reduced hunger and reduced subsequent energy and intakes.	Capsaicin acts by stimulating catecholamines secretion from the adrenal medulla, mainly through sympathetic activation of the central nervous system. As a result of β-adrenergic stimulation, capsaicin may increase in thermogenesis.	Hursel & Westerterp-Plantenga, 2010; Kovacs & Mela, 2006; Smeets & Westerterp-Plantenga, 2009
Garcina cambogia extract (hydroxycitric acid)	Pericarp rinds of *G. cambogia*, native to Southeast Asia	Hydroxycitric acid (HCA)	The pericarp rinds of *G. cambogia* have been used for centuries in domestic cooking and reported to make meals more satiating, without any adverse effects. HCA may decrease fatty acid synthesis and reduce appetite, but further investigation in humans is needed to determine its role in weight loss, efficacy and safety.	HCA inhibits adenosine triphosphate-citrate lyase, an extra-mitochondrial enzyme that may inhibit *de novo* lipogenesis and reduce appetite.	Poddar *et al.*, 2011; Tucci, 2010
Citrus aurantium extract	Fruits of *citrus aurantium* (bitter orange)	Alkaloids such as *p*-octopamine and synephrine	The extract exerts adrenergic agonist activity (similar effects as adrenaline) and may increase energy expenditure and decrease food intake due to decreased gastric motility. There are safety concerns with the consumption of this extract, similar to the concerns with ephedrine. In 2004, US FDA has banned the use of ephedrine due to its pharmacological effects.	Thermogenesis and decreased gastric motility.	Poddar *et al.*, 2011; Tucci, 2010

Table 7 *(Continued)*

Ingredient	Source	Composition or active constituents	Effect(s)	Possible mechanism	Reference
Hoodia gordonii (Hg) Hoodia P57 extract, Hoodia pure®, Hoodia MAX®, Pure Hoodia®, RapidSlim SX®, Hooderma®, Hoodia-HG57®	*Hoodia gordonii*, member of the milkweed family	Steroid glycosides	Although based on limited research evidence, *Hg* products have been marketed and promoted for its appetite suppressing and weight loss effects. Recent study on 15-day repeated consumption of *Hg* purified extract found no significant difference in *ad libitum* energy intakes and body weights, from placebo. Instead, *Hg* purified extract was less tolerated than placebo, because of episodes of nausea, emesis and disturbances of skin sensation. The *Hg* purified extract group had increased blood pressure, pulse, heart rate, bilirubin, and alkaline phosphatase.	Hoodia P57 extract has been reported to increase ATP content in hypothalamic cells, which correlates with a decrease in appetite *i.e.* through a central nervous system mechanism and possibly an additional peripheral mechanism.	Blom *et al.*, 2011; Tucci, 2010
Caralluma fimbriata (Cf) SLIMALUMA® (Gencor Pacific Group)	*Caralluma fimbriata*, a cactus of the family Asclepiadaceae	Pregnane glycosides, flavone glycosides, megastigmane glycosides, bitter principles, saponins and other flavonoids	Appetite suppression and reduced lipogenesis actions probably due to the pregnane glycosides. Two-month administration of *Cf* extracts was shown to reduce appetite, body weight and waist circumference.	*Cf* acts through a central nervous system mechanism which regulates appetite; pregnane glycosides and its related molecules act by amplifying the signalling of the energy sensing function in the hypothalamus.	Tucci, 2010

2.4 Summary and recommendations

The literature review has provided valuable information for the development of a satiety beverage model. Differences between satiation and satiety were defined. Satiation is the process or feeling of fullness during a meal or eating episode which leads to the termination of eating, whereas satiety is the process or feeling of fullness after a meal that persists, inhibiting further eating and delaying hunger (Blundell *et al.*, 2010). Satiation is measured through the measurement of *ad libitum* food intake of particular test foods/meals. Satiety can be quantified through the measurement of *ad libitum* food intake as well as through self-reported measures of appetite, such as visual analogue scales (VAS). It allows subjects to rate and record their feelings of hunger, fullness, satiety, desire to eat and prospective consumption (Benelam, 2009; Blundell *et al.*, 2010).

Most appetite studies focus on the measurement of satiety, of which the preload study design is often used to measure the effects of a particular variable(s) on the short-term (usually 4 hours) regulation of appetite and food intake. In the current work, variables of interest would be the type and concentration of phytochemicals and fibres to be used in the beverages. The review also indicated important considerations and confounding factors of satiety studies. It is important to consider issues such as matching the test and control preloads for taste, texture and other sensory properties, standardizing the preloads in terms of their energy, nutritional and macronutrient contents, and whether the preload is suitable for satiety measurement at that time of the day. Confounders such as body mass index, gender, age and dietary habits of subjects should also be considered.

Several satiety studies have shown evidence that proteins and specific fibres (viscous or gelling) have a greater impact on satiety than other macronutrients (Blundell *et al.*, 2010). Energy from proteins seems to have a larger effect on satiety than that from carbohydrates or fats (Benelam, 2009; Kleef *et al.*, 2011). Viscous fibres such as pectin and alginates appear to be more satiating than soluble fibres (Benelam, 2009; Wanders *et al.*, 2011). The effects of proteins and/or fibre on satiety are heavily dependent on the types and amounts used and the background food matrix (Benelam, 2009; Blundell *et al.*, 2010). The review focused on fibres, proteins, plant- and lipid-based ingredients for enhancing satiety. Study designs and findings of some published satiety studies involving pectins, alginates and beta-glucans in beverages were summarized in the review.

Based on the literature review and suggestions from the project team, recommendations for the development work include the following:

1. To use the preload study design to measure satiety through self-reported measures of appetite (using VAS).
2. To use a within-subject, repeated measures, completely balanced, crossover and randomized experimental design.
3. To focus on the characterization and evaluation of two viscous fibres, pectin and alginate for the beverage model. Exclude β-glucans as a suitable type for beverage application was unavailable at that time of project.
4. To standardize the preloads (test beverages) for energy, protein, sugars and fibre contents (non-variables).
5. To use the same type and quantity of protein in the beverages (non-variable).
6. To reduce the effects of confounding factors as much as possible.

Chapter 3
Materials and Methods

3.1 Introduction

This chapter describes the materials and methods of the development work, which comprised of three phases:

1. Characterization of viscous fibres
2. Beverage formulation and production
3. Satiety measurement trial.

3.2 Characterization of viscous fibres

3.2.1 Materials

A total of seven different pectin and alginate ingredients were evaluated and characterized to determine their suitability of use in the beverage. The ingredients or materials that were used are shown in Table 8 below.

3.2.2 Methods

3.2.2.1 Preparation of viscous fibre solutions

Solutions of viscous fibres were prepared at concentrations of 1% and 2% (w/w) with deionized water. The preparation methods were in accordance to suppliers' recommendations:

1. Pectins – solutions were prepared by slow addition of the pectin powder to hot water of 80–85°C under vigorous agitation, using an IKA propeller mixer at 500 rpm for 30 minutes, followed by constant agitation on a magnetic stirrer for another 2 hours.
2. Alginates – solutions were prepared by slow addition of the alginate powder to water of ambient temperature under vigorous agitation, using an IKA propeller mixer at 500 rpm for 30 minutes, followed by constant agitation on a magnetic stirrer for another 2 hours.

Original weights of the solutions were corrected at the end of mixing, to compensate for any water losses via evaporation. This ensured the required concentration of viscous fibre was achieved.

Table 8 List of materials used in the characterization of viscous fibres

Material	Supplier	Information
Pectin Classic AF-101	Herbstreith & Fox	Degree of esterification: >77%
GRINDSTED® Pectin AMD 780	DuPont Nutrition and Health	High-ester, with sugar, 47g fibre per 100g. Uses: yogurt / acidified / milk-fruit / nutritional drinks
GRINDSTED® Pectin LA410	DuPont Nutrition and Health	Low-ester (31%), amidated (19%), high calcium-reactive, with 37g sugar and 57g fibre per 100g. Uses: spreadable low-sugar jam, yogurt fruit, other fruit systems
DARILOID® QH sodium alginate blend	FMC / Hawkins Watts	Medium guluronic acid (G), with sugar, dextrin and trisodium phosphate, viscosity of 3% solution: 500–1100 cP. Uses: chocolate sauce, fruit toppings and syrups
KELCOSOL® sodium alginate	FMC / Hawkins Watts	Low G, viscosity of 1% solution: 1000–1500 cP. Uses: fruit preparations
Protanal® IC2053 alginate blend	FMC / Hawkins Watts	Medium G, with sugar and tetrasodium pyrophosphate, Viscosity: 25–55 cP (method 2174)
Protanal® LF120 alginate	FMC / Hawkins Watts	High G, viscosity of 1% solution: 200–400 cP. Uses: fruit preparations
Hydrochloric acid, 1N	Sigma-Aldrich	To decrease pH of the solutions
Glucono-delta-lactone (GDL), food-grade	Hawkins Watts	To slowly decrease pH of the solutions
Tricalcium phosphate, FCC	Budenheim / Sherratt	Calcium source; insoluble under neutral pH, soluble under acidic pH

3.2.2.2 Rheological measurements

Rheological measurements of the solutions were carried out using an Anton Paar Physica MCR 301 Rheometer in the Food Characterization Laboratory, Massey University (photograph shown in Figure 6). The Concentric Cylinder (CC27, SN23944) geometry was set up to measure viscosity over a range of shear rates (0.01–500/s) at 20°C and 37°C. Detailed settings of the rheometer are shown in Figure 7 below. pH of the solutions were also measured.

Pectin and alginate (2% w/w) solutions were also prepared to determine their reactivity / sensitivity to acids. The solutions were pH-adjusted to 2.0 using 1N hydrochloric acid, with the intention to mimic typical acidic stomach conditions. For solutions that remained liquids (non-gelling), rheological measurements were performed at 37°C. As

for solutions that gel instantaneously when hydrochloric acid was added, the glucono-delta-lactone (GDL)-acidification method was used instead. When GDL is exposed to water, it slowly hydrolyzes into gluconic acid, which decreases the pH of the system. GDL was added to pectin and alginate solutions at 0.5M and 1.0M, respectively. Formulations of the GDL-acidification methods are shown in Table 9. A higher concentration of GDL was added to alginate solutions as they inherently have higher (alkaline) pH than pectins, thus more GDL was required to decrease the pH to ~2.5 after 90 minutes. GDL was mixed with the solution for 1 minute and a sufficient amount was immediately loaded into the geometry for rheological measurements. Gelation profiles, elastic modulus (G') and viscous modulus (G''), of the solutions at 37°C were recorded over 3 hours. Detailed settings of the rheometer are shown in Figure 8 below. The remaining solution was placed in a water-bath of 37°C and pH was recorded every 10 minutes for 3 hours.

Further tests were carried out to determine the reactivity / sensitivity of the pectins and alginates to divalent calcium ions (Ca^{2+}). Tricalcium phosphate is insoluble in water at neutral pH, but becomes soluble when acid is present *i.e.* under acidic pH conditions. The procedure was similar to the acidification method described above but with 0.4% tricalcium phosphate (equivalent to 0.16% Ca^{2+}) added to the solution prior to mixing with GDL.

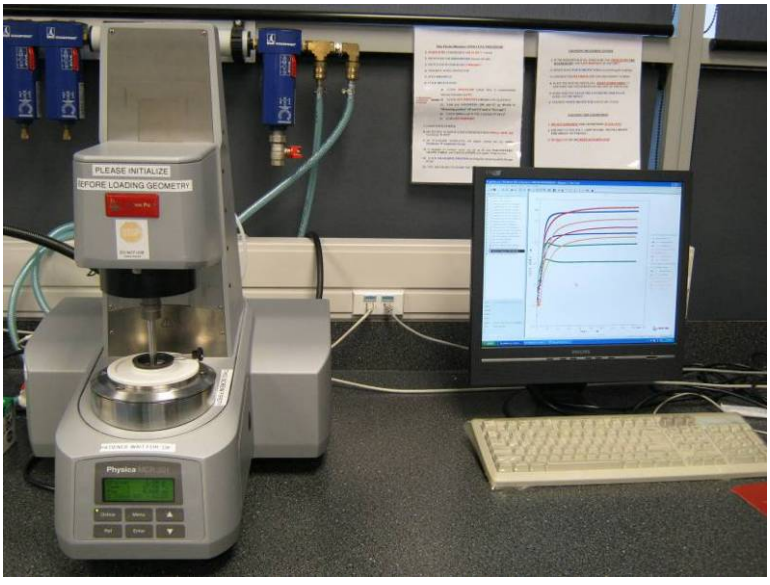


Figure 6 Anton Paar Physica MCR 301 Rheometer

Table 9 Formulations of the GDL-acidication method

	Acidification method and formulation in percentage (%)			
	Pectin		Alginate	
Ingredient	**0.5M GDL**	**$Ca_3(PO_4)_2$, 0.5M GDL**	**1M GDL**	**$Ca_3(PO_4)_2$, 1M GDL**
Pectin or alginate	2	2	2	2
GDL ($C_6H_{10}O_6$)	8.9	8.9	17.8	17.8
Tricalcium phosphate ($Ca_3(PO_4)_2$)	0	0.4	0	0.4
Water	89.1	88.7	89.1	88.7

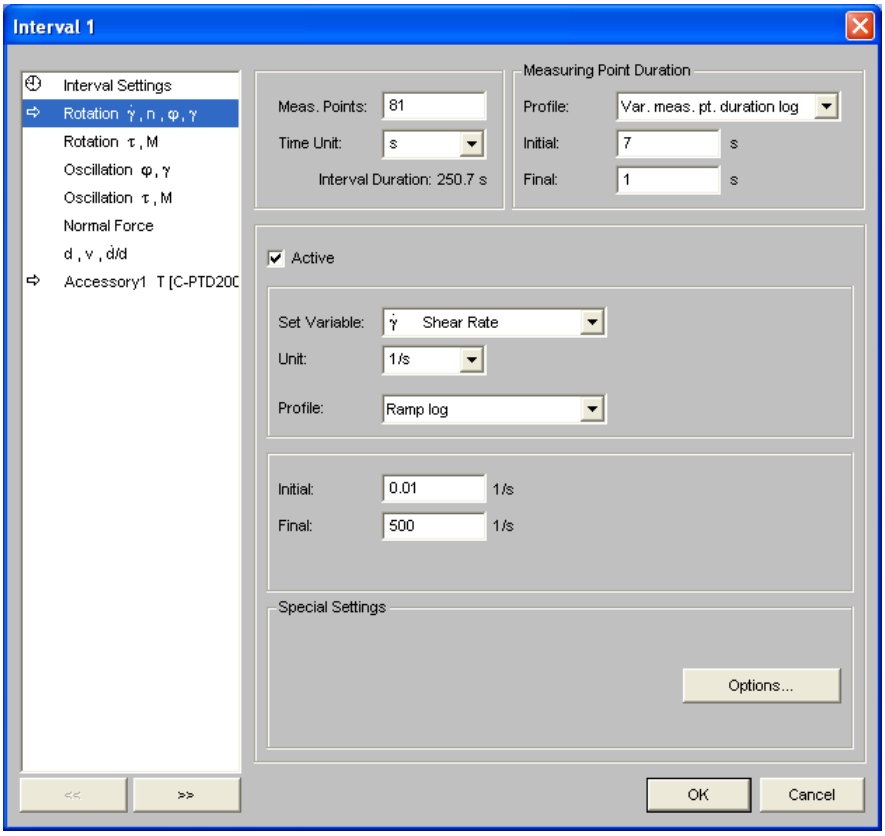


Figure 7 Settings of the rheometer for viscosity measurements

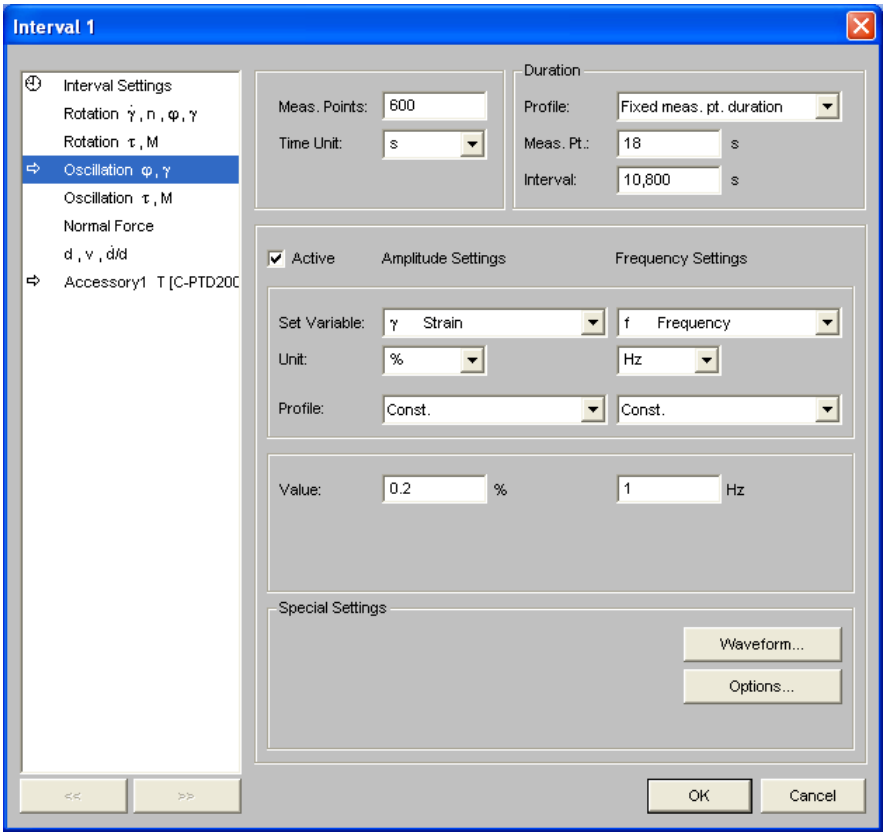


Figure 8 Settings of the rheometer for gelation (small deformation oscillatory) measurements

3.3 Beverage formulation and production

3.3.1 Materials

The project focused on developing a neutral (non-acidic), non-dairy, soy protein-based beverage model for the incorporation of potential appetite control ingredients such as viscous fibres, quercetin and fruit extract. The identity of the fruit extract used in this project will be kept confidential due to commercial reasons. After the characterization work, only a pectin (GRINDSTED® Pectin LA 410) and an alginate (Protanal® LF120) were further evaluated in the beverage formulations. Information of the materials and ingredients that were used are shown in Table 10.

3.3.2 Methods

3.3.2.1 Beverage processing

Laboratory and pilot plant trials of the beverage formulation work were carried out in the Food Development Laboratory (Plant and Food Research) and Food Pilot Plant

(Massey University), respectively. The processing method for beverages was adapted from Paulsen *et al.* (2005) with modifications.

As shown in the process flowchart (Figure 9) below, potassium citrate was first dissolved in water. It functions as a chelating agent; binding to free divalent cations in the water making them unavailable to interact with proteins therefore causing poor hydration and/or aggregation of proteins (Paulsen *et al.*, 2005). Isolated soy protein was slowly added to the water and mixed for about an hour at ambient temperature. Since the batch size for pilot plant trials was bigger, heating water to 40–50°C was necessary for better hydration of the soy protein.

The hydrocolloids, carboxymethylcellulose (CMC), alginate and pectin, were prepared as solutions, in concentrations of 2% w/w for CMC and alginate, and 7% w/w for pectin. Depending on the usage level of hydrocolloid (%) required in each formulation, the amount of hydrocolloid solution was calculated and added in accurately. After mixing all ingredients together, the beverage mixture was heated to 50°C and homogenized at 250/50 bar pressure, using the steam-jacketed cooker (as a water-bath) and APV Rannie 2-stage homogenizer, respectively. This step was only applicable to pilot plant trials. For laboratory trials, pasteurization of beverages was carried at 90°C with a holding time of 2 minutes. This was done in a boiling water-bath and constant stirring was applied. After heating, the beverages were cooled to 10°C in an ice water-bath before filling into clean bottles and kept in the chiller. As for pilot plant trials, the beverages were heat-treated using the plate-heat exchanger with an ultra-high temperature (UHT) of 140°C and holding time of 5 seconds, followed by rapid cooling to 10°C and aseptic filling into bottles within the laminar flow cabinet. The products were kept in a chiller of 4°C.

3.3.2.2 Rheological measurements

Viscosity (shear rates: 0.01–500/s, at 20°C) and gelation profiles (G' and G'', at 37°C, 3 hours) of the beverages were measured using the same rheometer settings and GDL-acidification method as described in Section 3.2.2.2. GDL was added at 0.5M concentration, which decreased pH to ~3 after 90 minutes. Viscosities of the beverages (20°C) were compared at a shear rate of 10/s, which is relevant to oral conditions (Peters *et al.*, 2011; Rao & Lopes da Silva, 2007)

Table 10 List of materials / ingredients used, their supplier and functionality

Material / ingredient	**Supplier**	**Functionality**
SUPRO® 760 IP isolated soy protein	Solae	Protein source, 88.1% protein
White sugar	Chelsea	Sugar source; sweetness
Fibruline® DS2 inulin	Cosucra / Salkat	Soluble fibre used for standardizing the fibre content in all beverages; 94% fibre
AQUALON® 7HOCF sodium carboxymethylcellulose (CMC)	Hercules / APS Food & Nutrition	Non-acid and -calcium reactive hydrocolloid to impart viscosity to the control beverage; 75% fibre
GRINDSTED® Pectin LA 410	Danisco / Dupont	Viscous fibre; 57% fibre; low-ester, amidated, high-calcium-reactive, standardized with sugar (37%)
Protanal® LF120 alginate	FMC BioPolymer / Hawkins Watts	Viscous fibre; min. 85% fibre; high guluronic acid (G) content, acid- and calcium-reactive
Tricalcium phosphate, micro fine powder, FCC, 40% Ca	Budenheim / Sherratt	Calcium source; insoluble under neutral pH, soluble under acidic pH
Potassium citrate	Hawkins Watts	Chelating agent to bind free divalent ions (e.g. Ca^{2+}) in the beverage (neutral pH)
Vanilla flavour 50190AB	Givaudan / GS Hall	Flavouring
Blueberry flavour QF18678	Givaudan / GS Hall	Flavouring
Food colourings (red, blue)	Queen Fine Foods	Colouring
Quercetin (high purity, >99.5%, food grade)	Quercegen Pharma LLC	Potential satiety ingredient
Isoquercetin (food grade)	Quercegen Pharma LLC	Potential satiety ingredient
Fruit extract (food grade)	–	Potential satiety ingredient
300ml HDPE bottles and caps	EPI Plastic	Packaging for the beverages

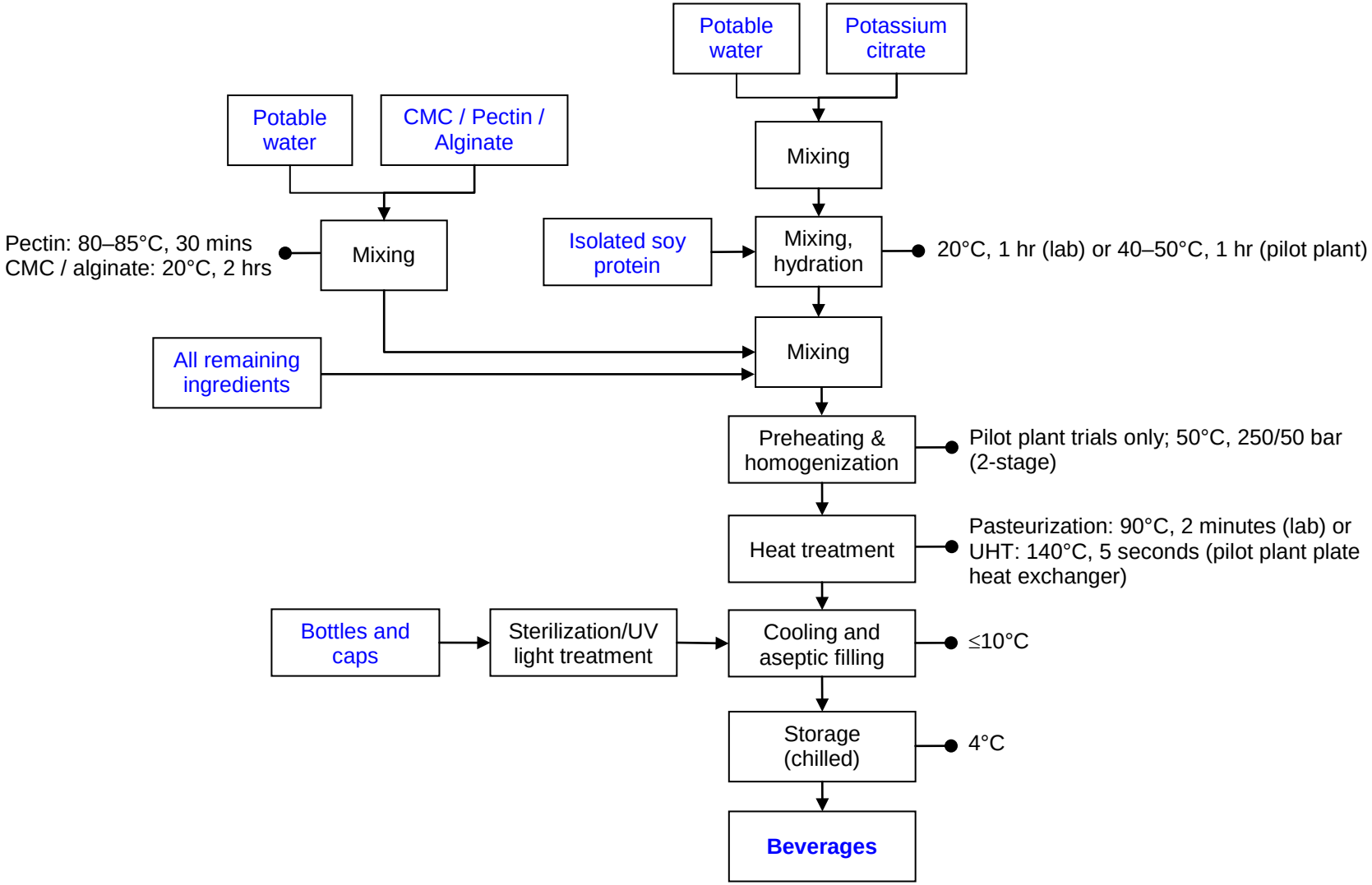


Figure 9 Process flowchart of the beverage model

3.3.2.3 Solids content and pH measurements

Solids content and pH of the beverages at 20°C were measured using a refractometer (ATAGO, model: Pocket PAL-1) and a pH meter (JENWAY, model: 3510), respectively.

3.3.2.4 Solubility tests

Several laboratory trials were carried out to determine a suitable method of incorporating quercetin / isoquercetin into the beverage, as they are insoluble in water. Thus, to compare the different methods in terms of the solubility of quercetin / isoquercetin, the beverages (10g sample in 15ml centrifuge tube) were centrifuged at 3000 rpm for 5 minutes using the Heraeus Multifuge IS-R centrifuge (located in the Food Chemistry Laboratory, Massey University). The relative centrifugal force (RCF) was calculated to be 1881.6. The amount of sedimentation (yellow-coloured particles of quercetin / isoquercetin) at the bottom of the tubes was observed visually and recorded.

3.4 Satiety measurement trial

3.4.1 Participants

The participants were volunteers recruited from Plant & Food Research in Palmerston North. An advertisement titled 'Seeking participants for a satiety measurement trial' (Appendix I) was used with several copies put up on notice boards around the institute, as well as a copy sent via electronic mail. The selection criteria were healthy male and female (not pregnant) individuals, aged 18–60 years with normal or overweight body mass index (BMI) of 18.5–30.0 kg/m^2. As it is important to consider the confounding factors in satiety studies, participants were excluded from the study if they were smoking, on medication, on a restraint diet and/or have food allergy / intolerance. All participants received a Participant Information Sheet and a Consent Form (Appendix II) and were given a verbal explanation of the trial.

A total of 28 participants were needed for the study based on statistical analysis (power of 80%). However, only 17 participants were recruited. Depending on their availability on the trial dates, they were either in Group A or Group B and each participant was randomly assigned with an identity code, for example A01, A02, B01, B02, *etc*. Of the 17 participants recruited, there were 2 dropouts (A05 and A06) before the study commenced, 1 participant (A01) withdrew after her 4th session due to gastrointestinal disturbances and headache, while another 2 participants (A02 and A08) completed all sessions but were unwell (possibly stomach bug or cold/flu) during 2 of their sessions.

Thus, only data of the 12 participants (6 females, 6 males) were collected. The group mean age and BMI were 41.2 years and 25.2 kg/m^2, respectively (Table 11).

Table 11 Age and BMI data of the participants

	Male, *n* = 6		**Female, *n* = 6**		**Group, *n* = 12**	
	Mean	**Range**	**Mean**	**Range**	**Mean**	**Range**
Age (years)	44	31–55	38.3	25–58	41.2	25–58
BMI (kg/m^2)	25.9	23.5–27.8	24.5	20.7–28.0	25.2	20.7–28.0

3.4.2 Test beverages and standard breakfast

From the development work, 6 beverages were finalized and 8 kg per batch each were produced for the satiety measurement trial. Descriptions of these test beverages are shown in Table 12. Formulations of the beverages can be found in Chapter 5, Section 5.2.10. Viscosity (shear rates: 0.01–500/s, at 20°C) and gelation profiles (G' and G'', at 37°C, 3 hours) of the beverages were measured using the same rheometer settings and GDL-acidification method as described in Section 3.2.2.2. GDL was added at 0.5M concentration, which decreased pH to ~3 after 90 minutes. Solids content and pH of the beverages were also measured. Samples were sent to AsureQuality for microbiological testing which ensured that the beverages were free from pathogens and safe for human consumption.

For the trial, each subject consumed all 6 test beverages once, over 6 separate morning sessions. The beverages were randomly assigned to the participants using a Latin square design. In this way, any potential aging effects (*e.g.* changes in viscosity and satiety effects) or carry-over effect of the beverages that were confounding to the study can be kept to a minimum. The actual quantities of beverages consumed were taken as the difference before- and after- consumption (*i.e.* weight of bottle with beverage minus weight of empty bottle). After consuming the test beverage (~250g), each participant consumed a standard breakfast which comprised of muesli (55g or about ½ cup) and either milk (125g) or soymilk (140g). The materials that were used are listed in Table 13. Presentation of the standard breakfast to the participants is shown in Figure 10.

Table 12 The test beverages

Test beverage	Labeled as – Beverage code:
1) Control (0.25% CMC)	543
2) CMC (0.25%) + fruit extract (0.2%)	786
3) Low level alginate / LLA (0.25% Protanal LF120)	694
4) Low level alginate / LLA (0.25% Protanal LF120) + fruit extract (0.2%)	127
5) High level alginate / HLA (0.5% Protanal LF120)	905
6) High level alginate / HLA (0.5% Protanal LF120) + fruit extract (0.2%)	281

Table 13 List of materials used in the satiety measurement trial

Material	Supplier	Purpose
Toasted Muesli Golden Oats and Fruit, Sanitarium	Pak n Save supermarket	Standard breakfast
Lite milk (1.5% fat), Homebrand	Countdown supermarket	Standard breakfast
Lite soymilk, Sanitarium	Countdown supermarket	Standard breakfast, dairy milk replacer
Clear plastic containers with dome lid, 250ml	Toops Wholesale Ltd	Container for muesli
White plastic cups, 200ml, Huhtamaki	Toops Wholesale Ltd	Container for milk or soymilk
Black/white paper cups, 115ml, Huhtamaki	Toops Wholesale Ltd	Container for plain water
Plastic dessert spoons, Toops	Toops Wholesale Ltd	Cutlery for eating breakfast

Figure 10 Presentation of the standard breakfast

3.4.3 Study design and procedures

The trial used a preload, single-blind, within-subject, repeated measures, completely balanced, crossover and randomized design. Each participant consumed all 6 beverages once *i.e.* they attended all 6 sessions. On those days before each session, they have to fast (no foods and drinks other than plain water) from 10 pm, refrain from alcohol consumption and strenuous physical activity. On the days of each session, they arrived at the trial location between 8.15 am to 9.15 am and be seated in the Sensory meeting room, where the Appetite rating form (Appendix III), Sensory evaluation form (Appendix IV), pen and timer were handed out to them. After understanding the instructions, each participant completed the baseline form for self-assessment of feelings of hunger, fullness, satisfaction (satiety), desire to eat and prospective food consumption, by means of marks on 100-mm line or visual analogue scales (VAS) scales.

Once the baseline VAS form was completed, each participant consumed a random-allocated 3-digit coded test beverage (Table 12) within 3 minutes and completed a sensory evaluation form rating its aroma, sweetness, thickness, flavour, aftertaste, colour and overall acceptability, by means of marks on 5-point just-about-right (JAR) scales. They then completed their second VAS form (time = 0), and subsequent VAS forms every 15 minutes for the first hour and every 30 minutes thereafter up to 4 hours. The participants used their timer to keep track of the time intervals required to fill up the forms.

After completing the 30-minute time interval VAS form, each participant consumed a standard breakfast (muesli and milk/soymilk) within 15 minutes. After the first hour, the participants returned to their workstations but were informed to do only paper or computer work and/or light laboratory work, avoiding strenuous physical work. They may consume plain water, limited to one cup (~100ml) per hour and only immediately after filling in the VAS form. Upon completion of the session, the forms were collected and the participants had their lunch as usual. They had a washout period of ≥3 days before returning to the trial location for subsequent sessions under the same conditions. Figure 11 below shows the timeline of a typical test session.

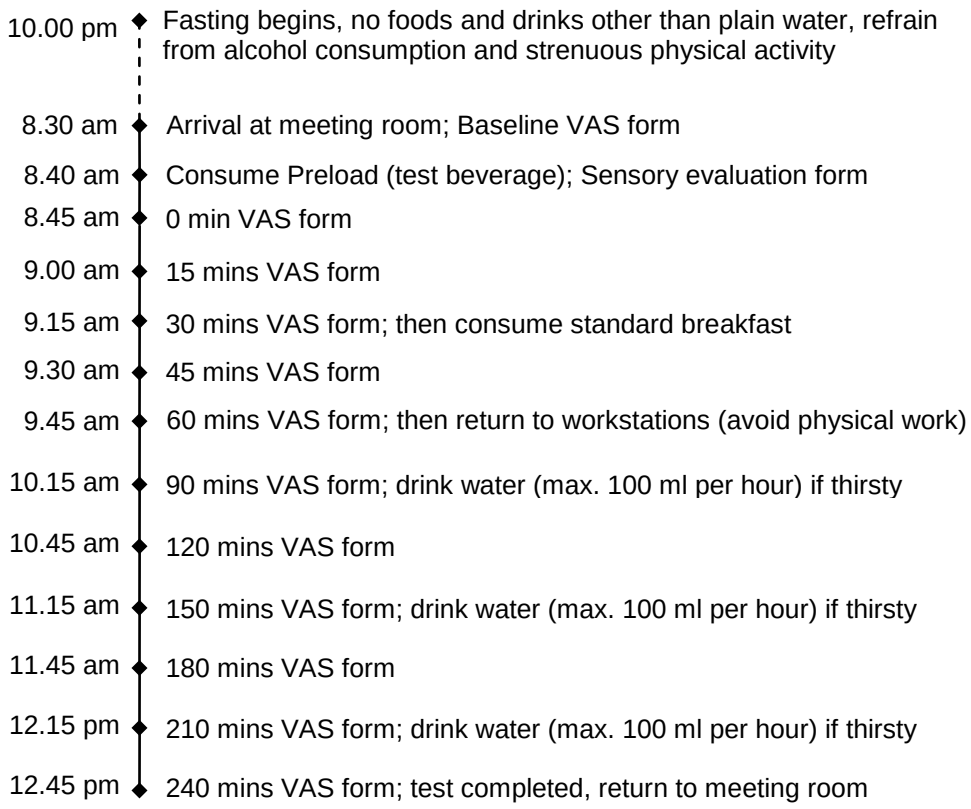


Figure 11 Timeline of a typical test session in the satiety measurement trial

3.4.4 Statistical data analyses

Data entry was done using Microsoft Office Excel 2003/2007. Statistical analyses and graphical representation of data were performed using Minitab 16 Statistical Software and SigmaPlot 12.0, respectively. The sensory evaluation JAR data were analyzed according to methods (ASTM standards) described by Rothman (2009) for JAR score means and by Parker (2009) for analysis of variance (ANOVA) to determine any differences between the mean scores (attributes) of samples (Parker, 2009; Rothman, 2009).

The appetite rating data was analyzed based on information described by Blundell *et al.* (2010) and with assistance by Mr. Duncan Hedderley (Statistician, Plant & Food Research). The appetite rating data (hunger, fullness, satisfaction, desire to eat and prospective food consumption) was analyzed using the mixed model approach (repeated measures analysis) of covariance (RMANCOVA), by the General Linear Model (GLM) in Minitab with baseline measurements as a covariate and participant or subject as a random factor. Results were expressed as means $\pm$ standard error of the mean (SE). Statistical differences between the means of test beverages were analyzed using the Tukey multiple comparison method at a confidence level of 95% (α-level of

0.05). Total areas under the curves (AUC) were computed using the macro function ‘Area below curves’ in SigmaPlot, which integrates area under curves using the trapezoidal rule. Analysis of covariance (ANCOVA) of the AUC data was also performed by the General Linear Model (GLM) in Minitab with baseline measurements as a covariate and subject as a random factor.

Chapter 4

Characterization of Viscous Fibres

4.1 Introduction

Two types of viscous fibres, pectin and alginate, were identified as satiety-enhancing ingredients that have been used in test beverages of several satiety studies. The type of pectin / alginate used in satiety studies is of importance, as not all pectin / alginate are the same. They can be chemically different, for example, in their molecular weight and methoxyl content (for pectins) or mannuronic acid to guluronic acid (M/G) ratios (for alginates). Different pectin / alginate can also differ in their physical properties *e.g.* viscosity of solutions, gel formation requirements, gel strength and stability, etc. Overall, the characteristics or properties of viscous fibres can in turn affect satiety responses in humans. Therefore, it is necessary to carry out characterization work in order to select the right type of pectin / alginate that would be suitable for use in the beverage model for satiety studies.

The aim of the current work was to determine suitable pectin and alginate for the next phase of the development work (beverage formulation and production). The ideal viscous fibre should not impart undesirable high viscosity and gelling to the beverage (during storage / before consumption), but should form a gel and contribute to gel formation and strength when the beverage is ingested (in the stomach). It is believed that gastric gelation can induce satiety, through the formation of a gel that has some strength (Hoad *et al.*, 2004; Peters *et al.*, 2011). Thus, viscous fibres will be selected based on their: (1) viscosity contribution to the beverage, (2) sensitivity to acids (low pH in stomach) and calcium ions (insoluble in the beverage, soluble at low pH) for gelation, and (3) gel strength.

The objectives of the characterization work were as follows:

1. To measure and compare the viscosity profiles of different pectins and alginates, thereby estimate the appropriate usage levels in the beverage model.
2. To compare their reactivity / sensitivity to calcium ions and acids; to determine the effects when pH is lowered to ≤ 3, intended to mimic acidic stomach conditions.

