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Effects of Hydrocolloids on Cooking Loss and Texture of Fermented Fava Bean

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Abstract

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In this study, the effects of three factors (m-% of SA, KG, and CA) to three response variables, (cooking loss, hardness, and toughness, coded y_1, y_2, and y_3, respectively) of patties made with fermented fava bean were studied. A CCC (central composite circumscribed) experimental design was employed to determine the individual effects and interactions of the three hydrocolloids. To protect trade secrets, the design variables were coded X_1, X_2 and X_3 when reporting the results. A texture analyser was used to measure hardness and toughness of the patties with a method that was developed for this purpose. Cooking loss was measured as weight loss of the patties when pan fried. Regression analysis was used to create mathematical models of the effects of the hydrocolloids to hardness and toughness of the patties.

Surprisingly, the hydrocolloids had no effect on cooking loss with the cooking method used. X_2 had clearly the strongest individual effect on both hardness and toughness. X_1 had a moderate individual effect on hardness and toughness. X_3 had a mild individual effect on hardness as well as a positive interaction with X_1 on hardness. In future studies, texture profile analysis could be used to reveal more detailed information on the effects of hydrocolloids to texture properties. It is possible that a different cooking method, for example oven baking, might highlight the effects on cooking loss as well. The analysis methods developed for this study can be used in future R&D tasks. The results of this study can be used as a reference in product development within MeEat Food Tech.

Keywords: fava bean, fermentation, hydrocolloids

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Tässä insinöörityössä tutkittiin kolmen suunnittelumuuttujan (konjac-hartsin, κ-karrageenin ja natriumalginaatin) vaikutuksia fermentoidusta härkäpavusta valmistettuihin pihveihin. Vastemuuttujat olivat paistohäviö (y_1), kovuus (y_2) ja sitkeys (y_3). Hydrokolloidien pää- ja yhteisvaikutukset selvitettiin CCC (central composite circumscribed) -koesuunnitelman avulla. Yrityssalaisuuksien suojelemiseksi suunnittelumuuttujat koodattiin (X_1, X_2, X_3) tuloksia esitettäessä. Pihvien kovuus ja sitkeys mitattiin rakenneanalysaattorilla käyttäen menetelmää, joka kehitettiin tätä työtä varten. Paistohäviö määritettiin mittaamalla pannulla paistettujen pihvien painohäviö. Matemaattiset mallit hydrokolloidien vaikutuksesta pihvien kovuuteen ja sitkeyteen luotiin regressioanalyysillä.

Hydrokolloideilla ei ollut vaikutusta paistohäviöön käytetyllä paistomenetelmällä. Muuttujista X_2 lisäsi selkeästi voimakkaimmin sekä kovuutta että sitkeyttä. X_1 vaikutti positiivisesti jonkin verran sekä kovuuteen että sitkeyteen. X_3:lla oli lievä positiivinen päävaikutus sekä yhteisvaikutus kovuuteen X_1:n kanssa. Jatkossa hydrokolloidien vaikutuksista rakenteeseen voitaisiin saada yksityiskohtaisempaa tietoa hyödyntämällä rakenneprofiilianalyysiä. On myös mahdollista, että eri paistomenetelmä, kuten uunipaisto, voisi korostaa hydrokolloidien vaikutusta paistohäviöön. Tätä insinöörityötä varten kehitettyjä menetelmiä voidaan hyödyntää tulevissa T&K-projekteissa. Tutkimuksen tuloksia voidaan käyttää lähdemateriaalina MeEat Food Techin tuotekehityksessä.

Avainsanat: härkäpapu, fermentointi, hydrokolloidit

Contents

List of Abbreviations

KGM Konjac glucomannan

FFB Fermented fava bean

ANF Antinutritional factor

SSF Solid state fermentation

SA Sodium alginate

CA κ-carrageenan

KG Konjac gum

G6PD Glucose 6-phosphate dehydrogenase

ADI Accepted daily intake

GDL D-glucono-δ-lactone

CCC Central composite circumscribed, a type of experimental design

1 Introduction

There are multiple motivations to reduce cooking loss and create a firmer texture to plant protein products. Generally, plant proteins lack the mouthfeel and texture of meat, specifically chewiness and firmness. When developing meat substitutes, it is therefore necessary to modify the texture of the products to resemble that of meat. [1, p. 20.] This can be achieved with hydrocolloids, processing methods such as extrusion or a combination of hydrocolloids and processing methods. [2, p. 1] Water content of a product affects its texture and cooking loss directly effects the profitability of a product: when products are sold by weight, any mass loss during processing reduces profitability. In this thesis cooking loss specifically refers to the water evaporated from the product during cooking.

Previously the individual effects of different hydrocolloids in modifying the texture and water retention of meat analogues have been studied. Majzoobi et al. [3, p. 1269] studied the individual effects of κ-carrageenan, konjac glucomannan (KGM) and xanthan gum in soy protein-based sausages. Water holding capacity was shown to increase and cooking loss was found to decrease with increasing concentrations of κ-carrageenan and KGM while the addition of xanthan gum decreased the water holding capacity and increased cooking loss. Texture profile analysis showed that KGM and κ-carrageenan increased hardness and chewiness while springiness and cohesiveness decreased or remained unchanged. Sensory evaluation of acceptability showed an increase in acceptability with KGM and κ-carrageenan. [3, p. 1271–1274.] Dinani et al. [2, p. 1] studied the effect of several hydrocolloids in a mixture of pea protein and wheat gluten. Low acyl gellan gum was found to increase tensile strength of fibrous meat analogues produced in a high-temperature shear shell the most while the highest water holding capacity was achieved with xanthan gum. [2, p. 1, 8, 10.] Although Xu et al. [4, p. 64] have studied the effect of lactic acid fermentation on the rheology of doughs made from fava bean flour, no literature was found regarding texture modification of fermented legumes with hydrocolloids.

