Improvement of physical manageability of refrigerated methylcellulose-containing vegetarian meat substitutes

Master thesis

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Abstract

The following work investigates the possibility of improving the structure of methylcellulose-containing vegan meat substitutes in terms of susceptibility to physical stress during handling at refrigerator and room temperatures. Attempts to combine the hot-setting gelling agent methylcellulose with another cold-setting hydrocolloid or starch were performed in order to create a structure that is rigid and manageable at refrigerator temperatures as well as when heated for consumption.

A total of 23 types of cold-setting hydrocolloids, mixtures thereof and starch alternatives were identified and ordered. Their cold gelling ability was thoroughly examined in a pure water solution followed by a methylcellulose solution. Trials to incorporate the cold-setting gelling agent into a basic vegetarian recipe were performed and finally evaluated using a texture analyzer. The measurements proved the existence of a significant improvement in cold structure for several different combinations of methylcellulose and hydrocolloids or starches with regards to susceptibility to physical stress. The improvement was most notable with the use of kappa-carrageenan, mixtures of kappa-carrageenan and locust bean gum, as well as a modified thin-boiling maize starch.

Foreword

The following master thesis is a completion to the Master of Science in engineering, biotechnology program at Lund University, Sweden. The work was carried out and compiled in Malmö and Lund during the first half of 2015. It was done in corporation with the Swedish vegan food manufacturer Anamma Foods and focuses on the improvement of the cold texture of their product range of shaped products with regards to susceptibility to physical stress.

I would like to acknowledge Mårten Hansson, Magnus Hansson and Christer Rydberg at Anamma Foods for the possibility of taking this project on, as well as for their helpfulness and good tutoring during the entire time of the research. I would also like to thank my supervisor Björn Bergenståhl at the department of food technology, engineering and nutrition at Lund University for excellent advisory and valuable insights. Last I thank Lars Nilsson, also at the department of food technology, engineering and nutrition at Lund University, for agreeing to serve as examiner of this master thesis.

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Introduction

Over the last few years the Swedish market for vegan and vegetarian food has increased a great deal. According to a recent study made in 2014 by one of the largest vegetarian food brands in Sweden, Hälsans Kök, 6 out of 10 Swedes wish to eat more vegetarian food [1]. The study claims that the reason for the increasing interest is people's desire to vary their diet to a further extent, as well as the health and environmental issues people find related to vegetarian food.

Vegetarian food is a hyponym for several types of diets, the most common being vegan, ovo-vegetarian, lacto-vegetarian and ovo-lacto-vegetarian. Vegan food is defined as foods being based entirely on plants. A vegan diet excludes all animal-derived ingredients such as all kinds of meat, fish and seafood as well as dairy, egg and honey [2]. Ovo-vegetarian food includes egg, while lacto-vegetarian food includes dairy. The combination of the two, ovo-lacto-vegetarian food, includes both egg and dairy.

The following research has been done in corporation with the Swedish vegan food manufacturer Anamma Foods. The company is a based in Malmö, with two close production facilities in Simrishamn and Vadensjö. Their product line consists solely of vegan products, most of which are soy protein based meat substitutes such as burgers, sausages and mince. Currently the products are exclusively sold as frozen food in the freezing counter of common grocery stores and are available in Sweden, Denmark, Norway and Finland. In 2014 the company had a sales number at 44 million SEK, making Anamma the largest manufacturer of vegan meat substitutes in Sweden.

With the product line being entirely vegan, Anamma has excluded the use of egg and egg white powder in their recipes, as opposed to many of their competitors which market their products as ovo-vegetarian. Excluding egg from shaped products such as burgers and sausages creates a great challenge when it comes to texture and binding of the products. Egg provides excellent irreversible structural characteristics to a product when heated, a feature that is difficult to mimic. Anamma have instead of egg made use of plant based alternatives, mainly the hydrocolloid methylcellulose, which provides similar function in their products when heated.

With the expanding vegetarian market Anamma Foods is now looking into the possibility of introducing a new line of refrigerated products. This puts new demands on the shaped products, requiring them to be firm and manageable at refrigerator and room temperatures. Current products retain their shape by the frozen state in which they are sold while methylcellulose provides structure and texture when the products are heated for consumption. The structure created by methylcellulose is however thermoreversible, meaning that it melts once the temperature of the product is lowered to intermediate temperatures such as in the refrigerator. The result is that at these temperatures products are perceived as soft, doughy and difficult to manage without the products falling apart. With Anamma's future goal of producing products which are to be sold refrigerated, these parameters first need to be fixed and the cold texture of their shaped products significantly improved.

Aim

The following research aims at improving the structure of Anamma Foods' shaped products in terms of susceptibility to physical stress during handling at refrigerator and room temperatures. Upon the introduction of a new line of refrigerated products, the company want cold products that are less fragile than existing products, and have good binding properties and manageability. Since Anamma is a completely vegan label, animal derived ingredients are precluded. A potential solution should be possible to incorporate into the existing production chain without too many alterations needed. There is also the economical aspect which requires that possible new ingredients will not increase the production price considerably.

To more precisely elaborate the problem and possible solutions, some fundamentals must first be outlined such as how to define a gel, the definition of hydrocolloids, the sol-gel transition, as well as the function of methylcellulose.

Background

Food texture is a sensory property which is perceived by humans through several of our senses, the most predominant being touch and feel. Textural properties of a product are often an important sign of food quality to consumers, and are therefore a key characteristic of the overall product [3, p. 260]. Textural preferences are subjective though, and highly dependent on the taster's own experiences and opinion [4, p. 725]. This of course makes it difficult to establish a universal concept of how the sensory properties of a product should be formulated in order to achieve consumer acceptance, and this is an important thing to remember during elaborations on food sensory characteristics.

This investigation focuses on the cold structure of Anamma's products. The products are not primarily intended for consumption during these temperatures but are to be cooked. What Anamma Foods wants to achieve however is products that are manageable enough for consumers to take out of the package and prepare for cooking in whatever way they want, without breakage of the structure of the product.

Food gels provide structure to numerous types of foods throughout the world. They contribute to and/or enhance the textural properties of for instance jam, jelly, confectionery products, yoghurt, cream cheese and ice cream, but also meat, poultry and, most vital for this research, vegetarian meat analogues [5] [6, p. 4].

There are several propositions on how to define a gel [7]. IUPAC, the International Union of Pure and Applied Chemistry, define a gel as a "non-fluid colloidal network or polymer network that is expanded throughout its whole volume by a fluid" [8]. A gel thus possesses a solid-like behaviour, meaning that it will not flow when subjected to stress. However gels have a finite yield stress, meaning that they will rupture when enough stress is applied, called yield point [9, p. 263]. Gels also differ in elasticity, which is the gels ability to return to its original shape after deformation, which only happens below its yield point. Finally gels may also vary in hardness or strength, comparing for instance jelly pudding to a hard boiled egg. A soft gel needs less force to deform or break than a harder gel.

Being solids is one of the two main characteristics of a gel. The second characteristic, also implicated by the IUPAC definition, is that gels are mixtures of at least two components where not all of them contribute to the solid-like behaviour [10, p. 159]. This implies that a solvent, most commonly water, is entrapped within the network formed by for instance a gelling polymer and the resulting structure is solid.

One major group of molecules which are able to form food gels are hydrocolloids. These are hydrophilic long chain polymers with a large number of hydroxyl groups which promote their affinity for water. They form colloidal solutions in water, hence their name [11]. They are extracted from for instance seeds, plants, algae and microbes and are most often derived in granular form. In food they have two main rheological functions; as thickening agents and as gelling agents. Not all hydrocolloids form gels, however all hydrocolloids have in common that they increase viscosity to some extent, though some more effectively than others [11].

Gel formation in a hydrocolloid solution is induced by one or several internal changes in the system that promotes the transformation from a liquid solution or suspension into a solid

[10, pp. 161-162]. The changes might be for instance a change in temperature, lowering of the pH, or introduction of cations. This triggers association of the polymer chains throughout the entire system, and a gel is formed. The term sol-gel transition is commonly used to describe the transition from a solution (sol) to a gel.

Gels formed by hydrocolloids belong to the class of physical gels, in the sense that the structure is created by polymer networks formed by physical aggregation [7]. These are made up of interactions such as hydrogen bonds, hydrophobic association, van der Waals forces and cation-mediated crosslinks [6]. The type of interaction varies depending on the hydrocolloid studied and its mechanism of gelation. Because of the low bond energy of these interactions, several adjacent bonds are needed in order to create a structure which is mechanically strong enough to create a gel. Such adjacent bonds together form what is called a junction zone, creating a much stronger attraction between polymers than would single non-adjacent bonds [10, pp. 162-163]. This is illustrated in figure 1.

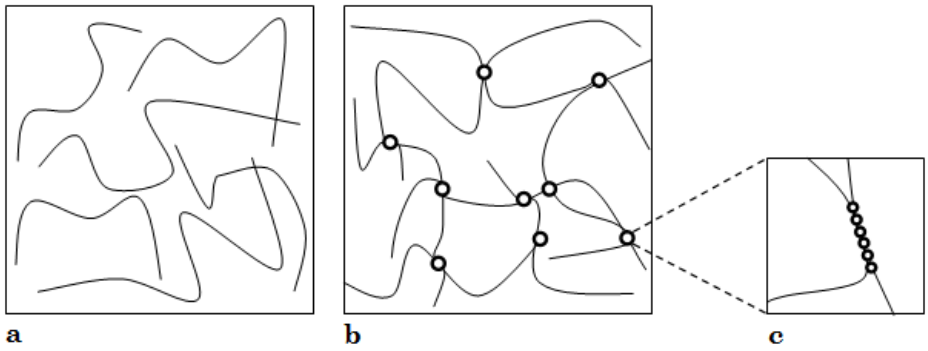

Figure 1: a) The dispersed non-gelled polymers with no bonds between the molecules. b) Gel formation due to the formation of junction zones between the polymers, entrapping the solvent in the network of the solutes. c) Enlargement of a junction zone. Inspired by [10, p. 163].

Most physical gels are thermoreversible as a result of the multiple weak interactions that build up the structural network. The junction zones can be disrupted by for instance temperature changes, with the destruction of the built up network as a result, the gel thus melts. When the temperature is restored however, the gel is rebuilt [10, p. 162]. Many hydrocolloids and starches will not dissolve until a dispersion of the hydrocolloid has been heated, and a gel will thus form upon cooling. These are called cold-setting gels. The more rare opposites, which dissolve under colder conditions and set upon heating, are called heat-setting gels.

The cellulose derivate methylcellulose is an example of a hydrocolloid which is able to form such heat-setting gels. As previously mentioned methylcellulose is the main ingredient responsible for texture and binding in Anamma's products. Cellulose in its native form has a structure made up entirely of anhydroglucose units as presented in figure 2. The absence of branching together with the large content of hydroxyl groups result in a highly crystalline structure caused by extensive hydrogen bonding between and within the polymers. Native cellulose is thus insoluble in water [12].