4.2 Results and discussion

The preparation and measurement methods have been described earlier (Chapter 3, Section 3.2.2). The pectins (Pectin Classic AF101, Pectin AMD780 and Pectin LA410) and sodium alginates (Dariloid QH, Kelcosol, Protanal IC2053 and Protanal LF120) evaluated are locally sourced, commercially available food ingredients.

4.2.1 Viscosity profiles and pH of the pectin and alginate solutions

Solutions of pectins and alginates were prepared in concentrations of 1% and 2% w/w. The pectin solutions were of acidic pH whereas the alginate solutions were of pH in the neutral to alkaline range (Table 14). Overall, the 2% pectin solutions were lower in pH (3.0 – 4.2) than the 1% pectin solutions (3.2 – 4.3). This is due to the acidic nature of pectin. Among the three pectins, Pectin LA410 (low methoxyl and amidated) was less acidic than Pectin AMD780 (high methoxyl) and Pectin Classic AF101 (high methoxyl). Amidated pectins are produced by reaction of high methoxyl pectin with ammonia. Thus, pH of pectins could possibly be altered during the reaction since ammonia is a weak base alkali leading to differences in the p*Ka* of the pectins. The alginate solutions (2%) were of pH in the range of 6.8 to 10. Dariloid QH and Protanal IC2053 had slightly higher pH than Kelcosol and Protanal LF120, probably due to variations in their production methods.

Viscosity curves of the pectin and alginate solutions are shown in Figure 12. All three pectin solutions appeared to be Newtonian *i.e.* viscosity is independent of shear rate and time. A higher concentration of pectin is required for it to exhibit non-Newtonian, pseudoplastic or shear-thinning behaviour (viscosity decreases with increasing shear rates) (Foster & Wolf, 2011). Viscosity data at various shear rates are shown in Table 14. For Newtonian liquids, the viscosity data obtained is known as ‘absolute viscosity’ whereas for non-Newtonian liquids, it is known as ‘apparent viscosity’ (given with the corresponding shear rate) (Rao, 2007b). Viscosities of these pectins did not seem to differ much, at these concentrations.

In contrast, viscosity differences among the four alginates were more evident. At both 1% and 2% concentrations, Kelcosol had the highest viscosity, followed by Protanal LF120, Dariloid QH and Protanal IC2053. This is in good agreement with the viscosity data from their product information sheets (Table 14); Kelcosol (1% solution: 1000–1500 cP) has the highest viscosity, followed by Protanal LF120 (1% solution: 200–400 cP), Dariloid QH (3% solution: 500–1100 cP) and Protanal IC2053 (viscosity 25–55 cP, unknown concentration). In a satiety study by Georg Jensen *et al.* (2012), viscosities of

alginates with different mannuronic:guluronic (M:G) acids ratio were measured. They found the alginate with a M:G ratio of 2.5 *i.e.* high M, low G to have higher viscosities at all concentrations tested (1–3% solutions) than alginates with lower M:G ratios of 0.8 and 1.3. Although the M:G acids ratio of these four alginates remained unknown, it did seem that low G alginates such as Kelcosol, have higher viscosity. However, the differences in viscosity could be due to differences in molecular weight and hydrodynamic radius (particle size) rather than the M:G ratio (Georg Jensen *et al.*, 2012).

Dariloid QH and Protanal IC2053 appeared to be Newtonian especially at low concentration of 1%, while Kelcosol and Protanal LF120 exhibited shear-thinning behaviour. The shear-thinning behaviour is observed with increasing concentration and is due to the interactions or re-alignment of alginate molecules when concentration exceeds the dilute regime (Foster & Wolf, 2011).

4.2.2 Reactivity of the pectins and alginates to acidification and calcium ions

Acidification of the pectin and alginate solutions was initially carried out using hydrochloric acid (1N). The method was appropriate for those materials that remained liquids *i.e.* non-gelling when pH is lowered to 2, enabling viscosity measurements to be taken. As for acid- and/or calcium-sensitive pectins and alginates, gelation or gel formation during the addition of hydrochloric acid was evident by a large increase in viscosity and gradually forming bits of gel as stirring was applied. Thus, a slow acidification using glucono-delta-lactone (GDL) was carried out to follow the development of structure with a gradual decrease in pH.

4.2.2.1 Pectins

Solutions of Pectin Classic AF101 and Pectin AMD780 remained as liquids when pH was lowered to 2. As shown in Figure 13, their viscosities were unaffected by the acidification process. As Pectin Classic AF101 and Pectin AMD780 have high methoxyl content, they are likely to form gels only under acidic and high soluble solids (>55%) conditions (EndreB & Christensen, 2009). Pectin Classic AF101 is recommended for use in jam, marmalades and fruit preparations, which have both acidic and high solids conditions for gelling. Uses of Pectin AMD780 includes yogurt-, acidified- and nutritional drinks, probably to impart viscosity, mouthfeel and/or stability and unlikely for gelling. Thus, Pectin Classic AF101 and Pectin AMD780 did not meet the selection criteria, were considered unsuitable for use in the beverage model.

Table 14 Product information, pH and viscosity data of the pectins and alginates

				Viscosity at various shear rates, measured at 20°C, unit: cP (1 Pa.s = 1000 cP), mean (*n* = 3)			
Viscous fibre	**Product information**	**Concentration**	**pH at 20°C**	**1/s**	**10/s**	**50/s**	**99/s**
Pectin Classic AF101	Degree of esterification: >77%. Uses: jam, marmalades and fruit preparations	1%	3.18	12.7	11.7	11.2	11.3
		2%	3.01	55.1	51.8	50.6	49.8
GRINDSTED® Pectin AMD 780	High-ester, with sugar, 47g fibre per 100g. Uses: yogurt / acidified / milk-fruit / nutritional drinks	1%	3.63	9.4	9.4	9.2	9.3
		2%	3.42	38.9	38.1	37.1	36.4
GRINDSTED® Pectin LA410	Low-ester (31%), amidated (19%), high calcium-reactive, with 37g sugar and 57g fibre per 100g. Uses: spreadable low-sugar jam, yogurt fruit, other fruit systems	1%	4.26	12.6	11.0	10.9	11.1
		2%	4.20	37.2	39.9	40.0	39.8
DARILOID® QH sodium alginate blend	Medium guluronic acid (G), with sugar, dextrin and trisodium phosphate, viscosity of 3% solution: 500–1100 cP. Uses: chocolate sauce, fruit toppings and syrups	1%	9.89	37.1	35.2	33.8	32.6
		2%	9.98	187.0	182.0	159.0	142.0
KELCOSOL® sodium alginate	Low G, viscosity of 1% solution: 1000–1500 cP. Uses: fruit preparations	1%	7.49	1543.3	942.7	520.0	382.3
		2%	7.65	11950	4735	2010	1340.0
Protanal® IC2053 alginate blend	Medium G, with sugar and tetrasodium pyrophosphate, Viscosity: 25–55 cP (method 2174)	1%	7.11	160.3	157.3	141.3	127.3
		2%	6.82	1400	1180	840.0	673.0
Protanal® LF120 alginate	High G, viscosity of 1% solution: 200–400 cP. Uses: fruit preparations	1%	9.66	19.2	18.4	18.3	18.0
		2%	9.67	91.8	90.3	84.3	78.3

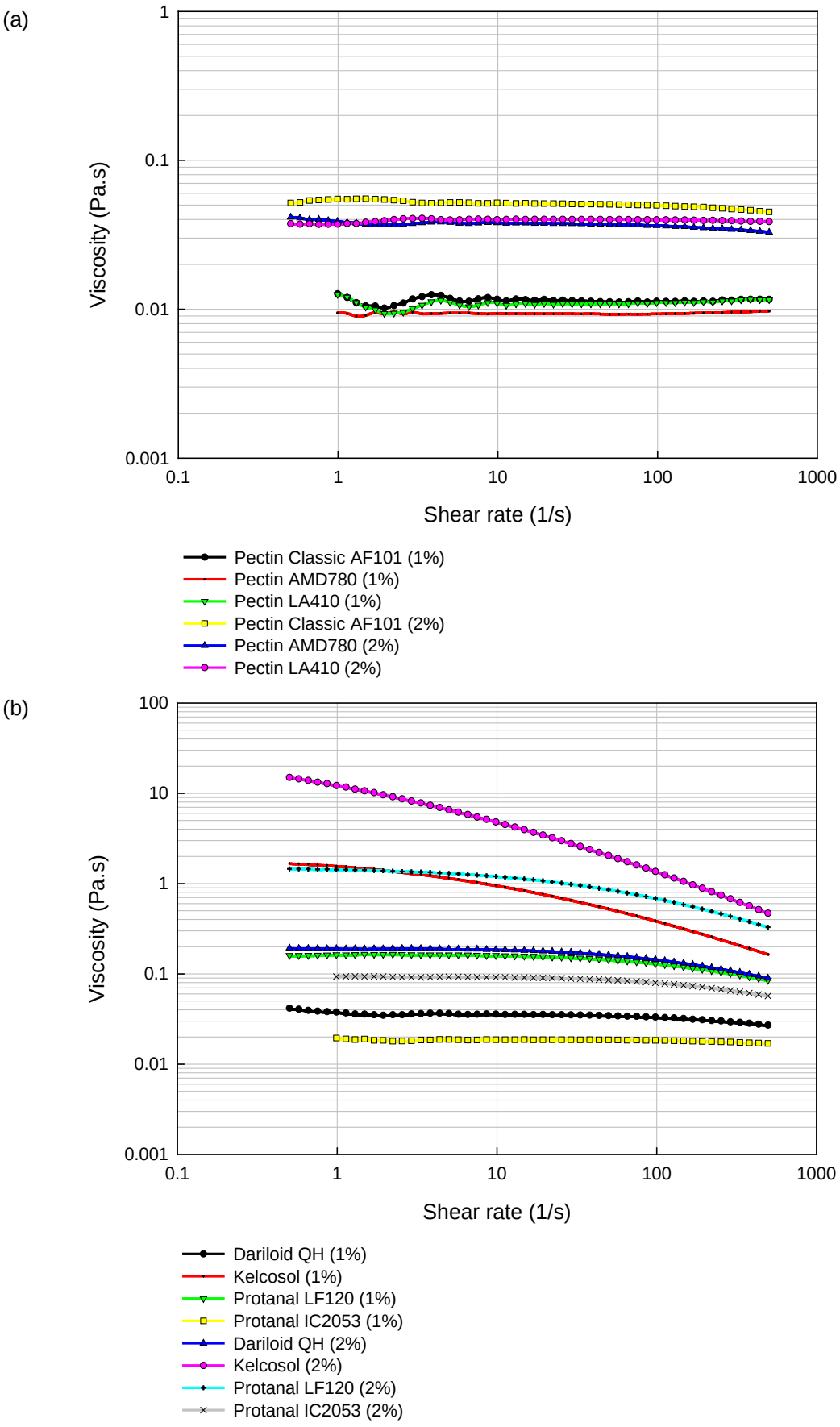
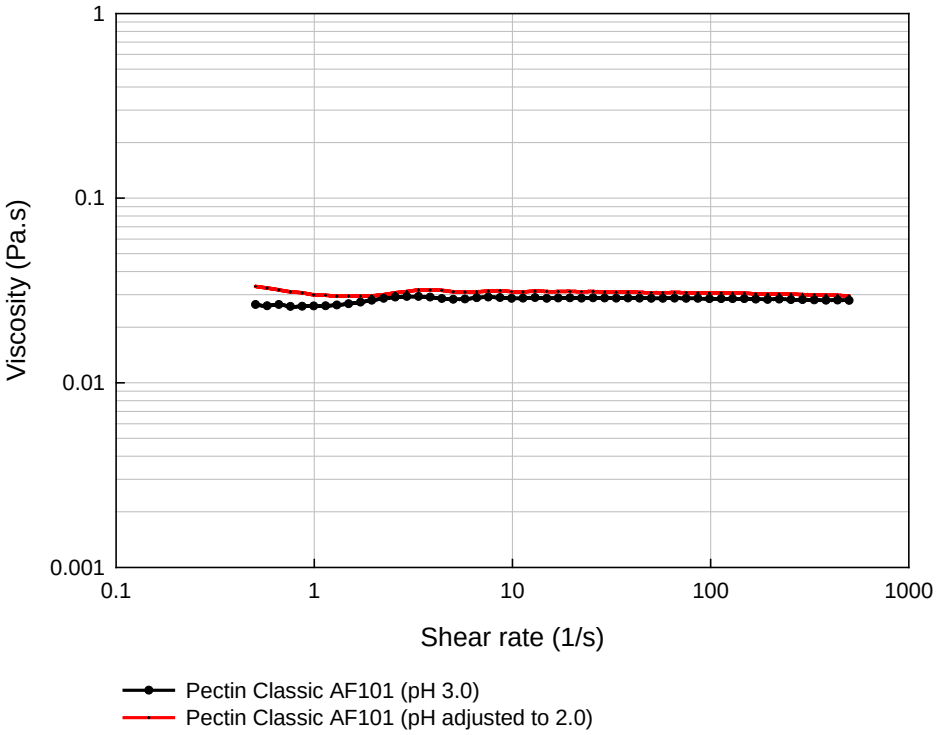


Figure 12 Viscosity curves of various (a) pectin solutions and (b) alginate solutions (1% and 2% w/w), measured at 20°C

(a)



(b)

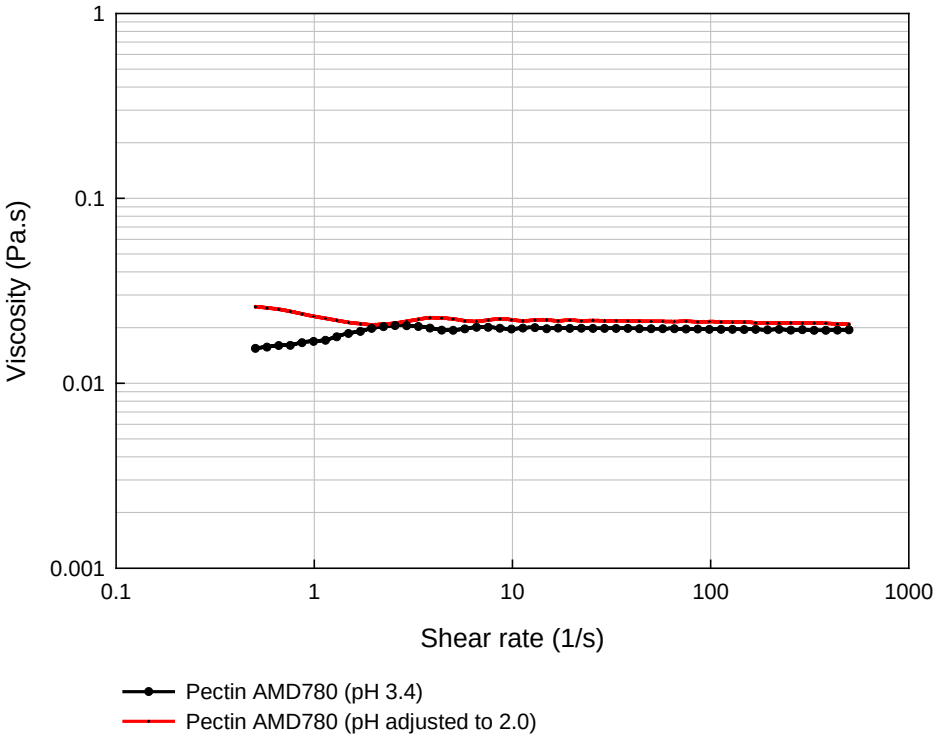


Figure 13 Viscosity curves of (a) Pectin Classic AF101 and (b) Pectin AMD780 solutions (2% w/w), measured at 37°C, with and without pH adjustment to 2.0

Gelation occurred in the low methoxyl (DE: ~31%), amidated (DA: ~19%) Pectin LA410 when hydrochloric acid was added. Thus, the GDL-acidification method was used to follow the gelation process. When dealing with a polymer solution, gelation refers to the transition from a liquid to a gel, known as the sol-gel transition (Lopes da Silva & Rao, 2007). It is where a continuous network of polymer molecules is formed. The small amplitude oscillatory shear technique is commonly used to measure the dynamic moduli during the gelation process, to identify the gel point, and to follow the continuous evolution of the viscoelastic properties of polymers. One of the definitions of gel point is the time at which elastic modulus (G') and viscous modulus (G'') crossover, at a given frequency (Lopes da Silva & Rao, 2007). The gelation profiles of Pectin LA410 are shown in Figure 14.

It is evident that Pectin LA410 showed sensitivity to acids and calcium ions, notably in the absence of other solids. During measurement of the pectin with tricalcium phosphate (TCP), the G' and G'' crossover (gelation) occurred before the sample was loaded. When GDL was added to the pectin solution with TCP, it slowly hydrolyzed into gluconic acid, which decreased the pH of the system. According to Le Chatelier's principle, the system responded to this reduction by producing more phosphate ions, thus the solid TCP will dissolves, releasing free calcium ions and the equilibrium will be shifted to the right. Figure 15 shows equations of the possible chemical reactions involved (ChemPRIME, 2010; Pocker & Green, 1973). With the free calcium ions and acidic condition, gelation of the calcium-reactive Pectin LA410 took place quickly. The gelation mechanism behind is the bridging between adjacent two-fold helical chains forming the so-called 'egg-box' junction zone structures (Thakur *et al.*, 1997). An illustration of the gelling mechanism is shown in Figure 16. Furthermore, the G' and G'' were significantly higher for Pectin LA410 with TCP than that without TCP. Clearly, the presence of calcium ions is an important factor for gel formation, structure and strength of Pectin LA410.

The G' and G'' crossover of the pectin solution (without TCP) was noted at ~28 minutes and at pH of approximately 2.7. G' and G'' of the pectin solution increased strongly as pH decreased to 2.4 at the end of 3 hours (Figure 14). Lootens *et al*. (2003) reported gelation of a similar low methoxyl (DE: 28.7%), amidated (DA: 18%) pectin with decreasing pH starting from pH ~3.4, in the absence of calcium. G' and G'' of the pectin increased strongly with decreasing pH until ~2.2. The authors suggested that further reduction of pH reduces the charge density of the pectin chains thereby reducing electrostatic repulsion. Conformational transition induces aggregation of the

pectin chains and that the amide groups reinforce the gel structure via hydrogen bonding. Furthermore, they found that in the presence of calcium ions and below pH ~3.5, G' and G'' are much higher than that in the absence of calcium ions. This shows calcium ions have a reinforcing effect on pectin gels (Lootens *et al.*, 2003).

In order to confirm that Pectin LA410 was suitable for the neutral beverage model, it was pH-adjusted to 7 using sodium hydroxide (NaOH, 1N). Pectin LA410, as well as Pectin Classic AF101 and Pectin AMD780, all remained liquids and their viscosities were measured (Figure 17). Increasing pH to 7 did not affect their viscosities. Therefore, Pectin LA410 was considered suitable for the next phase of the development work.

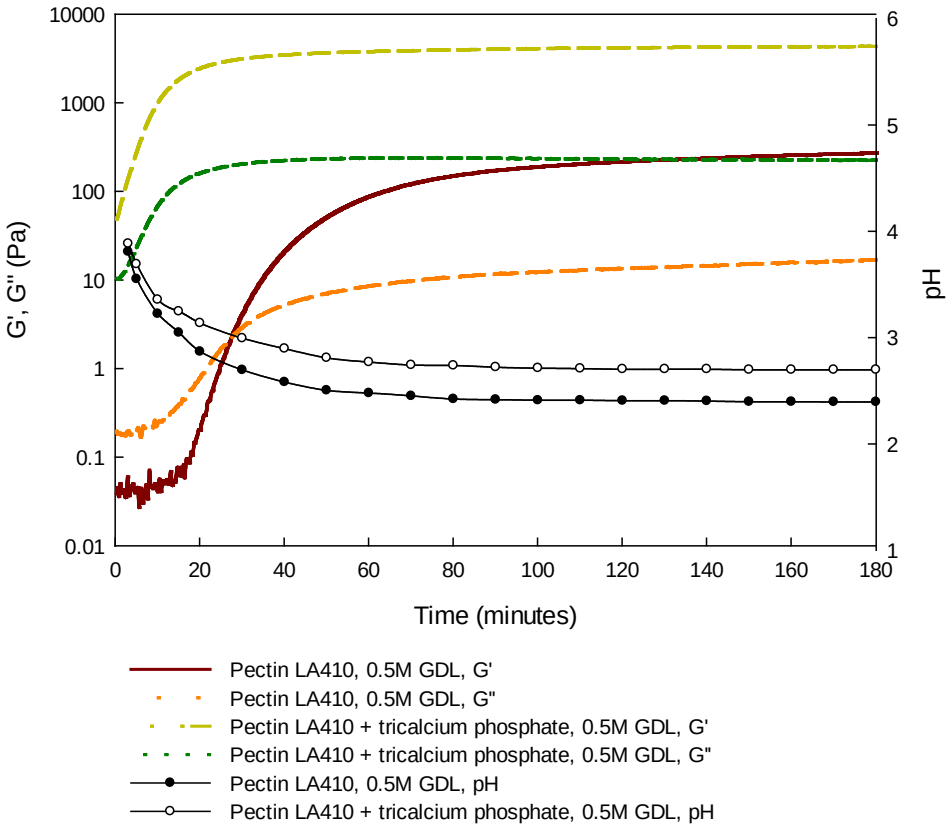


Figure 14 Gelation profiles of Pectin LA410 (2% w/w), changes in G' and G'' correlation to lowering of pH by 0.5M GDL, tricalcium phosphate was used at 0.4% w/w (equivalent to 0.16% Ca^{2+}), measured at 37°C, 1 Hz and 0.2% strain

D-glucono-delta-lactone + water Ý D-gluconic acid Ý D-gluconate ion + hydrogen ion

$C_6H_{10}O_6 + H_2O$ Ý $C_6H_{12}O_7$ Ý $C_6H_{11}O_7 + H^+$

When acid is added or present in the solution,

Tricalcium phosphate Ý calcium ions + phosphate ions

$Ca_3(PO_4)_2$ (s) Ý $3Ca^{2+}$ (aq) + 2PO□(aq)

H^+ ion (from GDL) + phosphate ion Ý Hydrogen phosphate ion

$H^+ + PO□$ Ý HPO‹ or $H_3O^+ + PO□$ Ý HPO‹ + H_2O

Figure 15 Equations of the chemical reactions involved

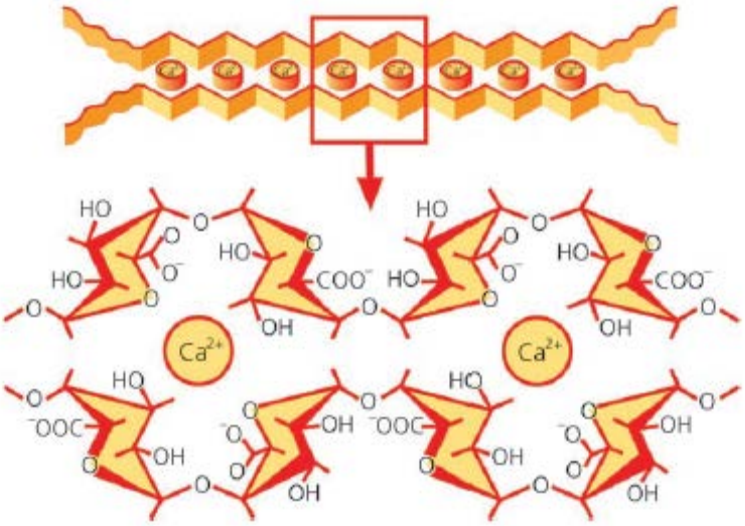


Figure 16 Gelling mechanism of low methoxyl pectins; complexing with calcium ions (Source: Herbstreith & Fox, 1999)

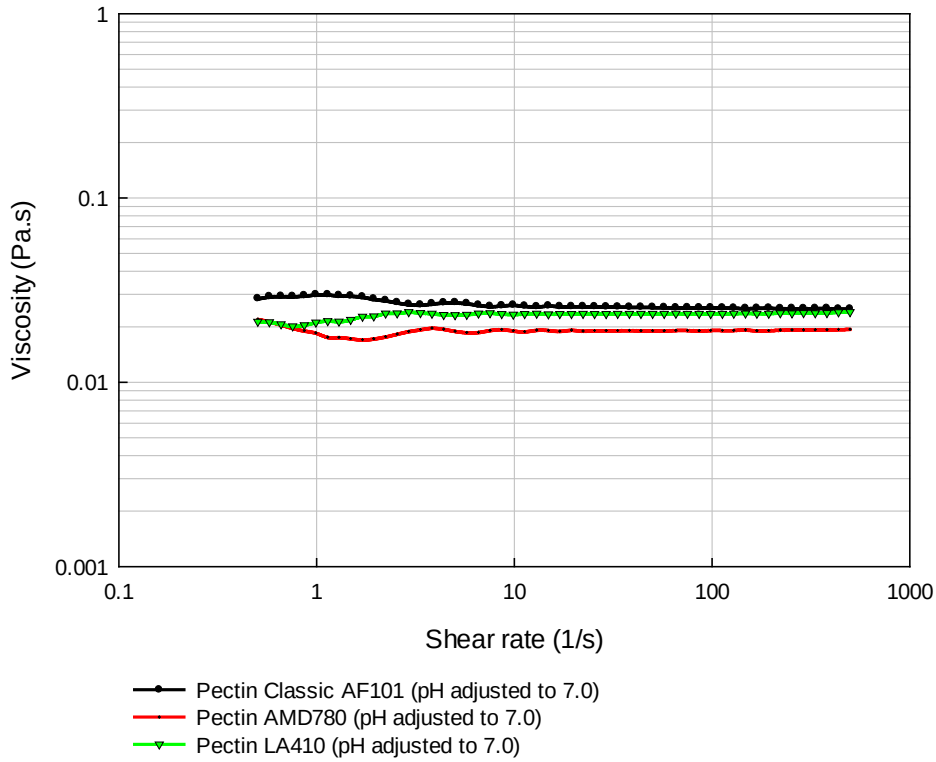


Figure 17 Viscosity curves of Pectin Classic AF101, Pectin AMD780 and Pectin LA410 solutions (2% w/w), measured at 37°C, with pH adjustment to 7.0 using NaOH (1N)

4.2.2.2 Alginates

Among the four alginates, only Kelcosol remained liquid when pH was lowered to 2 using hydrochloric acid. At pH 2, the viscosity of Kelcosol solution was higher, below 10/s, but lower above this rate, compared to the viscosity at pH 7.6 (without pH adjustment) (Figure 18). Since Kelcosol remained a viscous liquid at acidic pH and based on supplier's information that Kelcosol is of low guluronic (G) acid, it was considered unsuitable for the beverage model. From the literature review, it was found that satiety studies specify the use of high G alginates and either those of high molecular weight or strong gelling type. In addition, high G alginates generally are highly sensitive to calcium ions and produced stronger gels than low G alginates (Draget *et al.*, 1994).

The dissociation constants, p*Ka* values for mannuronic and guluronic acid monomers are 3.38 and 3.65, respectively (Draget, 2009; Haug, 1964). Alginate solutions may behave in two different ways when their pH is lowered, (1) instantaneous precipitation of alginic acid molecules, caused by an abrupt decrease in pH, or (2) formation of an 'alginic acid gel' achieved by a slow and controlled release of protons / H^+ ions (Draget, 2009). Although hydrochloric acid was added slowly to the alginate solutions and signs of gelling were obvious, it is likely that some precipitation of alginic acid molecules also

occurred. Therefore, the preparation of alginic acid gel should be performed with pH lowered in a controlled fashion, for example using GDL (Draget, 2009). Thus, the GDL-acidification procedure should allow a good comparison of the gelation profiles of different alginates.

Acidification of Protanal IC2053 solution (2%) was initially carried out using 0.5M GDL. As seen in Figure 19(a), the G' and G'' crossover did not occur until ~117 minutes, pH ~2.9. Moreover, G' (elasticity or gel properties) barely dominated over G'' (viscous properties). Clearly, a higher concentration of GDL was needed to trigger the gelation process earlier and obtain a stronger gel by reaching a lower pH (<3). Thus, 1M GDL was used for subsequent acidification of alginates. The final pHs obtained were around 2.4 – 2.5, somewhat similar to that of Pectin LA410 with 0.5M GDL. When 1M GDL was used, the G' and G'' crossover of Protanal IC2053 occurred at ~27 minutes. The pH was between 2.8 (at 30 minutes) and 3.0 (at 20 minutes), which seems to match the 'gelation' pH of the earlier test. As pH decreased further, G' strongly increased and was significantly higher than G''.

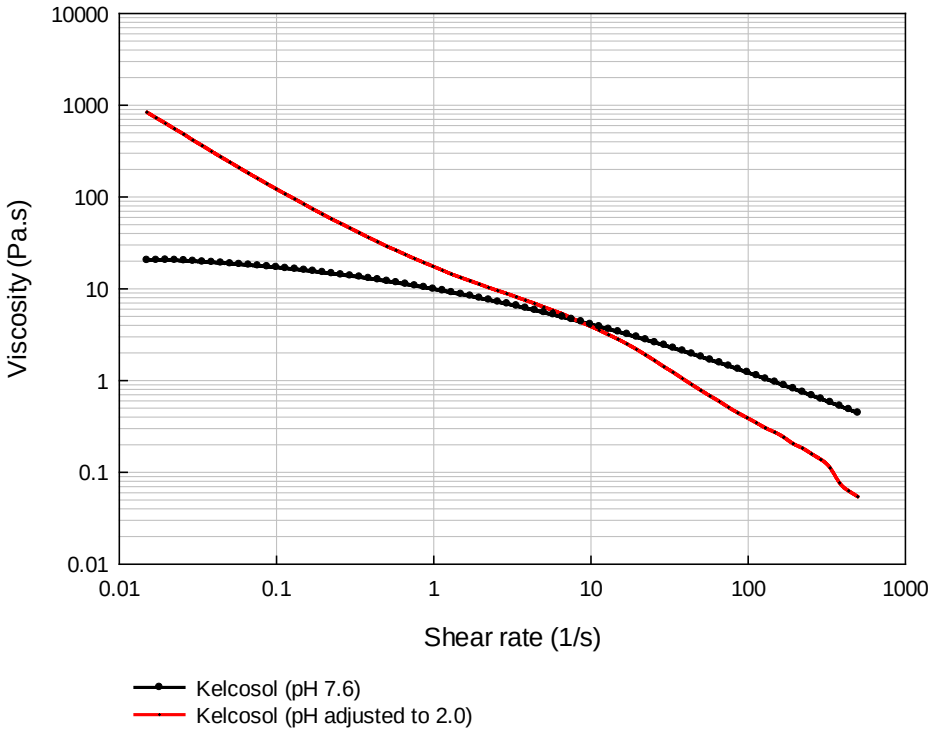
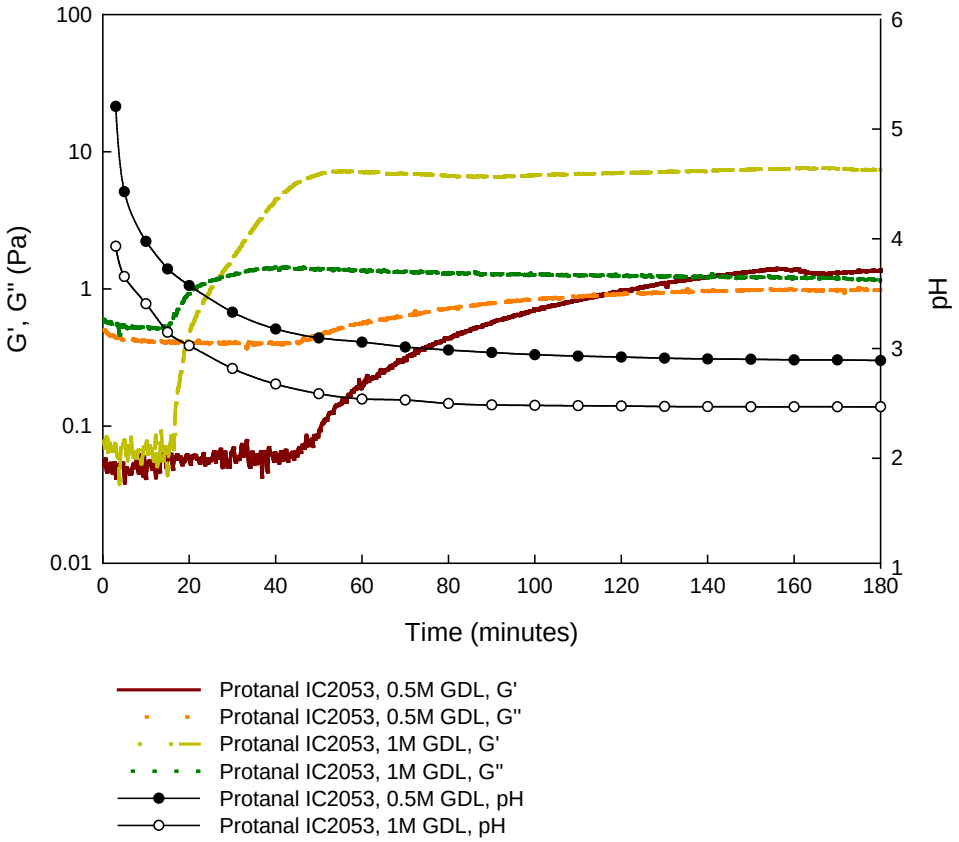


Figure 18 Viscosity curves of Kelcosol solutions (2% w/w), measured at 37°C, with and without pH adjustment to 2.0

(a)



(b)

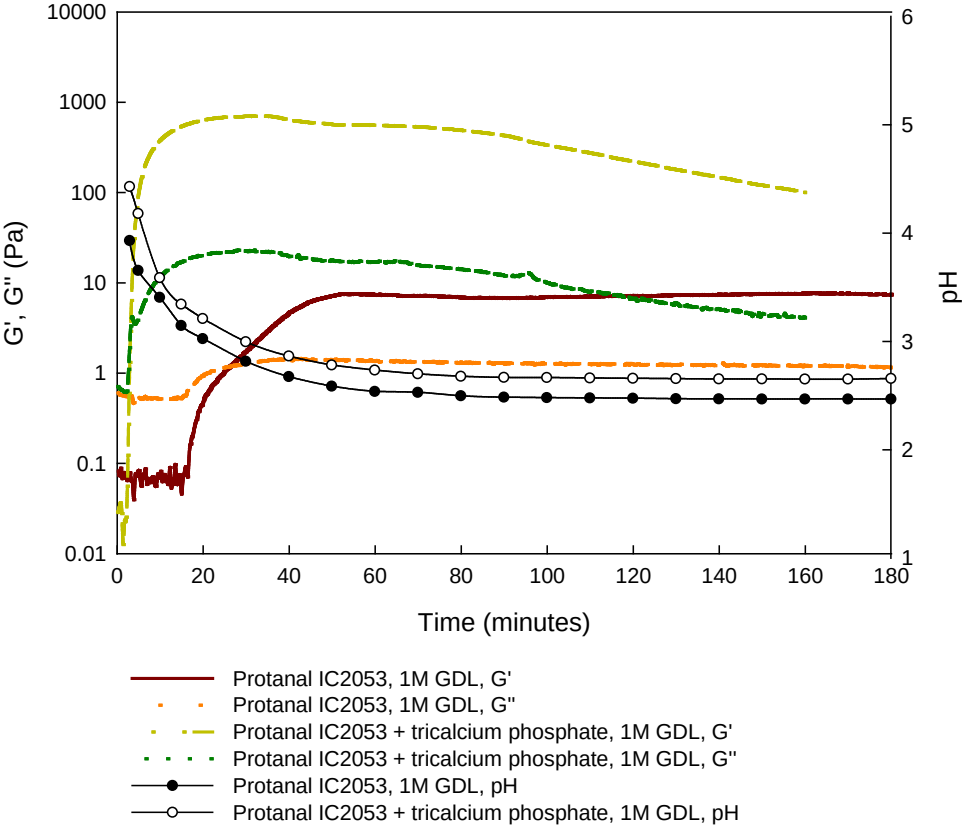


Figure 19 Gelation profiles of Protanal IC2053 (2% w/w), changes in G' and G'' correlation to lowering of pH (a) by either 0.5M or 1M GDL, and (b) by 1M GDL, tricalcium phosphate was used at 0.4% w/w (equivalent to 0.16% Ca^{2+}), measured at 37°C, 1 Hz and 0.2% strain

The same acidification method was applied to Protanal IC2053 but with TCP, which is similar to the 'internal setting' technique for producing alginate gels. As seen in Figure 19(b), the G' and G'' crossover occurred at ~3.3 minutes, pH of ~4.4. It implies that Protanal IC2053 was highly sensitive to calcium ions. G' and G'' increased strongly in the first 30 minutes, but gradually decreased after 35 minutes of the test. Being sensitive to calcium (Ca^{2+}) ions, it is likely that gelation of Protanal IC2053 and other alginates is produced by the binding of Ca^{2+} ions to G-blocks of alginate molecules, a phenomenon often explained by the egg-box model (Figure 20). In this case, a 'Ca-alginate gel' was formed, instead of an 'alginic acid gel'. However, as pH continued to decrease, the Ca-alginate gel might be converted to an alginic acid gel by exchanging the ions with protons (H^+ from acid) (Draget *et al.*, 1994). Draget *et al.* (1994) found that Ca-alginate gels lose a substantial part of their strength when converted to alginic acid gels. Thus, the decrease in G' and G'' after 35 minutes is probably due to the increase in acidity as H^+ is released from GDL. Another possibility is that the rapid acidification has led to localized instantaneous *i.e.* 'chaotic' gelation or precipitation, resulted in network rearrangements of inhomogeneous gels and hence loss of gel strength (Smith & Miri, 2011).

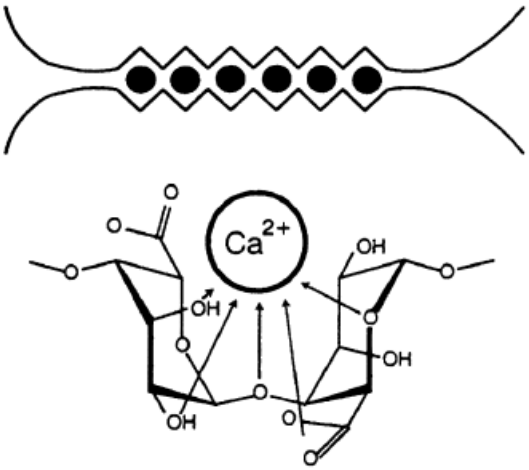


Figure 20 The egg-box model for binding of divalent cations e.g. Ca^{2+} to homopolymeric blocks of α-L-guluronic residues, and a probably binding site in a GG-sequence (Source: Draget *et al.*, 2005)

Acidification of Dariloid QH solution (2%) was also carried out using 1M GDL. The gelation profiles are shown in Figure 21. The G' and G'' crossover of Dariloid QH occurred at ~53 minutes, at pH of ~2.5, which was a longer time and lower pH than for Protanal IC2053. On the other hand, acidification of Dariloid QH with TCP produced very rapid G' and G' crossover, within the first minute. However, the G' and G''

decreased after 3 minutes. Similar to Protanal IC2053, it is probably due to the increase in acidity, which disrupted the gel network formed by Ca^{2+}.