Fava bean (*Vicia faba*) is a legume with a high protein content. The protein content of fresh fava bean is approximately 9 % making it an important source of human and animal nutrition. [5, p. 330; 6.] In this study, the individual and combined effects of three hydrocolloids, konjac gum (KG), κ-carrageenan (CA), and sodium alginate (SA), on cooking loss, hardness and toughness of patties made from fermented fava bean, oil and water were studied. The purpose was not to develop a product; therefore the taste of the patties was not focused on. The research questions of this thesis were the following:

- How do the selected additives affect cooking loss of FFB patties in different concentrations in combinations as well as individually?
- How do the selected additives affect the hardness of FFB patties in different concentrations in combinations as well as individually?

The topic was suggested by MeEat Food Tech Oy, a Finnish company developing and selling FFB and products containing FFB as well as other plant proteins under the brand name Muu. [7; 8; 9.]

2 Fermented legumes

To protect trade secrets, the specific process which MeEat uses to ferment fava beans will not be reviewed. Instead, fermentation of fava beans and other legumes will be covered generally. Legumes are a good source of protein with many benefits compared to animal products, including smaller water and land area use and high fibre content. Legumes are also recommended by the Finnish National Nutrition Council as a source of protein. [10; 11.] Use of legumes as human food is limited by their often-unpleasant sensory qualities and antinutritional factors (ANF), which cause indigestion [12, p. 4].

In fermentation, the fermenting organism breaks down the substrate it is growing on and induces changes in organoleptic, nutritional, and structural qualities through enzymatic activity. Fermentations can be roughly divided into two categories: solid state (SSF) and submerged fermentations. As the name implies, in submerged fermentations the substrate is submerged in liquid. In SSF, the substrate consists of solid particles with sufficient moisture for

microbial activity. [12, p. 1, 4.] In this thesis, the focus will be on SSF. Fermentation with mono or cocultures of various bacteria and fungi can be used to improve sensory qualities and digestibility of legumes. The most common microbes utilised are *Bacillus* spp., *Lactobacillus* spp., *Rhizopus* spp. and *Aspergillus* spp., the first two being bacteria and the last two being fungi. The selection of products manufactured globally from different legumes with different microbes is vast. [13, p. 10–12; 12, p. 1.] To give a general picture of SSF processes, two very different types of products are reviewed: *natto* of Japanese origin and *tempe* of Indonesian origin.

2.1 Effect of fermentation on nutritional quality of legumes

In this chapter effects on ANFs will be described for processes utilising lactic acid bacteria, *Bacillus* spp., and *Rhizopus oligosporus*. The specific effects of fermentation to nutritional qualities of legumes depend on the raw material, fermenting organism, and process conditions. [12, p. 4–9.] Nutritional differences between boiled, unfermented fava bean and FFB will be presented.

2.1.1 Antinutritional factors

Legumes contain ANFs, which need to be removed through processing to improve nutrient availability and avoid indigestion or even life-threatening conditions in the case of vicine and convicine. These ANFs include some oligosaccharides, tannins, lectins, trypsin inhibitors, phytic acid and, in the case of fava bean, vicine and convicine. [14, p. 531; 12, p. 11; 15, p. 1.] Raffinose, stachyose, and verbascose are oligosaccharides that ferment in the large intestine and cause flatulence. Tannins crosslink proteins reducing their digestibility. [12, p. 11–12.] Lectins are carbohydrate-binding proteins that can block the absorption of minerals or even cause red blood cells to clump together [16]. Trypsin inhibitors block the action of trypsin, a digestive protease, leading to poor protein availability in legumes [17, p. 17]. Phytic acid complexes among other minerals with calcium, iron, magnesium, and zinc reducing their digestion [12, p. 12–13].

Apart from lectins, the content of all the mentioned ANFs is reduced by lactic acid fermentation, although the efficiency is dependent on the specific bacteria, legume and fermentation process [12, p. 11–13]. *Rhizopus oligosporus* does not metabolise raffinose or stachyose and therefore does not reduce flatulence caused by these oligosaccharides [18, p. 619; 19, p. 250–251]. Fermentation with *R. oligosporus* decreases the content of phytic acid and increases the content of tannins, apparently due to hydrolysis of tannin polymers. The content of trypsin inhibitors has also been found to increase due to proteases that release bound trypsin inhibitors to a soluble form. The released trypsin inhibitors become heat sensitive and can subsequently be denatured by heating. [14, p. 537–538.] The exact temperature and heating time required to inactivate trypsin inhibitors depends at least on the type of heat treatment, legume, and possible pre-processing such as soaking. In unfermented soybeans, boiling at 100 ºC for 9 minutes inactivates most of the trypsin inhibitors. [17, p. 18–20.] Fermentation with *Bacillus* bacteria has been shown to increase availability of minerals and decrease the content of trypsin inhibitors in soybean [20, p. 521; 14, p. 538]. Soaking and cooking of legumes are steps that are in common between all fermentation processes. They have been shown to decrease the content of phytic acid, lectins, tannins, raffinose and stachyose. [21, p. 162; 16; 19, p. 250–251.]

2.1.2 Nutritional changes in fava bean

Although the Muu Fava™ process is a trade secret, the nutritional values of FFB are naturally publicly available. The nutritional values of dried and boiled fava bean and FFB are listed in Table 1.

Table 1. Nutritional values of boiled fava bean and MUU Fava™.