Each anhydroglucose unit of the cellulose polymer contains three functional hydroxyl groups, one or several of which may be substituted through alkali treatment.

Methylcellulose is formed when a methyl group is used as substituent. The introduction of methyl groups into the cellulose chains promotes the solubility of methylcellulose in cold water because of steric hindrance. The earlier tight structure of native cellulose is loosened up, giving rise to hydrogen bonds between the non-substituted hydroxyl groups on the cellulose backbone and water [13, p. 63].

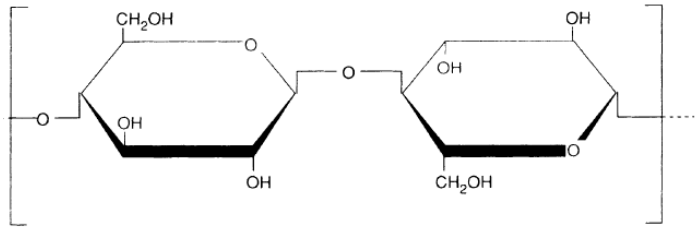

Figure 2: The structure of cellulose. Each ring structure makes up one anhydroglucose unit, which is connected to the following unit through a β-1,4-glycosidic linkage. The number of repeating units depicts the degree of polymerization. Picture from [14, p. 712].

There are basically two criteria which determine the characteristics of the methylcellulose polymer; the degree of substitution (ds), and the degree of polymerisation (dp) [15, p. 712]. The degree of substitution refers to the average number of hydroxyl groups substituted per anhydroglucose molecule, thus having a theoretical maximum of 3.0 [12]. The degree of polymerisation refers to the average chain length of the polymers, which is commonly described as the molecular weight of the polymer chains or the number of repeating anhydroglucose units. Accordingly numerous different types of methylcellulose with different properties can be achieved, depending on ds and dp. The viscosity of a methylcellulose solution for instance is highly dependent on dp, besides actual concentration, and can range from only a few cps to several thousand cps [15, p. 712].

The mechanism by which methylcellulose gelation is achieved is still somewhat unclear, and different proposals have been reported. One common theory is that when in solution, hydrophobic methyl groups along the methylcellulose polymers are surrounded by cage-like structures of water molecules [16]. With increasing temperature, the cage structure is disrupted and the polymers gradually lose their water of hydration. At the gelation point, association between polymers occurs due to extensive hydrophobic associations between exposed hydrophobic segments [17]. The hydrophobic associations are highly favoured by elevated temperatures and strong gels are able to form [4, p. 73].

Another theory suggests that gelation of methylcellulose solution resembles that of globular proteins [18]. This would imply that there exist small regions of unsubstituted cellulose units, along with longer highly substituted segments. When in liquid state, small stretches of unsubstituted cellulose units are bundled together, while highly substituted regions may associate in hydrophobic clusters. Heating causes structural rearrangements which allow association of hydrophobic segments on different strands, building up the gel network. The system is completely reversible however, thus when the temperature is restored the system goes back into solution.

In practice, this is the crucial point that distinguishes gels made using methylcellulose from gels made using egg protein. Once an egg protein gel has set, it will not revert to its liquid

state despite changes in temperature. The reason is that gels created using egg protein have, in addition to the weak interactions described earlier, also covalent linkages which are much stronger bonds [10, p. 162]. These types of bonds are not as readily affected by temperature changes and the result is thermoirreversible gels. Methylcellulose gels on the other hand are completely thermoreversible, and will go back into a viscous liquid solution upon cooling.

Approach

Based on the fact that at low temperatures, methylcellulose dissolved in water creates a viscous solution, it might be possible to introduce a second hydrocolloid or starch which gels upon cooling. The non-gelled methylcellulose might then exist dissolved in the continuous phase inside the voids of the structural network created by the aggregated cold-setting gelling agent. This requires a functioning coexistence of methylcellulose and (at least) one other cold-setting hydrocolloid or starch in the same system. The resulting mixed system would then show a solid-like behaviour at both high and low temperatures, making Anamma's products manageable throughout the entire temperature range.

Reviews on combinations of methylcellulose or similar heat-setting gelling agents and one or several cold-setting gelling agents are scarce. A thorough practical investigation will therefore be performed in order to assess whether it is possible to create a methylcellulose mixed gel in practice. The first stage is to find and identify cold-setting hydrocolloids and starches that theoretically could work in this combination in order to make a first selection.

About 30 different hydrocolloids are available for commercial use in food in the world today. The major part of them and their origins are presented in table 1 below [6, p. 2].

Table 1: Commercially available hydrocolloids for food use and their origins

Botanical	**Microbial**
Gum arabic	Xanthan gum
Karaya gum	Curdlan
Gum ghatti	Dextran
Tragacanth gum	Gellan gum
Pectin	**Animal**
Guar gum	Gelatin
Locust bean gum	Caseinate
Tara gum	Chitosan
Tamarind gum	Whey protein
Cassia gum	Egg protein
Xyloglucan	**Cellulosics**
Konjac mannan	Methyl cellulose
Algal	Hydroxypropyl cellulose
Agar	Hydroxypropyl methyl cellulose
Carrageenan	Methyl ethyl cellulose
Alginate	Carboxymethyl cellulose

Out of these, only a minor part is able to form gels. Hydrocolloids from animal sources are not an alternative, others (for instance curdlan and xyloglucan) are not permitted for food use in the European Union. Meanwhile, some hydrocolloids show synergistic effects when combined which can result in what is called mixed gels. A classic example is the combination of xanthan gum and locust bean gum [19]. When combining the two, a strong synergy is shown and firm gels are able to form, despite the fact that none of the hydrocolloids are particularly good gelling agents when used on their own. To date, the mechanism behind the interaction between the two hydrocolloids is still not fully understood, despite the fact that the phenomenon has been known for decades [20].

Following an extensive literature study, theoretically suitable cold-setting gelling hydrocolloids and known mixed gelling hydrocolloids are summarized in table 2.

Table 2: Cold-setting gelling hydrocolloids and mixed gelling hydrocolloids theoretically suitable for the application

Hydrocolloid	Basic mechanism of gelation
Agar	Mixture of agarose and agaropectin. Agarose is the component responsible for gelation. Gel formation upon cooling is a result of formation of double helices which aggregate solely through formation of hydrogen bonds. Gels have a high hysteresis [21, p. 92].
Kappa-carrageenan	Gelation upon cooling is induced by a coil-helix transition and aggregation between helices through physical cross-links promoted by the binding of potassium ions. Exhibits some hysteresis [22] [15, pp. 173-174].
Alginate	Irreversible gels form independent of temperature. Gelation is induced by the introduction of polyvalent cations, most noticeably calcium which cross-links molecules. The addition of calcium has to be controlled in order to form homogenous gels [23, pp. 817-818].
Low acyl gellan gum	Gels are formed upon cooling in the presence of salts. Exact mechanism is under debate but general consensus is that gelation is a result of formation of helices with subsequent aggregation promoted by cations which lower electrostatic repulsion between chains [24] [25, p. 209].
Low methoxyl pectin	Gels formed through interaction between pectin chains and calcium ions. Control of the availability of calcium is needed by the use of sequestrants [26, p. 280].
Kappa-carrageenan/konjac mannan	Proposed mechanism is intermolecular binding between carrageenan helices and the backbone of the konjac mannan chains or possibly solely the unsubstituted regions of the chains [27]. Ratios vary between 30/70 and 70/30, with increasing elasticity with increasing konjac mannan content.
Kappa-carrageenan/locust bean gum	Mechanism and ratios similar to that of konjac mannan [27]. LBG needs higher temperatures than carrageenan alone to dissolve completely and gel upon gelation [28, p. 243].
Xanthan gum/locust bean gum	Mechanism of gelation is under debate [20]. Ratios vary between 40/60 and 60/40 according to manufacturer recommendations.

Hysteresis is the difference in temperature needed for setting or melting of the gel [8]. In the case of agar for instance, which have a very high hysteresis, the gel can set around 38 °C, but will not melt again until heated to a temperature of around 85 °C [21].

Carrageenan is also available in a form called Processed *Eucheuma* seaweed (abbreviated PES), which can be described as a type of semi-refined carrageenan. PES contains the same gelling polymer, with the main difference being the way in which the powder is extracted from the raw material [15, pp. 167-168]. Extraction of PES is cheaper, but as a result the product contains more residues than refined carrageenan. Residues consist of acid insoluble material, mainly cellulose. In practice, the main difference is that gels created by PES are more opaque and may give a slight flavour taint. It is also possible to experience small differences in gel characteristics compared to refined carrageenan, why both types will be examined in the following experiments. In the USA, no distinction is made between carrageenan and PES, in the European Union however carrageenan is known as E407, while PES is denoted E407a.

Another segment of potential cold-setting gelling agents are starches. Starch is a carbohydrate consisting of the same anhydroglucose units that build up cellulose [29, p. 110]. The crucial point that differentiates starch from cellulose is the stereochemistry of the glycosidic bonds between each anhydroglucose unit, in starch being denoted α and in cellulose β. Starch originates from plants where it serves as energy storage present in the form of granules. Starch consists of two basic polymers; amylose which is essentially linear, and highly branched amylopectin. The ratio between the two is highly dependent on the origin of the starch.

Production of cold-setting gels using starch is initiated by heating starch dispersions in water. Heating causes the granules to absorb water, swell and eventually break causing leakage of amylose into the surrounding solution. Both amylose and amylopectin are able to form network structures upon cooling as a result of extensive hydrogen bonds between polymers [30] [31]. This causes an increase in crystallinity and eventual gel formation, the phenomenon is known as retrogradation. Amylose tends to retrograde and set considerably faster than amylopectin, which takes longer time to crystallize, possibly due to the much higher presence of branching [31]. As a consequence, it often takes time before final gel strength is achieved.

Although starch has several useful properties already in its native form, it is often modified in numerous ways in order to alter its properties in a number of different ways. Interesting modifications for this application include for instance acid thinned or thin boiling starches, which has been hydrolyzed through acid treatment. This results in partial breakdown of the starch granules and increased ratio of smaller linear molecules. This promotes the formation of strong gels after heat treatment compared to native starches [29, pp. 115-119].

The large diversity among starches, due to differences in origin as well as type of modification, makes it hard to assess all the possible alternatives available. Therefore distributors were consulted in order to find potential solutions within the large range of commercially available starches.

A total of 15 different manufacturers and distributors were contacted for ordering of samples. The reason for the research was presented to each manufacturer along with the first selection of hydrocolloids that had been made. Following a discussion about possible

solutions hydrocolloid samples were then chosen in accordance with our joint proposal. Starch samples were chosen in accordance with manufacturer recommendations.