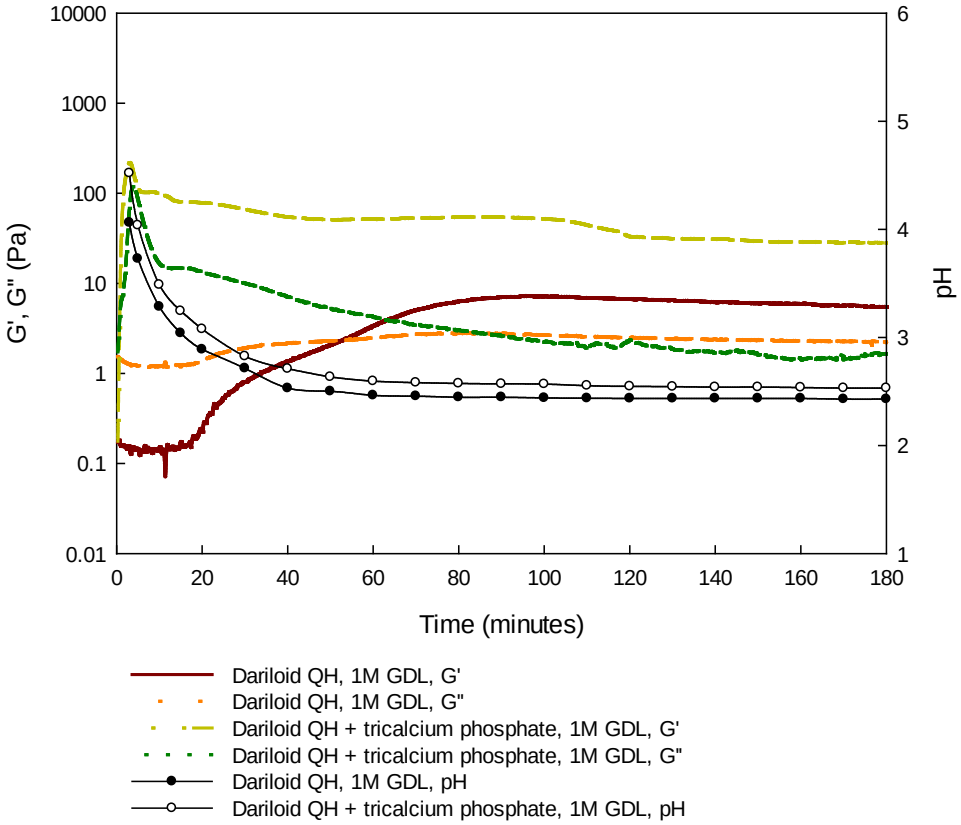


Figure 21 Gelation profiles of Dariloid QH (2% w/w), changes in G' and G'' correlation to lowering of pH by 1M GDL, tricalcium phosphate was used at 0.4% w/w (equivalent to 0.16% Ca^{2+}), measured at 37°C, 1 Hz and 0.2% strain

Higher acid and calcium sensitivities were observed in Protanal LF120. The G' and G'' crossover of the Protanal LF120 solution occurred at ~11 minutes and pH of ~3.2 (Figure 22). During measurement of the alginate with TCP, the G' and G'' crossover (gelation) occurred before the sample was loaded. Among the alginates measured, Protanal LF120 had the highest G' and G'' values. The strong-gelling property of Protanal LF120 is probably contributed by its high G content. As explained by the egg-box model, binding of Ca^{2+} ions is to G-blocks of the alginate molecules. Thus, a higher proportion of G-blocks, in the presence of Ca^{2+} ions, would increase the rate of gelation and result in higher gel strength (Draget, 2009).

The gelation profiles of Protanal LF120 began to decrease after ~45 minutes. The measurement was continued until the end of 3 hours but the data thereafter was considered unreliable. When the inner cylinder was raised from the outer concentric cylinder, a smooth and rigid gel formed around the inner cylinder was seen (as

photographed in Figure 23). The tests were repeated and the same gelation profiles and observations were obtained. It is thought to be a phenomenon known as the slip effect. During the gelation of Protanal LF120, a thin-layer of low-viscosity fluid is likely to have formed at the boundary solid-fluid (gel) interface that in turn contributed to lower viscosity values. Thus, the sudden 'break' or drop in the flow curves of Protanal LF120 is considered an indication of slip (Miri, 2011; Rao, 2007c). Formation of the thin-layer of low-viscosity fluid could be due to the increase in acidity (disrupts the gel network) and/or syneresis (separation of liquid from a gel). According to Draget (2009), observations have shown that gels produced by internal setting are more exposed to syneresis than gels by diffusion setting. Another explanation for the sudden 'break' or drop in the flow curves of Protanal LF120 is due to the rapid and/or further acidification that resulted in spontaneous gel shrinkage within the measurement cell, causing the gel to lose mechanical contact and stress signal with the measurement cell and rheometer (Matia-Merino *et al.*, 2004).

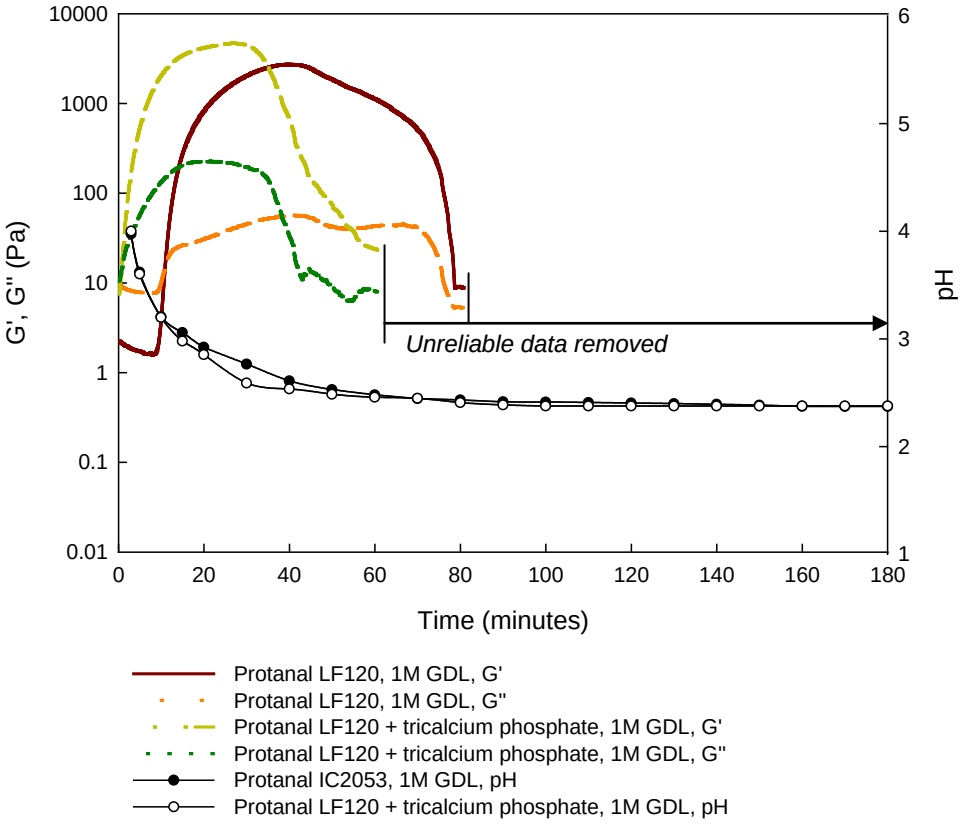


Figure 22 Gelation profiles of Protanal LF120 (2% w/w), changes in G' and G'' correlation to lowering of pH by 1M GDL, tricalcium phosphate was used at 0.4% w/w (equivalent to 0.16% Ca^{2+}), measured at 37°C, 1 Hz and 0.2% strain

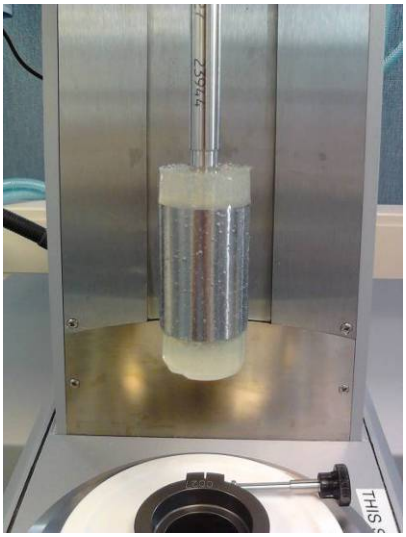


Figure 23 The slip effect phenomenon observed in Protanal LF120

The evolutions of pH and elastic modulus (G') of the alginates and Pectin LA410 are compared in Figure 24. Firstly, the rates of GDL-acidification were comparable for those tests without TCP. This could imply good repeatability of the experimental procedures. As for tests with TCP, pH curves of Protanal LF120 and Dariloid QH were slightly lower than those of Protanal IC2053 and Pectin LA410. This could be due to differences in their chemical structures / composition or possibly due to experimental variations. For example, the amount of TCP present in a sample (loaded into the geometry) might be different in the next sample, as TCP is insoluble initially, thus sample homogeneity cannot be assumed.

Secondly, in tests without TCP, Protanal LF120 had the highest acid sensitivity, followed by Protanal IC2053 / Pectin LA410 and Dariloid QH, based on their G' and G'' crossover time of about 11 minutes, 27 / 28 minutes and 53 minutes, respectively. After the gel point, Protanal LF120 had the highest G' profile (for ~45 minutes), followed by Pectin LA410, Protanal IC2053 and Dariloid QH.

Thirdly, in tests with TCP, the alginates and Pectin LA410 were found to be highly sensitive to calcium ions. Similarly, Protanal LF120 exhibited the highest G' profile (for ~35 minutes), followed by Pectin LA410, Protanal IC2053 and Dariloid QH. Thus, having met the selection criteria, Protanal LF120 and Pectin LA410 were considered more suitable for the next phase of the development work.

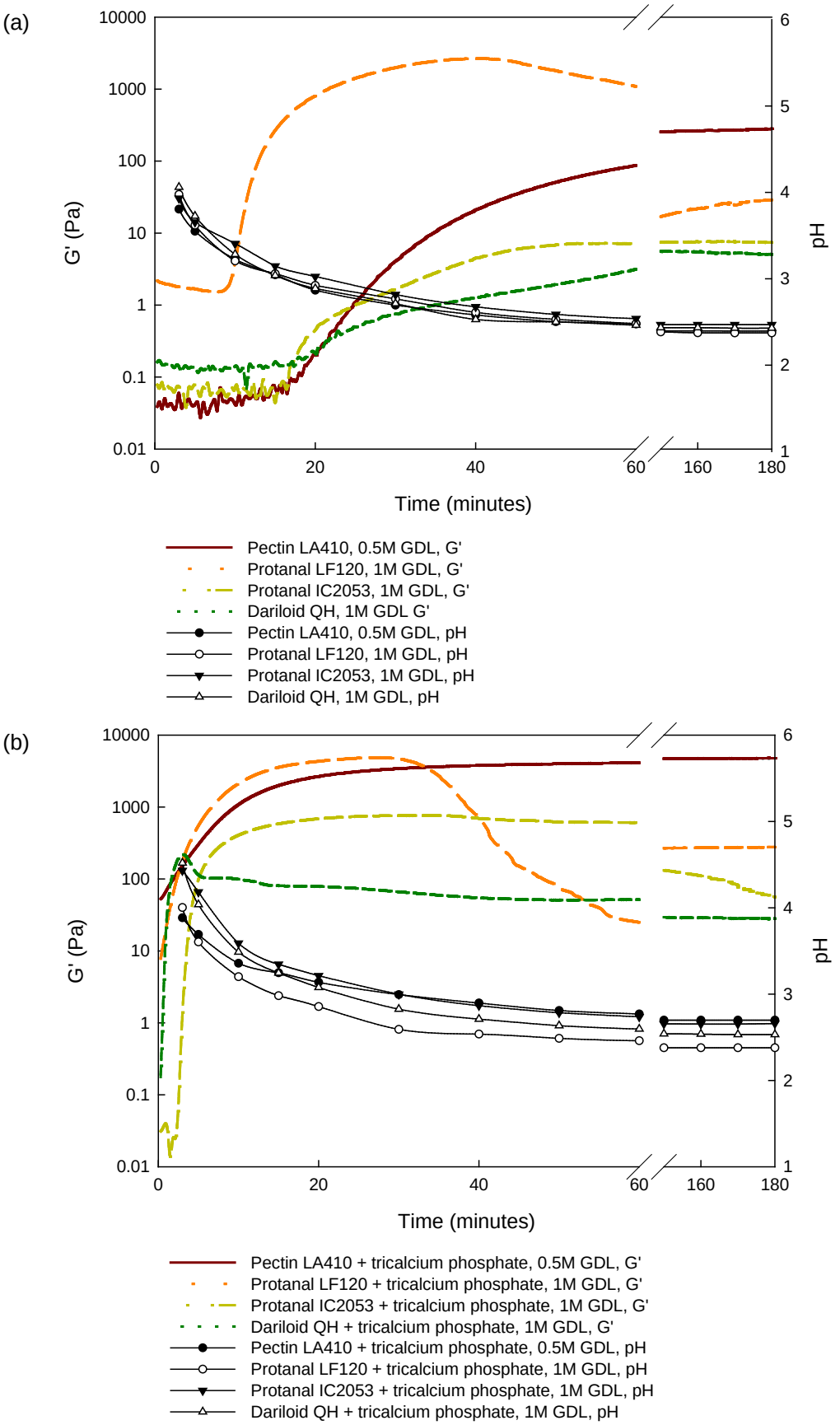


Figure 24 Gelation profiles of the pectin and alginates (2% w/w), changes in G' correlation to lowering of pH by GDL, (a) without and (b) with tricalcium phosphate used at 0.4% w/w (equivalent to 0.16% Ca^{2+}), measured at 37°C, 1 Hz and 0.2% strain

4.3 Conclusion

The characterization work has led to a conclusion that Pectin Classic AF101, Pectin AMD 780 and Kelcosol alginate were unsuitable for further work in the beverage model, since they lacked sensitivity to acids. Although Protanal IC2053 and Dariloid QH alginates were acid- and calcium-sensitive, they were considered less suitable as their gelation profiles (G' and G'') were lower than those of Protanal LF120 alginate and Pectin LA410. Having met the selection criteria of: (1) providing viscosity to beverage, (2) showing sensitivity to acids and calcium ions resulting in gelation, and (3) exhibiting higher gel strength (presumably in the stomach), Protanal LF120 and Pectin LA410 will be further evaluated in the beverage model. In conclusion, the aim and objectives of this chapter have been achieved. Formulation and production of the beverage model *i.e.* beverages with viscous fibres and plant extracts will be presented in the following chapter.

Chapter 5

Beverage Formulation and Production

5.1 Introduction

After determining the suitability of viscous fibres (Protanal LF120 alginate and Pectin LA410), the development work proceeded to establish a formulation for the satiety beverage model. For the beverage model, three product concepts were proposed and described in Table 15. As a pilot study and with commercial justification, it was decided to use Concept 2 (partial-meal replacer beverage), which alternatively can be used as Concept 3 (supplement beverage). The beverage was preferred to be non-dairy, soy-based (using isolated soy protein), fruit-flavoured (*e.g.* strawberry, blueberry, boysenberry) and of non-acidic / neutral pH.

Table 15 Beverage concepts (Adapted from Kleef *et al.*, 2011)

Concept 1: Meal replacer beverage	**Concept 2: Partial-meal replacer beverage**	**Concept 3: Supplement beverage**
Replaces a meal at the same volume, weight and energy density level.	Replaces the drink component (e.g. milk / juice) of a meal, but with lower energy density.	Taken between meals to curb hunger and is satiating to reduce food intake in following meal.
A satiety enhancing beverage that is designed to replace existing products or a meal within the normal diet. It contains an active ingredient to ensure prolonged feeling of fullness, delaying the next eating occasion and/or reduce subsequent food/energy intake.	A satiety enhancing beverage that is designed to replace existing product(s) of a meal at the same volume and weight, but with lower energy density and contains an active ingredient to ensure prolonged feeling of fullness, delaying the next eating occasion and/or reduce subsequent food/energy intake	A satiety enhancing beverage as a supplement that is taken outside the meal occasions or as a snack to specifically target reducing total food/energy intake. Innovative formats e.g. mini-drinks.

The aim of the current work was to formulate and produce beverages for the satiety measurement trial. The objectives were as follows:

1. To establish a base formulation and process for the beverage model.
2. To formulate with potential appetite control ingredients such as alginate, pectin, quercetin and fruit extract:
    a. To determine the low and high levels of Protanal LF120 and Pectin LA410 in the beverage model using rheological measurements.

b. To show proof of concept that phytochemical extracts (quercetin and fruit extract) can be incorporated into the beverage model.

3. To determine the effects of potassium citrate in the beverage model.
4. To determine to effects of ultra-high temperature (UHT) processing on the beverages

5.2 Results and discussion

The methods of beverage processing and evaluation, as well as the materials used have been described earlier (Chapter 3, Section 3.3).

5.2.1 Commercial beverage as benchmark

The benchmark for the beverage model was a commercially available breakfast drink. The commercial beverage (CB) contained 320 kJ (energy), 3.6g protein, 1.5g fat, 7.4g sugar, 1.5g fibre, 60mg sodium, 210mg potassium and 160mg calcium, per 100ml. It had pH and solids content of 6.85 and 17.5°B, respectively. Viscosity of the CB was measured at 4°C and 20°C (Figure 25). It exhibited a shear-thinning behaviour, probably contributed by the hydrocolloids (cellulose, CMC, carrageenan) present in the beverage. The viscosity at 4°C is higher than that at 20°C, which is expected and known as the temperature dependence of the viscosity of fluids (Rao, 2007a) .

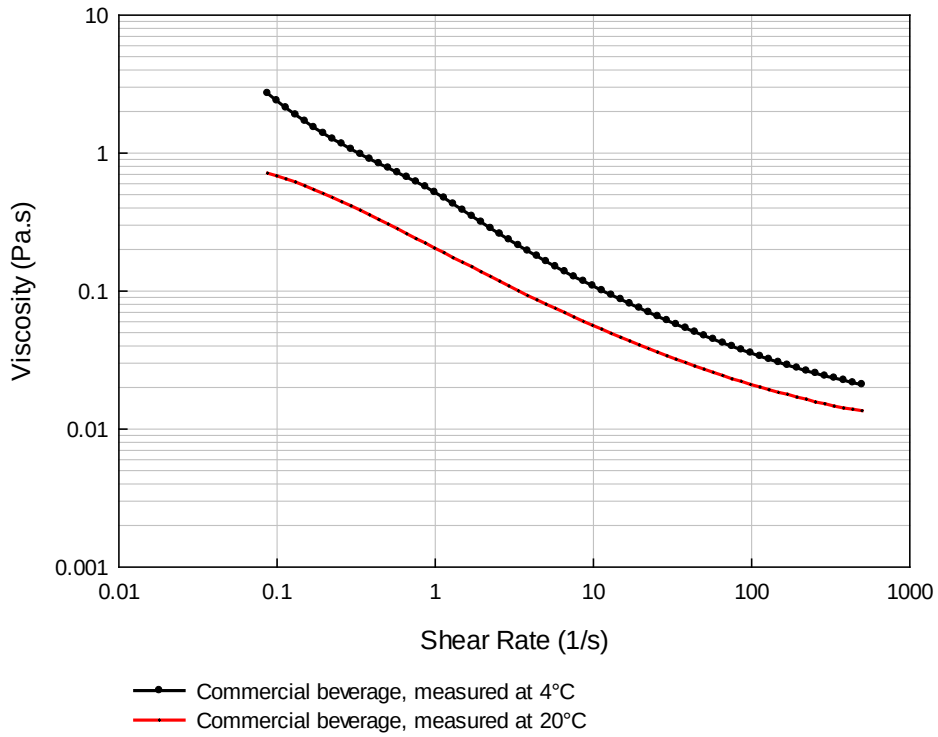


Figure 25 Viscosity curves of the commercial beverage, measured at 4°C and 20°C

5.2.2 Basis of the formulation

Based on the nutritional composition of the CB, usage levels of ingredients for the beverage model were determined, as presented in Table 16.

Table 16 Usage levels of ingredients for the beverage model

Nutritional composition	Target quantity per 100g	Key contributing ingredient(s)	Usage level in beverage model
Protein	3.6g	Isolated soy protein, 88.1% protein	4.1%
Fat, total	1.5g	None, beverage without added fat	0
Fat, saturated	0.2g		
Carbohydrates	11.0g	White sugar Pectin LA410 (37% sugar)	7.4% The amount of sugar contributed by Pectin LA410 will be deducted from the white sugar
Sugars	7.4g		
Dietary fibre	1.5g	Protanal LF120 (~85% fibre) Pectin LA410 (~57% fibre) Inulin (~94% fibre)	Inulin is used to standardize dietary fibre contents of all beverages to 1.5%
Sodium	60mg	Isolated soy protein, Protanal LF120	The amount of sodium depends on the amount of Protanal LF120 used
Potassium	210mg	Potassium citrate (38% potassium)	0.5%
Calcium	160mg	Tricalcium phosphate (40% calcium)	0.4%

Inulin, a soluble fibre, was used for standardization of dietary fibre contents in the beverages. Recent studies found soluble fibres including inulin have no effect on satiety or hunger, even when large amounts were fed (Wanders *et al.*, 2011). Other ingredients such as flavours and colourings *e.g.* vanilla flavour, blueberry flavour, red colouring and blue colouring, were used according to suppliers' recommendations or as required. Usage levels of these ingredients were often very low, thus their contribution to the nutritional content of the beverage was considered negligible.

5.2.3 Use of carboxymethylcellulose (CMC) in the control formulation

As with other experimental work, it is necessary to have a 'control' for good comparison of results and observations. From initial laboratory trials, the control beverage had very low viscosity compared to the CB and beverages with alginate / pectin (Figure 26). Thus, in order to match the viscosity of CB, particularly at shear rate of 10/s, it was

necessary to incorporate a hydrocolloid in the beverage. For comparing viscosities of beverages, a shear rate of 10/s is relevant to oral (swallowing) conditions (Peters *et al.*, 2011; Rao & Lopes da Silva, 2007).

CMC was considered as a suitable hydrocolloid for the following reasons: (1) it is a soluble fibre not known to affect satiety (Wanders *et al.*, 2011), (2) acidification or lowering of pH of CMC solutions could cause viscosity loss (Hercules Incorporated, 1999), instead of gelation, and (3) gelation of CMC solutions occurs with certain salts of trivalent metals, such as aluminum (Hercules Incorporated, 1999) and not with divalent metal ions including Ca^{2+}. A sample of AQUALON® 7HOCF sodium CMC was received from APS Food & Nutrition for the development work.

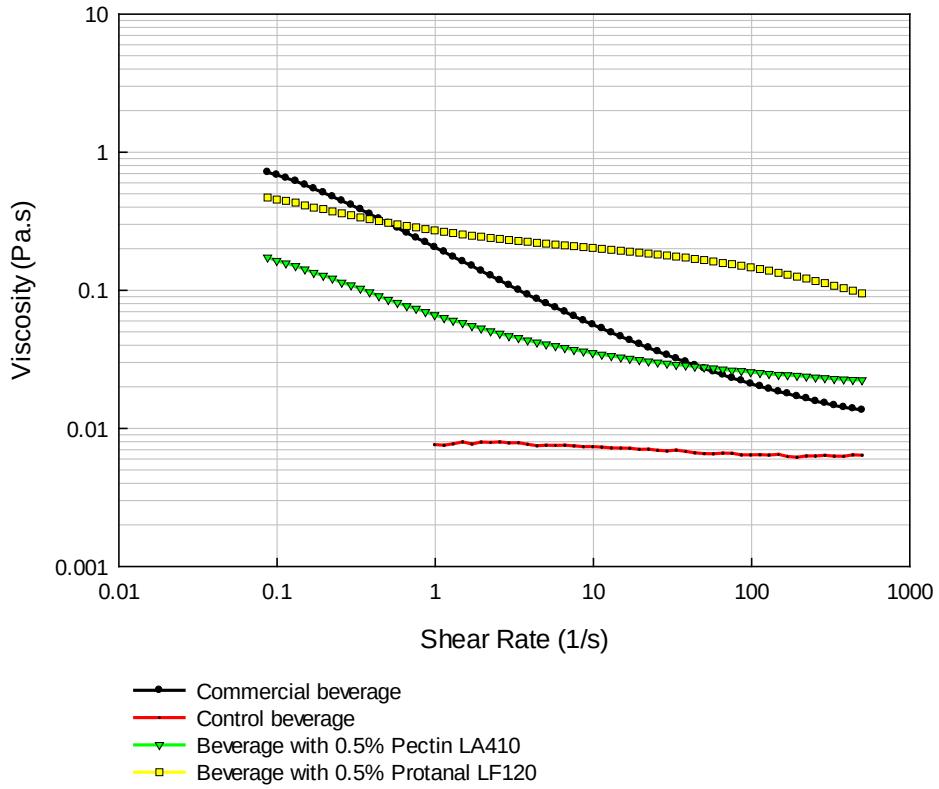


Figure 26 Viscosity curves of the control beverage (initally without hydrocolloid), commercial beverage and beverages with 0.5% Pectin LA410 / Protanal LF120, lab trial (LT) 3/5/12, measured at 20°C

Laboratory trials were carried out to determine the usage level of CMC in the beverage, using the CB's apparent viscosity of 56.4 cP (10/s, 20°C) as the target. Various levels of CMC were evaluated and the viscosity curves and data are shown in Figure 27 and Table 17, respectively. It was found that the apparent viscosity of the beverage with

0.15% CMC (averaged of 2 trials: 55.2 cP, 10/s) was a close match to that of the CB. Thus, it was decided to use 0.15% CMC in the control formulation.

It is known that GDL is commonly used in the manufacture of soybean curd or tofu from soymilk. Thus, gelation of the beverage model was expected, when pH was lowered with GDL, due to the presence of soy proteins in the formulation. As noted in Figure 28, the G' and G'' crossover of the beverages occurred within 2 minutes of the test. The pH curve of beverage with 0.3% CMC is slightly lower than that of beverage without hydrocolloid during the first 60 minutes of the test. This could be due to experimental variations, since original pH values of the beverages did not differ. Nevertheless, the results confirmed that the use of CMC in the control beverage would not substantially affect gelation and gel strength of the beverages, when pH was lowered to ~3 after ~90 minutes.

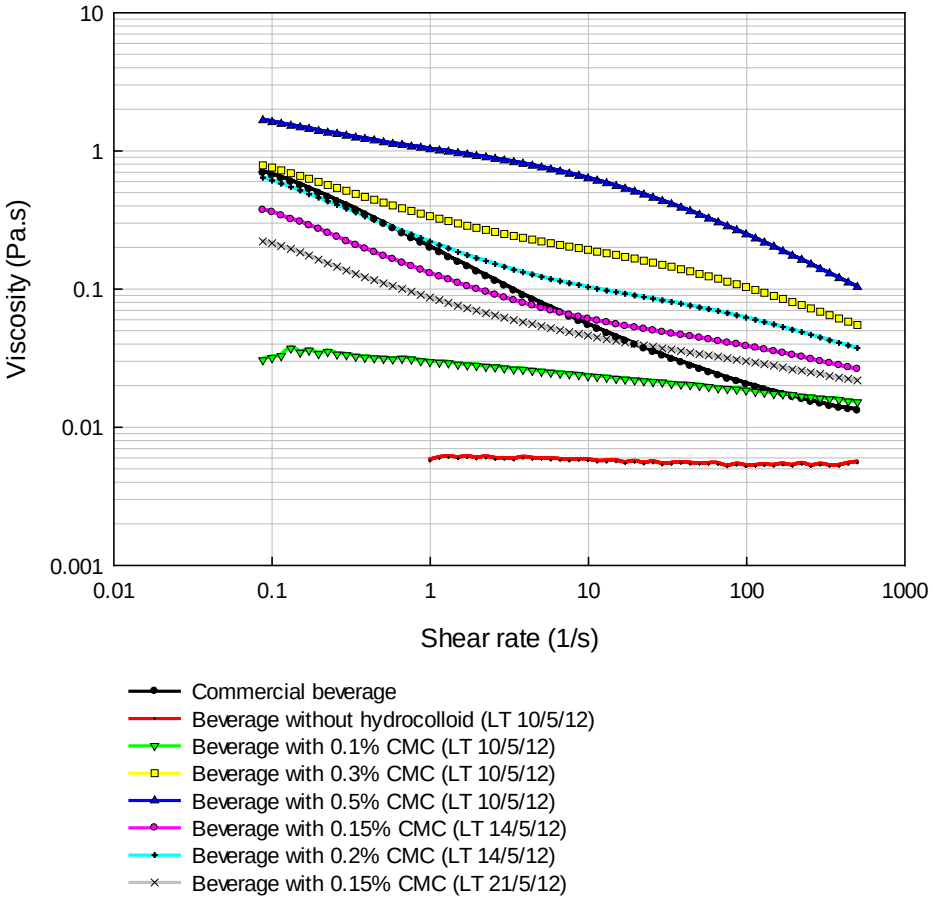


Figure 27 Viscosity curves of the commercial beverage, beverage without hydrocolloid, and beverages with various levels of CMC, measured at 20°C

Table 17 Viscosity data of beverages with various levels of CMC, beverages without hydrocolloid and the commercial beverage

Date of lab trial (LT)	Beverage	Viscosity at 10/s, 20°C (Pa.s)	Viscosity at 10/s, 20°C (cP)
–	CB	0.0564	56.4
LT 10/5/12	With 0.1% CMC	0.0242	24.2
LT 21/5/12	With 0.15% CMC	0.0477	47.7
LT 14/5/12	With 0.15% CMC	0.0627	62.7
LT 14/5/12	With 0.2% CMC	0.1070	107.0
LT 10/5/12	With 0.3% CMC	0.1990	199.0
LT 10/5/12	With 0.5% CMC	0.654	654.0
LT 3/5/12	Without hydrocolloid	7.37×10^{-3}	7.37
LT 10/5/12		5.93×10^{-3}	5.93
LT 14/5/12		6.55×10^{-3}	6.55
LT 21/5/12		6.56×10^{-3}	6.56

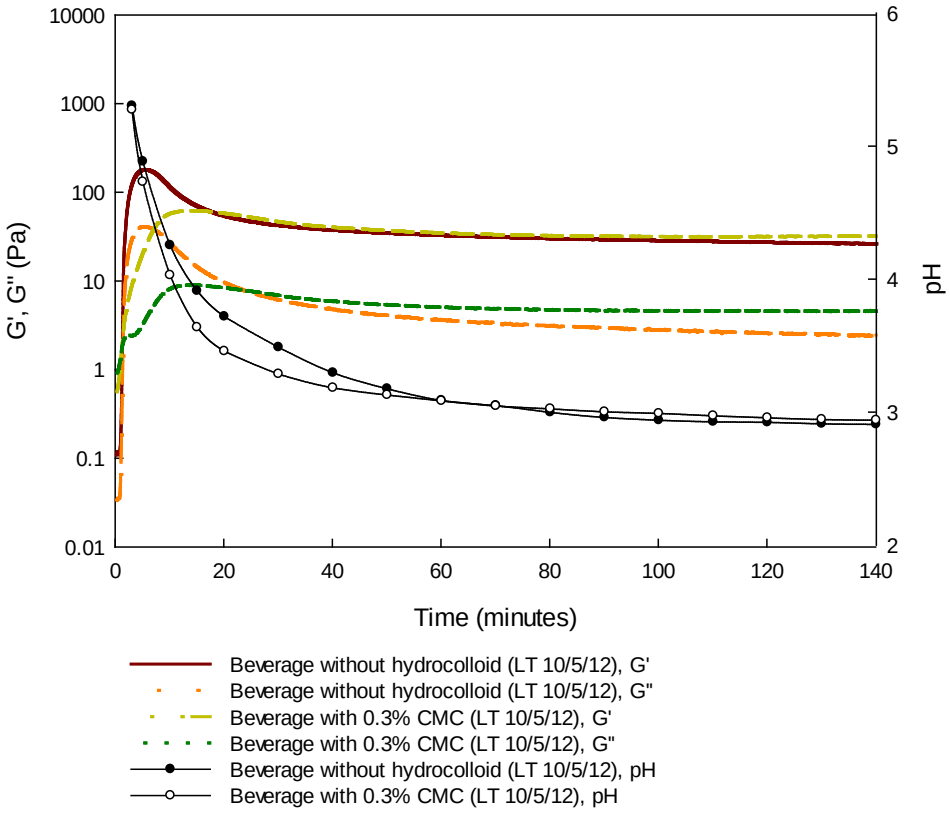


Figure 28 Gelation profiles of beverage without hydrocolloid and beverage with 0.3% CMC, changes in G' and G'' correlation to lowering of pH by 0.5M GDL, measured at 37°C, 1 Hz and 0.2% strain

5.2.4 Determining the usage levels of Protanal LF120 in the beverage model

Laboratory trials were carried out to determine the low and high levels of Protanal LF120 alginate in the beverage model. The low level alginate (LLA) beverage should closely match the viscosity (at 10/s) of the CB and the control beverage with 0.15% CMC. As for the high level alginate (HLA) beverage, the alginate level should be high enough to affect satiety (to be measured), but not affecting its sensorial acceptability.

Various levels of Protanal LF120 were evaluated and the viscosity curves and data are shown in Figure 29 and Table 18, respectively. As expected, higher usage levels of alginate yield higher viscosity in the beverages. It was thought that the apparent viscosity (66 cP, 10/s, 20°C) of beverage with 0.2% Protanal LF120 was similar to that of the CB, thus that level was used for the LLA beverage. Feedback from colleagues during informal sensory evaluation reported that beverages with 0.5% Protanal LF120 and above were perceived to be too thick in the mouth (unacceptable), whereas the thickness of beverage with 0.4% Protanal LF120 (viscosity of 179 cP, at 10/s, 20°C) was acceptable. Thus, it was decided that the HLA beverage will contain 0.4% Protanal LF120.

Table 18 Viscosity data of beverages with various levels of Protanal LF120, and the commercial beverage (CB)

Date of lab trial (LT)	CB / Beverage with *x*% Protanal LF120	Viscosity at shear rate 10/s (Pa.s)	Viscosity at shear rate 10/s (cP)
–	CB	0.0564	56.4
LT 14/5/12	0.2%	0.0661	66.1
LT 21/5/12	0.2%	0.0655	65.5
LT 14/5/12	0.25%	0.0834	83.4
LT 10/5/12	0.3%	0.104	104.0
LT 21/5/12	0.4%	0.179	179.0
LT 3/5/12	0.5%	0.203	203.0
LT 14/5/12	0.5%	0.287	287.0
LT 10/5/12	0.6%	0.425	425.0
LT 10/5/12	0.8%	0.814	814.0
LT 3/5/12	1%	0.889	889.0

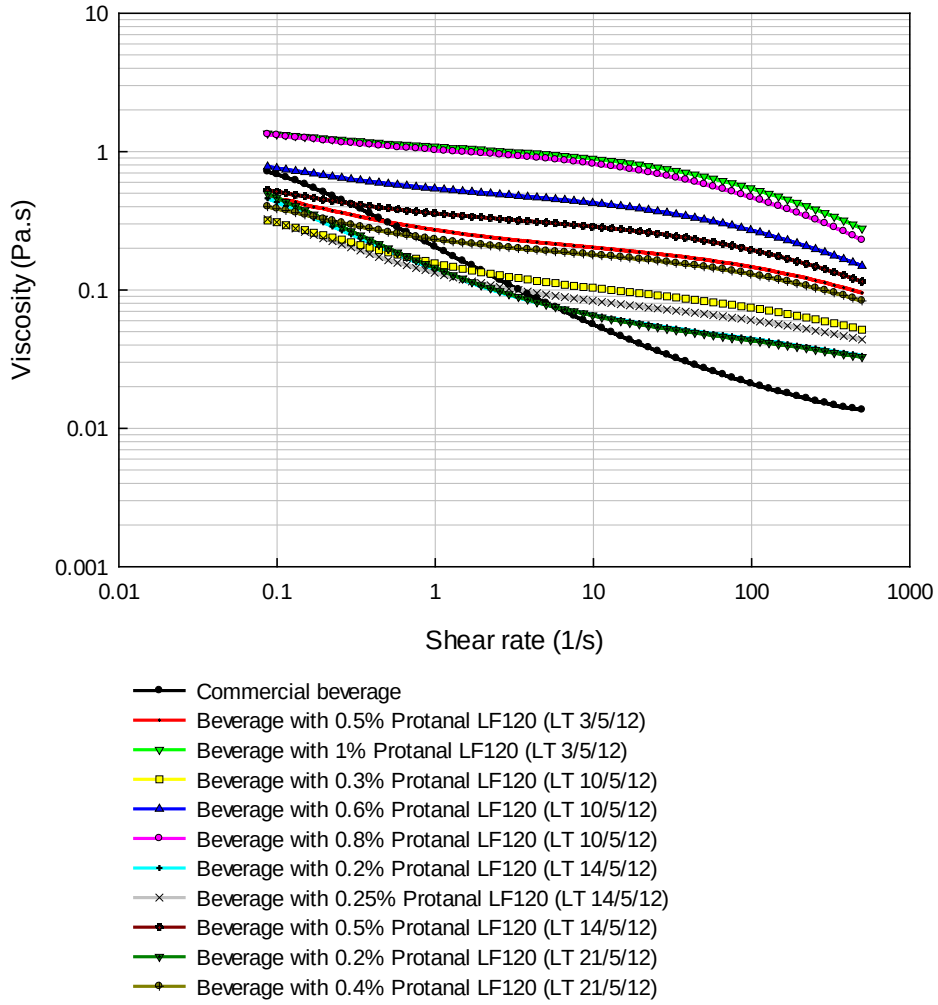


Figure 29 Viscosity curves of the commercial beverage and beverages with various levels of Protanal LF120, measured at 20°C

5.2.5 Determining the usage levels of Pectin LA410 in the beverage model

A fresh, new sample of Pectin LA410 was obtained from DuPont Nutrition and Health. From the product information sheet, the sugar content of the new sample (37g sugars per 100g) was different from the old sample (25g sugars per 100g). Thus, it was necessary to compare their viscosities. Solutions of Pectin LA410, both old and new samples, were prepared in concentrations of 1%, 1.5% and 2% according to the method in Section 3.2.2.1. As noted from the viscosity curves (Figure 30) and data (Table 19), the new Pectin LA410 was significantly of lower viscosity than the old sample. The viscosity difference seems to increase by ~5% with every 0.5% increase in pectin concentration.