Nutritional value per 100 g	**Fava bean, dried, boiled [22]**	**MUU Fava™ [23]**
Energy	427 kJ	415 kJ
Fat (saturated)	0.4 g (<0.1 g)	1.6 g (0.3 g)

Protein	7.6 g	17.4 g
Carbohydrates (sugars)	14.1 g (1.8 g)	6.1 g (0.5 g)
Fibre	5.4 g	5.6 g
Salt	0 g	0.1 g
Iron	1.5 mg	3.5 mg

During Muu Fava™ fermentation carbohydrates are consumed, and proteins and fat are produced. Total energy content remains practically the same. A considerable difference can also be seen in the iron content, which more than doubles.

Compared to other legumes, fava beans contain two additional ANFs, which need to be removed through processing: vicine and convicine. Vicine and convicine are glycosides, which, when hydrolysed, release divicine and isouramil. If ingested by an individual with a genetic glucose 6-phosphate dehydrogenase enzyme (G6PD) deficiency, these compounds cause a condition called favism, where the individuals red blood cells rupture. In ethnically Finnish population, G6PD deficiency has only been detected in a few family lines, but in Mediterranean, Middle Eastern and Asian populations it is more common. [15, p. 1; 24.] Concentrations of vicine and convicine have been shown to reduce significantly when soaked for 16 hours in a 1 % acetic acid solution. [25, p. 1056–1057]. Rizzello et al. [15, p. 6, 8] found that fermentation of fava bean flour by *Lactobacillus plantarum* degraded vicine and convicine and their hydrolysis products divicine and isouramil efficiently and considerably more compared to spontaneous lactic acid fermentation.

2.2 Effect of fermentation on technical properties of legumes

Research on the effect of fermentation on the technical properties (water and fat retention, emulsifying ability) of fava beans was not found. Instead, these effects in other legumes will be reviewed. Through modification of protein structure, fermentation influences the water and oil retention and emulsifying

properties of legumes. In lactic acid fermentation, microbial proteases lyse the legume proteins to smaller peptides. This may expose previously inaccessible hydrophilic sites, which interact with water. Lactic acid fermentation with various bacteria has been shown to increase water retention in pea, lupin, and chickpea proteins. [12, p. 17–18.] In a tempe-style process using *R. oligosporus* Barinderjeet et al. [26, p. 1768] noticed an increase in the water retention of chickpeas and a reduction in pigeon peas and soybeans. Similarly, the oil retention capacity is altered due to changes in protein structure, although oil retention is dependent on the accessibility of hydrophobic sites in proteins. Whether the effect of fermentation on oil retention is reducing or increasing, depends on the fermenting organisms and the legume. [12, p. 17–18.]

In an oil-in-water emulsion, proteins that have both hydrophobic and hydrophilic sites, can adsorb at the interface of an oil droplet and water and stabilise the emulsion 27, p. 711]. Once again, the effect of fermentation on the emulsifying properties of legumes is dependent on the fermenting organism, legume, and process conditions. Lactic acid fermentations have produced varied results. [12, p. 18.] Barinderjeet at al [26, p. 1768–1769] observed a significant improvement in emulsifying properties of chickpeas, pigeon peas and soybeans in their tempe-style *R. oligosporus* fermentation. Improvement of emulsifying properties has been linked to the formation of small protein molecules during fermentation, which adsorb at the interface of oil droplets and water. Conversely, deterioration of emulsifying properties is thought to be caused by the aggregation of proteins, which weakens their amphiphilic quality. [26, p. 1769; 12, p. 18.]

2.3 Examples of fermented legumes

2.3.1 Natto

Natto is a Japanese dish made with fermented soybeans. Modern commercial natto is produced using a monoculture of *Bacillus subtilis* var. *natto*. After fermentation, the soybeans are covered in a characteristic sticky mucus (Figure 1) [13, p. 10, 12].

Figure 1. Fermented natto [28].

The preparation of natto begins by soaking soybeans in 20 ºC water for 16–20 hours or until the mass of the beans has increased two-fold. After soaking, the beans are cooked by steaming, cooled to 50 ºC and inoculated with a pure inoculum of *B. natto* spores. Before fermentation the pH of steamed soybeans is approximately 6,8. The beans are fermented at 40 ºC usually for 20–30 hours. Natto is typically fermented in the styrofoam trays in which it is also sold (Figure 1). [29, p. 415; 30, p. 168; 31, p. 4418.] Quality natto is viscous, soft and has a slight flavour of ammonia [30, p. 170]. Kim et al. [32, p. 2008] analysed 21 commercial natto products and found water activities between 0.93–0.97, and pH values of 6.75–7.47. The increase in pH during fermentation is associated with the activity of proteolytic enzymes of *B. subtilis*. Degradation of soy protein increases the content of soluble nitrogen, such as ammonia, increasing pH and contributing to the development of the typical flavour of natto. [33, p. 1199.]

Nattokinase is a protease produced by *B. subtilis* during natto fermentation, that can dissolve blood clots. Nattokinase has been shown to withstand the

conditions of the human digestive tract and absorb whole into the blood stream. Nattokinase is claimed to be an effective way to thin the blood, prevent blood clots and reduce blood pressure. [34, p. 1–3; 35, p. 100–102.] Furthermore, nattokinase extracted from natto was accepted as a novel food in the EU in 2017 [36].

During natto fermentation, the intense microbial activity creates ideal conditions for the formation of biogenic amines. [31, p. 4415; 37, p. 2]. Biogenic amines are derivatives of ammonia, that in sufficiently high amounts cause symptoms such as sweating, headache and diarrhoea. Besides natto, other high-risk products include fish and matured cheese. [38, p. 404; 39.] Histamine and tyramine are considered to be the most toxic of the biogenic amines and therefore most important in regard to food safety. Tsai et al. [40, p. 1029] analysed biogenic amine contents from 39 commercial natto products and found at most 46 mg of histamine and 5 mg of tyramine in 100 g of natto. The European Food Safety Authority [37, p. 3] has stated that there is insufficient data to determine safe intake levels for biogenic amines, but for healthy individuals 50 mg of histamine or 600 mg of tyramine per meal has not caused symptoms. The fairly high histamine concentrations found by Tsai et al. [40, p. 1029] nonetheless indicate that the formation of biogenic amines is a risk factor in natto production and needs to be taken into account.