After consulting several manufacturers, it was concluded that gellan gum was not going to be included in the trials. According to manufacturer specifications, solubility of gellan gum is highly dependent on ion concentration, in particular calcium. With calcium concentrations as low as 0.01% the temperature must be raised to near 100 °C for complete dissolution, with even greater temperatures needed for higher calcium concentrations. Further there is a need for sequestrants for controlling the gelling behaviour and a thorough experimental plan must be developed in order to find a suitable optimum between ions and gel strength. Tests involving gellan gum thus comes with a great amount of workload, and it was concluded that the time is better spent on other, more easily manageable, hydrocolloids.

For similar reasons, pectin was also excluded from the experimental plan. Sequestrants may be needed in combination with pectin, and set and melting point is dependent on calcium level as well as pH and solid content. Preferably the outcome of this research should be versatile and usable in several different recipes. Pectin gels though may need adjustments each time an ingredient is changed, removed or added.

Since samples are exclusively delivered in powder form, the first requirement for gel formation using any of the hydrocolloids or starches is proper dispersion and dissolution of the solutes in the solvent. In this research, when possible, this was achieved by dry-blending, which involves mixing the powder of the hydrocolloid or starch with other dry ingredients such as spices, salt or protein isolates. Dry-blending separates the granules of hydrocolloids or starches to allow thorough wetting of particles.

Besides the criterion of proper dispersion, a minimum concentration unique for each hydrocolloid and starch exists below which gel formation will not take place [10, pp. 160-161]. The reason for this is that the concentration of the polymer needs to be high enough so that it spans the entire medium and is able to interact and form branching points between polymers, resulting in a gel network. The increase in viscosity that all hydrocolloids have in common is a result of no or only partial aggregation between polymers.

The theoretical minimum concentration needed for gel formation for a specific molecule differs between each type of hydrocolloid and starch. Generally higher concentrations are needed for starch samples than for hydrocolloids to induce gel formation. Increasing concentrations within one type of hydrocolloid or starch often result in higher gel strengths.

In order to introduce a new cold gelling compound into Anamma's existing recipes every sample's own gelling capacity will first be examined in pure water, without the influence of other factors such as the methylcellulose, salts and oil. Once that is established, a simple system consisting of only the new gelling compound, methylcellulose and water will be studied. If the new gelling compound proves to improve the cold gel strength of this system, it will be tested in a simple shaped vegetarian burger recipe. Finally, the gel strength of the different samples will be measured using a texture analyzer in order to receive quantitative data for the final discussion.

Materials and methods

The experimental part of this research was carried out in Anamma Foods' research kitchen in Malmö. A total of 23 samples were received for evaluation; 10 different samples of hydrocolloids and 13 samples of different starches. The samples were all received in granular form. Actual manufacturer names and real names of the products will be coded in this research according to table 3 and 4 below.

Table 3: Coded names and type of hydrocolloids used in the experiments

Name	Content
C1	Kappa-carrageenan with potassium chloride
C2	Processed Eucheuma Seaweed (PES) with potassium chloride
C3	Processed Eucheuma Seaweed (PES)
Al1	Na-Alginate, disodium pyrophosphate, calcium sulphate
Al2	Na-Alginate, tetrasodium diphosphate, disodium phosphate, calcium sulphate
Ag	Agar agar obtained from marine algae of the family *Gelidiaceae*
C/K	Mixture of PES/konjac mannan with potassium chloride
X/LBG	Mixture of xanthan gum/locust bean gum (LBG)
C/LBG1	Mixture of PES/locust bean gum (LBG) with potassium chloride
C/LBG2	Mixture of kappa-carrageenan/locust bean gum (LBG) with potassium chloride

Table 4: Coded names and types of starches used in the experiments

Coded name	Type of starch
S1	Thin-boiling maize starch
S2	Thin-boiling maize starch
S3	Thin-boiling maize starch
S4	Modified sago starch
S5	Speciality maltodextrin derived from waxy maize
S6	Native potato starch
S7	Native maize starch
S8	Speciality starch derived from tapioca
S9	Native pea starch with high amylose content
S10	Thin-boiling maize starch
S11	Native waxy maize starch
S12	Acetylated di-starch adipate
S13	Acetylated di-starch adipate based on waxy maize

The sample C/LBG2 was pre-blended from the manufacturer, the rest of the mixtures were prepared manually. An initial assessment of different ratios and the resulting gels in water was done within the recommended ratio intervals. X/LBG ratio was determined to 50/50 while C/K and C/LBG1 ratios were determined to 60/40 for a suitable balance between elasticity and gel strength. These ratios were used throughout all trials.

The experimental work was divided into four basic stages as below.

Stage 1: Study on the cold gelling capacity of the hydrocolloids/starches in water on their own.

Stage 2: Study on the cold gelling capacity of the hydrocolloids/starches in a methylcellulose solution.

Stage 3: Introduction of the mixture of methylcellulose and new cold gelling compound into a basic vegetarian shaped burger recipe, including hydrated soy protein, vegetable oil, spices and salt and evaluation of the result.

Stage 4: Selection and preparation of final samples for measurements of the cold gel strength and elasticity using a texture analyser.

During the first two stages evaluation of gel strength and elasticity was performed without the use of any measuring instruments. Instead samples were given values based on how they were perceived by the evaluator. The parameters evaluated were gel strength and elasticity, each given a value between 1 and 5 for every sample, with 5 corresponding to high gel strength and high elasticity, respectively. For the third stage samples were simply denoted either "pass" or "fail" depending on whether the cold structure was perceivably different from a reference sample as judged by the evaluator. Evaluation of samples that passed was done in the fourth and final stage using a texture analyzer.

The materials used include basic kitchen equipment such as pots, pans and plastic bowls for mixing. For temperature measurements a basic meat thermometer was used. A Mettler PE3600 balance with a precision of 0.01 gram was used for all weighing. In the fourth and final stage a Stable Micro Systems TA.XT2i Texture Analyzer was used.

Stage 1

The theoretical minimum concentration needed for gel formation in water was determined in accordance with recommendations from the manufacturers. This concentration was used as a starting point and corresponds to the concentration later termed "C1".

Experiments were carried out through dispersing a specific amount of hydrocolloid or starch during agitation in 100 g of water with a temperature of 10 °C. The hydrocolloid dispersions were heated in a pot with lock on the stove until a temperature of 95 °C was achieved. The solutions were then poured into moulds where they were left for cooling in an ice bath. For starch samples the temperature was raised to 80 °C and kept there for 8 minutes then poured into moulds where they were left for cooling in an ice bath.

Samples were left in the refrigerator overnight and hydrocolloid samples were assessed the day after. Starch samples were assessed three days after because of their tendency to retrograde. Each sample concentration was ranked by the evaluator on a scale from 1-5 with regards to gel strength and elasticity. Based on the outcome of the first set of experiments, the procedure was repeated two more times with increasing concentrations. The concentrations tested are listed in table 5 and 6.

Table 5: Hydrocolloid concentrations evaluated after gelation in pure water

Hydrocolloid	C1 (%)	C2 (%)	C3 (%)
C1	0.60	1.50	2.00
C2	0.60	1.50	2.00
C3	0.60	1.50	2.00
Ag	0.40	1.50	2.00
C/K	0.50	1.25	2.00
X/LBG	0.40	1.25	2.00
C/LBG1	0.50	1.25	2.00
C/LBG2	0.70	1.25	2.00

Table 6: Starch concentrations evaluated after gelation in pure water

Starch	C1 (%)	C2 (%)	C3 (%)
S1	10.0	12.5	15.0
S2	20.0	22.5	25.0
S3	10.0	12.5	15.0
S4	10.0	12.5	15.0
S5	20.0	17.5	22.5
S6	3.00	6.00	9.00
S7	2.00	4.00	6.00
S8	2.00	4.00	6.00
S9	10.0	12.5	15.0
S10	10.0	12.5	15.0
S11	5.00	10.0	15.0
S12	5.00	10.0	15.0
S13	5.00	10.0	15.0

Stage 2

In order to be able to compare how different hydrocolloids and starches functions together with methylcellulose, a standard methylcellulose solution with a fixed concentration of methylcellulose in water was produced. This standard was determined based on common concentrations used in Anamma's current products. The methylcellulose solution was prepared using the hot/cold technique as described below.

A fixed amount of water was heated in a pot on the stove. When the water had reached a temperature of 70 °C it was taken off the stove and the methylcellulose powder was added during agitation. The dispersion was cooled in an ice batch during constant agitation to 27 °C, causing a significant increase in viscosity. The dispersion was put in the refrigerator until a temperature of 6 °C had been reached. Agitation was no longer needed since the high viscosity prevented granules from sedimenting. The solution became clear and highly viscous, indicating that the methylcellulose had dissolved completely.

The hydrocolloid or starch was added as a powder and dispersed thoroughly in 100 g of the cold methylcellulose solution during vigorous agitation by hand. The concentration of

hydrocolloid or starch used was based on the outcomes of the first stage. An amount of 15 g of the dispersion was put on a warm frying pan, causing the methylcellulose to gel instantly on the surface, thus keeping the dispersion from flowing out. The samples were heated for 4 minutes and were flipped every minute. They were then left in the refrigerator for cooling. Four reference samples were also made for comparison, containing no added hydrocolloid or starch to the methylcellulose solution.

Alginate samples were prepared in the same manner (*Al1* and *Al2*), but these were never heated since gel setting is independent of temperature. Instead the dispersions were poured into moulds and left in the refrigerator for cooling. Hydrocolloid samples were assessed the day after. Starch samples were assessed three days after making. Once again each sample was ranked on a scale from 1-5 with regards to cold gel strength and elasticity. The concentrations tested in the second stage are listed in table 7 and 8.

Table 7: Hydrocolloid concentrations evaluated after gelation in methylcellulose solution

Hydrocolloid	C1 (%)	C2 (%)	C3 (%)
C1	1.00	2.00	3.00
C2	1.00	2.00	3.00
C3	1.00	2.00	3.00
Al1	3.00	5.00	7.00
Al2	3.00	5.00	7.00
Ag	1.00	2.00	3.00
C/K	1.00	2.00	3.00
X/LBG	1.00	2.00	3.00
C/LBG1	1.00	2.00	3.00
C/LBG2	1.00	2.00	3.00

Table 8: Starch concentrations evaluated after gelation in methylcellulose solution

Starch	C1 (%)	C2 (%)	C3 (%)
S1	10.0	12.5	15.0
S2	20.0	22.5	25.0
S3	10.0	12.5	15.0
S4	10.0	12.5	15.0
S5	3.00	6.00	9.0
S6	2.00	4.00	6.0
S7	2.00	4.00	6.0
S8	10.0	12.5	15.0
S9	10.0	12.5	15.0
S10	5.00	10.0	15.0
S11	5.00	10.0	15.0
S12	5.00	10.0	15.0
S13	10.0	12.5	15.0

Stage 3

A basic vegetarian burger recipe was developed in which the new combinations of methylcellulose and hydrocolloids/starches could be tested in an actual product. The recipe of this sample burger is presented in table 9 below.