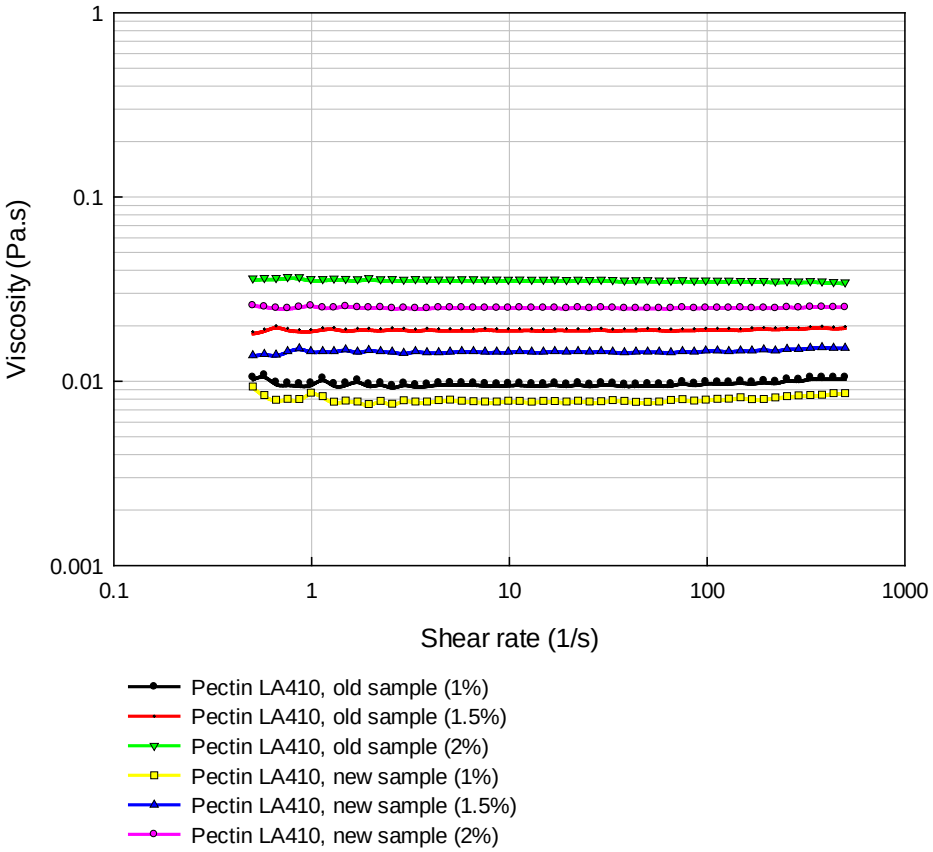


Figure 30 Viscosity curves of Pectin LA410 solutions (1%, 1.5% and 2% w/w), old vs. new samples, measured at 20°C

Table 19 Viscosity data of Pectin LA410 solutions, old and new samples

	Viscosity (10/s, 20°C) of Pectin LA410 solutions (cP)		
Concentration	**Old sample**	**New sample**	**Percentage (%) difference**
1%	9.4	7.6	19.5%
1.5%	18.7	14.0	25.1%
2%	34.5	24.2	29.9%

Various levels of Pectin LA410 were evaluated in the beverage model. Similarly, the low level pectin (LLP) beverage should closely match the viscosity (at 10/s) of the CB and the control beverage with 0.15% CMC, and the high level pectin (HLP) beverage, should be with pectin level high enough to affect satiety, but not affecting its sensorial acceptability. Based on the viscosity differences (pectin solutions) and viscosity data of the old Pectin LA410 in beverages (data not shown), it was estimated that the LLP beverage and the HLP beverage should contain 1.3–1.4% and 2.8–2.9% Pectin LA410, respectively. As shown in Figure 31, viscosity of the beverage with 1.4% Pectin LA410 (59.9 cP, 10/s, 20°C) was a close match to the CB. On the other hand, the viscosity of

the beverage with 2.8% Pectin (209 cP, 10/s, 20°C) was slightly higher than the viscosity of the HLA beverage (179 cP, 10/s, 20°C). Nevertheless, it was decided that the LLP and the HLP beverages will contain 1.4% and 2.8% Pectin LA410 (new sample), respectively.

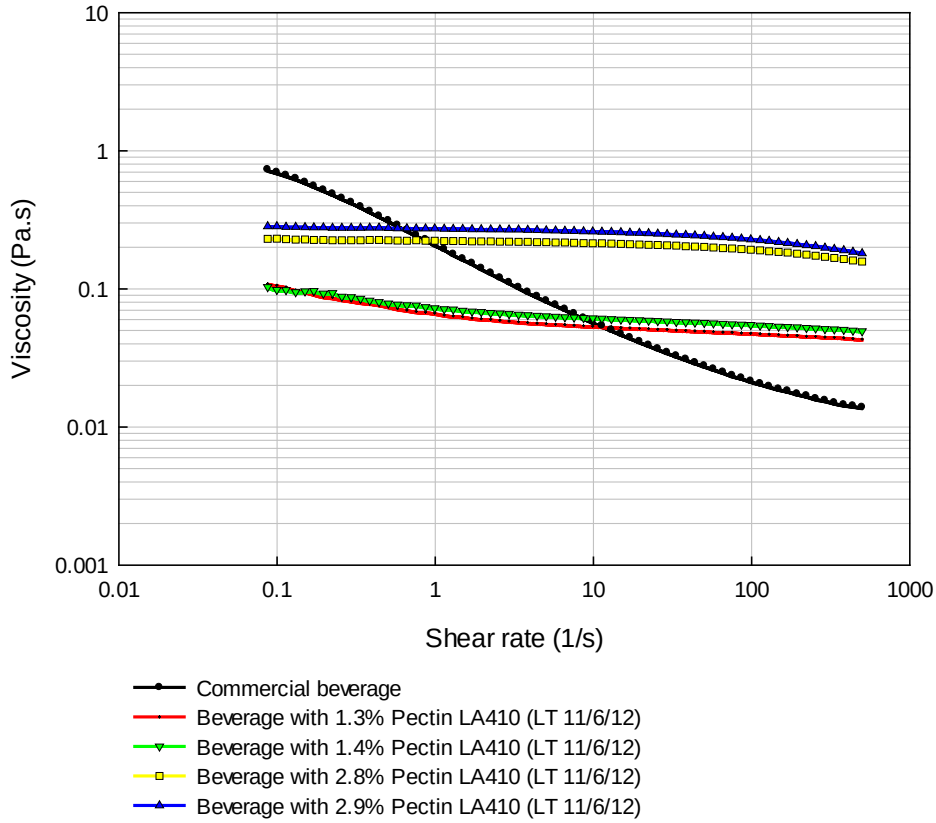


Figure 31 Viscosity curves of the commercial beverage and beverages with various levels of Pectin LA410 (new sample), measured at 20°C

5.2.6 Effects of potassium citrate in the beverage

Laboratory trials were carried out to investigate whether potassium citrate has any significant effects on the viscosity and gelation of the beverage model. It was thought that since alginate and pectin depend on calcium (Ca^{2+}) ions for gelation, the presence of potassium citrate (chelating agent) could mean a reduction or elimination of any free Ca^{2+} ions, thereby affecting gelation and gel strength. On the other hand, use of chelating agent(s) is recommended when processing beverages containing soy proteins. Chelating agents bind to free divalent cations such as Mg^{2+} and Ca^{2+} present in the process water and other ingredients, preventing divalent cations from reacting with soy proteins, otherwise poor hydration and protein aggregation will occur (Mai & Lo, 2004; Paulsen *et al.*, 2005).

Beverages were prepared with and without potassium citrate in the laboratory, using portable water as per industrial practices. As shown in the process flowchart of beverages (Chapter 3, Figure 9), potassium citrate was dissolved in water before the hydration of soy proteins takes place. In trials without potassium citrate, hydration of the soy proteins was slower (more time was needed to disperse a spoonful of the soy protein powder before another spoonful can be added) and a thicker mixture was obtained at the end of mixing. It is likely that divalent cations (from water) had reacted with the soy proteins, which resulted in poorer hydration and possibly aggregation of proteins.

As shown in Figure 32, the viscosity curves of beverages with 0.4% Protanal LF120, with and without potassium citrate are comparable. This was rather unexpected knowing the calcium-sensitivity of Protanal LF120, but it is likely that other factors such as concentrations (alginate, Ca^{2+}) and pH, would contribute to the effect when no potassium citrate was present. In contrast, the viscosities of beverages with Pectin LA (2.2% and 2.9%), without potassium citrate were much higher than those of beverages with potassium citrate. The effect of without potassium citrate *i.e.* increased viscosity seems more prominent in beverages with 2.2% and 2.9% Pectin LA410. This could be attributed to the higher usage levels of Pectin LA410 (several times higher than Protanal LF120) and/or lower pH (~6.5, 20°C) of the beverages (~7.4 for beverages with Protanal LF120).

From Figure 33, differences in the gelation profiles (G' and G'') of beverages with and without potassium citrate, were noticeable. Beverages without potassium citrate showed slightly earlier G' and G'' crossover, higher G' and G'' for the first 100 minutes or so for Protanal LF120, and higher G' and G'' throughout the test for Pectin LA410. Clearly, in beverages without potassium citrate and with pH decrease, there were more free Ca^{2+} ions to trigger the gelation of alginate and pectin as well as build stronger gel networks. Nevertheless, gelation still occurred in beverages with potassium citrate. It is likely that there were free Ca^{2+} ions that will interact with the pectin and alginate. Chelation is an equilibrium reaction; there are always some free metal ions as well as chelated metal ions. Binding of Ca^{2+} to citrate decreases the concentration of free Ca^{2+} and the equilibrium will shift to the right with further dissolution of TCP and release of free Ca^{2+} ions (Kirk-Othmer Food and Feed Technology, 2007). Moreover, with the presence of H^{+} ions (from GDL), the system responses to the reduction by producing more phosphate ions, thus solid TCP will dissolve and release free Ca^{2+} ions

(ChemPRIME, 2010). These chemical reactions that could occur in the beverage model during GDL-acidification are presented in Figure 34.

Based on the findings, the use of chelating agent(s) is necessary when processing beverages containing soy proteins. Addition of potassium citrate standardized the amount of free metal ions (including calcium) in the beverages, thus any effect or interaction will be at kept at a minimum at neutral pH. This is important to the current work, particularly when comparing the viscosity of different beverages. Furthermore, the use of chelating agent(s) at an adequate quantity is necessary for a soy-based beverage to be stable and have consistent quality during its intended shelf-life (Mai & Lo, 2004; Paulsen *et al.*, 2005).

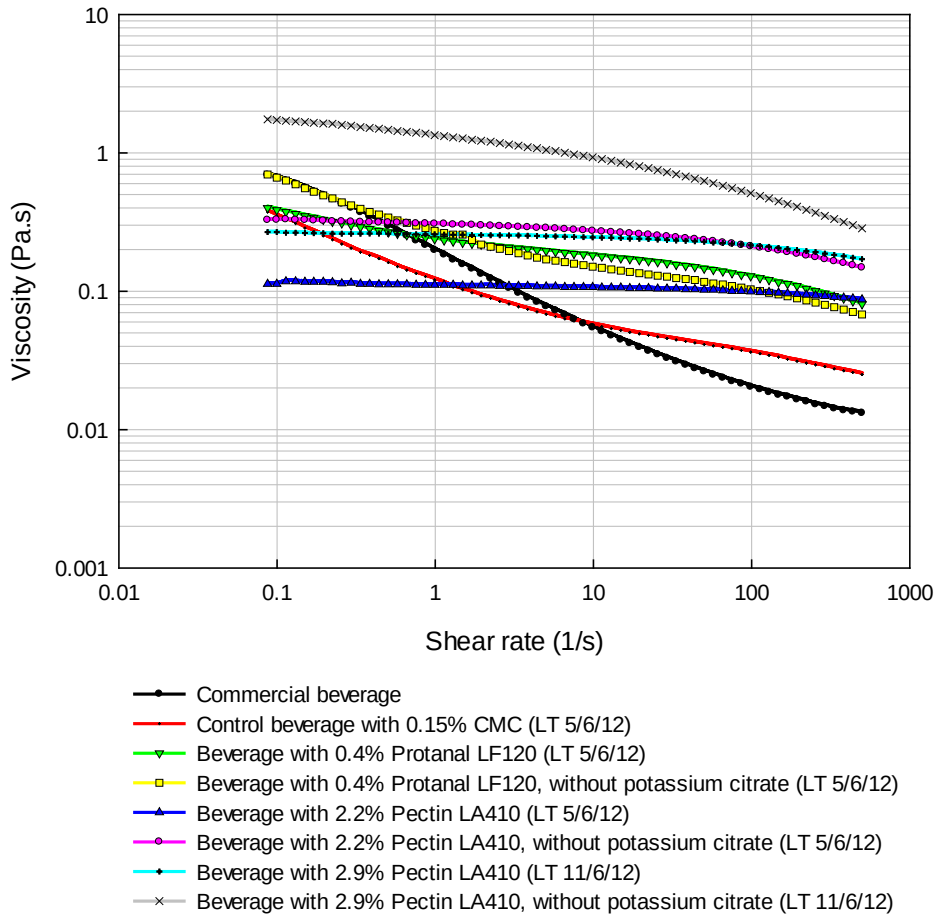


Figure 32 Viscosity curves of beverages with and without potassium citrate, measured at 20°C

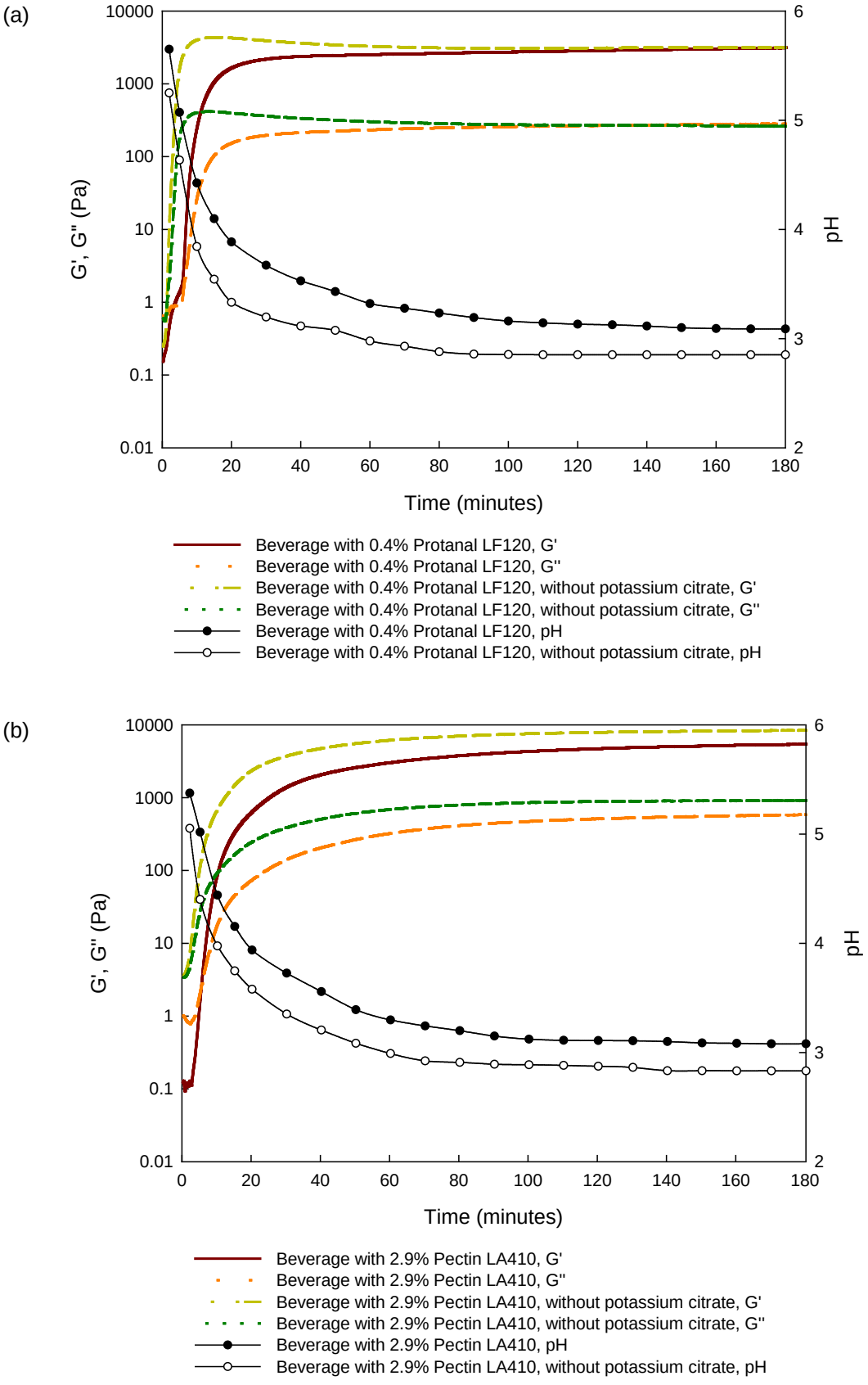


Figure 33 Gelation profiles of beverages (a) with 0.4% Protanal LF120 and (b) 2.9% Pectin LA410, with and without potassium citrate, changes in G' and G'' correlation to lowering of pH by 0.5M GDL, measured at 37°C, 1 Hz and 0.2% strain

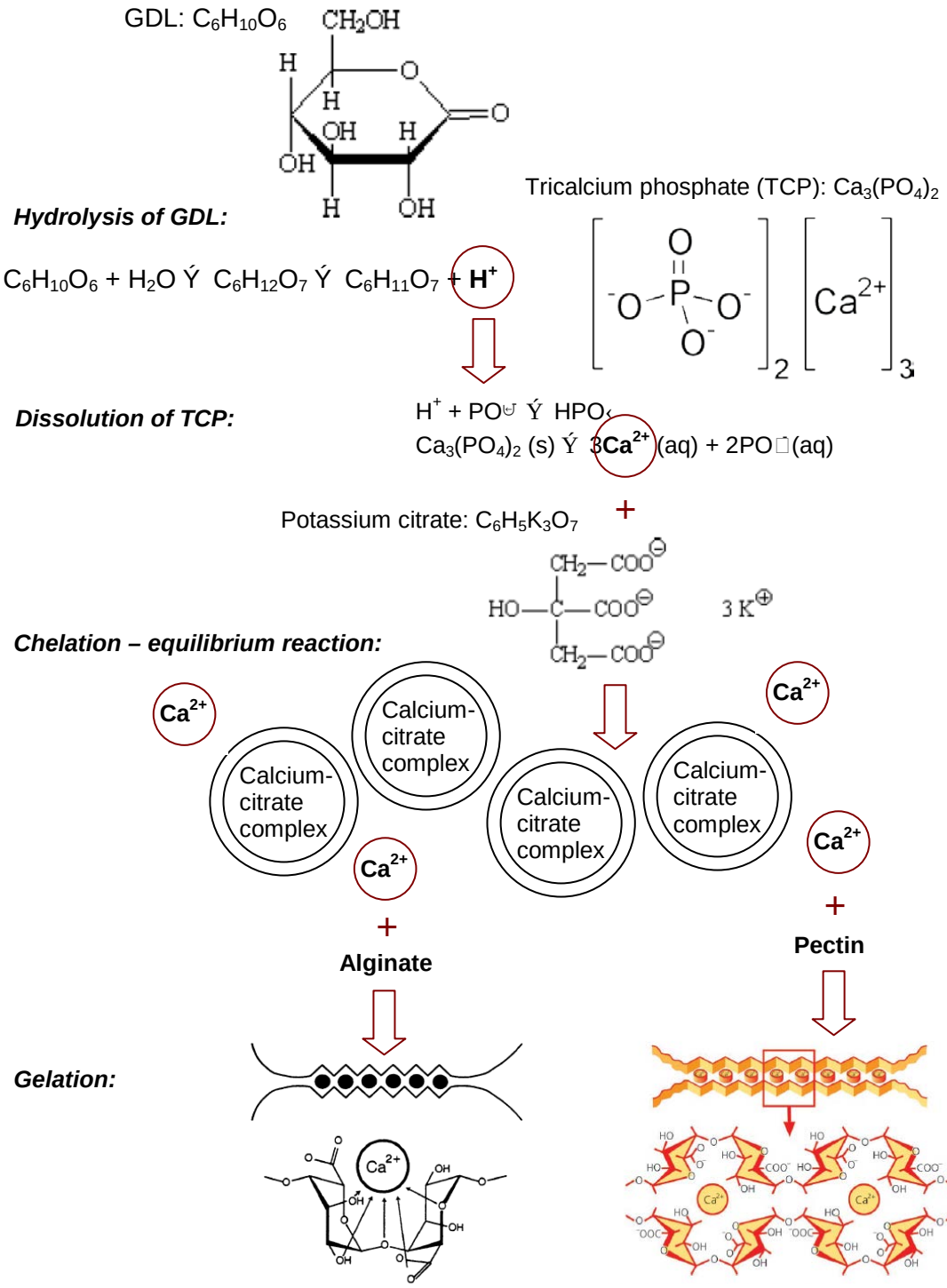


Figure 34 Diagram showing the chemical reactions that could occur in beverages with potassium citrate, tricalcium phosphate, and alginate or pectin, during acidification by GDL.

5.2.7 Effects of ultra-high temperature (UHT) processing on the beverages

After establishing the base formulation and process for the beverage model in the laboratory, further development work was conducted in the food pilot plant. It was to determine whether UHT-processing has any effects on the viscosity of the beverages.

Pilot plant trials were carried out to produce the following beverages:

1. Control beverage (0.15% CMC)
2. LLA beverage (0.2% Protanal LF120)
3. HLA beverage (0.4% Protanal LF120)
4. LLP beverage (1.4% Pectin LA410)
5. HLP beverage (2.8% Pectin LA410)

Viscosity of these beverages were measured and compared to those from the laboratory trials (pasteurization). From the viscosity data (10/s, 20°C) shown in Table 20, it was obvious that UHT-processing has affected the viscosities of all beverages. For the control beverage with CMC, viscosity was significantly lower with UHT-processing than with pasteurization, 11.8 cP and 47.7 cP (10/s, 20°C), respectively. Viscosity curves of control beverages with CMC are shown in Figure 35. During the pilot plant trial, a sample of the control beverage was taken before the preheating step. Viscosity of the sample was 110 cP (10/s, 20°C), which was higher than that after UHT-processing. Clearly, the high temperature (140°C, 5 seconds) and possibly high shear (of the plate heat exchanger and homogenizer) of UHT-processing affected the viscosity of the beverages with CMC considerably. Apparently, higher levels of CMC are recommended during scale-up for production, as heating at high temperatures could degrade CMC and permanently reduce viscosity (Hercules Incorporated, 1999). As measured, viscosity of the control beverage with UHT-processing remained unchanged after storage of 15 days. In a subsequent pilot plant trial, the level of CMC was increased to 0.25% and viscosity of the beverage after heating was 35.7 cP (10/s, 20°C). Although slightly lower than the viscosity of the commercial beverage, it was considered acceptable for the beverage model.

Table 20 Viscosity data of beverages from laboratory trials and pilot plant trials

	Viscosity (10/s, 20°C) of the beverages (cP)	
Beverage	**Laboratory trials (pasteurization)**	**Pilot plant trials (UHT-processing)**
Control beverage (0.15% CMC)		
▪ Before preheating	–	110.0
▪ After heating	47.7	11.8
▪ Stored for 15 days	–	11.2
Control beverage (0.25% CMC)		
▪ After heating	–	35.7
LLA beverage (0.2% Protanal LF120)		
▪ Before preheating	–	85.7
▪ After heating	65.5	21.9
▪ Stored for 15 days	–	23.1
LLA beverage (0.25% Protanal LF120)		
▪ After heating	–	37.3
HLA beverage (0.4% Protanal LF120)		
▪ Before preheating	–	252.0
▪ After heating	179.0	81.9
▪ Stored for 15 days	–	100.0
HLA beverage (0.5% Protanal LF120)		
▪ After heating	–	160.0
LLP beverage (1.4% Pectin LA410)		
▪ Before preheating	–	86.6
▪ After heating	59.9	13.3
▪ Stored for 15 days	–	14.3
LLP beverage (1.6% Pectin LA410)		
▪ After heating	–	21.4
HLP beverage (2.8% Pectin LA410)		
▪ Before preheating	–	304.0
▪ After heating	209.0	36.3
▪ Stored for 15 days	–	59.9
HLP beverage (3.2% Pectin LA410)		
▪ After heating	**–**	57.5

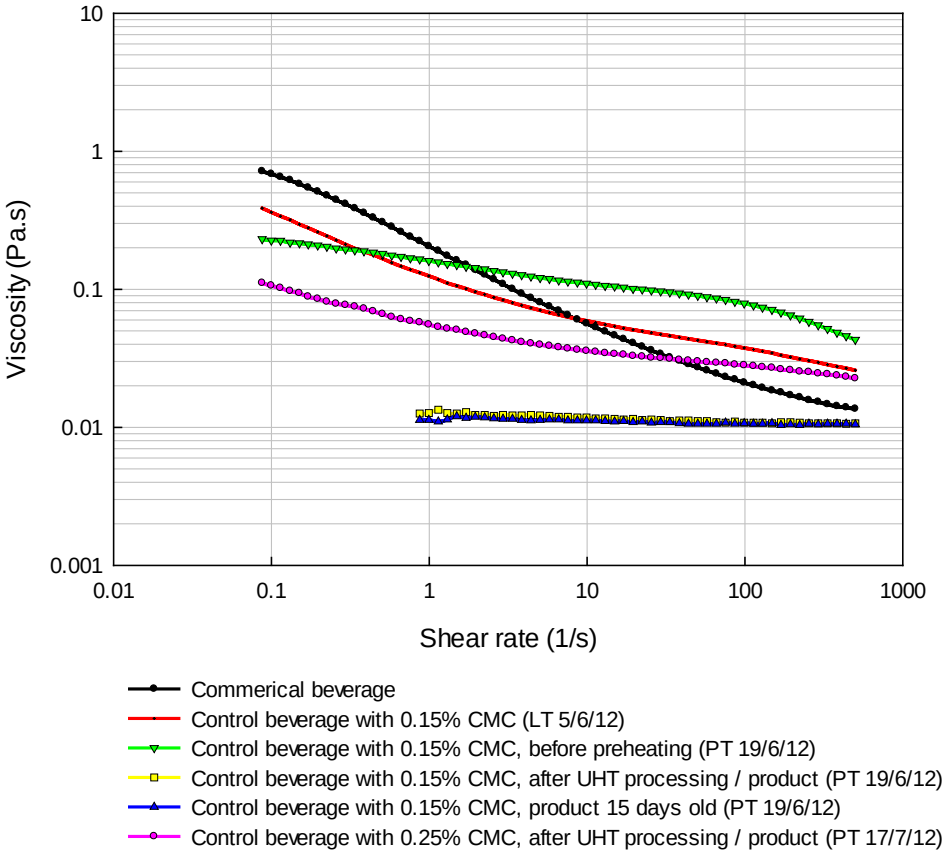
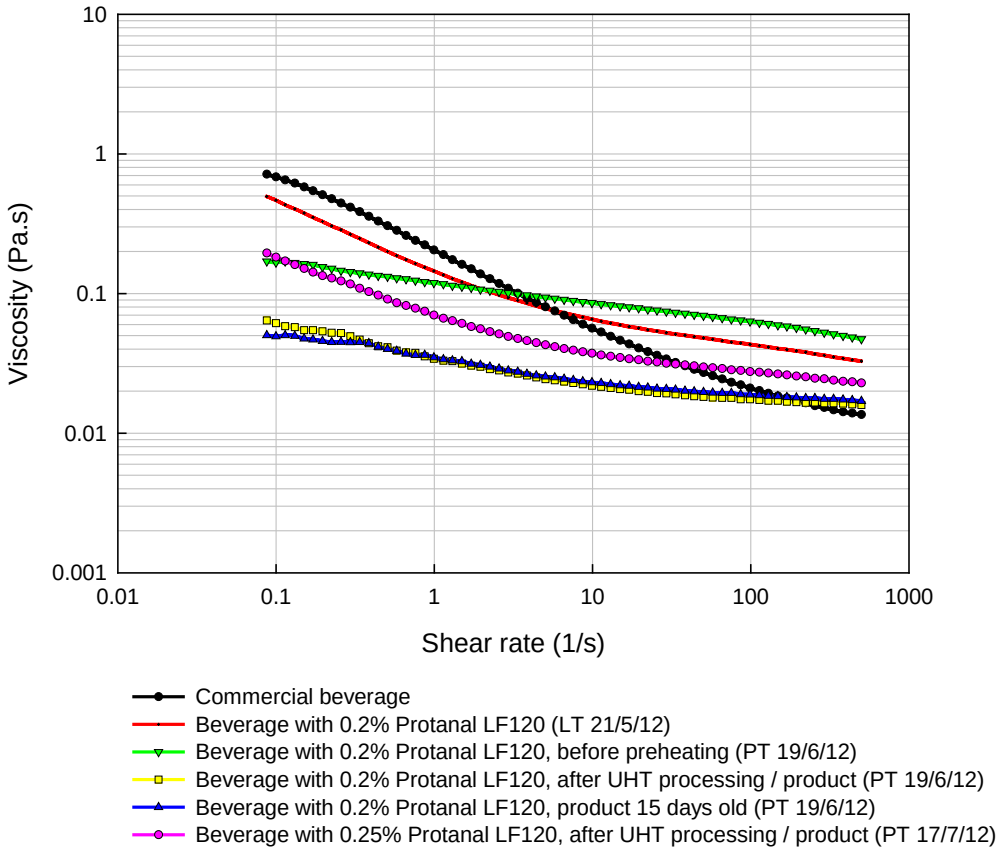


Figure 35 Viscosity curves of commercial beverage and control beverages with pasteurization in lab trial (LT) vs. UHT-processing in pilot plant trial (PT), measured at 20°C

Similarly, UHT-processing has resulted in a reduction in the viscosities of the LLA and HLA beverages. Viscosity curves of the beverages are shown in Figure 36. The viscosity of the LLA beverage with UHT-processing was 21.9 cP (10/s, 20°C), while that with pasteurization was 65.5 cP (10/s, 20°C). Thus, the level of Protanal LF120 was increased to 0.25% in a subsequent pilot plant trial and the resulting viscosity was 37.3 cP (10/s, 20°C), which was comparable to the control beverage with 0.25% CMC. Lower viscosity was also obtained for the HLA beverage with UHT-processing (81.9 cP) than with pasteurization (179 cP). Thermal processing such as heating (high temperatures), autoclaving and sterilization of alginate solutions often cause polymer breakdown, depolymerisation, loss of viscosity and possibly reduced gel strength (Draget, 2009). In a subsequent pilot plant trial, Protanal LF120 was increased to 0.5% and the beverage viscosity was 160 cP (10/s, 20°C). Feedback from colleagues (informal sensory evaluation) was obtained; they thought that a further increase in the thickness of the beverage could reduce its acceptability / palatability. Thus, 0.5% Protanal LF120 was used for the HLA beverage.

(a)



(b)

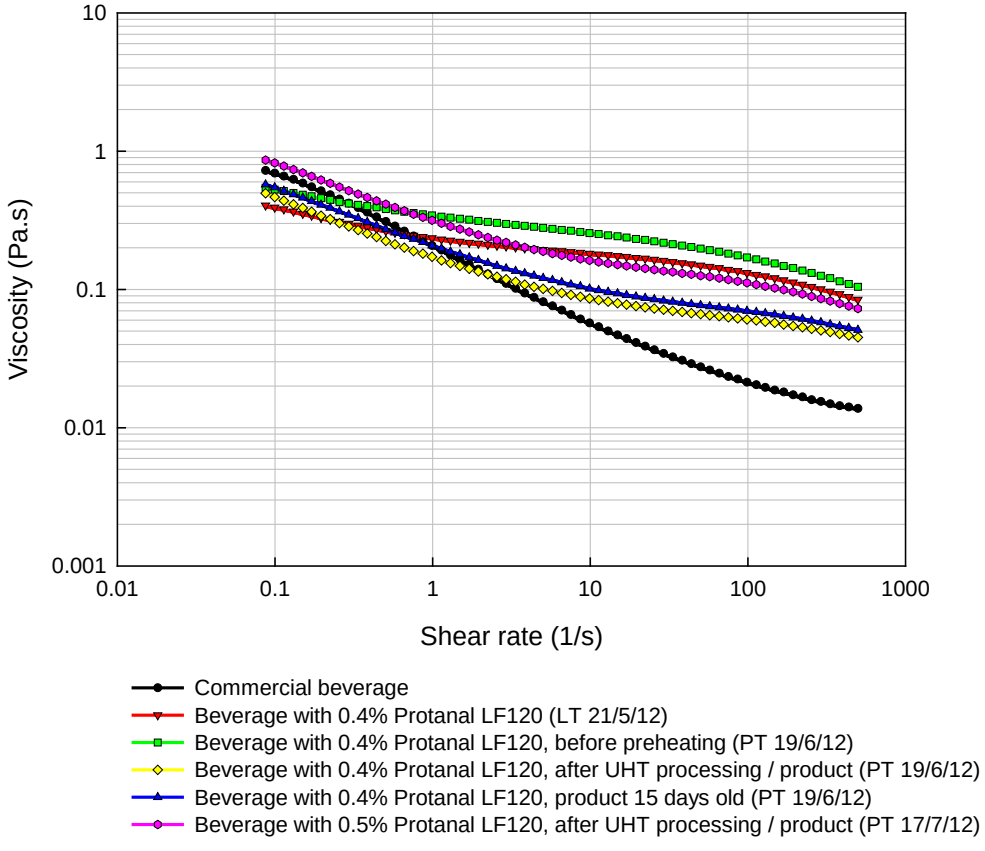


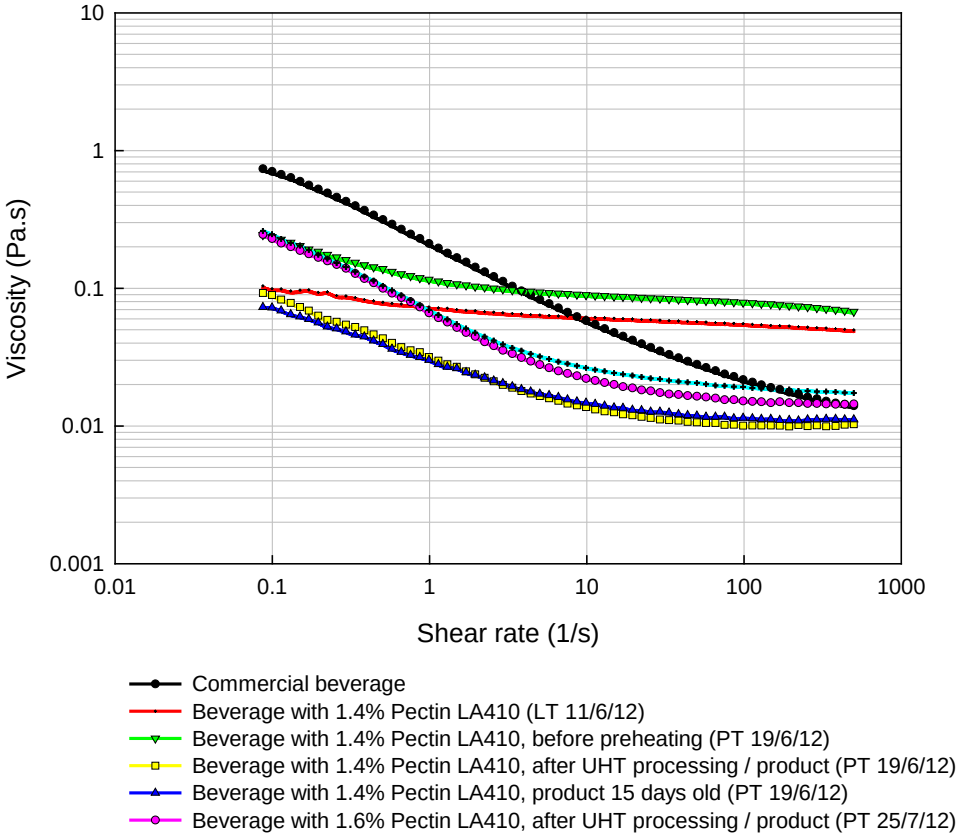
Figure 36 Viscosity curves of beverages with (a) low level alginate and (b) high level alginate, pasteurization in lab trial (LT) vs. UHT-processing in pilot plant trial (PT), measured at 20°C

The effect of UHT-processing is also evident in the LLP and HLP beverages. Viscosity curves of the beverages are shown in Figure 37. For the LLP beverage, viscosity was significantly lower with UHT-processing than with pasteurization, 13.3 cP and 59.9 cP (10/s, 20°C), respectively. Also for the HLP beverage, viscosity was significantly lower with UHT-processing (36.3 cP) than with pasteurization (209 cP). The viscosity drop is likely due to thermal degradation (depolymerization) of pectin molecules at high temperatures and neutral pH (Thakur *et al.*, 1997). In subsequent pilot plant trials, attempts were made to increase the viscosity of the beverages by increasing the usage levels of Pectin LA410 but did not seem feasible. Viscosities of the beverages with Pectin LA410 were too low – to match the viscosities of the control beverage and the LLA beverage, ~2.8% Pectin LA410 would be needed in the LLP beverage, and to match the viscosity of HLA, an unknown high level of Pectin LA410 would be needed for the HLP beverage. Using Pectin LA410 at high levels would also increase the fibre content of the beverage. For example if Pectin LA410 is used at 5%, its fibre contribution will be 3.15g per 100g of the beverage, which is higher than the target for the beverage model (1.5g fibre per 100g beverage). Therefore, Pectin LA410 is probably unsuitable for the beverage model; even at high usage levels, its viscosity remained low and its stability towards UHT-processing was inadequate.