2.3.2 Tempe

Tempe is a traditional Indonesian dish, which is most often made with yellow soybeans. Many variations made with other legumes, wheat, barley, or coconut exist. The fermenting organisms are members of the *Rhizopus* genus of filamentous fungi, of which *Rhizopus microsporus* var. *oligosporus* is the most common. The process conditions vary according to raw material and scale of production. After fermentation, the raw material is surrounded by a white mycelium forming a dense cake (Figure 2). Tempe is spiced and cooked before consumption. [18, p 609–610, 612–613, 619.] Here a process using soybeans

as a substrate and *Rhizopus oligosporus* as the fermenting organism is described.

Figure 2. Fermented tempe [41].

As with natto, the process of manufacturing tempe starts by soaking the dehulled soybeans for a minimum of 12 hours at 28 ºC. During soaking, the pH of the soybeans is lowered to 4.5–5.5 through spontaneous lactic and acetic acid fermentations. Although the bacteria responsible for acidification mostly die when the beans are cooked before inoculation, acidification is an important step that helps reduce growth of contaminants during fermentation. [18, p. 616–618] The soaked and cooked soybeans are cooled, surface dried and inoculated with a pure *R. oligosporus* starter culture. *R. oligosporus* is the dominant organism during fermentation, but significant amounts of bacteria and yeasts are also present since the substrate is not sterile. The beans are packed tightly into a fermentation vessel and covered with a perforated material to achieve low-oxygen conditions. Perforation of the cover is crucial since the oxygen concentration should remain between 0.4 and 2 volume-% and the CO_2 concentration below 16 volume-%. Growth of *R. oligosporus* stops at a CO_2

concentration of 35 volume-%. Excessive oxygen concentration may cause undesired sporulation of the fungus as well as overheating of the substrate due to rapid fungal growth. Overheating favour thermophilic bacteria and may result in poor quality tempe. The fermentation lasts for 1-2 days at 30–40 ºC. [29, p. 416; 18, p. 615, 623; 42, p. 793]

During fermentation, *R. oligosporus* modifies the soybean substrate with a wide array of enzymes that degrade carbohydrates, proteins, and fat into lower molecular weight compounds. *R. oligosporus* uses oleic acid as an important source of energy. As a result of enzymatic activity, free amino acid and free fatty acid contents are increased, while hemicellulose content is reduced. pH of the substrate will rise from 4.5–5.5 to 6–7. [18, p. 616, 623–625.] Sparringa et al. [43, p. 680] concluded that there are unknown mechanisms for alkalinisation during the growth phase of *R. oligosporus* in tempe. Nevertheless, protein metabolism resulting in the formation of ammonia and oxidation of organic acids are important mechanisms for pH elevation during tempe fermentation. After the active growth stage, pH elevation has been shown to be fully caused by ammonia production. [18, p. 623–625; 43, p. 677–678, 680.] Good quality tempe is firm, has a pH between 6 and 7, approximately 60 % moisture and a mild odour. Fresh tempe should be consumed soon after fermentation as it will eventually degrade due to enzymatic activity. Ammonia production and elevation of pH will continue during storage and a flavour of ammonia will be detectable at pH values 7,5–8. [18, p. 611–612, 616, 624; 43, p. 677.]

Similarly to natto, the conditions during tempe fermentation are ideal for the production of biogenic amines. [37, p. 2] Nout et al. [44, p. 300] analysed commercial and laboratory made tempe and found the total amount of biogenic amines to be 1000–4000 mg per kilogram of dry matter. Assuming a moisture content of 60 % and 150 g serving size, this would correspond at most to approximately 360 mg of total biogenic amines in one meal. Considering the negligible levels of histamine Nout et al. [44, p. 298–299, 301] detected, this level of biogenic amines would stay well within the levels where no adverse health effects have been found in healthy individuals [37, p. 3].

3 Effect of hydrocolloids on cooking loss and texture of foods

Hydrocolloids are defined as hydrophilic high molecular weight polymers, which form colloids in aqueous solutions, swell and increase the viscosity of the solution. In sufficiently high concentrations, individual hydrocolloid molecules can link to form gels. Hydrocolloids are sourced from plants, algae, animals, and bacteria. [45, p. 2; 46.] By volume, the most important hydrocolloids commercially are animal-based gelatin with a 46 % share of the global market, plant-based guar gum with a 12 % share, and algae-based carrageenan with a 9 % share [47, p. 5]. The properties of the three hydrocolloids selected for this study, konjac gum (E425i), κ-carrageenan (E407) and sodium alginate (E401), will be reviewed in the following subsections.

3.1 Konjac

Konjac (*Amorphophallus konjac*) is a plant originating in Southeast Asia. Konjac glucomannan is a polysaccharide that makes up 8–10 % of the mass of the plant's tubers (Figure 3). The tubers are processed into a powder, of which KGM is the main component. KGM comprises of two monomers: D-glucose and D-mannose linked by β-1,4 glycosidic links. Additionally, the polymer contains approximately one acetyl group for every 19 sugar residues. [48, p. 889–890, 892, 895.]

Figure 3. Konjac tuber [49].