Table 9: Recipe for sample burger to use for evaluation of hydrocolloid and starch samples

Ingredient	Weight (g)
Methylcellulose solution	48.4
Hydrated textured soy protein	40.4
Spices and salt	2.20
Vegetable oil	9.00
TOTAL	100

For the third stage, the amount of hydrocolloid or starch added on top of the recipe is expressed as a percentage of the methylcellulose solution in the recipe, since this was the relation that had been used in the previous stages. For instance, adding 10% of starch to the above recipe would correspond to an actual weight of 4.84 g.

The samples were prepared by manually dry-blending the powder of the hydrocolloid or starch with the spices and salt in the recipe in order to assure proper dispersion. The dry-blend was added to the methylcellulose solution in a plastic bowl and mixed thoroughly by hand. The hydrated textured soy protein was added to the dispersion which was mixed again. Last the vegetable oil was added to the batter and mixed in by hand. An amount of 75 g $\pm$ 2 g was added to a mould in the shape of a burger providing samples with a thickness of approximately 13 mm and a diameter of 80 mm. The preparation procedure deviates only for alginate samples (Al1 and Al2). For these, the powder was instead added to the batter after all other ingredients had been mixed together since it is desirable to delay the gelation as much as possible in industrial use. These samples were also the only ones which were not heated.

The samples were laid out on bakery paper on a baking tray and covered with a second sheet of bakery paper followed by a layer of tin foil for oven applications. This was done to prevent water evaporation during heating to the largest extent possible. Samples were placed in the oven at 125 °C with a meat thermometer placed in the middle of each sample. Hydrocolloid samples were heated until a centre temperature of 82 °C was reached and starch samples were heated until a centre temperature of 75 °C was reached. The samples were then left for cooling in room temperature and an hour later placed in the refrigerator for cooling and storing. A reference sample containing no added hydrocolloid or starch was also made for comparison.

Hydrocolloid samples were assessed the day after, starch samples were assessed after three days. Concentrations tested in the third stage are presented in table 10 and 11. For this evaluation, samples were only denoted either “pass” or “fail” by the evaluator since a more thorough assessment of gel strength and elasticity of passed samples was performed in the fourth final stage.

Table 10: Hydrocolloid concentrations evaluated after gelation in burger recipe

Hydrocolloid	C1 (%)	C2 (%)	C3 (%)
C1	1.00	2.00	3.00
C2	1.00	2.00	3.00
C3	2.00	3.00	-
Al1	3.00	5.00	7.00
Al2	5.00	7.00	-
Ag	2.00	3.00	-
C/K	1.00	2.00	3.00
X/LBG	2.00	3.00	-
C/LBG1	1.00	2.00	3.00
C/LBG2	2.00	3.00	-

Table 11: Starch concentrations evaluated after gelation in burger recipe

Starch	C1 (%)	C2 (%)	C3 (%)
S1	10.0	12.5	15.0
S2	20.0	22.5	25.0
S3	10.0	12.5	15.0
S4	10.0	12.5	15.0
S6	9.00	-	-
S7	6.00	-	-
S8	6.00	-	-
S9	10.0	12.5	15.0
S10	10.0	12.5	15.0
S11	10.0	15.0	-
S12	5.00	10.0	15.0
S13	10.0	15.0	-

Stage 4

Based on the results from the third stage, final samples were chosen and prepared for evaluation using a texture analyzer. The selection was based on performance in third stage as well as market price and similarities between samples. The making of samples was done in the same manner as described in the third stage, with the only difference being the method of measuring temperature in the samples. The placement of a thermometer in the sample causes a tear in the middle of the sample, which could later influence the measurements using the texture analyzer. Therefore, instead an identical “dummy” was made in which the thermometer was placed in the centre.

The dummy was placed right next to the real sample in the oven to get an as accurate estimation of the centre temperature of the real sample as possible. When the temperature had reached 82 °C for hydrocolloid samples and 75 °C for starch samples, the samples were taken out from the oven and the dummy was discarded. Duplicates were made for each sample.

Two reference samples were made containing no additional hydrocolloid or starch for comparison. One of the references was heated to 75 °C for comparison with starch samples, and one was heated to 82 °C for comparison with hydrocolloid samples. Duplicates were made for these as well.

An alginate sample in which spices and salts were excluded from the recipe was also made in order to study if the addition of salts may influence the gelling behaviour of alginate.

An additional series of samples were made in order to determine the temperature dependence of mixed carrageenan/LBG-samples. Five identical samples were made containing the same amount of C/LBG1 and placed in the oven next to dummies with thermometers for determination of centre temperatures of samples. Once the temperature had reached 65 °C, the first sample was taken out and left for cooling. The procedure was repeated for temperatures 70, 75, 80 and 85 °C. Duplicates were once again made for each sample.

Samples were made during a period of three days, beginning with starch samples on the first day. All texture measurements were performed on the fourth day.

The texture analysis was performed using a Stable Micro Systems TA.XT2i Texture Analyzer. Two different probes were used, one with a diameter of 7 mm and a rounded top and a second one with a diameter of 36 mm and a flat top. The instrument pressed a total of 10 mm into the sample and moved with a constant distance of 0.2 mm each second, continuously adjusting the force by which it pressed in order to keep a constant rate. 200 points per second were recorded and the raw data was given as force (N) per distance (mm).

Samples were taken from the refrigerator and immediately placed in the instrument. Samples were thus approximately at refrigerator temperatures during the measurements. Three measuring points was used on each burger sample for the 7 mm probe. Including duplicates, this provided six measurements for each sample. The 7 mm probe was used on all samples. The 36 mm probe was used as an additional evaluation after the 7 mm probe had been used, with one measuring point on each burger sample, thus in total two measurements per sample including duplicates. Due to time shortage however, the 36 mm probe was not used on all samples. Probe placements on samples are illustrated in figure 3.

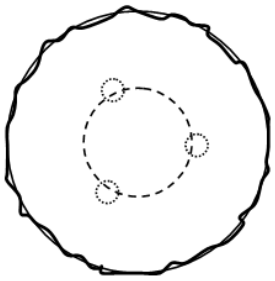

Figure 3: Probe placement on burger samples as seen from above. Small dotted circles show the 7 mm probe and the big dashed circle show the placement of the 36 mm probe.

A summary of the different samples made during the fourth and final stage is presented in table 12 below, along with designated names for each final sample. The concentration percentage of hydrocolloid/starch once again refers to a percentage of the methylcellulose solution in the recipe. For clarity, the actual mass of added hydrocolloid/starch is also presented. Measured centre temperature for each sample is presented and the last two columns denote which probe was used for each sample.

Table 12: Samples, concentrations and temperatures evaluated using the Stable Micro Systems TA.XT2i Texture Analyzer along with probes used for each sample

Name	Sample	C (%)	m (g)	T (°C)	7 mm	36 mm
S1	S1	13.0	6.29	75	X	X
S3	S3	13.0	6.29	75	X	X
S10	S10	13.0	6.29	75	X	X
S12	S12	13.0	6.29	75	X	
S4	S4	13.0	6.29	75	X	
S9	S9	13.0	6.29	75	X	
Ref75	Reference	-	-	75	X	X
Al1	Al1	5.00	2.42	-	X	
Al12	Al1 without salts	5.00	2.42	-	X	
C/L1.5	C/LBG1 (60/40)	1.50	0.73	82	X	X
C/L2.0	C/LBG1 (60/40)	2.00	0.97	82	X	X
C/L2.5	C/LBG1 (60/40)	2.50	1.21	82	X	X
C/L3.0	C/LBG1 (60/40)	3.00	1.45	82	X	X
C/L3.5	C/LBG1 (60/40)	3.50	1.69	82	X	X
Ref82	Reference	-	-	82	X	X
C/L65	C/LBG1 (60/40)	2.50	1.21	65	X	X
C/L70	C/LBG1 (60/40)	2.50	1.21	70	X	X
C/L75	C/LBG1 (60/40)	2.50	1.21	75	X	X
C/L80	C/LBG1 (60/40)	2.50	1.21	80	X	X
C/L85	C/LBG1 (60/40)	2.50	1.21	85	X	X
C1.5	C2	1.50	0.73	82	X	X
C2.5	C2	2.50	1.21	82	X	

Results

The results from the first stage are presented in tables 13 and 14. Each sample was given a value between 1 and 5 for gel strength (GS) and elasticity (E), with 5 corresponding to a high gel strength and elasticity, respectively. Gel strength is here defined as the gels ability to resist rupture when a pressing force is applied on the sample. Elasticity is defined as the gels ability to return to its original shape after deformation, below its yield point. For samples that does not have a value for gel strength and elasticity it was not possible to induce any gel formation. Values in each table are relational to each other.

Table 13: Hydrocolloid concentrations with assigned gel strength (GS) and elasticity (E) as estimated by the evaluator on a scale between 1 and 5 after gelation in water, with 5 corresponding to high gel strength and elasticity, respectively

Hydrocolloid	C1 (%)	GS	E	C2 (%)	GS	E	C3 (%)	GS	E
C1	0.60	3	2	1.50	5	2	2.00	5	2
C2	0.60	3	2	1.50	5	2	2.00	5	2
C3	0.60	1	2	1.50	2	2	2.00	3	2
Al1	1.50	-	-	3.00	-	-	4.50	-	-
Al2	1.50	-	-	3.00	-	-	4.50	-	-
Ag	0.40	3	2	1.50	5	2	2.00	5	2
C/K	0.50	2	3	1.25	4	3	2.00	4	3
X/LBG	0.40	1	4	1.25	2	5	2.00	2	5
C/LBG1	0.50	1	3	1.25	3	4	2.00	3	4
C/LBG2	0.70	1	3	1.25	3	4	2.00	3	4

Table 14: Hydrocolloid concentrations and assigned gel strength (GS) and elasticity (E) as estimated by the evaluator on a scale between 1 and 5 after gelation in water, with 5 corresponding to high gel strength and elasticity, respectively

Starch	C1 (%)	GS	E	C2 (%)	GS	E	C3 (%)	GS	E
S1	10.0	3	2	12.5	4	1	15.0	4	1
S2	20.0	4	1	22.5	4	1	25.0	4	1
S3	10.0	4	1	12.5	5	1	15.0	5	1
S4	10.0	3	2	12.5	4	2	15.0	4	2
S5	20.0	4	1	17.5	5	1	22.5	5	1
S6	3.00	-	-	6.00	-	-	9.00	-	-
S7	2.00	-	-	4.00	-	-	6.00	-	-
S8	2.00	-	-	4.00	-	-	6.00	-	-
S9	10.0	4	2	12.5	5	2	15.0	5	2
S10	10.0	4	2	12.5	5	2	15.0	5	1
S11	5.00	-	-	10.0	-	-	15.0	-	-
S12	5.00	-	-	10.0	3	2	15.0	3	2
S13	5.00	-	-	10.0	-	-	15.0	-	-

The results from the second stage are presented in tables 15 and 16. Once again each sample was given a value from 1 to 5 for gel strength (GS) and elasticity (E), with 5 corresponding to a high gel strength and elasticity, respectively. A value of 1 on gel strength corresponds to no perceivable difference in gel strength from a reference methylcellulose solution without any hydrocolloid or starch added.