Based on the results, beverages to be included in the satiety measurement trial were as follows:

1. Control beverage (0.25% CMC)
2. LLA beverage (0.25% Protanal LF120)
3. HLA beverage (0.5% Protanal LF120)

(a)



(b)

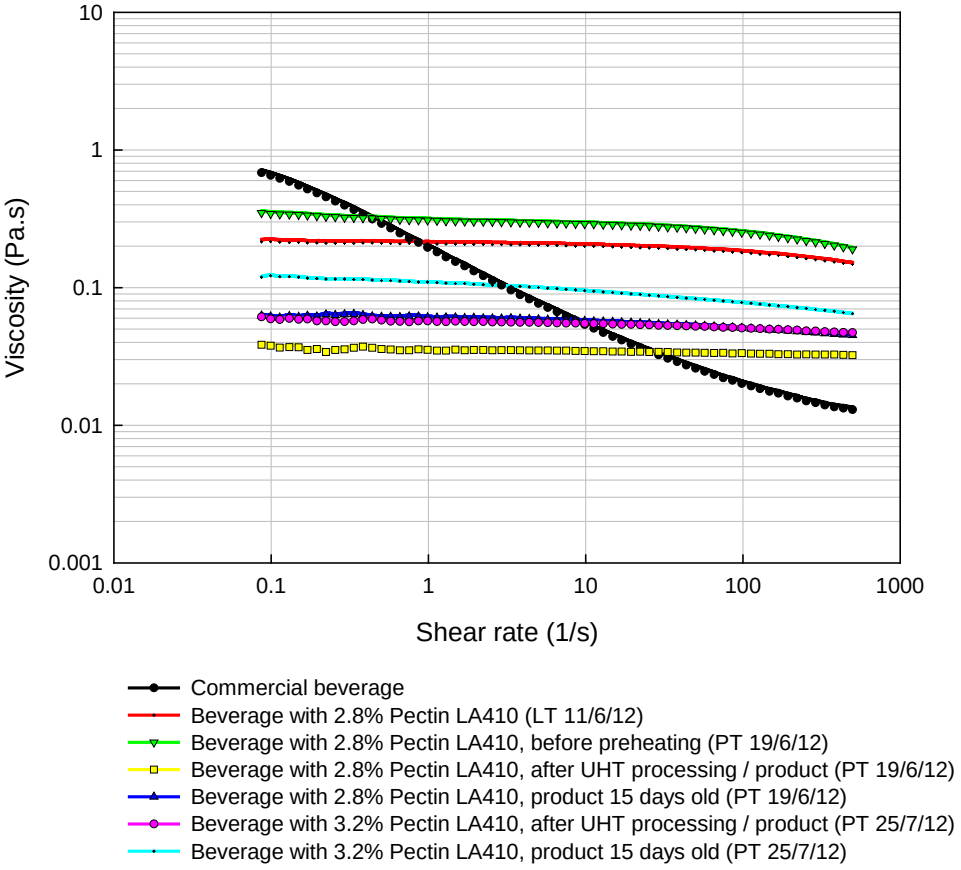


Figure 37 Viscosity curves of beverages with (a) low level pectin and (b) high level pectin, pasteurization in lab trial (LT) vs. UHT-processing in pilot plant trial (PT), measured at 20°C

5.2.8 Evaluation of quercetin and isoquercetin in the beverage model

Quercetin is one of the compounds showing positive results in *in-vitro* satiety tests (Ingram & Lo, 2012; Sergent *et al.*, 2012). Thus, one of the objectives is to show proof of concept that phytochemical extracts suc h as quercetin can be incorporated into the beverage model. The high-purity quercetin (>99.5%, food grade) and isoquercetin (food grade) were sourced from Quercegen Pharma LLC, USA. The intended / proposed uses of the high-purity quercetin include sports and energy drinks, cereal and energy bars, grain-based beverages, fruit-flavoured drinks, fruit smoothie drinks and dietetic or low-calorie soft candies. In beverages, quercetin can be used at 125–250mg per serve (240ml or g). The usage levels of quercetin in food products are limited by: (1) its poor solubility (insoluble in water, slightly soluble in alcohol, soluble in glacial acetic acid and aqueous alkaline solutions), (2) its characteristic bitter taste (similar to other flavonoids), astringency (mouth drying) and throat irritation, and (3) its bright yellow colour that might be undesirable in foods (Quercegen Pharma LLC, 2010). Nevertheless, several laboratory trials were carried out to determine a suitable method for incorporating quercetin into the beverage model.

Beverages were prepared according to the method described earlier (Chapter 3, Section 3.3.2.1) but with a modification to the mixing step. Quercetin / isoquercetin mixtures were incorporated into the rest of the beverage mixture under high speed mixing at 3500 rpm for 2 minutes using the Silverson L4RT mixer. It was thought that high speed mixing could improve their dispersibility and reduce the amount of sedimentation. Quercetin / isoquercetin were used at 0.1% w/w in the beverages (250mg in a 250g-serve). Descriptions and results of the experiments are summarized in Table 21 below.

From Expt. 6A, it was found that aqueous alkaline solutions could increase the solubility of quercetin; however the increase in pH of the beverage was undesirable. This method was unsuitable as it was also noted that the intended use of the quercetin does not include any beverage categories with a pH above 7.5 (Quercegen Pharma LLC, 2010). Sedimentation (bright yellow particles) was seen in all tests, slightly lesser when quercetin was mixed with oil (1.5%) and when ascorbic acid (0.1%) was used.

Colour changes (browning development) were obvious in beverages with quercetin / isoquercetin, especially after heating. It was thought to be the degradation of quercetin / isoquercetin due to oxidation and thermal processing. Flavonoids including quercetin

are susceptible to degradation under thermal processing, with higher rates of degradation in the presence of oxygen and/or alkaline pH (Buchner *et al.*, 2006; Makris & Rossiter, 2002). In Expt. 6A and 6B, when ascorbic acid (0.1%) was used along with quercetin / isoquercetin, the colour change was reduced; however it was limited to beverages before heating. Higher levels (0.2% and 0.3%) of ascorbic acid were tested in Expt. 6C. Although the colour change can be reduced with higher levels of ascorbic acid, it was still limited to beverages without heating. The effect of ascorbic acid could be limited by the pH of the beverages, since ascorbic acid is an antioxidant only in acidic conditions. As shown in Table 21, the pH of beverages decreases with higher levels of ascorbic acid. This probably resulted in phase separation of the beverages. Thus, it did not seem feasible to use higher levels of ascorbic acid.

In Expt. 6C, comparison of 'open' heating (current method) and 'closed' heating was also made. In 'closed' heating, beverages were filled into Schott bottles, capped and heated without stirring, which might reduce the amount of oxygen in the beverage, hence reducing the colour change. The 'closed' heating set of beverages have slightly lighter colour than the corresponding 'open' heating ones. However, this method did not prove to prevent the colour change nor increase solubility of quercetin / isoquercetin. Likewise, results from Expt. 6D showed that using gentle mixing as opposed to high speed mixing, did not change the colour of beverage after heating. In other words, reducing the amount of air / oxygen in the beverage did not reduce the extent of colour change.

Another possibility of the colour change (browning development) is due to interactions of quercetin / isoquercetin with other ingredients in the beverage. According to Makris & Rossiter (2002), the presence of iron (Fe^{2+})- or copper (Cu^{2+})-citrate complexes could contribute to a more rapid destruction of flavonols and browning development. It is possible that the beverages contained these complexes (due to the process water and potassium citrate) as well as calcium (Ca^{2+})-citrate complexes (due to tricalcium phosphate and potassium citrate). However, further tests in Expt. 6E showed that the colour change in beverages with quercetin was not related to the presence of potassium citrate and/or tricalcium phosphate.

Another ingredient that could react with quercetin / isoquercetin is the isolated soy protein. Indeed, when isolated soy protein was excluded, beverages with quercetin / isoquercetin did not have the obvious colour change or browning (Expt. 6F). The colour

change is therefore, likely due to the phenol-protein reaction, which is the oxidation of phenolic compounds to their respective quinones, because of their electrophilic character capable of reacting with nucleophilic substances (selected protein side chains) (Rohn *et al.*, 2006). Formation of quinone intermediates can be caused by: (1) the catalyzation by enzymes (basis of enzymatic browning reactions), (2) the heat treatment during food processing, where a thermal-induced oxidation takes place (the formation of semiquinones and quinones either to dark-coloured phenolic polymers or to a degradation of the flavonol structure), and/or (3) higher pH, which could accelerate the formation of quinone intermediates, although the reaction occur over a wide range of pH (4 – 10) (Buchner *et al.*, 2006; Rohn *et al.*, 2006).

The beverage model was considered unsuitable for the incorporation of quercetin / isoquercetin due to interactions with the soy proteins, which resulted in undesirable colour change. In addition, solubility of quercetin / isoquercetin was poor in this beverage model. Another beverage model without soy proteins, without any other proteins, or possibly with other source or type of proteins, is necessary for future evaluation work on quercetin as well as other phenolic compounds.

Table 21 Descriptions and results of the evaluation of quercetin and isoquercetin in the beverage model

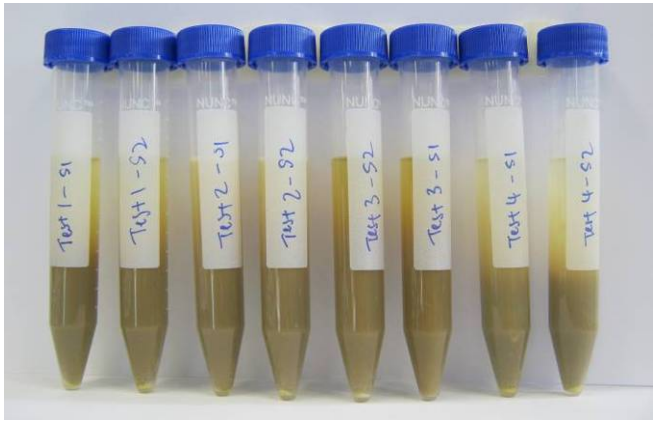
Test description	Observations	Key findings	Solubility test – photograph(s)
Expt. 6A – To check the solubility of quercetin in the beverage			
T1: Quercetin was mixed with the remaining water T2: Quercetin was mixed with oil (1.5%) T3: Quercetin was mixed with water and pH adjusted to 10 using 0.5N NaOH solution T4: Ascorbic acid (0.1%) was mixed with the remaining water until dissolved and quercetin was mixed in	T1, T2 and T4 – insoluble quercetin (bright yellow) particles seen; lesser quercetin particles seen in T3 mixture which changed from yellow to dark orange colour. T4 beverage before heating maintained its light yellow colour whereas T1, T2 and T3 changed to dull yellow-brown colour. All beverages changed to a dull greenish-brown colour after heating. T2 and T3 had slightly lesser sedimentation than T1 and T4.	Alkaline solution probably increased the solubility of quercetin; however final beverage pH was 8.0 which is undesirable. Ascorbic acid could help to reduce the colour change of the beverage before heating but not after heating.	 **Photograph 1** From left to right: T1 to T4, after heating, in duplicates, after centrifugation

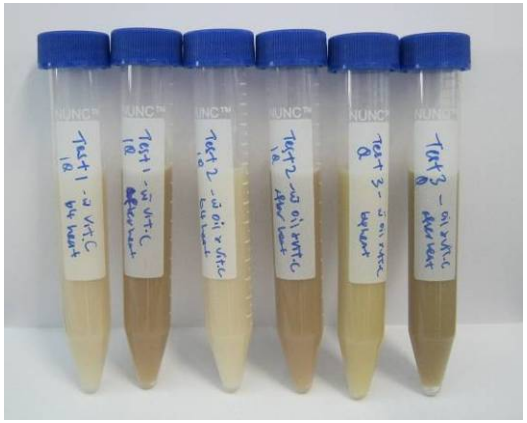
Table 21 *(continued)*

<table>
<tr><td colspan="4">Expt. 6B – To determine the effect of ascorbic acid on quercetin / isoquercetin in the beverage</td></tr>
<tr><td>T1: Ascorbic acid (0.1%) was mixed with the remaining water until dissolved and isoquercetin was mixed in

T2: Ascorbic acid (0.1%) was mixed with the remaining water; isoquercetin was mixed with oil (1.5%), then combine under magnetic stirring

T3: Ascorbic acid (0.1%) was mixed with the remaining water; quercetin was mixed with oil (1.5%), then combine under magnetic stirring</td><td>All samples, similar to T4 of Expt. 6A, maintained its light yellow colour before heating. They also changed to a dull greenish-brown colour after heating.

More sedimentation was seen in T3 (quercetin) than T2 and T1 (isoquercetin).</td><td>Using oil in the formulation did not seem to improve solubility of quercetin / isoquercetin.

Ascorbic acid could help to reduce the colour change of the beverage before heating. However, at 0.1% its effect is limited to the beverage before heating.</td><td> 
Photograph 2 From left to right: T1 (before heating / BH), T1 (after heating / AH), T2 (BH), T2 (AH), T3 (BH), T3 (AH), before centrifugation.

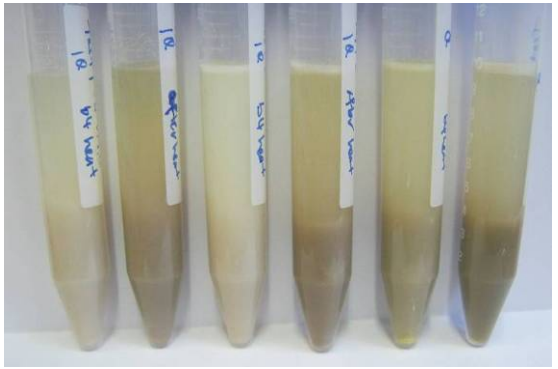
 
Photograph 3 Same as in Photograph 2, after centrifugation</td></tr>
</table>

Table 21 *(continued)*

<table>
<tr><td colspan="4">Expt. 6C – To evaluate the effect of ascorbic acid with increasing levels in the beverage; to compare any differences between ‘open’ heating and ‘closed’ heating. Note: ‘open’ heating (current method) – beverage in a stainless beaker was constantly stirred while heating; ‘closed’ heating – beverage was filled into Schott bottles and heated without stirring, assuming lesser amount of air/O_2 goes into the beverages</td></tr>
<tr><td>T1: Isoquercetin was mixed with the remaining water

T2: Ascorbic acid (0.1%) was mixed with the remaining water until dissolved and isoquercetin was mixed in

T3: Ascorbic acid (0.2%) was mixed with the remaining water until dissolved and isoquercetin was mixed in

T4: Ascorbic acid (0.3%) was mixed with the remaining water until dissolved and isoquercetin was mixed in</td><td>With higher levels of ascorbic acid, colour of the beverages (both before and after heating) was lighter.

The closed heating beverages have slightly lighter colour than corresponding open heating ones.

pH decreased with higher levels of ascorbic acid; T1 (7.3), T2 (6.8), T3 (6.3), T4 (6.1).

Phase separation noted in T2, T3 and T4 beverages left standing.

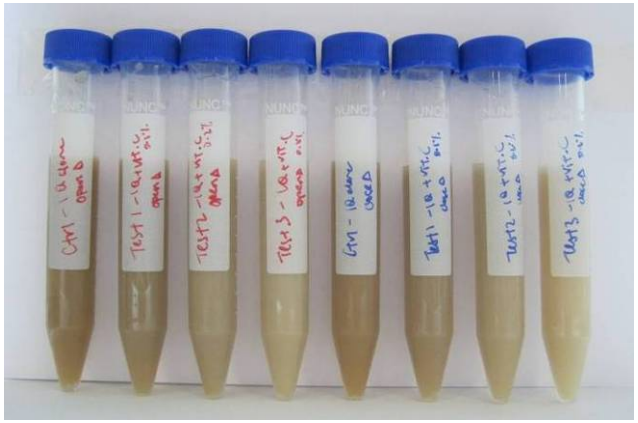
The amount of sedimentation between the beverages was not significantly different.</td><td>The colour change can be reduced with higher levels of ascorbic acid. However, it is limited to beverages without heating. The resulting decrease in pH is likely causing the phase separation in the beverages.</td><td> 
Photograph 4 From left to right: ‘open’ heating set – T1 to T4, ‘closed’ heating set – T1 to T4, before centrifugation</td></tr>
</table>

Table 21 *(continued)*

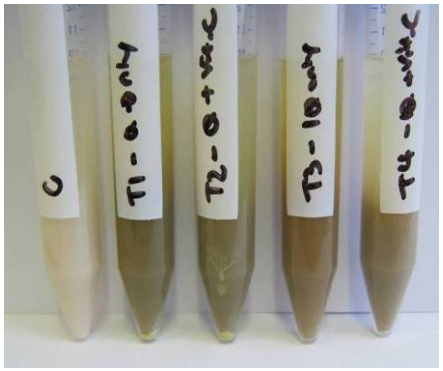
<table>
<tr><td colspan="4">Expt. 6D – To eliminate high speed mixing (Silverson) from the process; use low speed, gentle mixing, assuming lesser amount of air/O_2 goes into the beverages</td></tr>
<tr><td>C: Control formulation i.e. without quercetin / isoquercetin
T1: Quercetin was mixed with the remaining water
T2: Ascorbic acid (0.1%) was mixed with the remaining water until dissolved and quercetin was mixed in
T3: Isoquercetin was mixed with the remaining water
T4: Ascorbic acid (0.1%) was mixed with the remaining water until dissolved and quercetin was mixed in</td><td>The colour change (browning development) was not improved with gentle and reduced amount of mixing.
Slightly more sedimentation than those with by high speed mixing (previous experiments).
Beverages with quercetin showed more sedimentation than those with isoquercetin.</td><td>Oxidation and browning development of quercetin / isoquercetin did not reduce with lesser amount of incorporated air in the beverage.</td><td> 
Photograph 5 From left to right: C, T1 to T4, after heating, after centrifugation</td></tr>
</table>

Table 21 *(continued)*

<table>
<tr><td colspan="4">Expt. 6E – To check whether the colour change (browning development) is related to potassium citrate / tricalcium phosphate in the beverage</td></tr>
<tr><td>T1: Quercetin was mixed with the remaining water

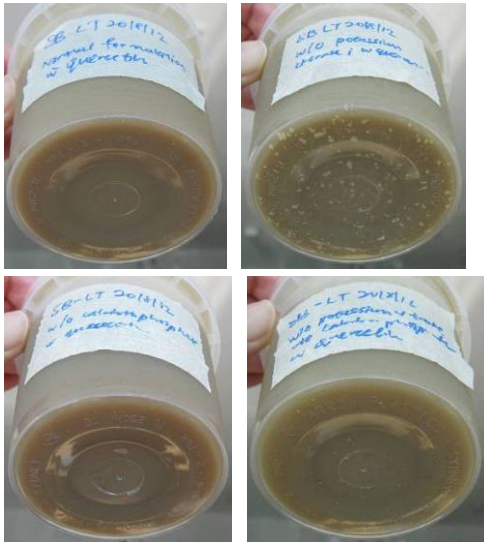
T2: Without potassium citrate; quercetin was mixed with the remaining water

T3: Without tricalcium phosphate; quercetin was mixed with the remaining water

T4: Without potassium citrate and tricalcium phosphate; quercetin was mixed with the remaining water</td><td>Similar colour change (browning development) was seen in all beverages.

Sedimentation: T2 > T4 > T3 ≈ T1

T2 with white- and yellow-coloured sediments, whereas the others with yellow-coloured sediments.</td><td>The colour change was not related to potassium citrate and tricalcium phosphate.

Without potassium citrate (chelating agent) in the beverage, quercetin + tricalcium phosphate formed more and bigger bits of sediments.</td><td> 
Photograph 6 Clockwise from top left: T1 to T4

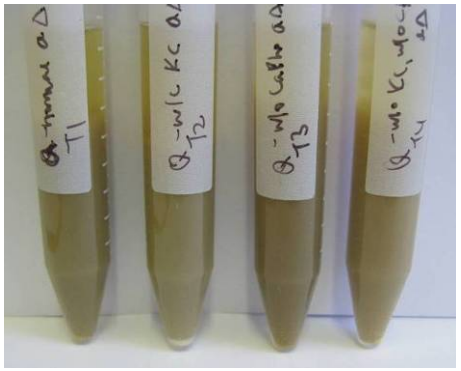
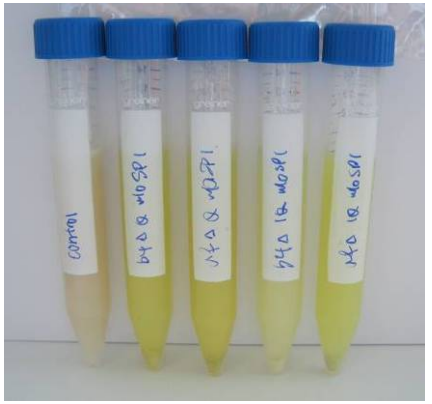
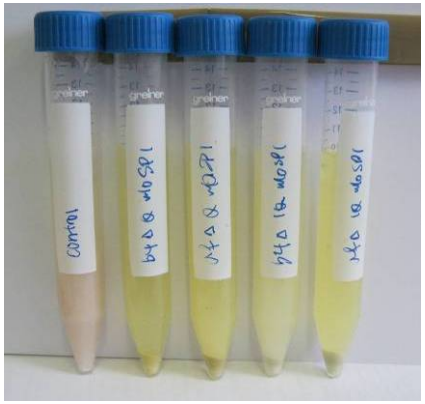
 
Photograph 7 T1 to T4, after centrifugation</td></tr>
</table>

Table 21 *(continued)*

<table>
<tr><td colspan="4">Expt. 6F – To determine whether the colour change (browning development) is related to isolated soy protein (ISP) in the beverage</td></tr>
<tr><td>C: Control formulation i.e. without quercetin / isoquercetin

T1: without ISP, quercetin was mixed with the remaining water

T2: without ISP, isoquercetin was mixed with the remaining water</td><td>Colour change did not develop in T1 and T2 after heating, very slight colour change – darker hue of yellow.

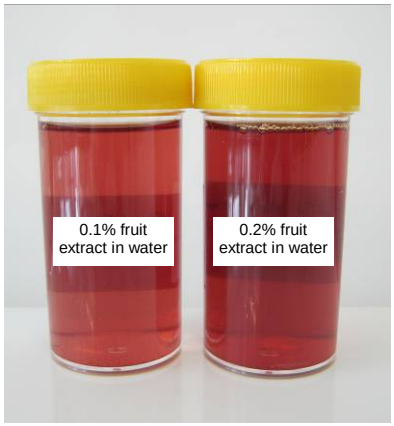
Sedimentation was noted in the T1 and T2.</td><td>The colour change was due to interactions of quercetin / isoquercetin with ISP in the beverage, which was accelerated by the heating process.</td><td> 
Photograph 8 From left to right: C (after heating), T1 (before heating), T1 (after heating), T2 (before heating), T2 (after heating), before centrifugation
 
Photograph 9 Same as in Photograph 8, after centrifugation</td></tr>
</table>

5.2.9 Evaluation of fruit extract in the beverage model

Another potential satiety ingredient is a fruit-based phytochemical extract (fruit extract), which also showed positive results in *in-vitro* satiety tests. According to the product information sheet, the fruit extract is 100% water soluble. Therefore, incorporating the fruit extract into the beverage model should be easier than the water-insoluble quercetin.

Laboratory trials were carried out to evaluate the fruit extract in the beverage model. As showed in Figure 38, both 0.1% and 0.2% fruit extract were fully soluble in water. The solutions were acidic, pH of 3.89 and 3.77, for 0.1% and 0.2% fruit extract, respectively. There was no obvious colour change in beverages with fruit extract before- and after- heating and no sedimentation of fruit extract was noted. However, incorporating the fruit extract into the beverage model changed the original colour of light yellow to a dull purple colour. Furthermore, vanilla flavour in beverages with fruit extract was perceived to be odd. Thus, the beverage model were modified for: (1) colour, by adjusting to purple colour using food colourings and (2) flavour, by changing from vanilla flavour to a blueberry flavour.

(a)



(b)

Figure 38 (a) Fruit extract in water, 0.1% (left) and 0.2% (right), (b) Beverages after centrifugation, from left to right: Control, 0.1% fruit extract (before heating), 0.1% fruit extract (after heating), 0.2% fruit extract (before heating) and 0.2% fruit extract (after heating)

Pilot plant trials were carried out using the LLA beverage (0.25% Protanal LF120) formulation, comparing these formulations: (1) with fruit extract, without colourings, (2) with fruit extract and colourings and (3) without fruit extract, with colourings. The blueberry flavour QF18678 was added at 0.075% to the beverages without fruit extract, whereas a higher level of 0.1% was added to beverages with fruit extract to mask the flavour of the fruit extract. Feedback from colleagues during informal sensory evaluation indicated that the colour of beverage (2) with fruit extract and colourings was

more appealing than that of beverage (1) with fruit extract, without colourings. The blueberry flavour was thought to suit the beverage better than vanilla flavour. As seen in Figure 39, colour differences were less obvious when the beverage was served in white, translucent bottles than in clear cups. The bottle was used as the serving format during the satiety measurement trial. However, it remains necessary to closely match the colour of the beverages to minimize/prevent the participants from being able to differentiate the beverages.

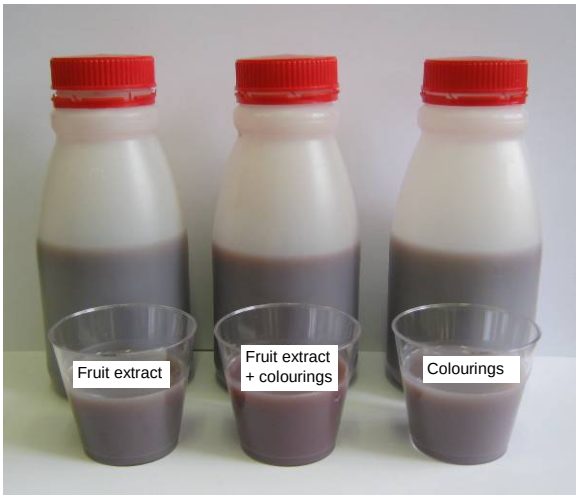


Figure 39 Pilot plant beverages, from left to right: (1) with fruit extract, (2) with fruit extract and colourings and (3) without fruit extract, with colourings.

5.2.10 Finalized formulation(s) of the beverage model

As a result, beverages to be used in the satiety measurement trial were as follows:

1. Control (0.25% CMC)
2. CMC (0.25%) + fruit extract (0.2%)
3. LLA (0.25% Protanal LF120)
4. LLA (0.25% Protanal LF120) + fruit extract (0.2%)
5. HLA (0.5% Protanal LF120)
6. HLA (0.5% Protanal LF120) + fruit extract (0.2%)

Formulations of these beverages are shown in Table 22 and the processing method is as described in Chapter 3, Section 3.3.2.1.

Table 22 Formulations of the beverages for satiety measurement trial

Formulations:	**Control (0.25% CMC)**	**0.25% CMC + fruit extract**	**LLA (0.25% Protanal LF120)**	**LLA (0.25% Protanal LF120) + fruit extract**	**HLA (0.5% Protanal LF120)**	**HLA (0.5% Protanal LF120) + fruit extract**
Beverage code:	**543**	**786**	**694**	**127**	**905**	**281**
Ingredient	%	%	%	%	%	%
Water	85.81	85.62	85.84	85.65	85.83	85.62
Soy protein isolate	4.10	4.10	4.10	4.10	4.10	4.10
Sugar	7.40	7.40	7.40	7.40	7.40	7.40
Inulin	1.40	1.40	1.37	1.37	1.14	1.14
CMC (Aqualon 7HOCF)	0.25	0.25	0	0	0	0
Protanal LF120 alginate	0	0	0.25	0.25	0.50	0.50
Blueberry flavour QF18678	0.075	0.10	0.075	0.10	0.075	0.10
Fruit extract	0	0.20	0	0.20	0	0.20
Food colourings - red	0.045	0.030	0.045	0.030	0.040	0.030
Food colourings - blue	0.020	0.003	0.020	0.003	0.015	0.003
Tricalcium phosphate	0.40	0.40	0.40	0.40	0.40	0.40
Potassium citrate	0.50	0.50	0.50	0.50	0.50	0.50
Total	100.0	100.0	100.0	100.0	100.0	100.0
Fibre (from inulin)	1.31	1.31	1.29	1.29	1.08	1.08
Fibre (from CMC)	0.19	0.19	0	0	0	0
Fibre (from alginate)	0	0	0.21	0.21	0.43	0.43
Water	45	45	45	45	45	45
Soy protein isolate	4.10	4.10	4.10	4.10	4.10	4.10
Potassium citrate	0.50	0.50	0.50	0.50	0.50	0.50
Sub-total	49.6	49.6	49.6	49.6	49.6	49.6
2% CMC/alginate solution	12.5	12.5	12.5	12.5	25	25
Other ingredients	9.34	9.53	9.31	9.50	9.07	9.28
Remaining water	28.56	28.37	28.59	28.40	16.33	16.12
Sub-total	50.4	50.4	50.4	50.4	50.4	50.4

5.3 Conclusion

The development work has showed that Pectin LA410 was unsuitable for further evaluation in the beverage model due to its low viscosity and poor stability in UHT-processing, even at high levels. Unlike fruit extract, attempts to incorporate quercetin and isoquercetin into the beverage model were unsuccessful because of their poor solubility and interactions with soy proteins. Thus, only Protanal LF120 alginate and fruit extract will be incorporated into the beverage model to test in the satiety measurement trial. In conclusion, the aim and objectives of this chapter have been achieved.

Chapter 6

Satiety Measurement Trial

6.1 Introduction

Having formulated the beverage model with the incorporation of a viscous fibre (Protanal LF120 alginate) and a phytochemical ingredient (fruit extract), the current work aimed to validate their satiety effects using *in-vivo* clinical trials. One of the common methods to measure satiety is through self-reported measures of appetite. Subjective appetite rating using visual analogue scales (VAS) which allows subjects to rate and record their feelings of hunger, fullness, satiety, desire to eat and prospective consumption were used. The satiety measurement trial used a preload, single-blind, within-subject, repeated measures, completely balanced, crossover and randomized design. The six test beverages of the trial were formulated based on the following hypotheses:

- H_1: Phytochemical (fruit extract) increases the satiety effect of the beverage.
- H_2: Higher viscosity (contributed by higher alginate level) increases the satiety effect of the beverage.
- H_3: Higher viscosity (contributed by higher alginate level) plus phytochemical (fruit extract) increase the satiety effect of the beverage.

In addition, the objectives of the satiety measurement trial are as follows:

1. To determine a methodology for satiety measurement and carry out a satiety measurement trial to validate the satiety effects of Protanal LF120 and fruit extract in beverages.
2. To evaluate the physical, chemical and sensorial properties of the beverages and correlate to subjective appetite data.

6.2 Results and discussion

The materials and methods of the satiety measurement trial have been described earlier (Chapter 3, Section 3.4).

6.2.1 Microbiological testing of the beverages

To ensure that the beverages were safe for human consumption in the satiety measurement trial, samples were sent to an external laboratory (AsureQuality) for microbiological testing. The test results are shown in Table 23. One of the beverages,

HLA (0.5% Protanal LF120) + fruit extract (Beverage code: 289) was tested and suspected to contain the bacteria, *Listeria monocytogenes*. It is highly unlikely for UHT-processed beverages produced in the food pilot plant to contain pathogenic microorganisms including *Listeria* due to stringent food hygiene and safety practices in place. Another sample of 289 was sent for retest and the result was negative for *Listeria*. Nevertheless, to eliminate any risks, another batch of beverage HLA (0.5% Protanal LF120) + fruit extract was produced and labeled as Beverage code: 281. As for the other beverages, the levels of microorganisms tested in 281 were below detectable limits. Therefore, the beverages were considered safe for human consumption in the satiety measurement trial.

6.2.2 Estimated nutritional contents of the test foods

The nutritional contents of the beverages were estimated; their nutritional information panels (NIP) and ingredient listings are shown in Figure 40. The beverages (250g or ml per serve) were closely matched for energy (489 – 494 kJ), protein (9g), fat (0.3g), sugars (18.6g), dietary fibre (3.8g), potassium (490mg) and calcium (414mg). Only the sodium content of HLA beverages (223mg/serve) was slightly higher than the control beverage (166mg/serve) and LLA beverage (170mg/serve). Protanal LF120 contains 8500mg sodium per 100g; thus with higher levels used in the beverage, the sodium content will be higher. The mean weight of beverage consumed by the participants was calculated to be 254.1±1.3g.

The standard breakfast comprised of muesli and either milk or soymilk. The amount of muesli (55g) is about ½ cup which is the recommended serving size. Due to lactose intolerance to dairy milk, participants A03, B02 and B08 were provided with soymilk (140g), while the others had milk (125g). The quantity of soymilk was higher than milk in order to match the energy, protein and fibre contents of the breakfast meals. As shown in Table 24, the energy, protein and fibre contents (particularly of importance in satiety studies) of various combinations of test beverage and breakfast have been closely matched. Consuming a test beverage followed by a standard breakfast would provide 1719±14 kJ of energy, which is about 20% of the daily intake based on an average adult diet of 8700 kJ.

Table 23 Microbiological test results of the beverages

Beverage	Beverage code	Aerobic plate count (cfu/ml)	Coliforms (cfu/ml)	E. coli (MPN/ml)	Yeasts and Moulds (cfu/ml)	S. aureus (cfu/ml)	Salmonella /25g	Listeria FDA /25g
Control (0.25% CMC)	543	<1	<1	<0.3	<1	<1	Not detected	Not detected
CMC (0.25%) + fruit extract (0.2%)	786	<1	<1	<0.3	<1	<1	Not detected	Not detected
LLA (0.25% Protanal LF120)	694	<1	<1	<0.3	<1	<1	Not detected	Not detected
LLA (0.25% Protanal LF120) + fruit extract (0.2%)	127	<1	<1	<0.3	<1	<1	Not detected	Not detected
HLA (0.5% Protanal LF120)	905	<1	<1	<0.3	<1	<1	Not detected	Not detected
HLA (0.5% Protanal LF120) + fruit extract (0.2%)	289	<1	<1	<0.3	<1	<1	Not detected	Suspicious (first test) Not detected (retest)
HLA (0.5% Protanal LF120) + fruit extract (0.2%)	281	<1	<1	<0.3	<1	<1	Not detected	Not detected

(a)

Nutrition information Serving size: 250 ml		
	Quantity per serve	Quantity per 100 ml
Energy (kJ)	489	196
Protein (g)	9.0	3.6
Fat, total (g)	0.3	0.1
Fat, saturated (g)	0.1	0.0
Carbohydrate (g)	18.6	7.4
Sugars (g)	18.6	7.4
Dietary fibre (g)	3.8	1.5
Sodium (mg)	166	66
Potassium (mg)	490	196
Calcium (mg)	414	166

Ingredients:
Water, sugar, soy protein, inulin, sequestrant (332), minerals (341), vegetable gum (466), fruit extract*, flavours, colours (124, 133)

**in CMC + fruit extract beverage only*

(b)

Nutrition information Serving size: 250 ml		
	Quantity per serve	Quantity per 100 ml
Energy (kJ)	492	197
Protein (g)	9.0	3.6
Fat, total (g)	0.3	0.1
Fat, saturated (g)	0.1	0.0
Carbohydrate (g)	18.6	7.4
Sugars (g)	18.6	7.4
Dietary fibre (g)	3.8	1.5
Sodium (mg)	170	68
Potassium (mg)	490	196
Calcium (mg)	414	166

Ingredients:
Water, sugar, soy protein, inulin, sequestrant (332), minerals (341), vegetable gum (401), fruit extract*, flavours, colours (124, 133)

**in LLA + fruit extract beverage only*

(c)

Nutrition information Serving size: 250 ml		
	Quantity per serve	Quantity per 100 ml
Energy (kJ)	494	198
Protein (g)	9.0	3.6
Fat, total (g)	0.3	0.1
Fat, saturated (g)	0.1	0.0
Carbohydrate (g)	18.6	7.4
Sugars (g)	18.6	7.4
Dietary fibre (g)	3.8	1.5
Sodium (mg)	223	89
Potassium (mg)	490	196
Calcium (mg)	414	166

Ingredients:
Water, sugar, soy protein, inulin, vegetable gum (401), sequestrant (332), minerals (341), fruit extract*, flavours, colours (124, 133)

**in HLA + fruit extract beverage only*

Figure 40 NIP and ingredients of the beverages – (a) Control and CMC + fruit extract, (b) LLA and LLA + fruit extract, (c) HLA and HLA + fruit extract, negligible nutritional contribution by fruit extract is assumed

Table 24 Estimated nutritional contents of various combinations of test beverage and breakfast

Test beverages	Control (0.25% CMC) / CMC + fruit extract		LLA (0.25% Protanal LF120) / LLA + fruit extract		HLA (0.5% Protanal LF120) / HLA + fruit extract	
Muesli with	Milk	Soymilk	Milk	Soymilk	Milk	Soymilk
Nutritional content						
Energy (kJ)	1729	1703	1731	1706	1734	1708
Protein (g)	19.0	19.0	19.0	19.0	19.0	19.0
Fat, total (g)	9.9	9.4	9.9	9.4	9.9	9.4
Fat, saturated (g)	3.0	2.0	3.0	2.0	3.0	2.0
Carbohydrate (g)	58.0	58.4	58.0	58.4	58.0	58.4
Sugars (g)	35.8	32.4	35.8	32.4	35.8	32.4
Dietary fibre (g)	8.0	8.0	8.0	8.0	8.0	8.0
Sodium (mg)	366	374	371	379	423	431
Potassium (mg)	683	683	683	683	683	683
Calcium (mg)	571	583	571	583	571	583

6.2.3 Solids content, pH and rheological properties of the beverages

The solids content and pH of the test beverages were measured to be 12.9±0.4°Brix and 7.3±0.1, respectively. Overall, the pH of beverages with fruit extract was slightly lower than that of corresponding beverages without fruit extract, which is probably due to the acidity of the fruit extract. As required, the viscosities of beverages 543, 786, 694 and 127 were in close agreement (32.7 – 37.6 cP, 10/s, 20°C). Similarly, beverages 905 and 281 had comparable viscosity, 151 cP and 140 cP (10/s, 20°C), respectively. The solids, pH and viscosity data of the beverages are shown in Table 25. The viscosity curves of the beverages are shown in Figure 41.

Gelation profiles of the test beverages, 543 and 786 (Figure 42a), 694 and 127 (Figure 42b) and 905 and 281 (Figure 43) were obtained using the GDL-acidification method. Beverages with fruit extract (786, 127 and 281) had similar G' and G'' profiles (virtually overlapping) as corresponding beverages without fruit extract (543, 694 and 905). There was good reproducibility in the production of beverages. As noted, the inclusion of fruit extract (0.2%) in the beverage did not affect its viscosity or gelation profiles.