The molecular weight of KGM is an important indicator of quality. The higher the molecular weight, the higher the viscosity of the solution is. Viscosity of pure KGM solutions is negatively affected by a pH below 4 but are unaffected by salt. Solution viscosity starts to rise sharply at KGM concentrations above 1 %: in pure water the viscosity rises from 32 000 cps at 1 % to 340 000 cps at 2 %. However, in the EU konjac has a specific maximum usage level of 1 % in food. [48, p. 896–899; 50, p. 39.] Thermally irreversible (does not melt when heated after gelling) KGM gel can be prepared by mixing a minimum of 0,5 % of konjac

in an alkaline calcium hydroxide solution. With κ-carrageenan, KGM has a synergistic effect. Together they form thermally reversible gels which are strengthened by the addition of approximately 1 % of salt although increasing concentrations weaken the gel. At a 3 % salt concentration, the gel does not form. Globally, konjac gels are used in a wide variety of products from dessert jellies and confectionery to pasta and beverages to retain moisture and improve texture. In meat products konjac has been used to retain moisture and as a fat replacer in low-fat meat products. [48, p. 891, 899–900.]

In the European Union, konjac (E425) is considered a food additive. Regulation No 1333/2008 on food additives makes a distinction between konjac gum (E425i) and KGM (E425ii). The difference between the two is in the washing method: konjac gum is produced by washing konjac flour with water whereas KGM is produced by washing with an aqueous ethanol solution resulting in a more concentrated, higher molecular weight product. Konjac gum has an average molecular weight of 200 000 g/mol – 2 000 000 g/mol and konjac glucomannan 500 000 g/mol – 2 000 000 g/mol. Konjac is not allowed to be used in jelly mini-cups, jelly confectionery or dehydrated foods, which rehydrate when ingested. Neither additive has an accepted daily intake (ADI) value, but a specific maximum level to be used in food is set at 10 g/kg individually or in combination. The maximum daily intake from all sources should stay below 3 g. Excessive consumption of konjac can cause digestive discomfort: during a 12-week human trial, a daily dosage of 3 g caused diarrhoea and constipation. [50, p. 39; 51, p. 1, 11–12.]

3.2 Carrageenans

Carrageenans are polysaccharides produced from the red algae *Euchema cottonii*, *E. denticulatum*, *Kappaphycus alvarezii* and *Gigartina* spp. The type of carrageenan varies between species and even individual plants. It is therefore essential to characterize the raw material before processing. The washed seaweed is treated with alkali to extract the carrageenan. Carrageenan is then precipitated from solution with isopropyl alcohol, dried and ground to a powder.

Several types of carrageenan powders are mixed to obtain a consistent quality and suitable properties for specific uses. [52, s. 165–167.]

Carrageenans consist of a galactose main chain with a varying amount of 3,6-anhydrogalactose and sulphate groups. The amount and location of the 3,6-anhydrogalactose and sulphate groups determine the solubility, melting and setting temperatures and the resulting texture of the carrageenan solution. There are three main types of carrageenans in the market: κ (kappa), λ (lambda) and ι (iota). ι- and κ-carrageenans produce thermally reversible gels, whereas λ-carrageenan gives viscosity to solutions. κ-carrageenan produces hard and brittle gels compared to the soft and elastic gels made from ι-carrageenan. Structurally, κ-carrageenan contains more 3,6-anhydrogalactose and less sulphate groups compared to ι-carrageenan, whereas λ-carrageenan does not contain 3,6-anhydrogalactose at all. [52, 164–165, 169.]

The hydration and gelling properties of κ-carrageenan are strongly affected by potassium ions. Potassium ions raise the hydration, setting and melting temperatures and of κ-carrageenan and increase the strength of the resulting gel. Setting temperature refers to the temperature at which the gel forms after hydration of the polysaccharide. There is a difference between setting and melting temperatures of κ-carrageenan gels: once the gel has set it will require a temperature of 5-20 ºC higher than the setting temperature to melt the gel. Depending on the concentration of potassium, the setting temperature can be between 40–70 ºC. When heated at pH <4,3, the strength of κ-carrageenan gels drops due to hydrolysis of the 3,6-anhydrogalactose link. [52, p. 170–174.]

In the EU, carrageenans (E407) are regulated as food additives. Carrageenans have an ADI of 75 mg/kg/day, but no specific maximum level, except in jellies and marmalades. Carrageenans are not allowed to be used at all in jelly mini-cups. In other foods, carrageenans can be used with the *quantum satis* principle, defined in Regulation No 1333/2008 on food additives [50, p. 39, 42; 53]:

> ‘*quantum satis*’ shall mean that no maximum numerical level is specified and substances shall be used in accordance with good manufacturing practice, at a level not higher than is necessary to achieve the intended purpose and provided the consumer is not misled. [50, p. 7]

3.3 Alginates

Alginates are polysaccharides found in brown algae, such as *Laminaria hyperborea* and *Macrocystis pyrifera*, that have water binding, gelling and viscosifying properties. Alginates make up as much as 40 % of the dry mass of the algae. The polysaccharide consists of 1,4-linked β-D-mannuronate and α-L-guluronate. [54, p. 807, 809–810, 55, p. 160–161.] Sodium, potassium, ammonium, and calcium salts of alginic acids (E401–E404) are used industrially and regulated as food additives in the EU. No ADI has been set for any alginate. Alginates are not allowed in jelly mini-cups and specific maximum levels have been set for jams and marmalades. [50, p. 355; 56; 57; 58; 59.]