Table 15: Hydrocolloid concentrations with assigned gel strength (GS) and elasticity (E) as estimated by the evaluator on a scale between 1 and 5 after gelation in a standardized methylcellulose solution, with 5 corresponding to high gel strength and elasticity, respectively

Hydrocolloid	C1 (%)	GS	E	C2 (%)	GS	E	C3 (%)	GS	E
C1	1.00	3	2	2.00	4	1	3.00	4	1
C2	1.00	3	2	2.00	4	1	3.00	4	1
C3	1.00	1	2	2.00	2	2	3.00	2	2
Al1	3.00	3	3	5.00	5	3	7.00	5	3
Al2	3.00	2	3	5.00	2	3	7.00	2	3
Ag	1.00	1	2	2.00	1	2	3.00	1	2
C/K	1.00	2	2	2.00	2	2	3.00	2	2
X/LBG	1.00	1	3	2.00	1	3	3.00	2	3
C/LBG1	1.00	2	2	2.00	2	3	3.00	3	3
C/LBG2	1.00	2	2	2.00	2	3	3.00	3	3

Table 16: Starch concentrations with assigned gel strength (GS) and elasticity (E) as estimated by the evaluator on a scale between 1 and 5 after gelation in a standardized methylcellulose solution, with 5 corresponding to high gel strength and elasticity, respectively

Starch	C1 (%)	GS	E	C2 (%)	GS	E	C3 (%)	GS	E
S1	10.0	3	2	12.5	3	1	15.0	3	1
S2	20.0	2	1	22.5	3	1	25.0	3	1
S3	10.0	3	1	12.5	3	1	15.0	4	1
S4	10.0	3	2	12.5	3	2	15.0	3	2
S6	3.00	-	-	6.00	-	-	9.0	-	-
S7	2.00	-	-	4.00	-	-	6.0	-	-
S8	2.00	-	-	4.00	-	-	6.0	-	-
S9	10.0	2	2	12.5	2	2	15.0	2	2
S10	10.0	2	1	12.5	3	1	15.0	3	1
S11	5.00	-	-	10.0	-	-	15.0	-	-
S12	5.00	-	-	10.0	2	2	15.0	2	2
S13	5.00	-	-	10.0	-	-	15.0	-	-

The results from the third stage are presented in tables 17 and 18. Samples which were perceivably different from a reference samples without any hydrocolloid or starch added were denoted “passed”, while the rest were denoted “fail”.

Table 17: Hydrocolloid concentrations evaluated in burger recipe and overall verdict of hydrocolloid sample based on the presence or absence (pass/fail, respectively) of perceivable differences from a reference sample as judged by the evaluator

Hydrocolloid	C1 (%)	C2 (%)	C3 (%)	Pass	Fail
C1	1.00	2.00	3.00	X	
C2	1.00	2.00	3.00	X	
C3	2.00	3.00	-		X
Al1	3.00	5.00	7.00	X	
Al2	5.00	7.00	-		X
Ag	2.00	3.00	-		X
C/K	1.00	2.00	3.00	X	
X/LBG	2.00	3.00	-		X
C/LBG1	1.00	2.00	3.00	X	
C/LBG2	2.00	3.00	-	X	

Table 18: Starch concentrations evaluated in burger recipe and overall verdict of hydrocolloid sample based on the presence or absence (pass/fail, respectively) of perceivable differences from a reference sample as judged by the evaluator

Starch	C1 (%)	C2 (%)	C3 (%)	Pass	Fail
S1	10.0	12.5	15.0	X	
S2	20.0	22.5	25.0		X
S3	10.0	12.5	15.0	X	
S4	10.0	12.5	15.0	X	
S6	9.00	-	-		X
S7	6.00	-	-		X
S8	6.00	-	-		X
S9	10.0	12.5	15.0	X	
S10	10.0	12.5	15.0	X	
S11	10.0	15.0	-		X
S12	5.00	10.0	15.0	X	
S13	10.0	15.0	-		X

Results from the final measurements using the texture analyzer are presented in table A1 (7 mm probe) and A2 (36 mm probe) in appendix. The results are presented as total work (mJ) performed by the probe when penetrating 10 mm into the sample. The work was achieved by calculating the area under each force (N) vs. distance (mm) graph.

For determination of significant differences between each sample, independent two-sample two-tailed t-tests were performed between each sample with the following hypothesis to be tested:

H_0: $\overline{X}_1 = \overline{X}_2$ ⇒ No significant difference between sample means
H_1: $\overline{X}_1 \neq \overline{X}_2$ ⇒ Significant difference between sample means

The t-values were calculated according to equation 1. Assumptions were made that the populations from which the samples were taken are normally distributed and that the standard deviation of the populations are equal. The latter was assumed since the same instrument was used for each sample and samples are of equal size and weight. If the calculated t-value was within the range of the critical value, the null hypothesis (H_0) was accepted, otherwise it was rejected. The results from the t-tests are presented in tables A3 and A4 in appendix.

$$t = \frac{\overline{X}_1 - \overline{X}_2}{\sqrt{\left(\frac{(N_1 - 1)s_1^2 + (N_2 - 1)s_2^2}{N_1 + N_2 - 2}\right)\left(\frac{1}{N_1} + \frac{1}{N_2}\right)}}$$

$\overline{X}$: Medelvärde
N: Frihetsgrader
s: Skattad standardavvikelse

Subscripts denotes sample number

Equation 1: Calculation of independent two-sample t-test

Discussion

The experimental work was divided into the four stages in order to more easily understand and visualize the gelling ability of each individual hydrocolloid and starch. Being able to study how the different samples performed in each stage makes it easier to discuss what might make some samples function well in the final product while some does not function as intended.

There are several reasons for not using a texture analyzer during the first three stages, the most significant being that samples forming strong enough gels suitable for this application are easily distinguished from the ones forming much too soft gels, or no gel at all. Further, measurements using a texture analyzer are very time consuming and the instrument was not readily accessible in the research kitchen. The values given for gel strength and elasticity during the first two stages are of course of quite poor quality from a quantitative point of view, but help to give an idea of the relational differences between samples.

For discussion of the results it is convenient to divide samples into related categories rather than stages. Carrageenan and agar samples will therefore be discussed first, followed by alginate, xanthan/LBG and starch samples. The final texture analysis will sum up the discussion.

Carrageenan and agar

From the results in table 13 it is possible to conclude that samples of kappa-carrageenan with added potassium chloride (*C1* and *C2*), as well as agar (*Ag*) were the ones that performed best with regards to gel strength. Sample *C3* did not perform as well as the other two samples of kappa-carrageenan and the obvious reason for this is the absence of potassium ions in this sample, which during gelation functions as cross-linking ion between helices formed upon cooling. The other two kappa-carrageenan samples had potassium chloride added from the manufacturer.

As suggested by the literature, *C1*, *C2* and *Ag* samples were however quite brittle, why they were given low values for elasticity all through the concentration range tested. This is the reason for the additional samples *C/K*, *C/LBG1* and *C/LBG2*, where konjac mannan is added to the former and locust bean gum to the two latter. The addition of these hydrocolloids to kappa-carrageenan induces, as previously explained, more elasticity to the gels while still providing good gel strength. The synergistic effect is most obvious for the addition of konjac mannan, however both types of mixed gels proved to perform well in the test.

Performance of samples *C1, C2, C3, C/K, C/LBG1* and *C/LBG2* in the second stage was quite consistent with how they performed in the first stage. There was a significant difference in all of these samples when compared to a reference without any hydrocolloid added, especially for samples *C1* and *C2*. The reference melts back into a viscous solution when cooled. The high viscosity provided by the methylcellulose makes it retain its shape somewhat, though there is basically no resistance when pressing on top of the sample. The mentioned hydrocolloids provided significantly more resistance to the samples as judged by the evaluator, resulting in firmer cold sample.

An interesting outlier however is the agar (*Ag*), which does not provide much structure at all to the sample and the very hard gel strength shown in the first stage is not noticeable. Agar in comparison to kappa-carrageenan needs higher temperatures to completely hydrate and properly gel upon cooling. However it is unlikely that this is where the problem is at, since this would result in a sample with a softer core and at least some gel formation in the outer parts of the sample. A more likely explanation can perhaps be attributed to the gelation mechanism of agar which is promoted by extensive hydrogen bonding between helices, compared to cross-linking ions in carrageenan. It might be that methylcellulose, which also may provide segments capable of hydrogen bonding, interferes with agar molecules and thus hinder the agar-agar associations, and therefore no structural network is built up.

In the third stage it was concluded that kappa-carrageenan without added potassium (*C3*) and agar (*Ag*) could be excluded from the final analysis, since neither did provide much structure at all to the cold burger samples. Remaining samples however proved to function well, with a more thorough assessment being discussed later in the fourth stage. However, not all samples that actually passed the third stage were assessed using the texture analyzer. The reason is mainly price differences. Samples *C1* and *C2* basically have the same functional ingredient (kappa-carrageenan), the difference being that *C2*, consisting of Processed Eucheuma Seaweed (PES), comes at approximately half the price of *C1*. Gel clarity is no necessity in this application, and possible flavor taints are believed to be easily covered in final recipes since the products in general contain quite dominant flavors, which is why *C2* was chosen for the final analysis.

Also the exclusion of *C/K* and *C/LBG2* from the final analysis is connected to pricing. Konjac mannan is a much more expensive ingredient than the more common locust bean gum and because of their similar performance in the second and third stage sample *C/LBG1* was chosen. *C/LBG2* came pre-blended from the manufacturer and in all stages performed quite equal to *C/LBG1* which was blended manually in a ratio of 60/40. The pre-blending from the manufacturer increase the price compared to manual blending.