Table 25 Solids content, pH and viscosity data of the test beverages

Beverage	Beverage code	Solids content (°Brix)	pH	Viscosity at 10/s, 20°C (cP)
Control (0.25% CMC)	**543**	13.1	7.37	34.5
CMC (0.25%) + fruit extract (0.2%)	**786**	13.2	7.18	32.7
LLA (0.25% Protanal LF120)	**694**	13.2	7.41	32.7
LLA (0.25% Protanal LF120) + fruit extract (0.2%)	**127**	13.2	7.17	37.6
HLA (0.5% Protanal LF120)	**905**	12.4	7.38	151.0
HLA (0.5% Protanal LF120) + fruit extract (0.2%)	**289**	12.4	7.12	146.0
HLA (0.5% Protanal LF120) + fruit extract (0.2%)	**281**	12.6	7.16	140.0

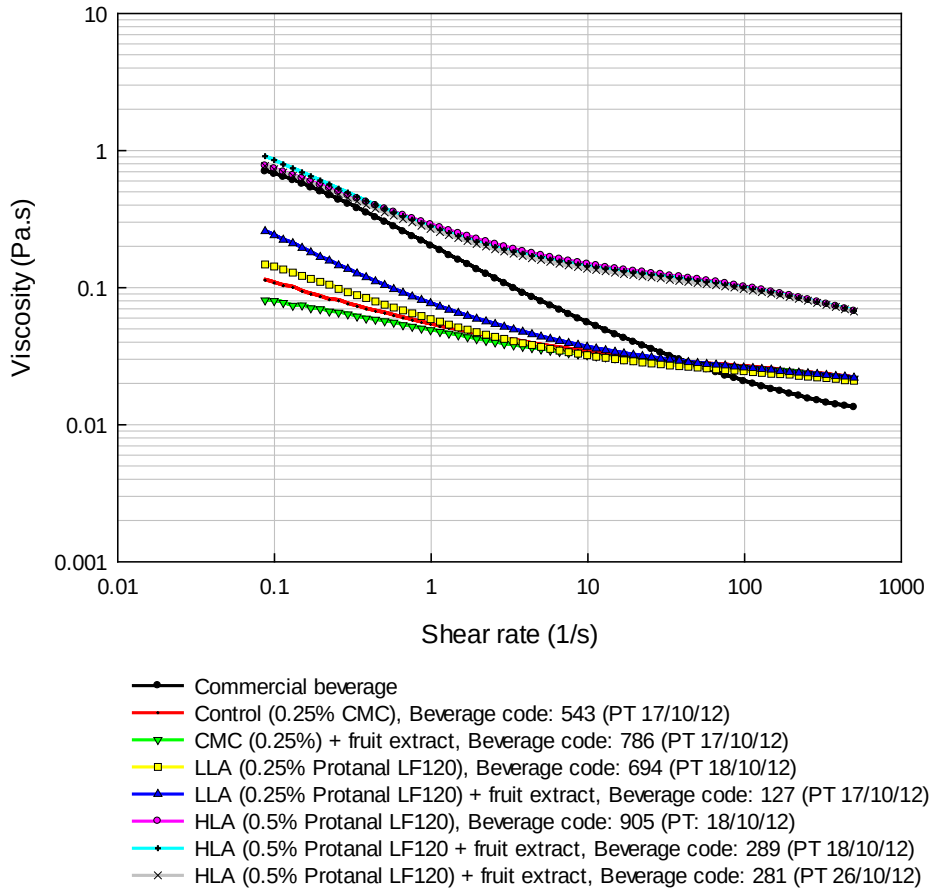


Figure 41 Viscosity curves of the commercial beverage and the test beverages (UHT-processed), measured at 20°C

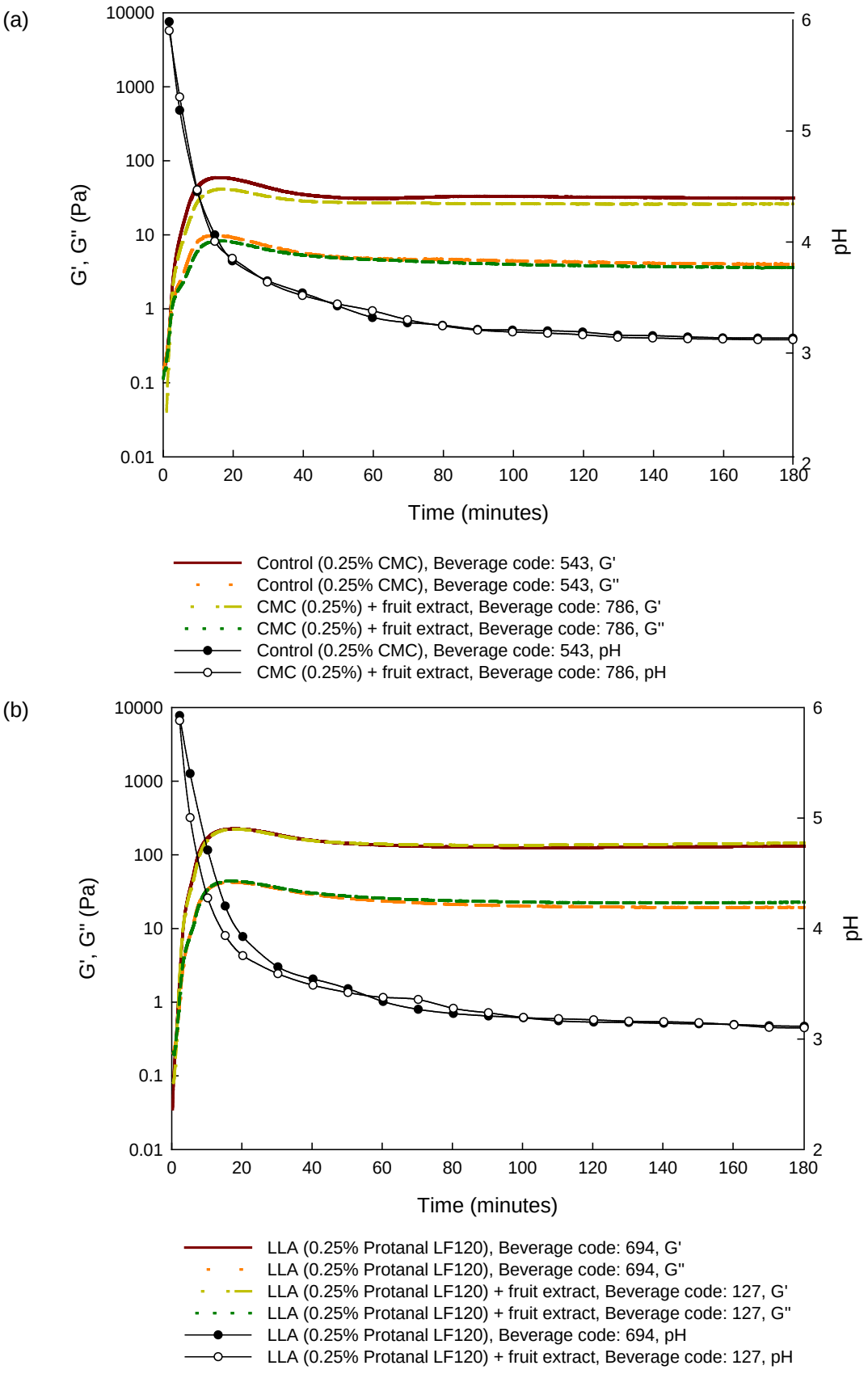


Figure 42 Gelation profiles of beverages (a) Control (694) / CMC + fruit extract (786) and (b) LLA (694) / LLA + fruit extract (127), changes in changes in G' and G'' correlation to lowering of pH by 0.5M GDL, measured at 37°C, 1 Hz and 0.2% strain

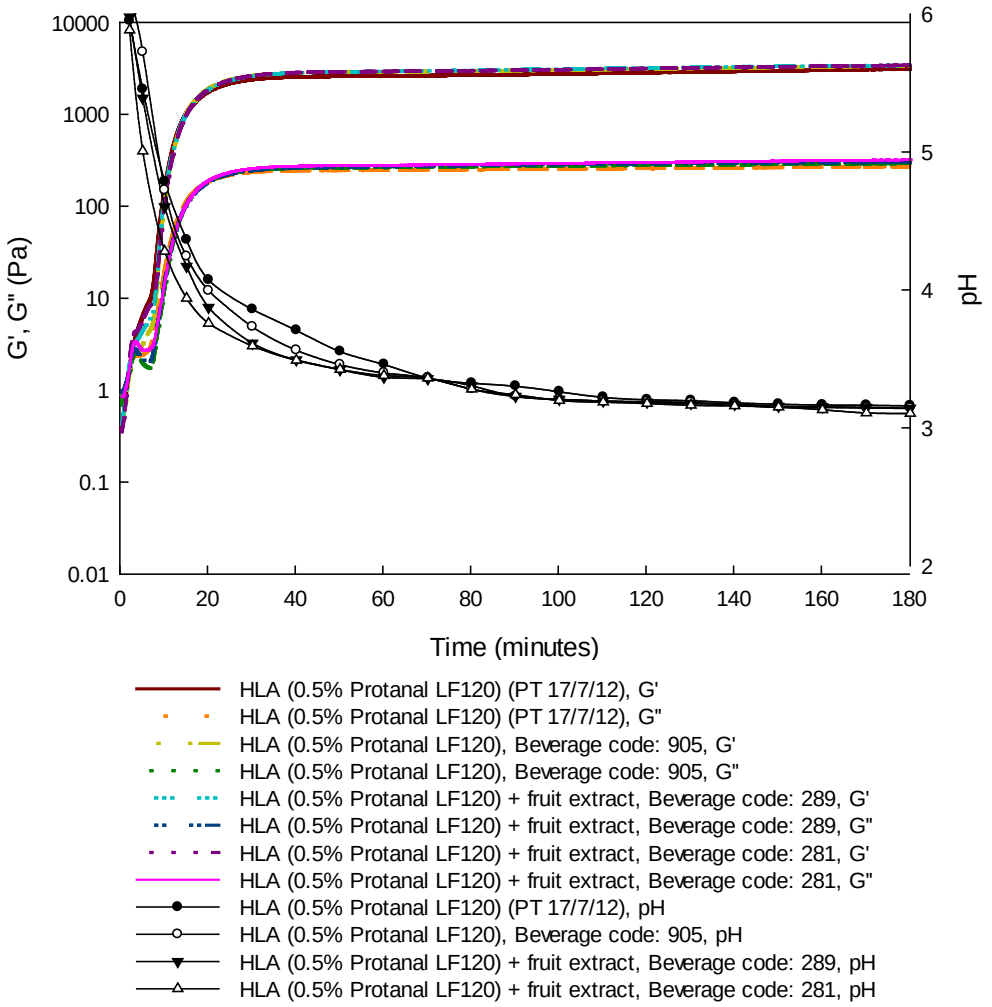


Figure 43 Gelation profiles of beverages HLA (905) / HLA + fruit extract (289 and 281), changes in changes in G' and G'' correlation to lowering of pH by 0.5M GDL, measured at 37°C, 1 Hz and 0.2% strain

The evolutions of pH and elastic modulus (G') of the beverages were compared and presented in Figure 44. As predicted, the G' of Control (543) / CMC + fruit extract (786) were lower than that of LLA (694) / LLA + fruit extract (127). Unlike CMC, Protanal LF120 at 0.25% contributed to higher G' *i.e.* gelation and gel strength of the beverages. As expected, the G' of HLA (905) / HLA + fruit (281) was higher than that of the LLA (694) / LLA + fruit (127), which was contributed by a higher alginate level.

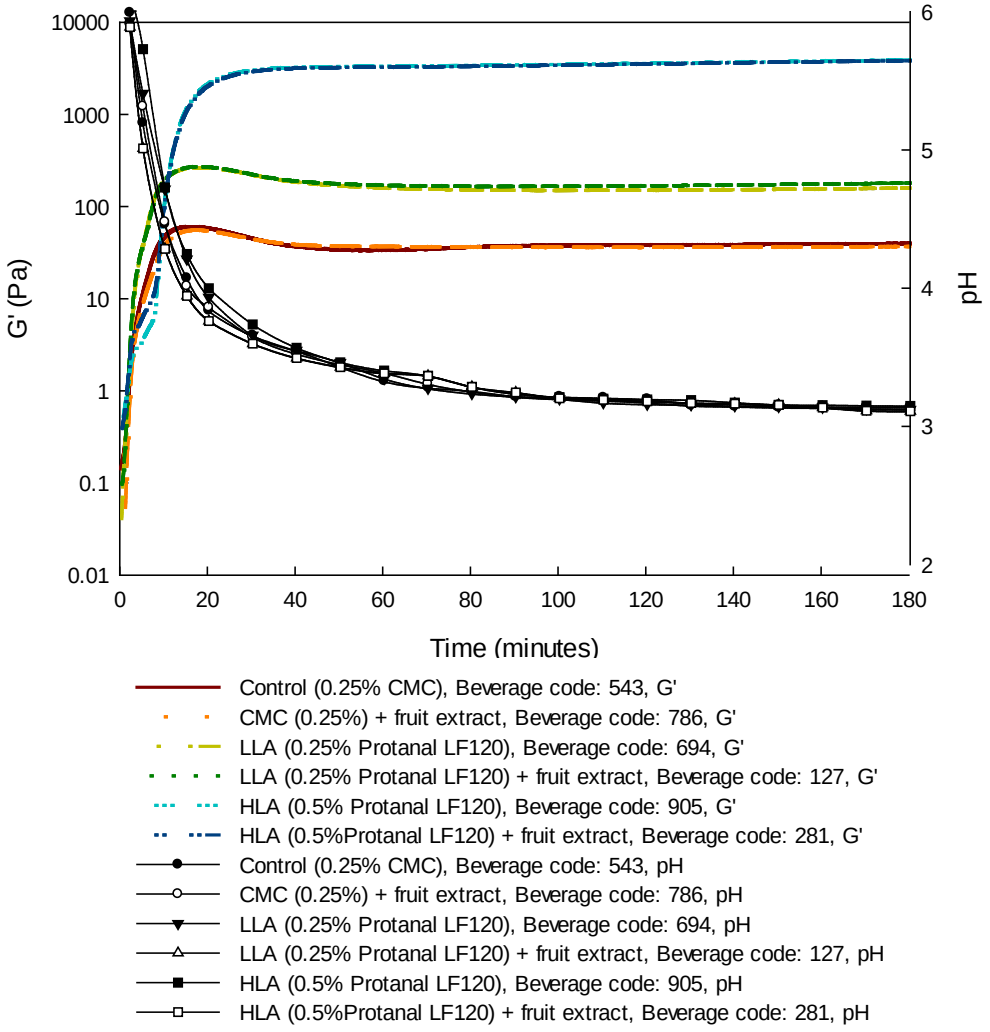


Figure 44 Gelation profiles of the test beverages, changes in changes in G' correlation to lowering of pH by 0.5M GDL, measured at 37°C, 1 Hz and 0.2% strain

6.2.4 Sensory evaluation of the beverages

The JAR score means and ANOVA results are shown in Table 26 and Figure 45. As anticipated, there is a significant difference (P <0.05) in the participants / subjects judging all attributes of the beverages. The result is typical for general consumers or untrained sensory panelists.

There were significant differences in the thickness and colour of the beverages (P <0.05). The subjects perceived beverages 905 (HLA) and 281 (HLA + fruit extract) to be thicker than the rest. Apparently, there is a correlation of the sensory data for thickness to the viscosity data. However, data analysis using Pearson correlation gave a correlation coefficient (r^2) of 0.588 (P < 0.01).

Colour of beverages was judged as darker for 127, followed by 281, 786, 905, 694 and 543. Beverages 127, 281 and 786 are those containing the fruit extract. Thus, colour matching to beverages without fruit extract proved to be difficult and needs to be improved. A photograph of the beverages in sampling cups is shown in Figure 46.

Table 26 JAR score means and ANOVA results of the beverages

Beverage	**Beverage code**	**Mean (*n* = 12)**	**Beverage *P*-value**	**Subject *P*-value**	**Beverage grouping (Tukey Method, 95.0% confidence)**
Aroma (Too weak – JAR – Too strong)					
Control (CMC)	543	3.1	0.516	0.000	A
CMC + fruit extract	786	3.0			A
LLA	694	3.1			A
LLA + fruit extract	127	3.3			A
HLA	905	3.1			A
HLA + fruit extract	281	3.0			A
Sweetness (Not sweet enough – JAR – Too sweet)					
Control (CMC)	543	3.4	0.078	0.015	A
CMC + fruit extract	786	3.1			A
LLA	694	3.0			A
LLA + fruit extract	127	3.0			A
HLA	905	3.2			A
HLA + fruit extract	281	3.3			A
Flavour (Too weak – JAR – Too strong)					
Control (CMC)	543	3.1	0.377	0.000	A
CMC + fruit extract	786	2.9			A
LLA	694	2.9			A
LLA + fruit extract	127	3.0			A
HLA	905	3.3			A
HLA + fruit extract	281	3.2			A

Table 26 *(Continued)*

Thickness (Too thin – JAR – Too thick)					
Control (CMC)	543	2.8	0.000	0.000	B
CMC + fruit extract	786	2.8			B
LLA	694	2.8			B
LLA + fruit extract	127	3.0			B
HLA	905	3.8			A
HLA + fruit extract	281	3.7			A
Aftertaste (None, slight, moderate, strong, very strong)					
Control (CMC)	543	3.1	0.118	0.000	A
CMC + fruit extract	786	2.7			A
LLA	694	3.3			A
LLA + fruit extract	127	2.9			A
HLA	905	2.7			A
HLA + fruit extract	281	2.9			A
Colour (Too light – JAR – Too dark)					
Control (CMC)	543	2.8	0.000	0.000	C
CMC + fruit extract	786	3.3			ABC
LLA	694	3.0			BC
LLA + fruit extract	127	3.6			A
HLA	905	3.2			ABC
HLA + fruit extract	281	3.5			AB
Overall acceptability (Dislike very much – Neither – Like very much)					
Control (CMC)	543	3.7	0.878	0.000	A
CMC + fruit extract	786	3.5			A
LLA	694	3.6			A
LLA + fruit extract	127	3.7			A
HLA	905	3.5			A
HLA + fruit extract	281	3.4			A

On the other hand, the subjects found aroma, sweetness, flavour, aftertaste and overall acceptability to be similar among the beverages. For aroma, sweetness and flavour, the means are at or around the “Just about right” value of 3. As for aroma, the means falls in the slight–moderate–strong categories while it is in the neither–like categories for overall acceptability.

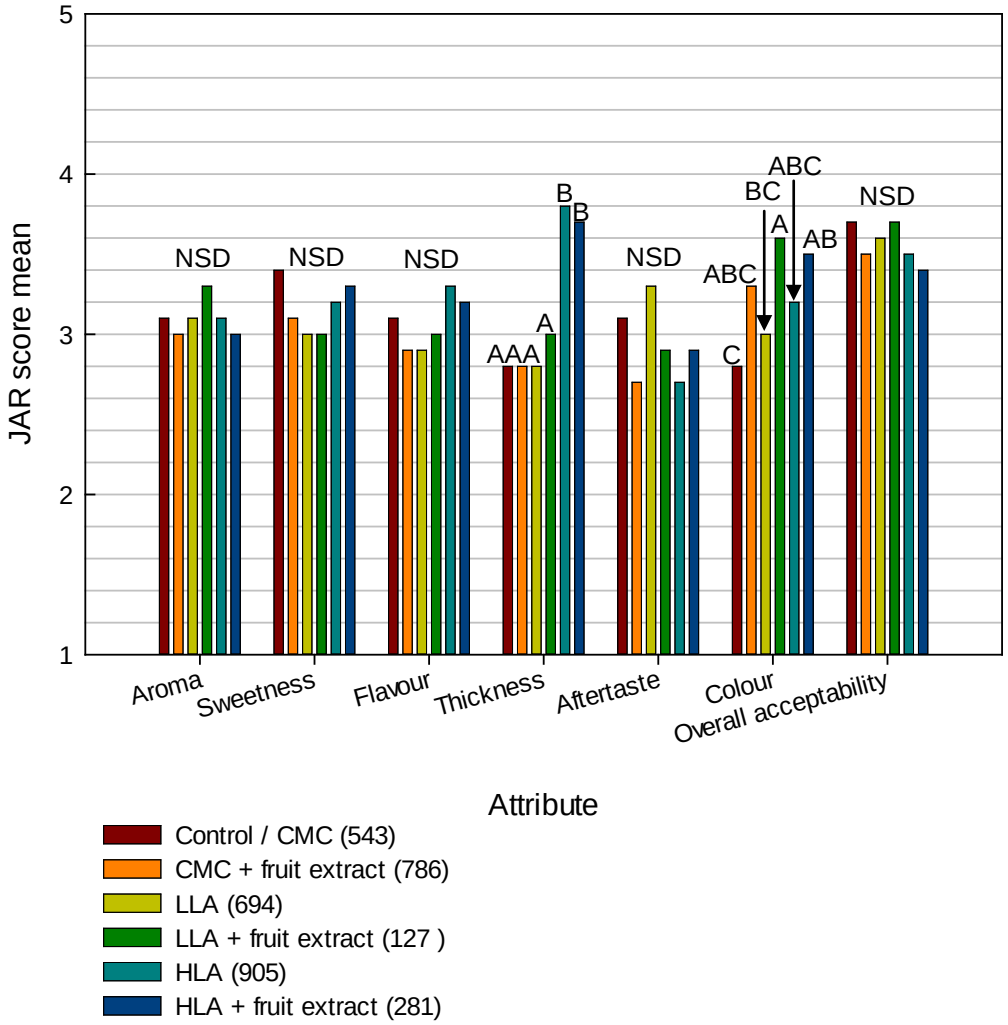


Figure 45 Histogram of JAR score means of the beverages, grouping using Tukey Method, 95.0% confidence, NSD: not significantly different

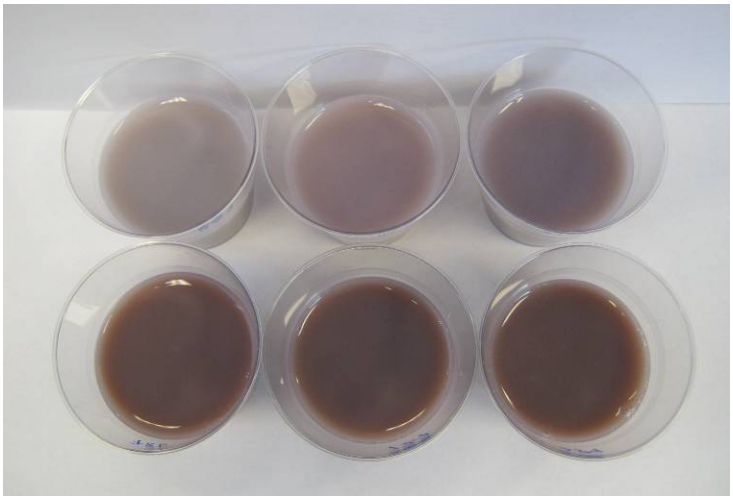


Figure 46 Top row left to right: beverages without fruit extract – 543, 694 and 905; bottom row left to right: beverages with fruit extract – 786, 127 and 281

6.2.5 Subjective appetite

A beverage with higher satiety effect is referred as having mean ratings of lower hunger, higher fullness, higher satiety, lower desire to eat and lower prospective food consumption. The appetite ratings data were analyzed using repeated measures analysis of covariance (RMANCOVA) and Tukey pairwise comparison method, between: (1) with and without fruit extract, (2) none, low and high alginate levels, and (3) the 6 beverages. According to Blundell *et al.* (2010), a 10% difference in mean ratings between foods is typically seen as a 'reasonable and realistic' difference.

6.2.5.1 Satiety effect of the fruit extract

As shown in Table 27 and Figure 47, only mean hunger rating was significantly different; beverages with fruit extract (786, 127 and 281) gave lower hunger ratings than those without fruit extract (543, 694 and 905) (P = 0.015). Since only 1 of the 5 satiety responses was found to be significant, the hypothesis H_1: phytochemical (fruit extract) increases the satiety effect of the beverage is rejected. Nevertheless, further testing is warranted as the satiety effect of the fruit extract could be dose-dependent. It would be worthwhile to incorporate higher levels *i.e.* above the current level of 0.2% in future testing.

Table 27 RMANCOVA results comparing beverages with and without fruit extract

With or without fruit extract in beverage	**RMANCOVA**		
	Mean ±SE (mm)	***P*-value**	**Beverage grouping (Tukey Method, 95.0% confidence)**
Hunger (not at all hungry – very hungry)			
With	53.1 ±1.1	0.015	B
Without	55.7 ±1.1		A
Fullness (not at all full – very full)			
With	45.7 ±1.2	0.156	A
Without	44.1 ±1.1		A
Satiety / satisfaction (completely empty – cannot take another bite)			
With	44.9 ±1.1	0.408	A
Without	44.0 ±1.0		A
Desire to eat (very weak – very strong)			
With	57.8 ±1.1	0.447	A
Without	58.6 ±1.1		A

Table 27 *(Continued)*

Prospective food consumption (nothing at all – a very large amount)			
With	57.3 ±1.1	0.178	A
Without	58.6 ±1.0		A

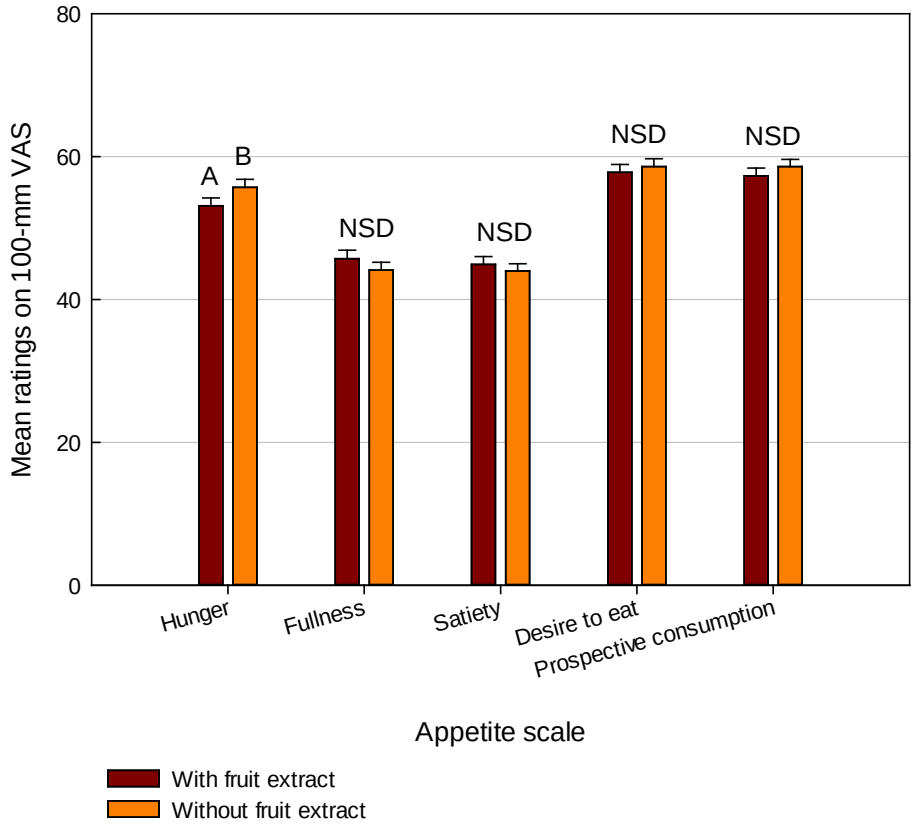


Figure 47 Histogram of appetite ratings; means ±SE, *n* = 12, grouping using Tukey Method, 95.0% confidence, NSD: not significantly different

6.2.5.2 Satiety effect of the alginate

Data analysis was also performed to determine the satiety effect of different alginate levels (none, low and high) in the beverages. Significant differences between low and high alginate levels (P <0.05) were found. As shown in Table 28 and Figure 48, the effects of high alginate levels (beverages 905 and 281) across all appetite scales are greater than low alginate levels (beverages 694 and 127). Differences in mean appetite ratings between low and high alginate levels were 6.9%, 8.3%, 10.6%, 6.3% and 6.7% for hunger, fullness, satiety, desire to eat and prospective food consumption ratings, respectively. Significant differences between none, low and high alginate levels was also found for fullness (P = 0.008) and desire to eat (P = 0.007) scales; beverages with none and low alginate levels were comparable while being significantly different from the high alginate levels beverages. Thus, the satiety effect of Protanal LF120 alginate

is presumably dose-dependent. These findings are consistent with those in the satiety study by Peters *et al.* (2011). As a result, the hypothesis H_2: higher viscosity (contributed by higher alginate level) increases the satiety effect of the beverage is accepted although further testing to confirm these results is recommended.

Table 28 Mean appetite ratings and RMANCOVA results comparing none, low and high alginate levels in the beverages

Beverage alginate level	**RMANCOVA**		
	Mean ±SE (mm)	***P*-value**	**Beverage grouping (Tukey Method, 95.0% confidence)**
Hunger (not at all hungry – very hungry)			
None (CMC)	54.9 ±1.3	0.012	AB
Low	56.1 ±1.3		A
High	52.3 ±1.4		B
Fullness (not at all full – very full)			
None (CMC)	43.9 ±1.4	0.008	B
Low	43.4 ±1.4		B
High	47.4 ±1.4		A
Satiety / satisfaction (completely empty – cannot take another bite)			
None (CMC)	44.2 ±1.3	0.001	AB
Low	42.3 ±1.3		B
High	47.0 ±1.4		A
Desire to eat (very weak – very strong)			
None (CMC)	59.1 ±1.3	0.007	A
Low	59.7 ±1.3		A
High	55.8 ±1.4		B
Prospective food consumption (nothing at all – a very large amount)			
None (CMC)	58.3 ±1.3	0.004	AB
Low	59.7 ±1.3		A
High	55.9 ±1.3		B

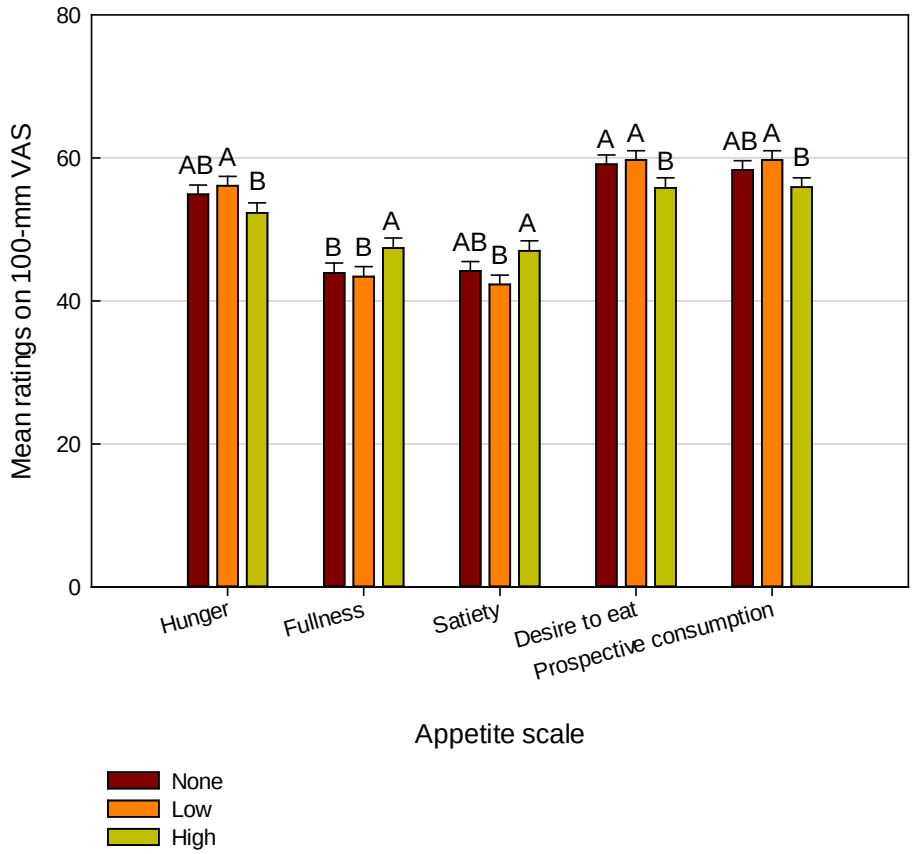


Figure 48 Histogram of appetite ratings; means ±SE, *n* = 12, grouping using Tukey Method, 95.0% confidence, NSD: not significantly different

6.2.5.3 Satiety effect of fruit extract + alginate

Another hypothesis of the study was H_3: higher viscosity (contributed by higher alginate level) plus phytochemical (fruit extract) increase the satiety effect of the beverage. As shown in Table 29 and Figure 49(a), significant differences (*P* <0.05) were observed across all appetite ratings of the 6 beverages; beverages 281 (HLA + fruit extract) and 694 (LLA) were found to be significantly different (*P* <0.05). Beverages 905 (HLA) and 694 (LLA) also differed significantly (*P* <0.05) for satiety and prospective food consumption scales. The data analysis showed that the interaction of alginate*fruit extract was not statistically significant. This implies that the higher satiety effect could be purely due to high alginate level in the beverage (accepted H_2). Therefore, it is appropriate to reject H_3, although further testing is recommended.

Analysis of covariance (ANCOVA) was performed on the total area under the curves (AUC) data but no significant difference was found among the beverages across all appetite scales. The mean total AUC data and histogram are shown in Table 29 and Figure 49(b), respectively. Generally, if a treatment effect is observed using RMANCOVA, then most likely a treatment effect will be observed when using AUC

analysis (as noted from several satiety studies). Thus, there is a contradiction with the data analysis. Differences between the beverages existed early in the experiment (first hour or so) and measurements were closer (every 15 minutes). This probably made a greater contribution to the repeated measures mean, which weights each observation equally unlike the AUC which gives less weight to observations that are closer together. In a situation where one treatment gives a smaller sustained satiety effect, while another gives a strong immediate satiety effect, the latter which disappears more quickly could possibly show up in the RMANCOVA (as a Time*Treatment interaction) but not in the AUC analysis (D. Hedderley, personal communication, May 20, 2013).

Table 29 Mean appetite ratings, RMANCOVA results and mean total AUC of the beverages

Beverage	**Beverage code**	**RMANCOVA**			**Mean total AUC ±SE ($\times 10^3$ mm.minute)**
		Mean ±SE (mm)	***P*-value**	**Grouping (Tukey method, 95.0% confidence)**	
Hunger (not at all hungry – very hungry)					
Control (CMC)	543	55.5 ±1.7	0.007	AB	13.1 ±0.9
CMC + fruit extract	786	54.3 ±2.0		AB	12.9 ±1.2
LLA	694	58.0 ±1.8		A	13.8 ±1.0
LLA + fruit extract	127	54.3 ±1.9		AB	12.7 ±1.2
HLA	905	53.7 ±2.0		AB	12.4 ±1.3
HLA + fruit extract	281	50.8 ±1.9		B	12.1 ±1.0
Fullness (not at all full – very full)					
Control (CMC)	543	44.1 ±1.9	0.018	AB	11.4 ±1.0
CMC + fruit extract	786	43.7 ±2.1		AB	11.2 ±1.3
LLA	694	42.1 ±1.9		B	10.7 ±1.2
LLA + fruit extract	127	44.7 ±2.1		AB	11.7 ±1.3
HLA	905	46.1 ±2.1		AB	11.9 ±1.3
HLA + fruit extract	281	48.6 ±2.0		A	12.3 ±1.2
Satiety / satisfaction (completely empty – cannot take another bite)					
Control (CMC)	543	44.2 ±1.8	0.007	AB	11.5 ±0.9
CMC + fruit extract	786	44.1 ±2.0		AB	11.2 ±1.2
LLA	694	41.2 ±1.7		B	10.4 ±1.0

Table 29 *(Continued)*

LLA + fruit extract	127	43.3 ±1.9		AB	11.3 ±1.2
HLA	905	46.7 ±1.9		A	11.8 ±1.0
HLA + fruit extract	281	47.3 ±2.0		A	12.1 ±1.1
Desire to eat (very weak – very strong)					
Control (CMC)	543	58.1 ±1.8	0.009	AB	13.8 ±0.8
CMC + fruit extract	786	60.0 ±2.0		AB	14.0 ±1.1
LLA	694	61.4 ±1.8		A	14.9 ±1.1
LLA + fruit extract	127	58.1 ±1.9		AB	13.3 ±1.1
HLA	905	56.4 ±2.0		AB	13.2 ±1.3
HLA + fruit extract	281	55.2 ±2.0		B	13.1 ±1.0
Prospective food consumption (nothing at all – a very large amount)					
Control (CMC)	543	58.1 ±1.8	0.004	AB	13.7 ±1.0
CMC + fruit extract	786	58.4 ±1.8		AB	13.7 ±1.2
LLA	694	61.7 ±1.7		A	14.9 ±1.1
LLA + fruit extract	127	57.8 ±1.9		AB	13.6 ±1.3
HLA	905	55.9 ±1.8		B	13.0±1.2
HLA + fruit extract	281	55.8 ±1.8		B	13.5 ±1.0

Beverages with grouping as ‘AB’ were not significantly different from those ‘A’ and ‘B’ beverages; which included the control beverage 534. The satiety effects of beverages were thought to correlate to its gelation profiles / elastic modulus (G'); beverage with higher G' would have higher satiety effect. However, beverages 543 (Control / CMC) and 786 (CMC + fruit extract) despite having lower G' than beverages 694 (LLA) and 127 (LLA + fruit extract) showed slightly higher (but statistically insignificant) satiety effect. The lack of correlation could be due the complexity of the human digestive system after ingestion of the beverages. The GDL-acidification method might not be a good representation of true stomach conditions. Other than pH and temperature, the composition (hydrochloric acid, pepsinogens / enzyme pepsin), mucus and water), quantity and strength of gastric juice in the stomach contribute to the complexity of gastric digestion of foods (Kong & Singh, 2008). Small G’ or gel strength differences between beverages can be detected by sensitive rheological measurements, but is likely to be masked by the complexity of gastric conditions, which is further complicated by possible variability among the subjects.

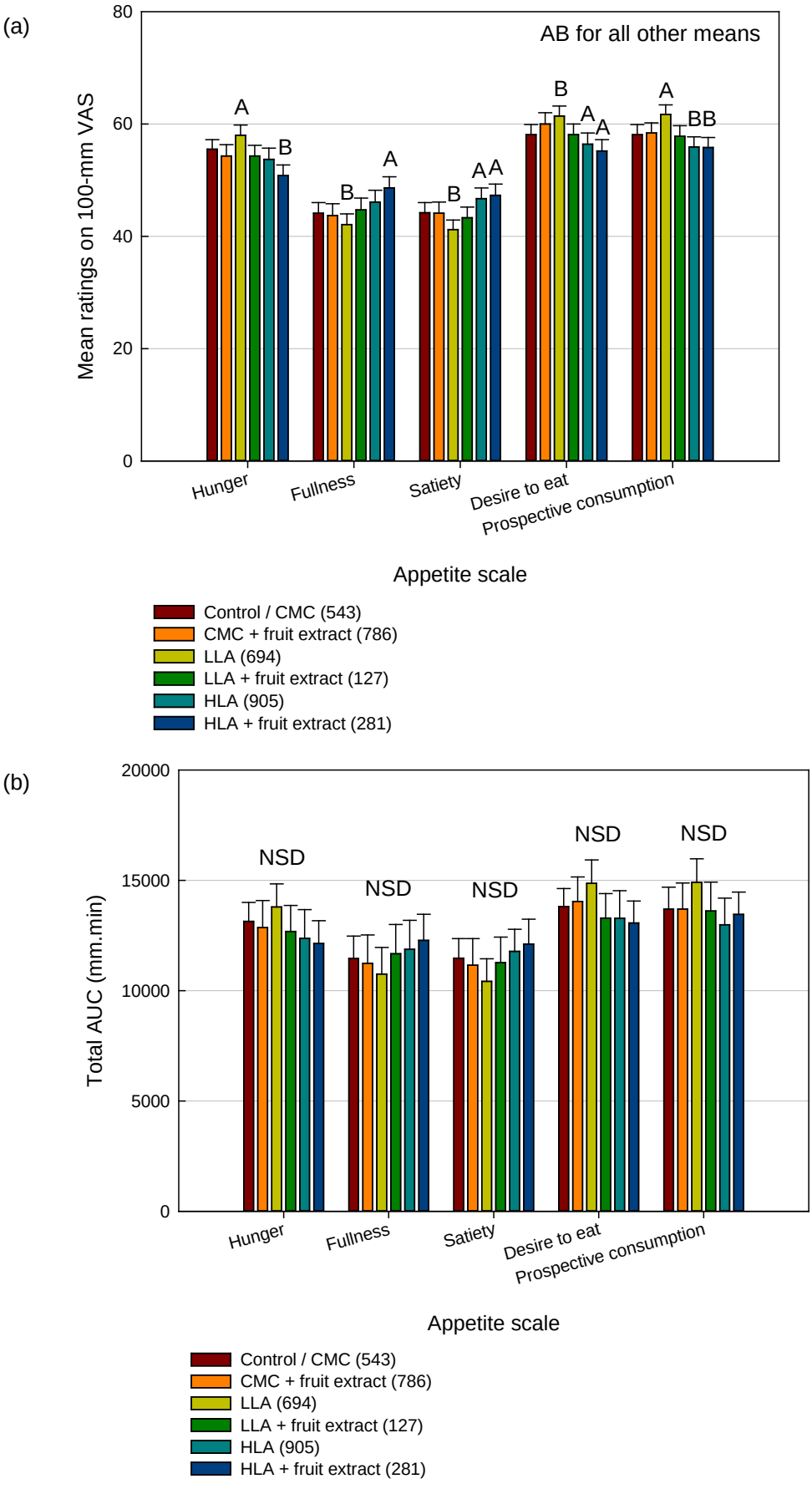


Figure 49 Histograms (a) Mean ratings (b) Total AUC; means ±SE, *n* = 12, grouping using Tukey Method, 95.0% confidence, NSD: not significantly different

Plots of mean ratings over time (4 hours) for the various appetite scales are shown in Figures 50 – 52. Compared to the other beverages, 905 (HLA) and 281 (HLA + fruit extract) gave lower hunger, higher fullness, higher satiety, lower desire to eat and lower prospective consumption ratings at time between 0 to 30 minutes. The higher satiety effects could be due to gelation of the beverage and/or formation of a stronger gel in the stomach after ingestion. The tested level of Protanal LF120 alginate (0.5%) in beverages 905 (HLA) and 281 (HLA + fruit extract) seems to be 'sufficient' for gelation under gastric conditions; hence the effect can be detected physiologically. Beverage 281 (HLA + fruit extract) showed slightly lower hunger, higher fullness and higher satiety than the others from time 45 to 120 minutes. Apparently, the effect could be attributed to the fruit extract since beverage 905 (HLA) did not show a similar trend. However, further testing is required to determine whether the effect is contributed by the fruit extract alone or is due to additive or synergetic effect with Protanal LF120.