3.3.1 Gelling mechanics

Alginate gels are cold setting and thermo-irreversible, meaning they do not require heating to gel and, once gelled, do not melt when heated. Despite being cold setting, gelling temperature effects the speed of gelling and the properties of the gel. Alginates gel in the presence of Mg^{2+}, Ca^{2+}, Sr^{2+} and Ba^{2+} ions by forming ionic crosslinks. Alginates have a stronger affinity for Ca^{2+} compared to Mg^{2+}, making Ca^{2+} the most suitable crosslinking ion for food applications. [54, p. 811, 817.] The length of the polymer and ratio of mannuronate and guluronate and their sequence vary between brown algae species and within an individual plant. Mannuronate and guluronate residues form blocks of varying length in the polymer: M-blocks consisting of purely mannuronate, G-blocks consisting of purely guluronate and MG-blocks consisting of alternating mannuronate and guluronate. All these structural factors affect the viscosity and gelling properties of the alginate. [54, p. 808–810.] Length of the polymer directly effects the molecular weight, which in turn effects viscosity and gel

strength. [54, p. 815.] Draget et al. [55, p. 174, 177] concluded that gel strength increases with increasing G-block length and guluronate content.

3.3.2 Alginates in food applications

Alginates gel almost instantly when mixed with Ca^{2+} most often resulting in a lumpy product if simply mixed. As mentioned earlier, the composition of the alginate affects the gelling properties; nevertheless to achieve homogenous gelling, Ca^{2+} needs to be introduced to the product in a controlled fashion. There are two main techniques with which this can be achieved: diffusion setting and internal setting. Briefly, in the diffusion technique Ca^{2+} is allowed to diffuse to the product from an external source inducing rapid gelation. This method is used for example in the manufacture of onion rings. [54, 817–820.] In this study the internal setting technique will be employed due to it being the simpler method of the two for this application.

In the internal setting technique, a poorly soluble source of Ca^{2+}, for example $CaCO_3$ or $CaSO_4$, is mixed with the product. In this study, $CaCO_3$ is used due to it being insoluble in the absence of acids and therefore offering a higher degree of control to the gelation compared to $CaSO_4$. [54, p. 821.] Calcium ions are released from $CaCO_3$ by the addition of acid, which triggers the following reaction:

$$CaCO_3\,(s) + 2\,H^+\,(aq) \longrightarrow Ca^{2+}\,(aq) + CO_2\,(g) + H2O\,(l)$$

Draget et al. [55, p. 160] used D-glucono-δ-lactone (GDL) and $CaCO_3$ to achieve homogenous gelling. GDL slowly hydrolyses to gluconic acid, which in turn donates a proton [55, p. 160; 60, p. 927]. The advantage of GDL compared other acids is the slow release of protons and an even higher degree of control over the speed of gelation. [55, p. 163.] It can be seen from the reaction equation of $CaCO_3$ and protons, that their molar ratios should be 1:2 to avoid acidification of the product. The optimal ratio of alginate and $CaCO_3$ depends on the guluronate content of the alginate and the concentration of non-gelling ions,

such as Na^+. Below this optimal level syneresis occurs. Above the optimal level, the gel begins to weaken. The mechanism behind this phenomenon is not fully understood, but the presence of sodium ions reduces the undesired effects of non-optimal concentrations of $CaCO_3$. [54, p. 821–822; 55, p. 173–174.]

4 Materials and methods

4.1 Preparing the FFB mass and patties

The fermented fava bean mass was made from FFB (MeEat Food Tech Oy, Finland), rapeseed oil (Keiju Rypsiöljy, Bunge Finland Oy), tap water and mineral salt (Pan Suola, Oriola Finland Oy) containing potassium chloride. Sodium alginate (SA, E401), κ-carrageenan (CA, E407) and konjac gum (KG, E425i) (all three provided by MeEat, no additional information available) were added according to the experimental design, along with $CaCO_3$ (provided by MeEat, no additional information available) and food grade 80 m-% lactic acid (Galacid Excel 80, Galactic, Belgium) to the masses containing SA. Vegetable oil was added to make the fat content of the mass resemble that of an actual product. Water was added to make the mass softer and easier to handle. Mineral salt was used to provide a source of K^+ for the gelation of CA. $CaCO_3$ and lactic acid were used to provide a source of Ca^{2+} for the gelation of SA. Different FFB batches were used in the three preliminary sets of experiments and in the main experiments. However, in the main experiments, a single batch (batch code 114110100, use-before-date 31.1.2024) was used to avoid the effect of batch variation on results.

A Create Chefbot Touch Cooking Robot (Woods and Go Desing, S.L., Spain) was used to homogenise the FFB masses. First, the FFB, water and mineral salt were homogenised for 30 seconds, along with SA, $CaCO_3$, CA and KG as required. SA was hydrated for a few minutes in tap water included in the recipe before adding it to the mass. This was done to prevent the negative impact of mineral salt to hydration of SA [54, p. 814]. Next, vegetable oil was added and homogenised for 30 seconds. Finally, to the masses containing SA, lactic acid

diluted in 10 ml of tap water included in the recipe, was added, and homogenised for 30 seconds. FFB was used at a temperature of 5 °C. All other ingredients were used at room temperature. The patties were made immediately. To obtain patties of even diameter and thickness, 30 g of FFB mass was weighed on a piece of baking paper and shaped using a circular mould with a diameter of 6 cm.

Figure 4. Fried patties.

The mass was soft and sticky when uncooked and crumbly when cooked. For this reason, the patties were pan fried on individual pieces of baking paper to prevent mass loss and ease handling. The patties were fried for 5 minutes per side on medium heat as shown in Figure 4.

4.2 Analysis of cooking loss

A VWR electrical precision balance was used to record the uncooked mass of the patties to 0,01 grams. After frying, the patties were cooled to room temperature and weighed again. Percentual cooking loss was calculated with the following formula:

$$Cooking\ loss = \frac{m_{raw} - m_{cooked}}{m_{raw}} * 100\% \quad (1)$$

m_{raw} is the raw mass of the patty (g)

m_{cooked} is the cooked mass of the patty (g)

4.3 Texture analysis

After weighing the room temperature patties, their hardness [kg] and toughness [kg*s] were analysed using a TA.XT Plus C texture analyser (Stable Micro Systems, United Kingdom). Settings of the analysis method are presented in Table 2. In developing the texture analysis method, a pre-made method from Stable Micro Systems' database was used as a starting point. During preliminary experiments, the parameters were adjusted to suit the needs of the FFB patties.