Alginate

Alginate samples are quite different from agar and carrageenan samples in the sense that gel formation is more or less independent of temperature. Alginate instead sets upon the introduction of calcium ions, which irreversibly cross-links alginate strands. The mechanism of gelation is often described using the egg-box model [32]. Binding of calcium is a very rapid and irreversible mechanism, why a direct mixing of readily soluble calcium salts such as calcium chloride and alginate in water would result in inhomogeneous gels. Instead, samples Al1 and Al2 both contain calcium sulphate which is poorly soluble in water, along with a complexing phosphate agent. The idea is that calcium is released slowly into the solution through the complexing agent, which acts as sequestrant. This method of controlling the gelation is called internal setting [23, p. 821].

For both alginate samples (*Al1* and *Al2*), it was not possible to induce any homogenous gel formation in water, regardless of the concentration tested. The believed reason for the alginate not to set is that the low viscosity of the water causes sedimentation of particles, because some gel formation had occurred at the bottom of the moulds. Supporting this theory is the fact that in combination with methylcellulose in the second stage, which

provides a considerable amount of viscosity to the cold solution, substantial cold gel strength was noticed when the samples were assessed the next day. Particularly *Al1* proved to function well with regards to gel strength, actually best of all hydrocolloids tested.

The time needed for these particular alginate gels to set has not been examined, even though this is a crucial aspect industrially. When preparing large batters for production of any shaped product, it would be vital that the gelation of alginate is delayed long enough for the entire batter to be shaped into products. The risk otherwise is that the gel sets too quickly, which might not only ruin the batch, but also processing equipment. Therefore, in the third stage alginate was added last, as would be suitable industrially. Once again *Al1* proved to work best in the third stage, compared to *Al2.* Therefore only sample *Al1* was used for the final evaluation.

Gel formation using alginate may also be influenced by the addition of salt and spices in the final recipe due to competition between binding sites on alginate, as suggested by the supervisor at Anamma. To assess whether this could be a problem, an alginate sample without the addition of salts and spices was also produced, denoted *Al12*.

Xanthan/LBG

The combination of xanthan and locust bean gum generally did not provide much useful results throughout stage 1, 2 and 3. Already in the first stage, gels produced were very weak. They exhibit quite good elasticity, but break easily when subjected to a fairly small amount of stress. In combination with the methylcellulose solution, some elasticity is retained, but the strength and ability to provide rigid structure to the sample is inadequate. Unsurprisingly, this holds true also for the burger samples in the third stage, and the samples was thus excluded from the final measurements.

The results are a little surprising, since earlier investigations suggest the formation of firm gels at the studied ratio (50/50) [33]. When consulting hydrocolloid manufacturers however, the information is contradictious. One part confirms the formation of gels that are very firm, while another states that the mixed gels are so elastic and non-brittle they are unpleasant to eat and seldom used in food applications. There may have been a misinterpretation of the description of the gel, or the specific xanthan or locust bean gum used was not optimal for the synergistic interaction, even though this was requested from the manufacturer that delivered the hydrocolloids.

Starch

Starch samples provided quite various results in the first stage. Some (*S1, S2, S3, S9* and *S10*) provided really hard gels over the entire concentration range tested, while for others (*S6, S7, S8, S11* and *S13*) it was not possible to induce any gel formation. Sample S5 provided more of a “spread” type of gel, without any ability to retain its shape once strained. It was therefore the only sample which was excluded immediately. It was in retrospect concluded together with manufacturers that some of the samples (*S6, S7, S8* and *S13*) received were not particularly good gel formers, but rather cold viscosity enhancers. Gelling starch samples are generally quite brittle with low elasticity, as indicated by values in table 14.

Combinations of the different starch samples and methylcellulose provided quite equal results, and the results were consistent throughout the third stage as well. Elasticity of samples is generally low, but with significant improvement of structure with regards to resistance to stress and manageability.

Texture analysis

From the raw data from the texture analysis it was possible to achieve the total work performed for each measurement. A typical graph obtained from one reading is presented in figure 4 below, which is the first measurement for sample C/L2.5. The area under the curve corresponds to the total work performed in millijoules.

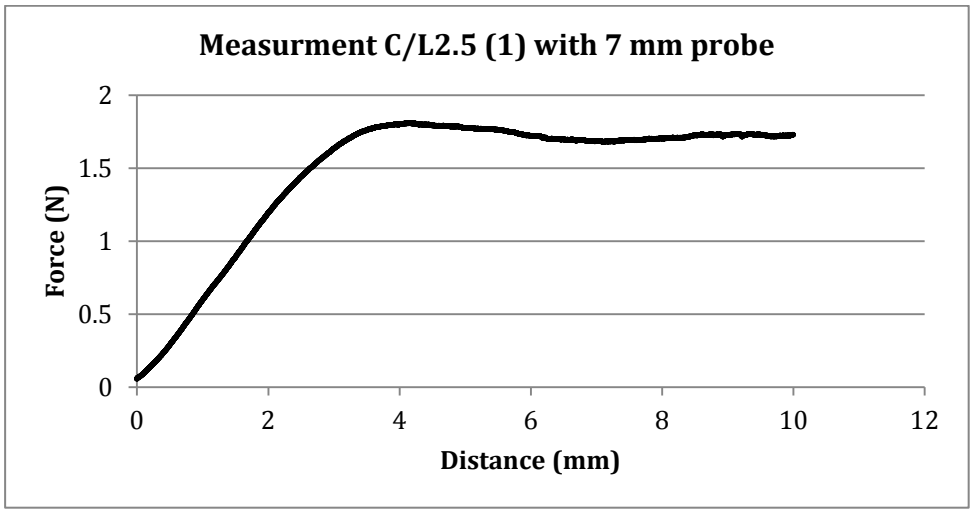

Figure 4: Raw data from measurement C/L2.5 (1) using 7 mm probe

From the curve in figure 4 it is also evident that it is possible to determine curve maximum as a parameter for each curve, which in the case of the above measurement is around distance 4.2 mm. However, after plotting curve maximum for each measurement against the total work (figure 5) it was concluded that the two parameters are highly correlated, and therefore only one parameter would be evaluated further.

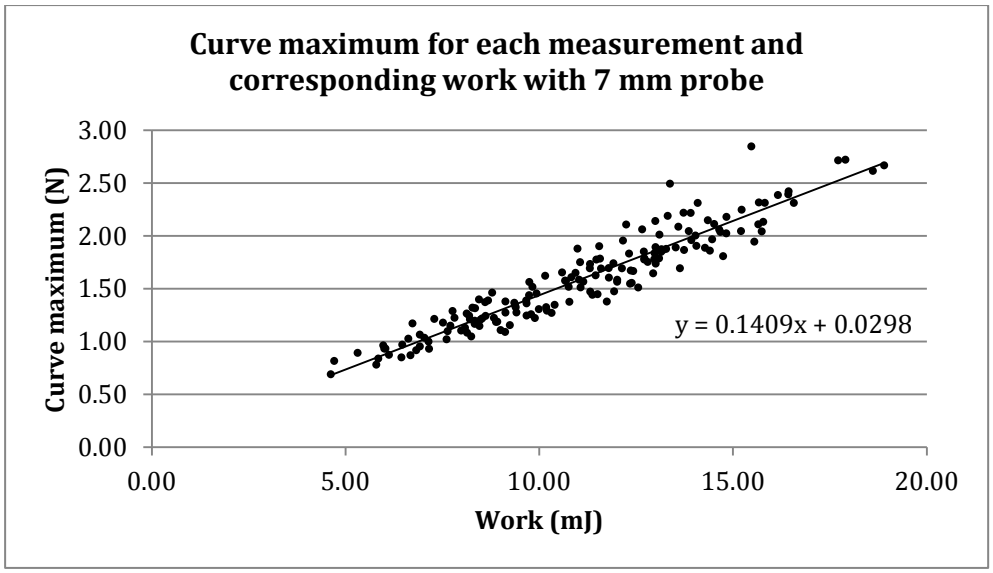

Figure 5: Correlation between curve maximum and total work for each measurement using 7 mm probe

The first thing to notice from the analysis using the 7 mm probe (Table A1) is that there is quite a large standard deviation even within measurements of the same burger sample. The first, second and third measurement is taken from the first burger sample, while the fourth, fifth and sixth measurement is taken from the duplicate burger. Looking at for instance sample *S3*, the work drops with 41% from the fourth to the sixth measurement, which seems an unreasonably huge variation within the same burger.

The theory for the large diversity is that samples are too inhomogeneous for the 7 mm probe to give truthful results. Samples contain roughly about 50% hydrocolloid solution as the continuous phase, and 40% hydrated textured soy protein as a dispersed solid phase, the remaining 10% being made up of vegetable oil and spices and salt. This is illustrated in figure 6 below, with the hydrated textured soy protein being illustrated by circles in the sample. Since the area by which the 7 mm probe presses is small relative to the total area of the burger sample, it may become important where the probe is placed on the sample.

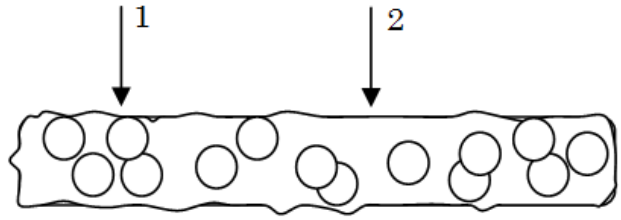

Figure 6: Illustration of differences in measurements due to probe location

If the probe was to be placed at position 1 in figure 6, which is a dispersed phase-dense region, it is reasonable to believe that the reading would be different from position 2, which is a continuous phase-dense region. It is reasonable to believe that position 2 would give a better estimation of the actual gel strength since the gel network is present in the continuous phase. In reality however it is impossible to tell where samples might be dispersed phase- or continuous phase-dense. The 7 mm probe covers only approximately 0.8% of the total surface area of the burger sample, why it is likely that this phenomenon has played a part in the readings.

This was noticed during ongoing experiments and this is the reason why the 36 mm probe was used as well, which covers 20% of the sample burger surface area. Even with this probe however, some considerable variations were achieved, but they are smaller. In table 19 the average coefficient of variance has been calculated for each probe, which is the average of the standard deviation divided by the mean of each sample. There are fewer degrees of freedom using the 36 mm probe because of fewer readings for each sample, but the calculation gives a notion on the performance of each probe.

Table 19: Average coefficient of variance for each probe used

Probe	Average coefficient of variance
7 mm	12.2%
36 mm	5.85%

Other parameters that might explain the large variance between readings are uneven distribution of heat inside the oven when heating the samples since there was no possibility to use a convection oven. This could imply that in some samples the temperature was not raised enough so that the hydrocolloid or starch was functionalized completely, or that some samples experienced more water evaporation than others, despite attempts to avoid this as far as possible.