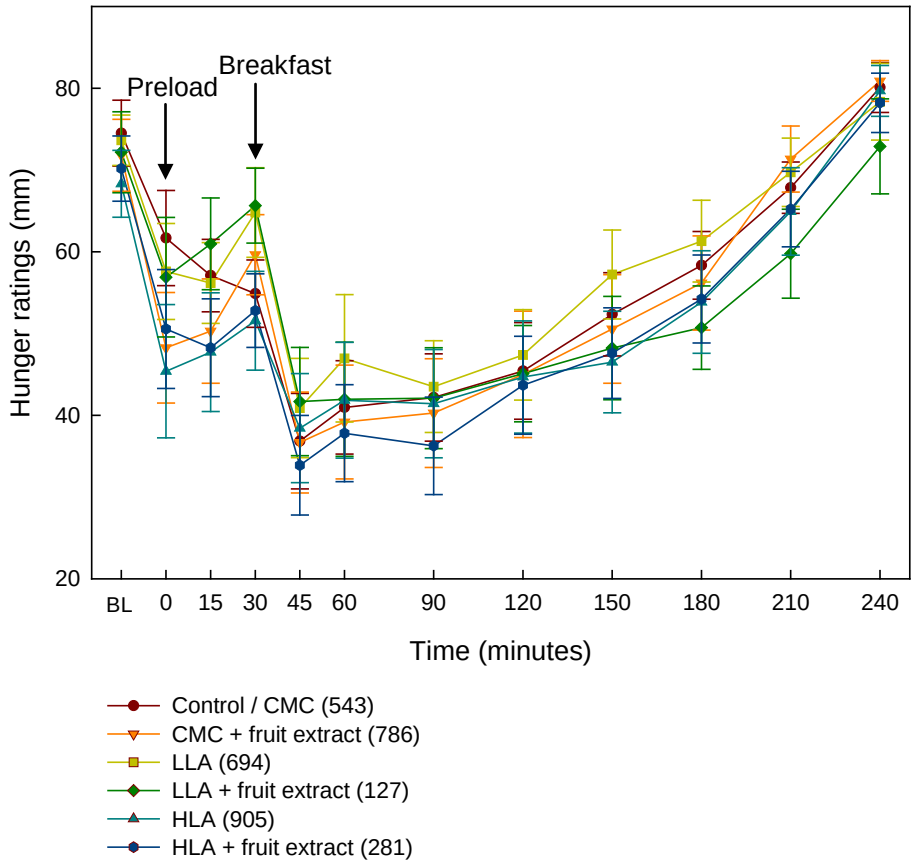


Figure 50 Hunger ratings, means ±SE, *n* = 12, after consumption of preload (test beverage) and breakfast. BL: baseline (~5 minutes before preload)

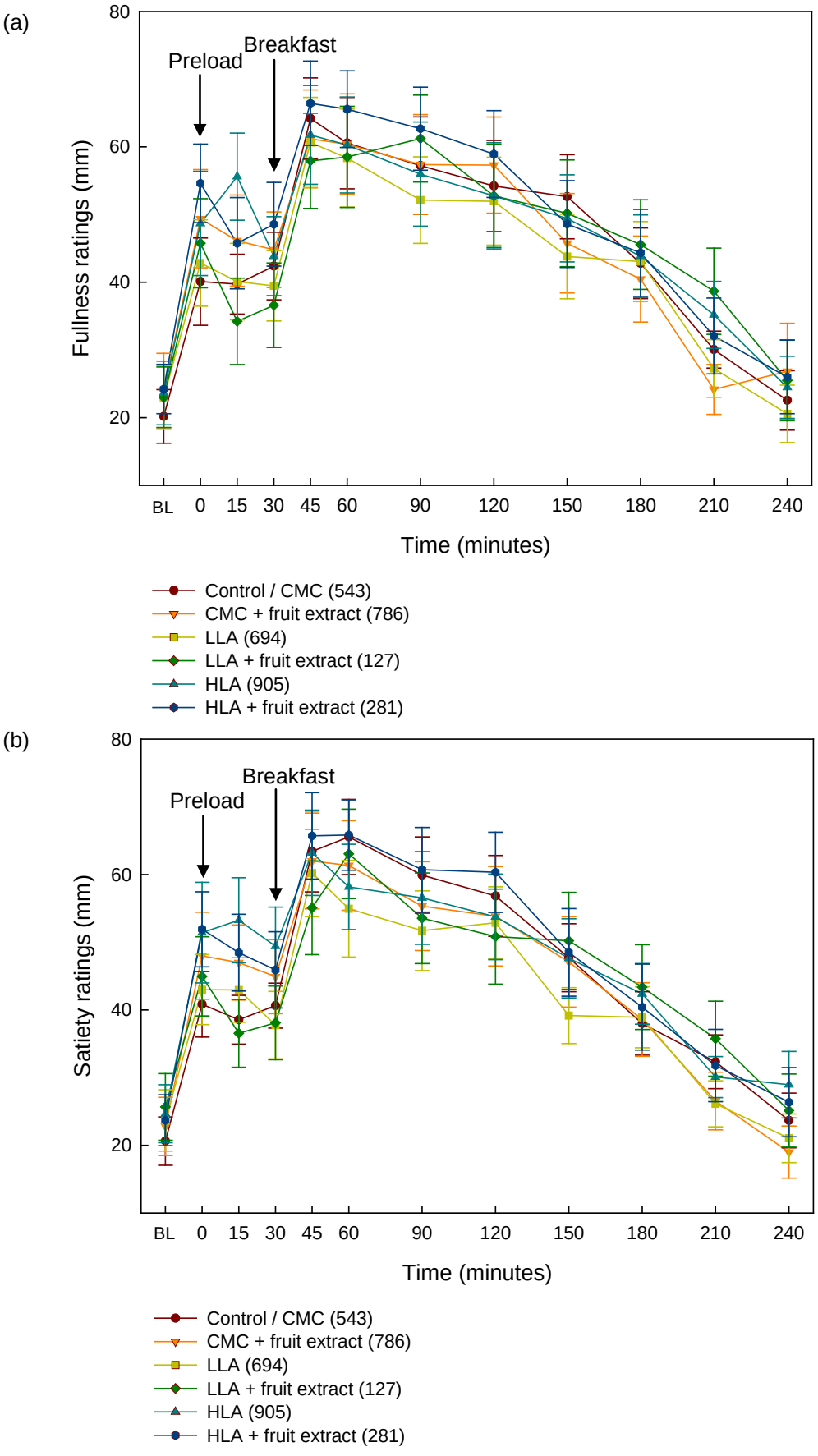
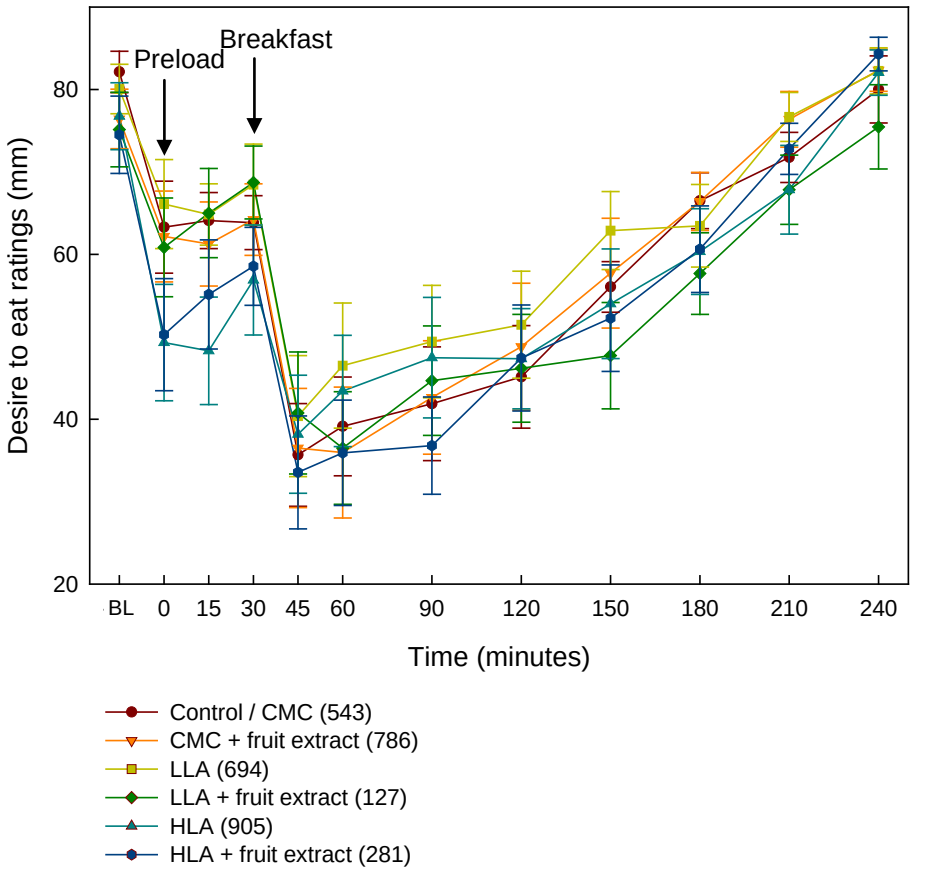


Figure 51 (a) Fullness and (b) Satiety ratings; means ±SE, *n* = 12, after consumption of preload (test beverage) and breakfast. BL: baseline (~5 minutes before preload)

(a)



(b)

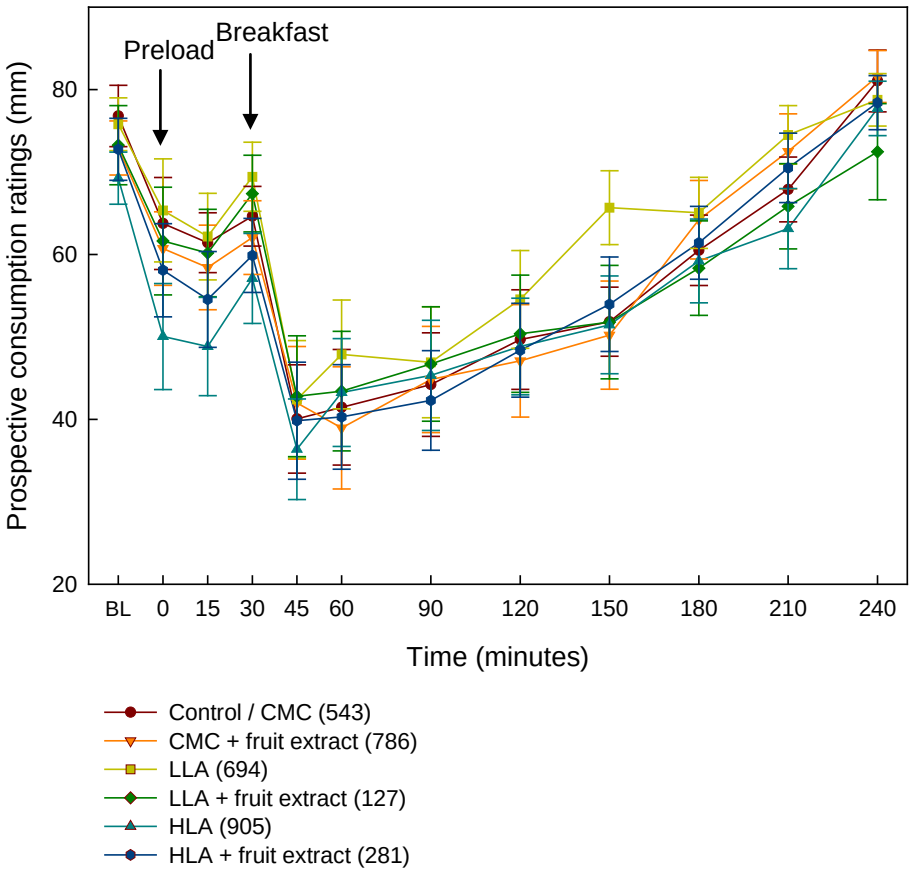


Figure 52 (a) Desire to eat and (b) Prospective consumption ratings; means ±SE, *n* = 12, after consumption of preload (test beverage) and breakfast. BL: baseline (~5 minutes before preload)

The Pearson correlation *P*-values for thickness (sensory attribute) and hunger, fullness, desire to eat and prospective consumption (but not satiety) ratings are smaller than 0.01, thus there is sufficient evidence at 99% confidence level that the correlations are not zero. This implies that thickness of the beverages could have influenced the appetite ratings of the subjects. There is also a possibility that the lower hunger, higher fullness, higher satiety, lower desire to eat and lower prospective consumption ratings of 905 (HLA) and 281 (HLA + fruit extract) at time between 0 to 30 minutes is due to learned responses or expectations (cognitive) of the subjects. Some of the subjects could differentiate the beverages in terms of their thickness and might have a lower hunger / higher fullness perception after consuming a thicker beverage.

6.2.5.4 Power analysis

Further statistical analysis was performed by D. Hedderley, a statistician at PFR. He fitted the repeated measures model using Genstat (VSNi Ltd, Hemel Hempstead, UK) following the recommendations of Littell and colleagues (Littell *et al.*, 1998). The analysis found significant alginate effects for desire to eat and satiety scales ($P = 0.005$ and 0.036 respectively), a near-significant alginate effect for fullness scale ($P = 0.064$) but no significant alginate effects for hunger or prospective consumption scales ($P = 0.334$ and 0.102 respectively); no significant fruit extract effects ($P = 0.550$ to 0.909) and no significant alginate*fruit extract interactions ($P = 0.324$ to 0.928). Power analysis using these results, assuming to detect a 10% difference in means, then power for the alginate main effect ranged from 49% to 63%; for the fruit extract main effect, power ranged from 63% to 78%. The number of subjects required to achieve 80% power for the main effects ranged from 13 to 28 (D. Hedderley, personal communication, May 20, 2013). This coincides with the initial target of 28 subjects. For future work, modification to the current experimental design is necessary to increase power of the study to 80%. With the high or required power, if the data still does not reveal any statistically significant results *i.e.* no meaningful difference or effect, then the study should be discontinued.

6.2.5.5 Sources of variation

The sources of variation including interactions taken in account were session, session*beverage, time, time*beverage, gender and subjects.

The subjects attended 6 sessions where they consumed the 6 different beverages in a randomized order. Data analysis using ANOVA and Tukey pairwise comparisons method (95% confidence level) did not find any significant differences in the mean

ratings between the 6 sessions across all appetite scales. In addition, there was no interaction present for session*beverage. Thus, potential aging / carry-over and unwanted effects confounding to the study were minimal (Blundell *et al.*, 2010).

As shown in Figures 50 – 52, the mean ratings are dependent on time points. For example, hunger ratings dropped after consuming the beverage and breakfast but gradually increased back to baseline over time. There is no interaction present for time*beverage, thus the effect of beverages was independent on individual time points.

There were 6 females and 6 males subjects in the trial. From the ANOVA results, all mean appetite ratings were significantly different (P <0.05) for gender. Overall, the male subjects gave higher hunger, desire to eat and prospective food consumption ratings and lower fullness and satiety ratings than the female subjects. An interval plot of the data is shown in Figure 53. Males generally have higher energy requirements than females, thus involving both gender in the trial could introduce certain variation to the results. Due to the low number of subjects in the trial, analysis of the data by gender was not attempted.

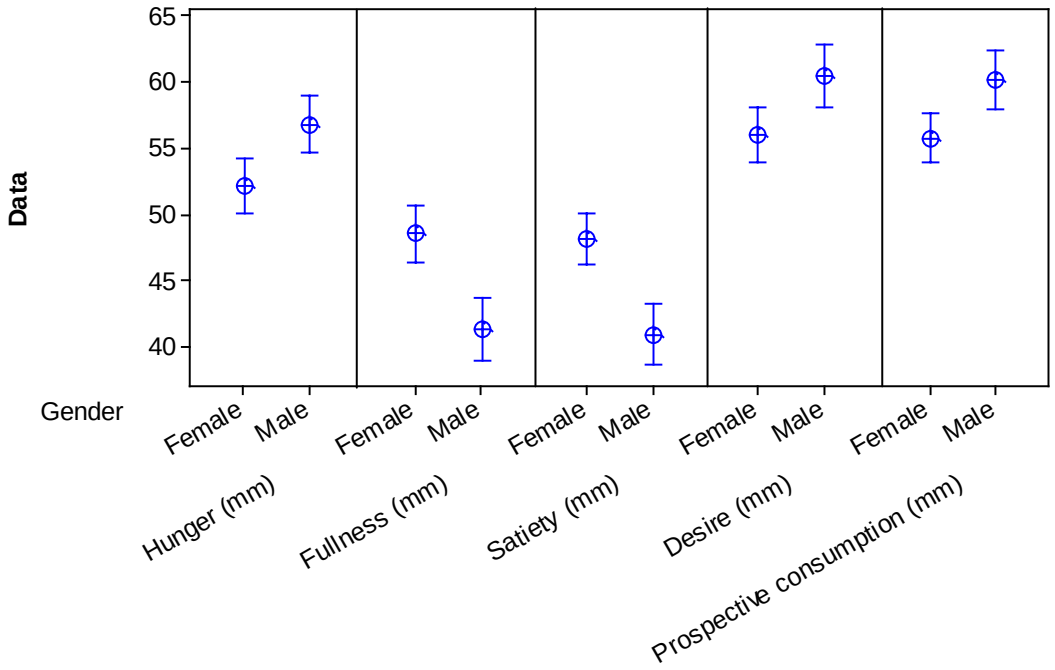


Figure 53 Interval plot of appetite rating data comparing females and males, 95% confidence interval for the mean

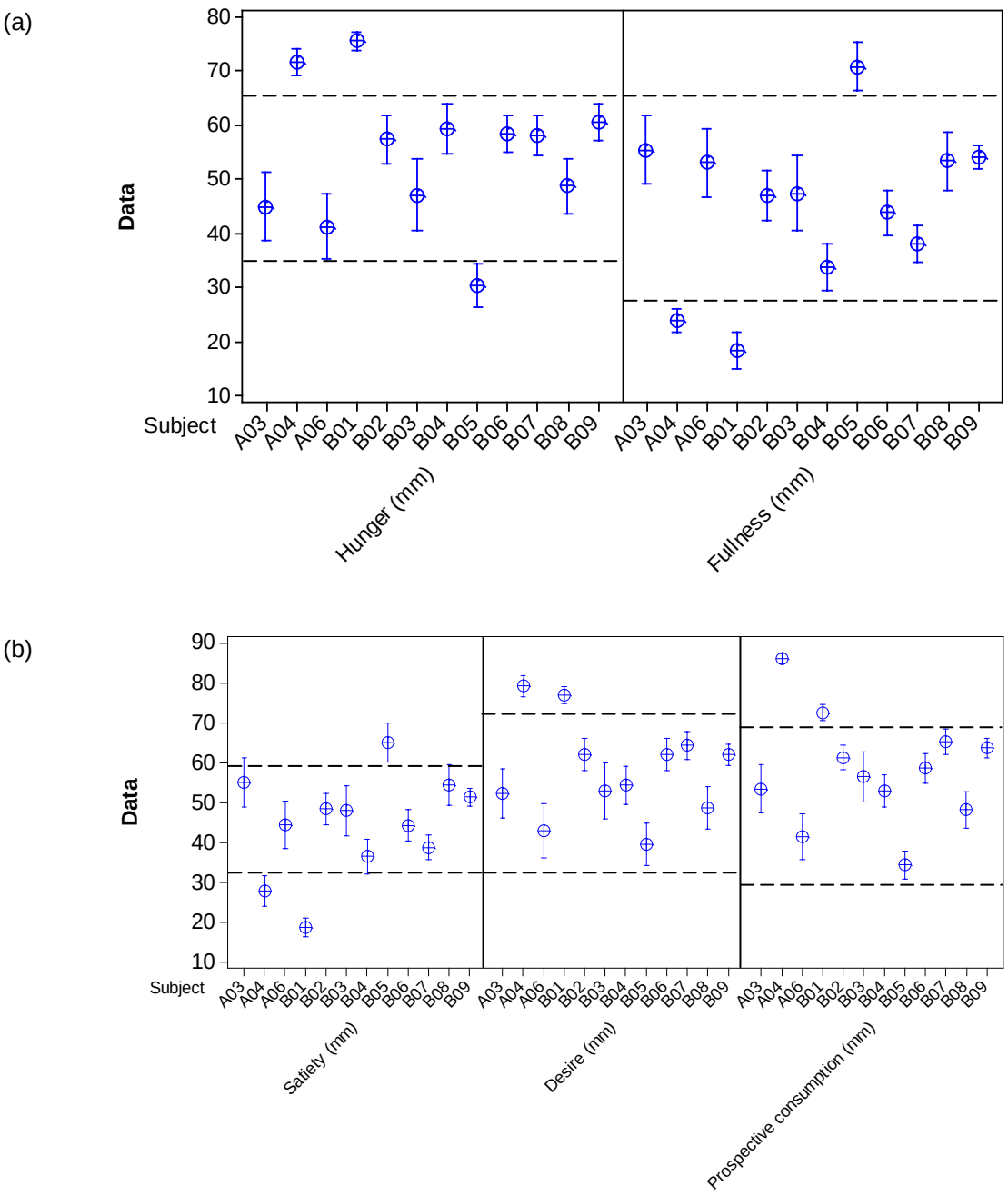


Figure 54 Interval plot of appetite rating data (a) hunger and fullness, (b) satiety, desire to eat and prospective consumption; comparing 12 subjects, 95% confidence interval for the mean

Another source of variation is among the subjects, which is inevitable considering heterogeneity of the human population (Blundell *et al.*, 2010). Significant differences (P <0.05) in their ratings across all appetite scales was found. For example, subject A04 and B01 gave higher hunger, desire to eat and prospective consumption and lower fullness and satiety ratings than others, whereas subject B05 gave ratings of the opposite. An interval plot of the data is shown in Figure 54. The analysis indicates the presence of subpopulations among the subjects *i.e.* those with higher hunger ratings and those with lower hunger ratings, than the majority. Further data analysis, for

example, the Principal Components Analysis could be performed to obtain other useful information.

6.3 Conclusion

The satiety measurement trial has provided valuable findings on the appetite control / satiety effects of Protanal LF120 alginate and fruit extract in the beverage model. It was found that the satiety effect of Protanal LF120 is dose-dependent; higher alginate level increased the satiety effect of the beverage. Current findings did not provide a conclusive validation on the satiety effect of the fruit extract, but suggest that the fruit extract is worth further investigation in relation to appetite control. Further investigation is warranted: (1) to incorporate higher levels of fruit extract in the beverage model to evaluate any dose-dependency and (2) to determine whether the effect is contributed by the fruit extract alone or is due to additive or synergetic effect with Protanal LF120 alginate, and (3) to modify the current experimental design to increase power of the study to 80% by increasing the number of subjects.

Chapter 7

Key Findings, Conclusions and Recommendations

The key findings and methodology of the current project work are summarized in Figure 55 below. Findings from the characterization work confirmed substantial differences among various pectins and alginates. Of interest, differences in their viscosity, sensitivity to acids and calcium ions for gelation, and gel strength generated, were observed in the seven pectins and alginates evaluated. The viscosity profiles did not differ much among the pectins. High-ester pectins, Pectin Classic AF101 and Pectin AMD 780, did not show any reactivity to acids, whereas the low-ester, amidated Pectin LA410 showed sensitivity to acids with onset of gelation and gel formation, when pH of its solution was lowered by acids. Typically, high-ester pectins form gels only under acidic and high soluble solids (>55%) conditions, whereas low-ester pectins form gels under a wider range of pH (3 – above 5) and soluble solids (10 – 80%) as well as in the presence of calcium ions (EndreB & Christensen, 2009).

Viscosity differences were more evident among the alginates. Kelcosol, low guluronic (G) acid content, had the highest viscosity but lacked sensitivity to acids. Protanal LF120 (high G), Protanal IC2053 (medium G) and Dariloid QH (medium G) showed sensitivity to acids with onset of gelation and gel formation. These alginates and Pectin LA410 showed faster onset of gelation and exhibited higher gelation profiles / gel strength when calcium ions were present during acidification. Having met the selection criteria: (1) providing viscosity to the beverage, (2) forming a gel at low pH in the presence of calcium (and presumably in the stomach), and (3) having the highest gel strength amongst those forming a gel, Protanal LF120 and Pectin LA410 were chosen and evaluated in the beverage model.

Recommendations for future work on the characterization of viscous fibres are as follows:

1. To use 0.5M instead of 1M GDL for the acidification of Protanal LF120. The rate of acidification could have been too fast with 1M GDL, resulting in chaotic gelation and therefore in a loss of gel strength with observed syneresis.
2. To source for and evaluate another pectin similar to Pectin LA410 (in terms of acid and calcium sensitivity) but of higher purity. Pectin LA410 is a blend containing 37% sugar, which is likely to have a significant effect on its physico-chemical properties.

Development of a Beverage Model to Test Appetite Control Food Ingredients

1. Characterization of viscous fibres

Rheological measurements: Viscosity at shear rates of 0.01–500/s, 20°C
Gelation profiles, G' and G'', 3 hours, 37°C – acid and calcium sensitivity by GDL-acidification method

- Pectin Classic AF101, Pectin AMD 780 and Kelcosol alginate – lacked sensitivity to acids
- Protanal IC2053 and Dariloid QH – acid and calcium sensitive but lower in G' and G''
- Pectin LA410 and Protanal LF120 – acid and calcium sensitive; higher in G' and G''

2. Beverage formulation and production

Product concept: Partial-meal replacer beverage
Commercial beverage as benchmark: (1) viscosity at 10/s, 20°C and (2) nutritional content used as basis of the formulation
Standardize energy, protein, fat, sugar and fibre contents
Processing method: Pasteurization (lab trials), UHT-processing (pilot plant trials)

Laboratory trials (pasteurization):

- Standardized fibre contents in all beverages with inulin
- Increased viscosity of the control beverage with CMC
- Included potassium citrate as chelating agent
- Other ingredients: isolated soy protein, sugar, calcium phosphate and flavor
- Determined usage levels of CMC, Pectin LA410 and Protanal LF120
- Incorporated fruit extract into the beverage model

Pilot plant trials (UHT-processing):

- Viscosity dropped in all beverages with CMC, Pectin LA410, Protanal LF120
- Unlike CMC and Protanal LF120, increasing usage levels of Pectin LA410 was not feasible due increased beverage fibre content (above the target of 1.5)
- Beverages – Control (0.25% CMC), low level alginate (LLA) and high level alginate (HLA) with 0.25% and 0.5% Protanal LF120, respectively

3. Satiety measurement trial

Participants: 6 females, 6 males (n = 12), mean age and BMI of 41.2 years and 25.2 kg/m^2, respectively
Study design: preload, single-blind, within-subject, repeated measures, completely balanced, crossover and randomized
Standard breakfast: Muesli and milk or soymilk, taken 30 minutes after preload (test beverage)
Sensory evaluation: JAR scales for aroma, sweetness, thickness, flavour, aftertaste, colour and acceptability
Subjective appetite: 100-mm VAS hunger, fullness, satiety, desire to eat and prospective consumption
Statistical data analysis: mean appetite ratings, RMANCOVA

- Thickness: beverages HLA and HLA + fruit extract were thicker than the others.
- Colour: beverages + fruit extract were darker than those without fruit extract
- Rejected H_1: Phytochemical (fruit extract) increases the satiety effect of the beverage.
  - Fruit extract decreased only the hunger response
- Accepted H_2: Higher viscosity (contributed by higher alginate level) increases the satiety effect of the beverage; 7% difference in mean hunger rating
- Rejected H_3: Higher viscosity (contributed by higher alginate level) plus phytochemical (fruit extract) increases the satiety effect of the beverage

Figure 55 Summary on the key findings and methodology of the project

3. To reduce the gelation measurement time (GDL-acidification method) from 3 hours to 2 hours, as it would be adequate for good comparisons between samples.

The development work has established a base formulation and processing method for the beverage model and has successfully incorporated Protanal LF120 and fruit extract. UHT-processing resulted in viscosity drop in the beverages. Unlike beverages with CMC (control) and Protanal LF120, it was not feasible to increase the usage levels of Pectin LA410, as that would exceed the fibre content target of 1.5% in the beverage. Due to its low viscosity and poor stability in UHT-processing (even at high levels), Pectin LA410 was excluded from further evaluation. Incorporation of quercetin and isoquercetin into the beverage model was unsuccessful because of their poor water-solubility and interactions with soy proteins. The water-soluble fruit extract was incorporated into the beverage model with acceptable colour and flavour modifications of the beverage model.

Recommendations for future work on the beverage formulation and production work are as follows:

1. To source for and evaluate another pectin similar to Pectin LA410 (in terms of acid and calcium sensitivity) but of higher purity as well as of higher heat stability.
2. Although UHT-processing was the preferred method for the beverages (in this project), it may be worthwhile to evaluate other heating methods *e.g.* pasteurization (using the pilot plant heat exchanger). Using lower heating temperatures might reduce viscosity losses in the beverages.
3. To determine any effects of UHT-processing on the fruit extract in the beverage model.
4. To develop another beverage model, without soy proteins or with other source or type of proteins that would not interact with quercetin / isoquercetin.
5. To develop another beverage model without sedimentation of quercetin / isoquercetin in the beverage as a concern. For example, a beverage model that is a powder mix containing quercetin / isoquercetin and to be consumed immediately after reconstitution.
6. Although the current blueberry flavour is acceptable, it may be worthwhile to evaluate other fruit flavours that will complement the fruit extract in the beverage better.

The satiety measurement trial has provided valuable findings on the appetite control / satiety effects of Protanal LF120 alginate and fruit extract in the beverage model. Firstly, the hypothesis H_2: higher viscosity (contributed by higher alginate level) increases the satiety effect of the beverage is accepted. Differences in mean appetite ratings between low and high alginate levels were 6.9%, 8.3%, 10.6%, 6.3% and 6.7% for hunger, fullness, satiety, desire to eat and prospective food consumption ratings, respectively. The satiety effect of Protanal LF120 alginate is presumably dose-dependent.

Secondly, the hypothesis H_1: phytochemical (fruit extract) increases the satiety effect of the beverage is rejected as the data did not reveal statistically significant results across all appetite scales (except for hunger). Further testing is warranted as the satiety effect of the fruit extract could be dose-dependent. It would be worthwhile to incorporate higher levels *i.e.* above the current level of 0.2% in future testing. In addition, modification to the current experimental design is necessary to increase power of the study to 80% by increasing the number of subjects.

Thirdly, the hypothesis H_3: higher viscosity (contributed by higher alginate level) plus phytochemical (fruit extract) increase the satiety effect of the beverage is also rejected. The data analysis showed that the interaction of alginate*fruit extract was not statistically significant, implying that the higher satiety effect could be purely due to high alginate level in the beverage. Nevertheless, further work is recommended to confirm this result as well as to test higher levels of fruit extract, determining whether an additive or synergetic effect exists with a higher level of fruit extract and high alginate level in the beverage.

Last but not least, recommendations for future work on the satiety measurement trial are as follows:

1. To modify the GDL-acidification method to improve the correlation between gelation profiles (G') and satiety effects (subjective appetite) of the beverages.
2. To incorporate higher levels of fruit extract in the beverage model *i.e.* above the current level of 0.2%, to test for dose-dependency and additive or synergetic effect with high alginate level.
3. To increase power of the study to 80% by having an adequate number of subjects.
4. To use a single-gender group of subjects to reduce potential variations, due to higher energy requirements by males than females.

5. To use a between-subjects design to reduce confounding effects of learned responses or expectations (cognitive) of the subjects. Examples: (1) due to viscosity differences: one group of subjects will receive the low viscosity beverage(s) while another group of subjects will receive the higher viscosity beverage(s); and (2) due to colour differences, one group of subjects will receive the beverage(s) without fruit extract while another group of subjects will receive the beverage(s) with fruit extract; and compare the results.
6. To use other statistical software that handle complex analyses more effectively *e.g.* Genstat or SAS for data analysis.
7. To test the satiety effect of pectin, if a suitable product is available.

In this project, the development work has formulated a satisfactory beverage model, determined its appropriate processing method and parameters and showed proof of concept that phytochemical (fruit extract) and viscous fibre (Protanal LF120 alginate) can be incorporated into the beverage model. In addition, a methodology for satiety measurement has been established and a trial was carried out to validate the satiety effects (subjective appetite) of fruit extract and alginate in beverages. Correlations were made to relate results of subjective appetite to the physico-chemical and sensorial properties of the beverages. Certainly, further work is warranted and recommendations have been made. In conclusion, the aim and objectives of the project have been achieved.

References

Anderson, G. H., & Moore, S. E. (2004). Dietary Proteins in the Regulation of Food Intake and Body Weight in Humans. *The Journal of Nutrition*, *134*(4), 974S-979S.

Barone Lumaga, R., Azzali, D., Fogliano, V., Scalfi, L., & Vitaglione, P. (2012). Sugar and dietary fibre composition influence, by different hormonal response, the satiating capacity of a fruit-based and a β-glucan-enriched beverage. *Food & Function*, *3*(1), 67-75.

Beck, E. J., Tosh, S. M., Batterham, M. J., Tapsell, L. C., & Huang, X.-F. (2009). Oat β-glucan increases postprandial cholecystokinin levels, decreases insulin response and extends subjective satiety in overweight subjects. *Molecular Nutrition & Food Research*, *53*(10), 1343-1351.

Benelam, B. (2009). Satiation, satiety and their effects on eating behaviour. *Nutrition Bulletin*, *34*(2), 126-173.

Blundell, J., De Graaf, C., Hulshof, T., Jebb, S., Livingstone, B., Lluch, A., Mela, D., Salah, S., Schuring, E., Van Der Knaap, H., & Westerterp, M. (2010). Appetite control: methodological aspects of the evaluation of foods. *Obesity Reviews*, *11*(3), 251-270.

Brownlee, I. A. (2011). The physiological roles of dietary fibre. *Food Hydrocolloids*, *25*(2), 238-250.

Cardello, A. V., Schutz, H. G., Lesher, L. L., & Merrill, E. (2005). Development and testing of a labeled magnitude scale of perceived satiety. *Appetite*, *44*(1), 1-13.

ChemPRIME (2010). Ksp and calcium fortification. Retrieved 15 March 2012, from http://wiki.chemprime.chemeddl.org/index.php/Ksp_and_calcium_fortification.

de Graaf, C., Blom, W. A., Smeets, P. A., Stafleu, A., & Hendriks, H. F. (2004). Biomarkers of satiation and satiety. *The American Journal of Clinical Nutrition*, *79*(6), 946-961.

Department of Health (1991). Food for Health: Report of the Nutrition Taskforce. Wellington, Department of Health.

Dettmar, P. W., Strugala, V., & Craig Richardson, J. (2011). The key role alginates play in health. *Food Hydrocolloids*, *25*(2), 263-266.

Di Lorenzo, C., Williams, C. M., Hajnal, F., & Valenzuela, J. E. (1988). Pectin delays gastric emptying and increases satiety in obese subjects. *Gastroenterology*, *95*(5), 1211-1215.

Draget, K. I. (2009). Alginates. In: G. O. Phillips, & P. A. Williams, *Handbook of hydrocolloids* (pp. 807-828). Boca Raton: Woodhead Publishing Limited.

Draget, K. I., Skjåk Bræk, G., & Smidsrød, O. (1994). Alginic acid gels: the effect of alginate chemical composition and molecular weight. *Carbohydrate Polymers*, *25*(1), 31-38.

Draget, K. I., Smidsrød, O., & Skjåk-Bræk, G. (2005). Alginates from Algae. *Biopolymers Online*: Wiley-VCH Verlag GmbH & Co. KGaA.

El Khoury, D., Cuda, C., Luhovyy, B. L., & Anderson, G. H. (2012). Beta Glucan: Health Benefits in Obesity and Metabolic Syndrome. *Journal of Nutrition and Metabolism*, *2012. Article ID 851362*, 28 pages.

EndreB, H.-U., & Christensen, S. H. (2009). Pectins. In: G. O. Phillips, & P. A. Williams, *Handbook of hydrocolloids* (pp. 274-297). Boca Raton: Woodhead Publishing Limited.

European Food Safety Authority (2012). EFSA Panel on Dietetic Products, Nutrition and Allergies. Scientific Opinion on Dietary Reference Values for protein *EFSA Journal*, *10(2):2557*, 66.

Flint, A., Raben, A., Blundell, J. E., & Astrup, A. (2000). Reproducibility, power and validity of visual analogue scares in assessment of appetite sensations in single test meal studies. *International Journal of Obesity*, *24*(1), 38-48.

Flood-Obbagy, J. E., & Rolls, B. J. (2009). The effect of fruit in different forms on energy intake and satiety at a meal. *Appetite*, *52*(2), 416-422.

Foster, T., & Wolf, B. (2011). Hydrocolloid Gums – Their Role and Interactions in Foods. In: I. T. Norton, F. Spyropoulos, & P. Cox, *Practical Food Rheology* (pp. 61-84): Wiley-Blackwell.

FSANZ (2001). Section 1.2.8 Nutrition information requirements. Food Standards Australia New Zealand.

Georg Jensen, M., Knudsen, J. C., Viereck, N., Kristensen, M., & Astrup, A. (2012). Functionality of alginate based supplements for application in human appetite regulation. *Food Chemistry*, *132*(2), 823-829.

Gómez, C., Navarro, A., Manzanares, P., Horta, A., & Carbonell, J. V. (1997). Physical and structural properties of barley (1 → 3),(1 → 4)-β-d-glucan. Part II. Viscosity, chain stiffness and macromolecular dimensions. *Carbohydrate Polymers*, *32*(1), 17-22.

Halton, T. L., & Hu, F. B. (2004). The Effects of High Protein Diets on Thermogenesis, Satiety and Weight Loss: A Critical Review. *Journal of the American College of Nutrition*, *23*(5), 373-385.