Table 2. Parameters of texture analysis method.

Parameter	**Value**
Probe	Blade set with knife
Platform	Heavy-duty platform
Load cell	5 kg
Test mode	Compression
Pre-test speed	2.00 mm/s
Test speed	5.00 mm/s
Post-test speed	10.00 mm/s

Target mode	Distance
Distance	25.000 mm
Trigger type	Auto (Force)
Trigger force	20.0 g

The developed method begins measuring force when the device detects a force of 20.0 g. The trigger force was set very low due to the softness of the patties. The blade proceeds for 25 mm after initiating force measurement and returns to starting position. Hardness was defined as the maximum force of shear, while toughness was defined as the work of shear. The analyses were conducted with a straight blade and a slotted guide, allowing the patty to be cut all the way through (Figure 5).

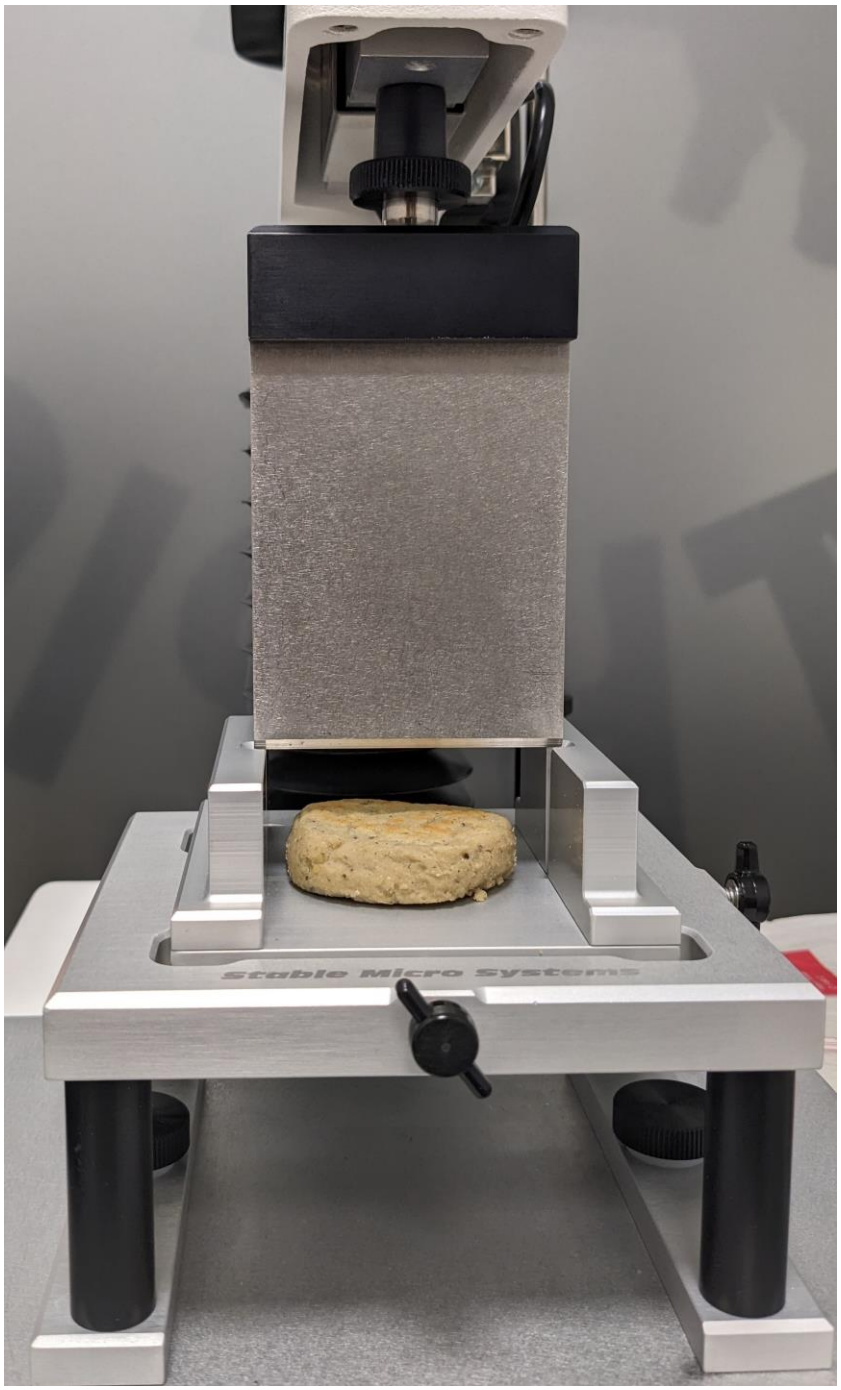

Figure 5. Texture analysis setup. Sample 3 from Experiment 6.

The straight blade was selected to obtain a representative result of bite force [61].

4.4 Preliminary experiments

Three sets of preliminary experiments were conducted to ensure the functionality of the mass without hydrocolloids and to determine their appropriate maximum concentrations. Criteria for a functional FFB mass, applied in every experiment, were as follows:

- homogenous mass,
- easy to shape into patties,
- easy to pan fry without breaking the patty and
- oil or water do not separate from the mass.

First, a suitable composition of the mass without additives was determined (Table 3).

Table 3. Recipe of FFB mass without additives.