In spite of some apparent sources of error, several useful results were achieved in the final measurements. Beginning with starch samples, it is evident that samples *S4* and *S9*, which is a modified sago starch and a native pea starch with high amylose content, functioned worst. The performance of the remaining starch samples (*S1, S3, S10* and *S12*) was quite equal, at least when evaluated using the 7 mm probe. The samples were remarkably stronger compared to the reference *Ref75* and all four samples show significant differences from this sample. With the 36 mm probe, only sample *S10* showed significant difference from the reference. Using the 36 mm probe, it was also possible to show the brittleness of starch gels that has been discussed previously. At least one of each starch sample cracked during measurements, giving rise to a curve similar to the one seen in figure 8 below. At a distance of approximately 5.3 mm there is a sudden drop in force, indicating rupture of the sample. This was only experienced with starch samples.

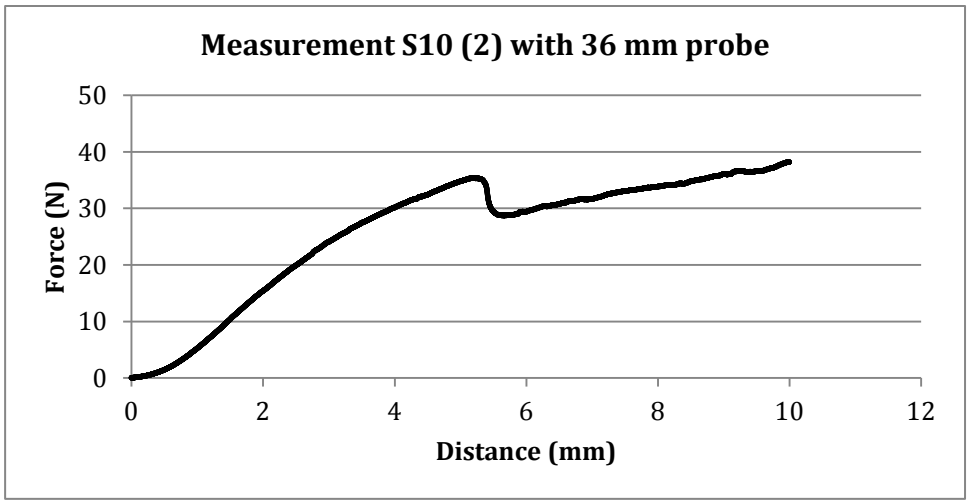

Figure 7: Raw data from measurement S10 (2) using 36 mm probe

All starch gels produced in these experiments have in general had very low elasticity, which could be disadvantageous in the final product. Not only does it make the products more prone to breaking when stress is applied, it is also possible that samples having some elasticity provide more of the impression of a juicy and appealing product. These are of course speculations, and a more thorough sensory evaluation from a costumer point of view would be needed to really confirm this theory.

The alginate samples (*Al1* and *Al2*) did both show a significant difference compared to the reference sample that had been heated to 75 °C, *Ref75.* These were only assessed using the 7 mm probe. Between the two alginate samples (with and without the addition of spices/salts), there is no significant difference though, implying that the addition of salt does not influence the gelling capacity of alginate. When compared to other samples such as *S1,*

S10, and the majority of the carrageenan-containing samples, the alginate samples are however considerably weaker in gel strength. An important aspect to keep in mind though is that the alginate samples have not been heated, and therefore it is possible to assume that all other samples may have experienced some water evaporation in comparison, probably making them a little firmer.

The excellent cold gelling capacity experienced from alginate in the second stage of the experiments is in other words not quite as obvious in the final assessments. There is a possibility that the alginate was not properly dispersed in the continuous phase since it was added to the recipe last, with the motivation as described earlier. It would have been interesting to see if the results would improve if the alginate powder would first be thoroughly dispersed in the methylcellulose solution, followed by the addition of the rest of the ingredients. This could lead to a better dispersion in the continuous phase, and a better gelling capacity may be experienced.

To sum up the alginate discussion, it is evident from the research that alginate does possess some interesting potential when it comes to cold-setting gels in combination with methylcellulose. Being the only hydrocolloid in this research where the need for heat is eliminated there is a great potential to cut production costs, even though it is possible that in the case of refrigerated products heat will be needed anyway for control of microbial growth. Further assessment of the use of alginate would though be needed in order to incorporate it into Anamma's products with satisfying results.

The most interesting samples in the research are probably carrageenan-containing samples. Once again quite large variations in measurements are seen, however there are some important aspects that with most certainty can be concluded. The first is the temperature dependence of kappa-carrageenan/LBG mixtures. It is evident from both measurements (7 mm and 36 mm probe) that samples heated to and above 80 °C (*C/L80* and *C/L85*) are significantly stronger than remaining samples (*C/L65, C/L70* and *C/L75*), as well as the reference *Ref82*. Locust bean gum will not dissolve completely until samples are heated sufficiently as suggested by several manufacturers, which obviously affects the gel formation in combination with kappa-carrageenan. No significant difference was detected with either probe when comparing *C/L80* to *C/L85,* indicating that heat treatment to 80 °C might be sufficient for the specific locust bean gum used.

Both samples containing only carrageenan (*C1.5* and *C2.5*) also showed a great improvement in gel strength with both probes compared to the reference *Ref82.* No significant difference in work can be shown in comparison to *C/L80* and *C/L85*, and a potential difference in elasticity between the two sets of samples, which could be expected, is hard to show quantitatively. Indeed a difference in elasticity was noticed during assessment of samples during stage 3, though it could not be demonstrated in the measurements. An estimation of the spring constant for each sample would be of help, but this is hard to achieve based on the measurements performed in this research due to the quite poor precision of the method used. Parameters such as the inhomogeneity of the samples and the friction against the probe would further interfere with the trueness of this estimation. The conclusion is that some difference in elasticity should be suspected, in favor of the samples containing locust bean gum.

Concerning samples *C/L1.5* to *C/L3.5*, these samples were included in order to determine the result of increasing concentration on gel strength. The ratio between PES and LBG was kept constant at 60/40. The measurements using the 7 mm probe show few interesting results however, the only significant differences shown being between the highest concentration, *C/L3.5,* and the lower concentrations *C/L1.5, C/L2.0* and *C/L2.5*. Measurements using the 36 mm probe show the same results, except for sample *C/L1.5* which shows no significant difference between any of the samples. This can be though be explained by the huge amount of variance within the measurement of this sample.

There is thus little that can be said about the relation between hydrocolloid concentration of C/LBG samples and resulting gel strength from the quantitative measurements performed in this research, which is a little unfortunate. It is possible that new measurements using the larger probe and more replicates would provide more information, but with the current analysis there is not much more to say about the relation. Sample *C/L2.5* and *C/L80* should theoretically have the same gel strength, since they have the exact same content, the only difference being the small difference in heat treatment, which is a centre temperature of 82 °C and 80 °C, respectively. Indeed, no significant difference is shown using the 7 mm probe, the 36 mm probe on the other hand does show a difference. This may be a result of the sources of error that was previously discussed, concerning heat distribution in the oven.

To sum up the discussion, it is evident that some vital conclusions can be drawn from the texture analysis. Even though the precision of the measurements clearly was quite poor, several significant differences of considerable magnitude were shown. By following the vertical column of *Ref75* and *Ref82* downwards in table A3 and A4, it is possible to make out the magnitude of the significant difference by looking at the size of the absolute value in each column. Samples which stand out are most notably *S10, C/L3.5, C/L80, C/L85, C1.5* and *C2.5*. All of these show a considerable increase in the work needed in order to penetrate 10 mm into the sample, which is attributed to each sample's ability to build up a structural network upon cooling and thus increase the rigidity, manageability and resistance to physical stress at refrigerator temperatures.

The fact that substantial brittleness could be shown in the thin-boiling maize starch sample (*S10*) makes it a little inferior to remaining samples. Also pure carrageenan samples (*C1.5* and *C2.5*) could lack some elasticity, even though this was not possible to show during measurements. Samples C/L80 and C/L85, having a PES/LBG ratio of 60/40 and a total hydrocolloid concentration of 2.5% with respect to methylcellulose solution content in samples, both provided great results in the final assessment.

Conclusion

It has been proved that it is possible to improve the structure of refrigerated methylcellulose-containing vegetarian meat substitutes with respect to susceptibility to physical stress. By combining the methylcellulose with either kappa-carrageenan, kappa-carrageenan/locust bean gum or thin-boiling maize starch the rigidity and manageability of the resulting product is significantly improved. The effect has been shown in a basic vegetarian burger recipe consisting of hydrated textured soy protein, vegetable oil and spices and salt, and can now with reference to the present study be tested in more advanced recipes.

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Appendix

Table A1: Total work performed by the texture analyzer when penetrating 10 mm into burger samples using the 7 mm probe, with mean and standard deviation (STD)

Name	Work (mJ)						Mean (mJ)	STD
	1	2	3	4	5	6		
S1	12.94	11.94	15.56	13.63	12.70	13.11	13.31	1.23
S3	10.99	15.23	15.67	15.75	12.39	9.25	13.21	2.75
S10	13.53	11.31	15.83	13.19	14.83	12.80	13.58	1.58
S12	10.59	13.86	15.78	12.25	13.93	13.32	13.29	1.75
S4	6.92	6.01	7.16	5.85	6.13	5.98	6.34	0.55
S9	8.89	8.20	9.68	9.74	7.71	8.46	8.78	0.82
Ref75	6.68	4.71	6.92	7.82	7.04	8.25	6.90	1.23
Al1	11.07	9.68	8.34	8.92	9.79	8.83	9.44	0.97
Al12	9.36	10.83	9.93	10.40	10.20	9.42	10.02	0.57
C/L1.5	12.71	13.91	14.09	10.67	11.48	12.02	12.48	1.36
C/L2.0	12.35	11.80	11.06	11.60	12.37	13.01	12.03	0.69
C/L2.5	12.43	11.32	12.01	13.01	12.98	14.84	12.76	1.20
C/L2.5	14.75	14.41	18.91	10.16	13.15	11.55	13.82	3.04
C/L3.0	15.21	12.97	13.28	14.68	14.35	14.52	14.17	0.86
Ref82	11.80	12.32	10.69	11.03	9.99	10.18	11.00	0.91
C/L65	7.61	5.80	6.47	5.32	4.62	6.04	5.98	1.02
C/L70	8.35	9.83	8.53	6.45	6.73	6.63	7.75	1.36
C/L75	7.30	8.28	7.64	9.13	8.13	8.09	8.09	0.63
C/L80	16.16	14.04	13.10	13.16	14.28	18.61	14.89	2.14
C/L85	14.65	14.47	16.57	11.46	11.92	13.59	13.78	1.89
C1.5	14.06	17.91	13.74	17.72	12.66	12.72	14.80	2.40
C2.5	16.44	16.43	15.48	13.00	12.17	15.66	14.86	1.83