Haug, A. (1964). *Composition and properties of alginates*. Norwegian Institute of Seaweed Research.

Herbstreith & Fox (1999). The Specialists for Pectin. Herbstreith & Fox KG Pektin-Fabriken.

Hercules Incorporated (1999). AQUALON Sodium Carboxymethylcellulose - Physical and Chemical Properties. Retrieved 3 May 2012, from http://www.ashland.com/Ashland/Static/Documents/AAFI/PRO-250-10H_Aqualon_CMC.pdf.

Hlebowicz, J., Darwiche, G., Björgell, O., & Almér, L.-O. (2008). Effect of Muesli with 4 g Oat β-Glucan on Postprandial Blood Glucose, Gastric Emptying and Satiety in Healthy Subjects: A Randomized Crossover Trial. *Journal of the American College of Nutrition*, *27*(4), 470-475.

Hoad, C. L., Rayment, P., Spiller, R. C., Marciani, L., Alonso, B. d. C., Traynor, C., Mela, D. J., Peters, H. P. F., & Gowland, P. A. (2004). In Vivo Imaging of Intragastric Gelation and Its Effect on Satiety in Humans. *The Journal of Nutrition*, *134*(9), 2293-2300.

Hogenkamp, P. S., Stafleu, A., Mars, M., Brunstrom, J. M., & de Graaf, C. (2011). Texture, not flavor, determines expected satiation of dairy products. *Appetite*, *57*(3), 635-641.

Hsu, C.-L., & Yen, G.-C. (2008). Phenolic compounds: Evidence for inhibitory effects against obesity and their underlying molecular signaling mechanisms. *Molecular Nutrition & Food Research*, *52*(1), 53-61.

Huffman, L., Jones, V., Hedderley, D., Jaeger, S., & Wallace, A. (2011). Consumer survey on food concepts (presentation slides). The New Zealand Institute for Plant & Food Research.

Hursel, R., & Westerterp-Plantenga, M. (2009). The Effects of Caffeine and Green Tea on Energy Expenditure, Fat Oxidation, Weight Loss, and Weight Maintenance. *Weight Control and Slimming Ingredients in Food Technology* (pp. 159-176): Wiley-Blackwell.

Ingram, J., & Lo, K. (2012). The role of plant components in modulating satiety (presentation slides). The New Zealand Institute for Plant & Food Research.

Juntunen, K. S., Niskanen, L. K., Liukkonen, K. H., Poutanen, K. S., Holst, J. J., & Mykkänen, H. M. (2002). Postprandial glucose, insulin, and incretin responses to grain products in healthy subjects. *The American Journal of Clinical Nutrition*, *75*(2), 254-262.

Juvonen, K. R., Purhonen, A.-K., Salmenkallio-Marttila, M., Lähteenmäki, L., Laaksonen, D. E., Herzig, K.-H., Uusitupa, M. I. J., Poutanen, K. S., & Karhunen, L. J. (2009). Viscosity of Oat Bran-Enriched Beverages Influences Gastrointestinal Hormonal Responses in Healthy Humans. *The Journal of Nutrition*, *139*(3), 461-466.

Kacinik, V., Lyon, M., Purnama, M., Reimer, R. A., Gahler, R., Green, T. J., & Wood, S. (2011). Effect of PGX, a novel functional fibre supplement, on subjective ratings of appetite in overweight and obese women consuming a 3-day structured, low-calorie diet. *Nutrition and Diabetes*, *1*, e22.

Kirk-Othmer Food and Feed Technology (2007). Kirk-Othmer Food and Feed Technology. vol. 1: John Wiley & Sons.

Kleef, v. E., Trijp, v. J. C. M., Borne, v. d. J. J. G. C., & Zondervan, C. (2011). Successful development of satiety enhancing food products: towards a multidisciplinary agenda of research challenges. *Critical Reviews in Food Science and Nutrition*.

Kong, F., & Singh, R. P. (2008). Disintegration of Solid Foods in Human Stomach. *Journal of Food Science*, *73*(5), R67-R80.

Kovacs, E. M. R., & Mela, D. J. (2006). Metabolically active functional food ingredients for weight control. *Obesity Reviews*, *7*(1), 59-78.

Littell, R. C., Henry, P. R., & Ammerman, C. B. (1998). Statistical analysis of repeated measures data using SAS procedures. *Journal of Animal Science*, *76*(4), 1216-1231.

Lootens, D., Capel, F., Durand, D., Nicolai, T., Boulenguer, P., & Langendorff, V. (2003). Influence of pH, Ca concentration, temperature and amidation on the gelation of low methoxyl pectin. *Food Hydrocolloids*, *17*(3), 237-244.

Lopes da Silva, J. A., & Rao, M. A. (2007). Rheological Behavior of Food Gels. *Rheology of Fluid and Semisolid Foods* (pp. 339-401): Springer US.

Lunn, J., & Buttriss, J. L. (2007). Carbohydrates and dietary fibre. *Nutrition Bulletin*, *32*(1), 21-64.

Lyly, M., Liukkonen, K.-H., Salmenkallio-Marttila, M., Karhunen, L., Poutanen, K., & Lähteenmäki, L. (2009). Fibre in beverages can enhance perceived satiety. *European Journal of Nutrition*, *48*(4), 251-258.

Mai, J., & Lo, G. S. (2004). Soy-Based Nutritional Beverages. In: F. Shahidi, & D. K. Weerasinghe, *Nutraceutical beverages : chemistry, nutrition, and health effects* Washington, DC: American Chemical Society.

Mälkki, Y., & Virtanen, E. (2001). Gastrointestinal Effects of Oat Bran and Oat Gum: A Review. *LWT - Food Science and Technology*, *34*(6), 337-347.

Matia-Merino, L., Lau, K., & Dickinson, E. (2004). Effects of low-methoxyl amidated pectin and ionic calcium on rheology and microstructure of acid-induced sodium caseinate gels. *Food Hydrocolloids*, *18*(2), 271-281.

Mattes, R. D., & Rothacker, D. (2001). Beverage viscosity is inversely related to postprandial hunger in humans. *Physiology & Behavior*, *74*(4-5), 551-557.

Mikkelsen, P. B., Toubro, S., & Astrup, A. (2000). Effect of fat-reduced diets on 24-h energy expenditure: comparisons between animal protein, vegetable protein, and carbohydrate. *The American Journal of Clinical Nutrition*, *72*(5), 1135-1141.

Miri, T. (2011). Viscosity and Oscillatory Rheology. In: I. T. Norton, F. Spyropoulos, & P. Cox, *Practical Food Rheology: An Interpretive Approach*: Wiley-Blackwell.

National Research Council (2005). 11 Macronutrients and Healthful Diets. *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients)*. Washington, DC: The National Academies Press.

Naturex Group (2008). What is Svetol®? Retrieved 1 March 2012, from http://www.svetol.com/.

NHMRC/MOH (2006). Nutrient Reference Values for Australia and New Zealand. Canberra, National Health and Medical Research Council & Wellington, Ministry of Health.

Paddon-Jones, D., Westman, E., Mattes, R. D., Wolfe, R. R., Astrup, A., & Westerterp-Plantenga, M. (2008). Protein, weight management, and satiety. *The American Journal of Clinical Nutrition*, *87*(5), 1558S-1561S.

Parker, M. J. (2009). Appendix J: Analysis of Variance (ANOVA). In: L. Rothman, & M. J. Parker, *Just-about-right (JAR) scales: design, usage, benefits, and risks*, vol. MNL63. Bridgeport, NJ: ASTM International.

Paulsen, P., Welsby, D., & Huang, X. (2005). Ready-To-Drink Soy Protein Nutritional Beverages. *Soy Applications in Food* (pp. 199-226): CRC Press.

Paxman, J. R., Richardson, J. C., Dettmar, P. W., & Corfe, B. M. (2008). Daily ingestion of alginate reduces energy intake in free-living subjects. *Appetite*, *51*(3), 713-719.

Pelkman, C. L., Navia, J. L., Miller, A. E., & Pohle, R. J. (2007). Novel calcium-gelled, alginate-pectin beverage reduced energy intake in nondieting overweight and obese women: interactions with dietary restraint status. *The American Journal of Clinical Nutrition*, *86*(6), 1595-1602.

Peters, H. P., Boers, H. M., Haddeman, E., Melnikov, S. M., & Qvyjt, F. (2009). No effect of added β-glucan or of fructooligosaccharide on appetite or energy intake. *The American Journal of Clinical Nutrition*, *89*(1), 58-63.

Peters, H. P. F., Koppert, R. J., Boers, H. M., Strom, A., Melnikov, S. M., Haddeman, E., Schuring, E. A. H., Mela, D. J., & Wiseman, S. A. (2011). Dose-Dependent Suppression of Hunger by a Specific Alginate in a Low-Viscosity Drink Formulation. *Obesity*, *19*(6), 1171-1176.

Pocker, Y., & Green, E. (1973). Hydrolysis of D-glucono-.delta.-lactone. I. General acid-base catalysis, solvent deuterium isotope effects, and transition state characterization. *Journal of the American Chemical Society*, *95*(1), 113-119.

Poddar, K., Kolge, S., Bezman, L., Mullin, G. E., & Cheskin, L. J. (2011). Nutraceutical Supplements for Weight Loss. *Nutrition in Clinical Practice*, *26*(5), 539-552.

Quercegen Pharma LLC (2010). GRAS notice for high-purity quercetin. Retrieved 7 August 2012, from http://www.accessdata.fda.gov/scripts/fcn/gras_notices/grn341-1.pdf: Quercegen Pharma LLC, USA.

Rao, M. A. (2007a). Flow and Functional Models for Rheological Properties of Fluid Foods. *Rheology of Fluid and Semisolid Foods: Principles and Applications* (pp. 27-58): Springer US.

Rao, M. A. (2007b). Introduction: Food Rheology and Structure. *Rheology of Fluid and Semisolid Foods: Principles and Applications* (pp. 1-26): Springer US.

Rao, M. A. (2007c). Measurement of Flow and Viscoelastic Properties. *Rheology of Fluid and Semisolid Foods: Principles and Applications* (pp. 59-151): Springer US.

Rohn, S., Petzke, K. J., Rawel, H. M., & Kroll, J. (2006). Reactions of chlorogenic acid and quercetin with a soy protein isolate – Influence on the in vivo food protein quality in rats. *Molecular Nutrition & Food Research*, *50*(8), 696-704.

Rothman, L. (2009). Appendix D: The Mean. In: L. Rothman, & M. J. Parker, *Just-about-right (JAR) scales: design, usage, benefits, and risks*, vol. MNL63. Bridgeport, NJ: ASTM International.

Sanaka, M., Yamamoto, T., Anjiki, H., Nagasawa, K., & Kuyama, Y. (2007). Effects of agar and pectin on gastric emptying and post-prandial glycaemic profiles in healthy human volunteers. *Clinical and Experimental Pharmacology and Physiology*, *34*(11), 1151-1155.

Schroeder, N., Gallaher, D. D., Arndt, E. A., & Marquart, L. (2009). Influence of whole grain barley, whole grain wheat, and refined rice-based foods on short-term satiety and energy intake. *Appetite*, *53*(3), 363-369.

Sergent, T., Vanderstraeten, J., Winand, J., Beguin, P., & Schneider, Y.-J. (2012). Phenolic compounds and plant extracts as potential natural anti-obesity substances. *Food Chemistry*, *135*(1), 68-73.

Slavin, J., & Green, H. (2007). Dietary fibre and satiety. *Nutrition Bulletin*, *32*, 32-42.

Smith, A. M., & Miri, T. (2011). Alginates in Foods. In: I. T. Norton, F. Spyropoulos, & P. Cox, *Practical Food Rheology: An Interpretive Approach*: Wiley-Blackwell.

St-Onge, M.-P. (2005). Dietary fats, teas, dairy, and nuts: potential functional foods for weight control? *The American Journal of Clinical Nutrition*, *81*(1), 7-15.

Stevenson, D. G., & Inglett, G. E. (2009). Cereal b-glucans. In: G. O. Phillips, & P. A. Williams, *Handbook of hydrocolloids* (pp. 615-652). Boca Raton: Woodhead Publishing Limited.

Stunkard, A. J., & Messick, S. (1985). The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. *Journal of Psychosomatic Research*, *29*(1), 71-83.

Sutton, K. (2012). Developing foods for appetite control - a combinatorial approach. In: *The NZIFST 2012 Conference*. Hamilton: The New Zealand Institute of Food Science & Technology Inc.

Tan, S. Y., Batterham, M., & Tapsell, L. (2010). Energy Expenditure Does not Differ, but Protein Oxidation Rates Appear Lower in Meals Containing Predominantly Meat versus Soy Sources of Protein. *Obesity Facts*, *3*(2), 101-104.

Thakur, B. R., Singh, R. K., Handa, A. K., & Rao, M. A. (1997). Chemistry and uses of pectin — A review. *Critical Reviews in Food Science and Nutrition*, *37*(1), 47-73.

Tiwary, C. M., Ward, J. A., & Jackson, B. A. (1997). Effect of pectin on satiety in healthy US Army adults. *Journal of the American College of Nutrition*, *16*(5), 423-428.

Tucci, S. A. (2010). Phytochemicals in the Control of Human Appetite and Body Weight. *Pharmaceuticals*, *3*(3), 748-763.

van Strien, T., Frijters, J. E. R., Bergers, G. P. A., & Defares, P. B. (1986). The Dutch Eating Behavior Questionnaire (DEBQ) for assessment of restrained, emotional, and external eating behavior. *International Journal of Eating Disorders*, *5*(2), 295-315.

Vitaglione, P., Lumaga, R. B., Montagnese, C., Messia, M. C., Marconi, E., & Scalfi, L. (2010). Satiating Effect of a Barley Beta-Glucan–Enriched Snack. *Journal of the American College of Nutrition*, *29*(2), 113-121.

Vitaglione, P., Lumaga, R. B., Stanzione, A., Scalfi, L., & Fogliano, V. (2009). β-Glucan-enriched bread reduces energy intake and modifies plasma ghrelin and peptide YY concentrations in the short term. *Appetite*, *53*(3), 338-344.

Vuksan, V., Panahi, S., Lyon, M., Rogovik, A. L., Jenkins, A. L., & Leiter, L. A. (2009). Viscosity of fiber preloads affects food intake in adolescents. *Nutrition, Metabolism and Cardiovascular Diseases*, *19*(7), 498-503.

Wanders, A. J., van den Borne, J. J. G. C., de Graaf, C., Hulshof, T., Jonathan, M. C., Kristensen, M., Mars, M., Schols, H. A., & Feskens, E. J. M. (2011). Effects of dietary fibre on subjective appetite, energy intake and body weight: a systematic review of randomized controlled trials. *Obesity Reviews*, *12*(9), 724-739.

Westerterp-Plantenga, M. S., Nieuwenhuizen, A., Tomé, D., Soenen, S., & Westerterp, K. R. (2009). Dietary Protein, Weight Loss, and Weight Maintenance. *Annual Review of Nutrition*, *29*(1), 21-41.

Zijlstra, N., Mars, M., de Wijk, R. A., Westerterp-Plantenga, M. S., & de Graaf, C. (2007). The effect of viscosity on ad libitum food intake. *Int J Obes*, *32*(4), 676-683.

Appendix I – Advertisement 'Seeking particpants for a satiety measurement trial'

Seeking participants for a satiety measurement trial

- Seeking healthy adults aged between 18 to 60 years, with normal body mass index (BMI) of 18.5 to 30 kg/m^2
- To participate in a trial measuring the satiety induced by various formulated beverages (and the standard breakfast).
- Only using simple line scales to rate your feelings of hunger, fullness, etc. No blood or tissue needed.
- The trial comprises of 6 morning sessions over 4 weeks in October— November 2012, at PFR Palmerston North
- Exclusions: pregnant, smoking, on medication, on a restraint diet and/or have food allergy or intolerance to soy, cereals, nuts and milk.

If you would like to participate or find out more about the study, please contact:

Irene Ho, Masters student
t. 021 208 1939 ✉ irene.ho@plantandfood.co.nz or
Lee Huffman, Project supervisor
t. 06 355 6153 ✉ lee.huffman@plantandfood.co.nz

This study does not require Health and Disability Ethics Committee (HDEC) review.

$$BMI = \frac{\text{Weight (kg)}}{\text{Height (m)} \times \text{Height (m)}}$$

Appendix II – Participant Information Sheet and Consent Form

Participant Information Sheet

Study title: Comparing the satiety effects of various formulated beverages in adults

Locality: Plant and Food Research, Palmerston North

Researchers: Irene Ho (Masters Student)
☏ 06 355 6055, 021 208 1939 ✉ irene.ho@plantandfood.co.nz

Lee Huffman (Science Group Leader)
☏ 06 355 6153 ✉ lee.huffman@plantandfood.co.nz

Lara Matia-Merino (Senior Lecturer)
☏ 06 356 9099 ✉ L.Matia-Merino@massey.ac.nz

About the study:
You have been invited to take part in this study to investigate the level of satiety induced by various formulated beverages. Satiety is the feeling of fullness after a meal that persists, inhibiting further eating and delaying hunger. It will be measured through self-reported measures of appetite i.e. using visual analogue scales (VAS) which allow subjects to rate and record their feelings of hunger, fullness, satiety, desire to eat and prospective food consumption. The aim of the study is to compare the satiety effects of various formulated (model) beverages. Results from the study will provide a better understanding on the formulation of the model beverage, in relation to the use of macro-nutrients and phytochemicals for satiety effects. The model beverage formulation and the satiety measurement protocol can be applied for future *in vivo* testing of other macro-nutrients and phytochemicals, which in turn may translate into information/advice on weight management and lifestyle changes, may contribute to the production of specialized food products, may increase consumer choices for healthier foods, and may contribute towards lowering levels of obesity and its associated health problems such as cardiovascular disease and type 2 diabetes.

In order to prevent biased evaluation, we are unable to disclose to you the macro-nutrients and phytochemicals used in the beverages. However, please be assured that the beverages use only food-grade ingredients, will be UHT-processed and packaged in a food-safe pilot plant, will be microbiologically tested to ensure free from pathogenic microorganisms, and are matched for energy, protein, sugars and fibre contents. A standard breakfast (commercially available muesli and milk) will also be consumed 30 minutes after consuming the beverage. Some food or ingredients may cause allergic reactions with certain groups of people. You are requested not to participate if you may be adversely affected by the following:

- Soybean and its products

- Cereals containing gluten
- Nuts
- Sesame seeds
- Dairy product – milk

Trial information / procedure:

- Subjects for the study need to be healthy male and female (not pregnant) individuals, aged 18–60 years with normal body mass index (BMI) of 18.5–30.0 kg/m^2.
- As it is important for us to consider the confounding factors in satiety studies, you may be excluded from the study if you are smoking, on medication, on a restraint diet and/or have food allergy / intolerance.
- For the study, each subject will consume 6 beverages *i.e.* to attend 6 sessions in the morning, about 4 hours per session. The sessions will be on specific weekdays over 4 weeks (session dates to be informed).
- On those days before each session, you have to fast (no foods and drinks other than plain water) from 10 pm, refrain from alcohol consumption and strenuous physical activity.
- On the days of each session, you will arrive at the PFR by 8.30 am and be seated in a quiet room. You will be asked to complete a baseline appetite rating form with visual analogue scales (VAS) for feelings of hunger, fullness, satisfaction, desire to eat and prospective food consumption.
- At 8.40 am, you will be given the test beverage (250 ml) to consume within 3 minutes and complete a sensory evaluation form to rate its aroma, sweetness, thickness, flavour, aftertaste, colour and overall acceptability. This is followed by completing VAS forms every 15 minutes for the first hour and every 30 minutes thereafter up to 4 hours.
- At 9.15 am, you will consume a standard breakfast (muesli and milk) within 15 minutes and complete further VAS forms up to 4 hours.
- After the first hour, you could return to your workstations to carry out daily work (paper/computer work, light laboratory work) but avoid strenuous physical work, and remembering to complete the VAS forms on time.
- You may consume plain water, max. 100 ml allowed each hour, but only immediately after filling in the VAS forms. Upon completion of the session at 12.45 pm, you can then have your usual lunch.
- Timeline of the trial is shown in Figure 1 below.
- You will have a break of at least 3 days and then return to the trial location to repeat another session under the same conditions.

- Participating in the study (6 sessions) will probably take up about 28 hours of your time. You may have to plan and reschedule your work in a way that would not affect your work or your involvement in the study.

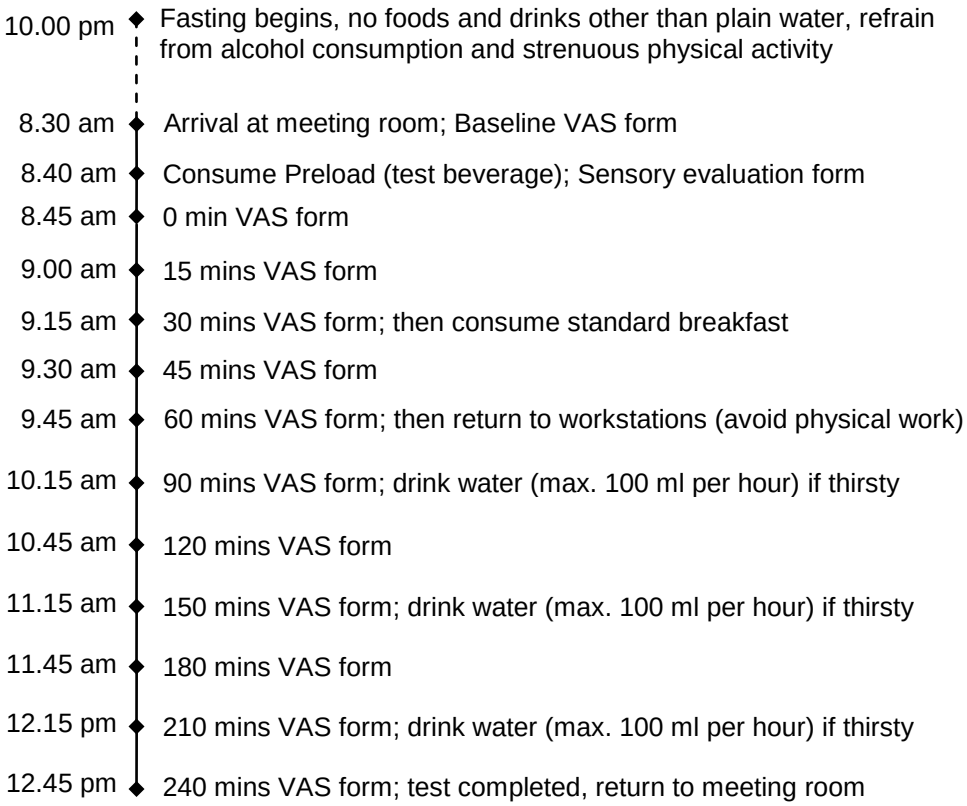


Figure 1. Timeline of satiety measurement trial

Possible risks

It is possible that some subjects may experience gastrointestinal disturbances such as nausea, stomach discomfort, abdominal bloating and flatulence, and general bodily symptoms such as headache, dizziness and fatigue. If you find any of these a problem or have other discomfort, please inform the researcher and you can stop your participation at any time during the trial.

Compensation

If you were injured in this study, which is unlikely, you would be eligible for compensation from ACC just as you would be if you were injured in an accident at work or at home. If you private health or life insurance, you may wish to check with your insurer that taking part in this study will not affect your cover.

Confidentiality

All documents (personal information, questionnaire, forms) will be filed in a folder marked ‘Confidential’ and kept in a locked cabinet at PFR by the coordinating researcher. It will be kept for a period of 10 years and eventually destroyed by a data

destruction company. Computer files containing health information or data will be password-protected and access is strictly by the researchers only. Results of the study will be published in internal report / Masters Thesis that do not identify individual participants; only pooled or group data will be reported.

Rights

This study does not require the Health and Disability Ethics Committee (HDEC) review. Nevertheless, the researchers have used HDEC Online Form to think through the ethical issues involved in the study. If you have any queries or concerns about your rights as a participant in this study, you can contact an independent health and disability advocate on:

☎ 0800 555 050
🖷 0800 2 SUPPORT (0800 2787 7678)
✉ advocacy@hdc.org.nz

If you have any questions, concerns or complaints about the study at any stage, please do not hesitate to contact:

Irene Ho ☎ 06 355 6055, 021 208 1939 ✉ irene.ho@plantandfood.co.nz or
Lee Huffman ☎ 06 355 6153 ✉ lee.huffman@plantandfood.co.nz

The researchers appreciate and thanks to all participants who voluntarily participate in this study.

Thank you very much!

Consent Form

Study title: Comparing the satiety effects of various formulated beverages in adults
Location: Plant and Food Research, Palmerston North
Date: October – November 2012

Declaration by participant:

	Please tick
I have read and understood the Participant Information Sheet. I have had the opportunity to ask questions and I am satisfied with the answers I have received. I understand that I may ask further questions at any time.	☐
I agree to voluntarily participate in this study.	☐
I understand I have the right to withdraw from the study at any time and to decline to answer any particular questions.	☐
I prefer to be named in the acknowledgement section of the researcher's report or thesis. or I prefer not to be named in the acknowledgement section of the researcher's report or thesis.	☐ or ☐
I have been given a copy of the Participant Information Sheet and Consent Form to keep.	☐

Participant's name: _____

Signature: _____ Date: _____

Declaration by member of research team:

I have given a verbal explanation of the research project to the participant, and have answered the participant's questions about it.	☐
I believe that the participant understands the study and has given informed consent to participate.	☐

Researcher's name: _____

Signature: _____ Date: _____

CONFIDENTIAL

Participant's personal information

Name :

Phone no. :

Email address :

Gender :

Age :

As measured by member of the research team:

Height :

Weight :

Body mass index:

Date :

Notes or comments for the research team (if any):

Appendix III – Appetite Rating Form

Appetite Rating Form

Name:

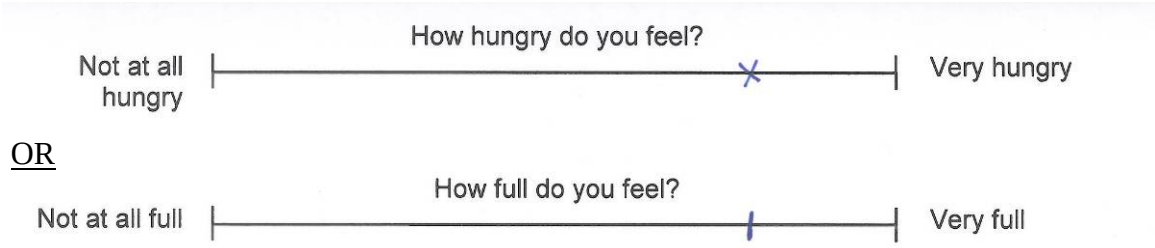
Participant ID:

Session:

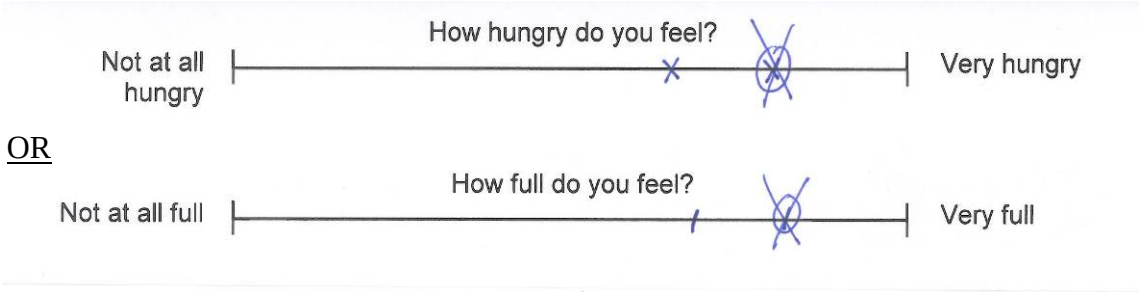
Beverage code:

Instructions

1. Please rate your feelings of appetite in response to the following questions and mark along the line scale with either a cross or a slash, for example:



2. If you want to change your rating, circle and cross out the previous rating, for example:



3. Complete the forms every 15 minutes for the first hour, and every 30 minutes for the next 3 hours.

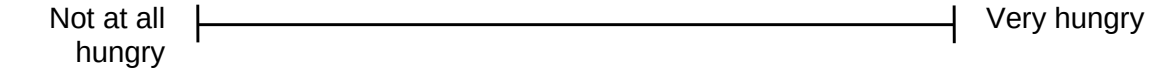
4. A comment section can be found on the last page; please let us know if you have any comments or felt any discomfort during the session.

Please turn over to the next page…

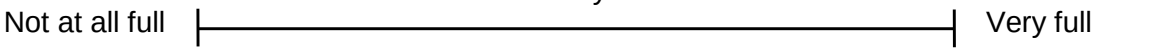
Participant ID: _____

Time interval: Baseline

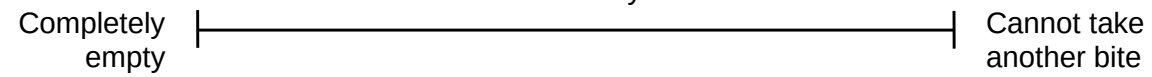
How hungry do you feel?



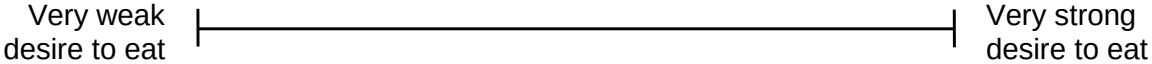
How full do you feel?



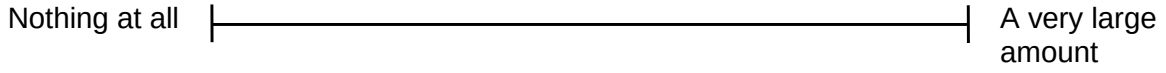
How satisfied do you feel?



How strong is your desire to eat?



How much do you think you can eat right now?



The Preload (test beverage) will be served shortly…

Please consume the beverage and complete the **Sensory Evaluation Form**…

Please turn over to the next page…

Participant ID: _____

Time interval: 0 minute; 8.45 am or the actual time is: _____

How hungry do you feel?

Not at all hungry — Very hungry

How full do you feel?

Not at all full — Very full

How satisfied do you feel?

Completely empty — Cannot take another bite

How strong is your desire to eat?

Very weak desire to eat — Very strong desire to eat

How much do you think you can eat right now?

Nothing at all — A very large amount

Please turn over to the next page…

Participant ID: _____

Time interval: 15 minutes; 9.00 am or the actual time is: _____

How hungry do you feel?

Not at all hungry — Very hungry

How full do you feel?

Not at all full — Very full

How satisfied do you feel?

Completely empty — Cannot take another bite

How strong is your desire to eat?

Very weak desire to eat — Very strong desire to eat

How much do you think you can eat right now?

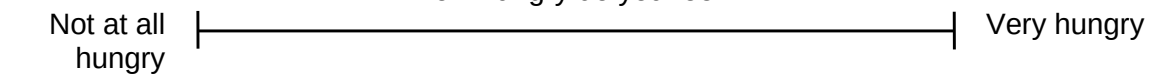
Nothing at all — A very large amount

Please turn over to the next page…

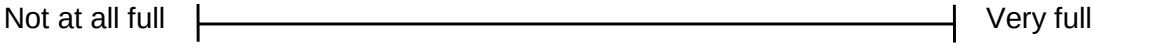
Participant ID: _____

Time interval: 30 minutes; 9.15 am or the actual time is: _____

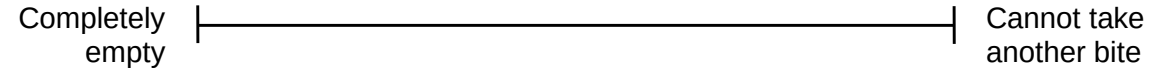
How hungry do you feel?



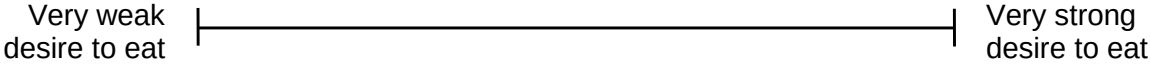
How full do you feel?



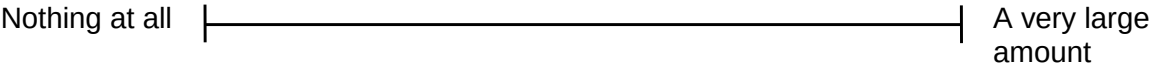
How satisfied do you feel?



How strong is your desire to eat?



How much do you think you can eat right now?



A breakfast (muesli + milk) will be served, please consume it within 15 minutes.

Please turn over to the next page…

Participant ID: _____

Time interval: 45 minutes; 9.30 am or the actual time is: _____

How hungry do you feel?

Not at all hungry — Very hungry

How full do you feel?

Not at all full — Very full

How satisfied do you feel?

Completely empty — Cannot take another bite

How strong is your desire to eat?

Very weak desire to eat — Very strong desire to eat

How much do you think you can eat right now?

Nothing at all — A very large amount

Please turn over to the next page…

Participant ID: _____

Time interval: 1 hour; 9.45 am or the actual time is: _____

How hungry do you feel?

Not at all hungry — Very hungry

How full do you feel?

Not at all full — Very full

How satisfied do you feel?

Completely empty — Cannot take another bite

How strong is your desire to eat?

Very weak desire to eat — Very strong desire to eat

How much do you think you can eat right now?

Nothing at all — A very large amount

Please turn over to the next page…

Participant ID: _____

Time interval: 1 hour 30 minutes; 10.15 am or the actual time is: _____

How hungry do you feel?

Not at all hungry — Very hungry

How full do you feel?

Not at all full — Very full

How satisfied do you feel?

Completely empty — Cannot take another bite

How strong is your desire to eat?

Very weak desire to eat — Very strong desire to eat

How much do you think you can eat right now?

Nothing at all — A very large amount

Please turn over to the next page…

Participant ID: _____

Time interval: 2 hours; 10.45 am or the actual time is: _____

How hungry do you feel?

Not at all hungry — Very hungry

How full do you feel?

Not at all full — Very full

How satisfied do you feel?

Completely empty — Cannot take another bite

How strong is your desire to eat?

Very weak desire to eat — Very strong desire to eat

How much do you think you can eat right now?

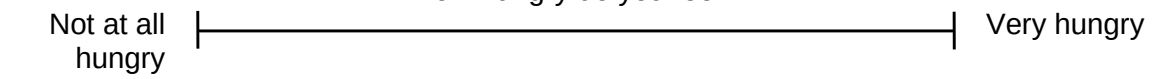
Nothing at all — A very large amount

Please turn over to the next page…

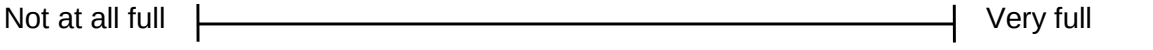
Participant ID: _____

Time interval: 2 hours 30 minutes; 11.15 am or the actual time is: _____

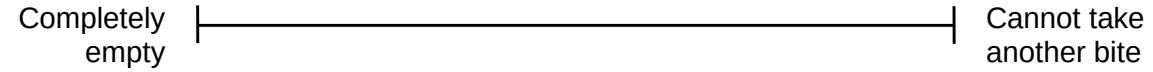
How hungry do you feel?



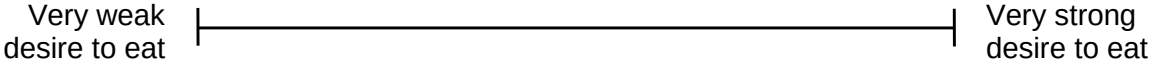
How full do you feel?



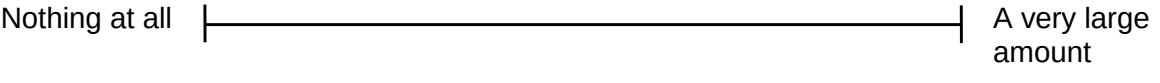
How satisfied do you feel?



How strong is your desire to eat?



How much do you think you can eat right now?



Please turn over to the next page…

Participant ID: _____

Time interval: 3 hours; 11.45 am or the actual time is: _____

How hungry do you feel?

Not at all hungry — Very hungry

How full do you feel?

Not at all full — Very full

How satisfied do you feel?

Completely empty — Cannot take another bite

How strong is your desire to eat?

Very weak desire to eat — Very strong desire to eat

How much do you think you can eat right now?

Nothing at all — A very large amount

Please turn over to the next page…

Participant ID: _____

Time interval: 3 hours 30 minutes; 12.15 pm or the actual time is: _____

How hungry do you feel?

Not at all hungry ——— Very hungry

How full do you feel?

Not at all full ——— Very full

How satisfied do you feel?

Completely empty ——— Cannot take another bite

How strong is your desire to eat?

Very weak desire to eat ——— Very strong desire to eat

How much do you think you can eat right now?

Nothing at all ——— A very large amount

Please turn over to the next page…

Participant ID: _____

Time interval: 4 hours; 12.45 pm or the actual time is: _____

How hungry do you feel?

Not at all hungry — Very hungry

How full do you feel?

Not at all full — Very full

How satisfied do you feel?

Completely empty — Cannot take another bite

How strong is your desire to eat?

Very weak desire to eat — Very strong desire to eat

How much do you think you can eat right now?

Nothing at all — A very large amount

Comments (if any):

_____

_____

_____

Please return to the meeting room with your forms.

Thank you very much!

Appendix IV – Sensory Evaluation Form

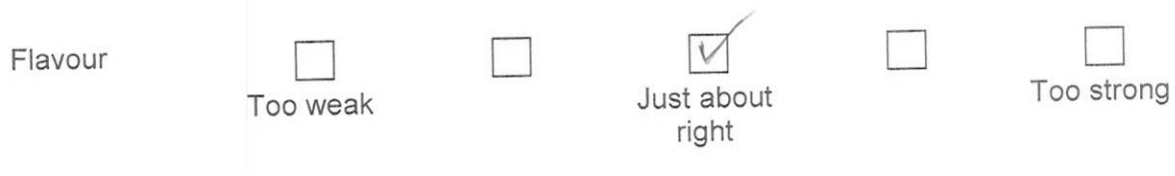
Sensory Evaluation Form

Instructions

5. Please consume the beverage all at once.

6. Indicate your rating for the following attributes with a tick in one of the boxes, for example:

Correct



7. Please don't tick anywhere outside the given boxes, for example:

Wrong

8. Write your comments (if any).

Please turn over to the next page…

Name: _____

ID: _____

Session: _____

Beverage code: _____

Aroma	☐ Too weak	☐	☐ Just about right	☐	☐ Too strong
Sweetness	☐ Not sweet enough	☐	☐ Just about right	☐	☐ Too sweet
Flavour	☐ Too weak	☐	☐ Just about right	☐	☐ Too strong
Thickness	☐ Too thin	☐	☐ Just about right	☐	☐ Too thick
Aftertaste	☐ None	☐ Slight	☐ Moderate	☐ Strong	☐ Very strong
Colour	☐ Too light	☐	☐ Just about right	☐	☐ Too dark
Overall acceptability	☐ Dislike very much	☐ Dislike	☐ Neither	☐ Like	☐ Like very much

Comments (if any):

_____

_____

_____