Table A2: Total work performed by the texture analyzer when penetrating 10 mm into burger samples using the 36 mm probe, with mean and standard deviation (STD)

Name	Work (mJ)		Mean (mJ)	STD
	1	2		
S1	*174.51	185.23	179.87	7.58
S3	216.25	*257.67	236.96	29.29
S10	*264.04	*258.98	261.51	3.58
Ref75	175.84	170.78	173.31	3.58
C/L1.5	226.52	292.06	259.29	46.34
C/L2.0	275.00	260.69	267.85	10.12
C/L2.5	282.39	273.90	278.14	6.00
C/L3.0	252.21	300.56	276.38	34.19
C/L3.5	339.96	329.36	334.66	7.49
Ref82	205.40	214.34	209.87	6.32
C/L65	140.79	153.67	147.23	9.11
C/L70	169.71	162.86	166.29	4.84
C/L75	169.83	185.07	177.45	10.77
C/L80	338.11	319.55	328.83	13.12
C/L85	293.35	323.47	308.41	21.30
C1.5	302.70	329.83	316.26	19.18

* Samples cracked during measurement

Table A3: Independent two-sample two-tailed t-test for 7 mm probe results

Name	S1	S3	S10	S12	S4	S9	Ref 75	Al1	Al12	C/L 1.5	C/L 2.0	C/L 2.5	C/L 3.0	C/L 3.5	Ref 82	C/L65	C/L70	C/L75	C/L80	C/L85	C1.5	C2.5
S1		-0.08	0.33	-0.03	-12.65	-7.52	-9.04	-6.06	-5.93	-1.11	-2.23	-0.78	0.38	1.39	-3.69	-11.24	-7.42	-9.26	1.57	0.50	1.35	1.72
S3	0.08		0.29	0.06	-6.00	-3.78	-5.13	-3.17	-2.78	-0.58	-1.02	-0.37	0.36	0.81	-1.86	-6.04	-4.35	-4.44	1.18	0.41	1.07	1.22
S10	-0.33	-0.29		-0.30	-10.59	-6.61	-8.18	-5.47	-5.18	-1.30	-2.21	-1.01	0.17	0.80	-3.46	-9.90	-6.84	-7.91	1.21	0.19	1.04	1.30
S12	0.03	-0.06	0.30		-9.27	-5.72	-7.32	-4.72	-4.34	-0.89	-1.64	-0.61	0.37	1.10	-2.84	-8.84	-6.11	-6.85	1.42	0.46	1.25	1.52
S4	12.65	6.00	10.59	9.27		6.05	1.03	6.80	11.31	10.26	15.78	11.93	5.94	18.68	10.69	-0.77	2.35	5.14	9.49	9.23	8.42	10.93
S9	7.52	3.78	6.61	5.72	-6.05		-3.12	1.28	3.06	5.73	7.46	6.74	3.93	11.11	4.45	-5.25	-1.58	-1.63	6.55	5.94	5.82	7.44
Ref75	9.04	5.13	8.18	7.32	-1.03	3.12		3.98	5.65	7.47	8.94	8.38	5.17	11.87	6.57	-1.42	1.13	2.12	7.95	7.46	7.18	8.86
Al1	6.06	3.17	5.47	4.72	-6.80	-1.28	-3.98		1.27	4.47	5.35	5.29	3.37	8.93	2.88	-6.03	-2.47	-2.86	5.70	5.00	5.08	6.42
Al12	5.93	2.78	5.18	4.34	-11.31	-3.06	-5.65	-1.27		4.09	5.49	5.06	3.01	9.79	2.23	-8.46	-3.76	-5.57	5.39	4.65	4.75	6.18
C/L1.5	1.11	0.58	1.30	0.89	-10.26	-5.73	-7.47	-4.47	-4.09		-0.73	0.38	0.99	2.57	-2.21	-9.38	-6.02	-7.19	2.33	1.36	2.06	2.56
C/L2.0	2.23	1.02	2.21	1.64	-15.78	-7.46	-8.94	-5.35	-5.49	0.73		1.30	1.41	4.75	-2.20	-12.05	-6.87	-10.37	3.13	2.12	2.72	3.55
C/L2.5	0.78	0.37	1.01	0.61	-11.93	-6.74	-8.38	-5.29	-5.06	-0.38	-1.30		0.79	2.33	-2.86	-10.57	-6.77	-8.47	2.13	1.11	1.86	2.35
C/L3.0	-0.38	-0.36	-0.17	-0.37	-5.94	-3.93	-5.17	-3.37	-3.01	-0.99	-1.41	-0.79		0.27	-2.18	-6.00	-4.47	-4.52	0.71	-0.03	0.62	0.72
C/L3.5	-1.39	-0.81	-0.80	-1.10	-18.68	-11.11	-11.87	-8.93	-9.79	-2.57	-4.75	-2.33	-0.27		-6.17	-15.01	-9.74	-13.95	0.77	-0.46	0.61	0.84
Ref82	3.69	1.86	3.46	2.84	-10.69	-4.45	-6.57	-2.88	-2.23	2.21	2.20	2.86	2.18	6.17		-8.99	-4.86	-6.44	4.10	3.23	3.63	4.62
C/L65	11.24	6.04	9.90	8.84	0.77	5.25	1.42	6.03	8.46	9.38	12.05	10.57	6.00	15.01	8.99		2.55	4.33	9.22	8.88	8.30	10.39
C/L70	7.42	4.35	6.84	6.11	-2.35	1.58	-1.13	2.47	3.76	6.02	6.87	6.77	4.47	9.74	4.86	-2.55		0.56	6.90	6.33	6.26	7.64
C/L75	9.26	4.44	7.91	6.85	-5.14	1.63	-2.12	2.86	5.57	7.19	10.37	8.47	4.52	13.95	6.44	-4.33	-0.56		7.48	6.98	6.63	8.58
C/L80	-1.57	-1.18	-1.21	-1.42	-9.49	-6.55	-7.95	-5.70	-5.39	-2.33	-3.13	-2.13	-0.71	-0.77	-4.10	-9.22	-6.90	-7.48		-0.96	-0.07	-0.03
C/L85	-0.50	-0.41	-0.19	-0.46	-9.23	-5.94	-7.46	-5.00	-4.65	-1.36	-2.12	-1.11	0.03	0.46	-3.23	-8.88	-6.33	-6.98	0.96		0.82	1.01
C1.5	-1.35	-1.07	-1.04	-1.25	-8.42	-5.82	-7.18	-5.08	-4.75	-2.06	-2.72	-1.86	-0.62	-0.61	-3.63	-8.30	-6.26	-6.63	0.07	-0.82		0.05
C2.5	-1.72	-1.22	-1.30	-1.52	-10.93	-7.44	-8.86	-6.42	-6.18	-2.56	-3.55	-2.35	-0.72	-0.84	-4.62	-10.39	-7.64	-8.58	0.03	-1.01	-0.05	

Critical t-value: 2.228. Level of significance: 0.05. Degrees of freedom: 10. Shaded numbers indicates that H_0 is accepted, i.e. there is no significant difference between means. A t-value has been calculated for each possible combination of samples, making it easy to compare each individual sample to every other sample. By following a vertical column downwards, significant differences are indicated by numbers that are not faded. Sample "S4" for instance, is significantly different from all samples except "Ref75" and "C/L65".

Table A4: Independent two-sample two-tailed t-test for 36 mm probe results

Name	S1	S3	S10	Ref75	C/L1.5	C/L2.0	C/L2.5	C/L3.0	C/L3.5	Ref82	C/L65	C/L70	C/L75	C/L80	C/L85	C1.5
S1		2.67	13.77	-1.11	2.39	9.84	14.37	3.90	20.54	4.30	-3.89	-2.14	-0.26	13.90	8.04	9.35
S3	-2.67		1.18	-3.05	0.58	1.41	1.95	1.24	4.57	-1.28	-4.14	-3.37	-2.70	4.05	2.79	3.20
S10	-13.77	-1.18		-24.65	-0.07	0.83	3.37	0.61	12.46	-10.06	-16.51	-22.37	-10.47	7.00	3.07	3.97
Ref75	1.11	3.05	24.65		2.62	12.46	21.22	4.24	27.48	7.12	-3.77	-1.65	0.52	16.17	8.85	10.36
C/L1.5	-2.39	-0.58	0.07	-2.62		0.26	0.57	0.42	2.27	-1.49	-3.36	-2.82	-2.43	2.04	1.36	1.61
C/L2.0	-9.84	-1.41	-0.83	-12.46	-0.26		1.24	0.34	7.51	-6.87	-12.53	-12.81	-8.65	5.21	2.43	3.16
C/L2.5	-14.37	-1.95	-3.37	-21.22	-0.57	-1.24		-0.07	8.33	-11.08	-16.97	-20.52	-11.55	4.97	1.93	2.68
C/L3.0	-3.90	-1.24	-0.61	-4.24	-0.42	-0.34	0.07		2.35	-2.71	-5.16	-4.51	-3.90	2.03	1.12	1.44
C/L3.5	-20.54	-4.57	-12.46	-27.48	-2.27	-7.51	-8.33	-2.35		-18.01	-22.47	-26.70	-16.94	-0.55	-1.64	-1.26
Ref82	-4.30	1.28	10.06	-7.12	1.49	6.87	11.08	2.71	18.01		-7.99	-7.74	-3.67	11.55	6.27	7.45
C/L65	3.89	4.14	16.51	3.77	3.36	12.53	16.97	5.16	22.47	7.99		2.61	3.03	16.08	9.84	11.26
C/L70	2.14	3.37	22.37	1.65	2.82	12.81	20.52	4.51	26.70	7.74	-2.61		1.34	16.44	9.20	10.72
C/L75	0.26	2.70	10.47	-0.52	2.43	8.65	11.55	3.90	16.94	3.67	-3.03	-1.34		12.61	7.76	8.92
C/L80	-13.90	-4.05	-7.00	-16.17	-2.04	-5.21	-4.97	-2.03	0.55	-11.55	-16.08	-16.44	-12.61		-1.15	-0.76
C/L85	-8.04	-2.79	-3.07	-8.85	-1.36	-2.43	-1.93	-1.12	1.64	-6.27	-9.84	-9.20	-7.76	1.15		0.39
C1.5	-9.35	-3.20	-3.97	-10.36	-1.61	-3.16	-2.68	-1.44	1.26	-7.45	-11.26	-10.72	-8.92	0.76	-0.39	

Critical t-value: 4.303. Level of significance: 0.05. Degrees of freedom: 2. Shaded numbers indicates that H_0 is accepted, i.e. there is no significant difference between means. A t-value has been calculated for each possible combination of samples, making it easy to compare each individual sample to every other sample. By following a vertical column downwards, significant differences are indicated by numbers that are not faded. Sample “S3” for instance, is significantly different only from sample “C/L3.5