Ingredient	**Mass (g)**	**Mass-%**
FFB	250	80.6 %
Rapeseed oil	30	9.7 %
Water	30	9.7 %
Total mass	310	-

Next, suitable concentrations of SA, $CaCO_3$ and lactic acid as well as appropriate methods to produce a homogenous mass with SA were developed. Since the ingredients and their concentrations vary between FFB masses, the total weight of each mass was set at 310 g. The combined mass of FFB, rapeseed oil and water in each mass was calculated by subtracting the mass of all other ingredients from the total mass of 310 g. The ratios of FFB, oil and water of their combined mass were set at 80.6 % for FFB and 9.7 % for oil and water based on the recipe of FFB mass without additives (Table 3). The formulae for calculating the mass of FFB, oil and water were as follows:

$$m_{FFB+O+W} = 310\ g - m_{MS+SA+CC+LA+KGM+CA} \quad (2)$$

$$m_{FFB} = 0.806 * m_{FFB+O+W} \quad (3)$$

$$m_{O,W} = 0.097 * m_{FFB+O+W} \quad (4)$$

where $_{FFB+O+W}$ is the combined mass of FFB, oil and water (g), $m_{O,W}$ is the mass of oil and water (g), and $m_{MS+SA+CC+LA+KGM+CA}$ is the combined mass of mineral salt, sodium alginate, $CaCO_3$, lactic acid, konjac glucomannan and κ-carrageenan (g).

Ratios of $CaCO_3$ to SA and lactic acid to $CaCO_3$ were based on the work by Draget et al. [55, p. 163], who used 30 mM GDL and 15 mM $CaCO_3$ for 1 % (w/v) SA solutions. The ratio of acid and $CaCO_3$ agrees with the reaction equation presented in chapter 3.3.1. In this study, lactic acid was used instead of GDL. Gluconic acid and lactic acid are both monoprotic, meaning they can be used in the same molar concentrations [55, p. 163; 38, p. 191]. For simplicity, the concentrations of $CaCO_3$ and lactic acid were converted directly from mol/l to mol/kg. Detailed calculations can be found in Appendix 1. SA was tested at two concentrations: 1 m-% and 2 m-%. 2-m% of alginate resulted in an excessively hard mass, while 1 m-% produced a mass that was firm and easy to handle. As mentioned earlier, the optimal ratio of $CaCO_3$ to SA depends on the guluronate content of the alginate [55, p. 173–174]. No information on the guluronate content of the alginate used was available. Therefore, higher $CaCO_3$ concentrations (0.5 m-%, 1.0 m-%) were tested. No beneficial effects were detected in cooking loss or texture and in fact the hardness and toughness of the patties appeared to be lower with 1 m-% $CaCO_3$ (Appendix 2). This observation would seem to agree with the results of Draget et al. [55, p. 173–174], who concluded that an excessive amount of $CaCO_3$ weakens the alginate gel. In conclusion, maximum concentrations of SA, $CaCO_3$ and lactic acid (80 m-%) were determined to be 1.0 m-%, 0.2 m-% and 0.3 m-%, respectively (Table 4).

Table 4. Recipe of FFB mass with 1 m-% sodium alginate.

Ingredient	**Mass (g)**	**Mass-%**
FFB	245	79.0

Rapeseed oil	29	9.5
Water	29	9.5
Mineral salt	2.0	0.6
Sodium alginate	3.0	1.0
$CaCO_3$	0.47	0.2
Lactic acid (80 m-%)	1.05	0.3

Finally, a third set of experiments was conducted to ensure the functionality of the mass with maximum amounts of all three hydrocolloids present simultaneously and to determine an estimate of experimental errors. Use of KG is limited to 1 m-% in the EU [50, p. 39]. CA has an ADI of 75 mg/kg/day [53]. For a person weighing 60 kg, this would mean a maximum daily intake of 4.5 g. With a concentration of 1 m-% of CA, this would be equivalent to consuming 450 g of the product in question. Daily intake should therefore stay well within the ADI with expected consumption. Therefore, SA, KG, and CA were all used at a level of 1 m-%. Three FFB masses with the same composition were prepared. Three patties were made from each mass, which were analysed for cooking loss, hardness, and toughness. Full results and the recipe of the mass can be found in Appendix 3. Results for each mass as well as the combined results are presented in Table 5.

Table 5. Texture and cooking loss with maximum concentrations of SA, KG, and CA (average ± standard deviation).

Property	**Mass I**	**Mass II**	**Mass III**	**Total**
Hardness (kg)	1.19 ± 0.06	1.29 ± 0.15	1.41 ± 0.10	1.30 ± 0.11
Toughness (kg*s)	3.13 ± 0.12	3.49 ± 0.11	3.51 ± 0.14	3.38 ± 0.21
Cooking loss (%)	9.1 ± 0.5	11.4 ± 1.0	10.9 ± 0.2	10.5 ± 1.2

The mass proved homogeneous, firm, and easy to handle. Preliminary estimates of experimental errors were determined, and the experiment proved repeatable. In conclusion, maximum concentrations were confirmed as 1 m-% for SA, CA, and KG.

4.5 Experimental design

A central composite circumscribed (CCC) design was selected for the main experiments. Lecturer Eija Koriseva from Metropolia UAS was consulted when choosing the appropriate experimental design. A CCC design is comprised of three parts: 2^N, or factorial, experiments, axial experiments, and centre point experiments for evaluating experimental error. In 2^N experiments, each factor has two levels. 2^N experiments reveal the main effects and interactions of the factors, but not possible quadratic effects. Since KG and CA form synergistic gels, it is plausible that quadratic effects are present. To evaluate these effects, axial experiments were required [62; 63] In this study, the effects of three factors (m-% of SA, KG, and CA) to three response variables, (cooking loss, hardness and toughness, coded y_1, y_2, and y_3, respectively) were studied. The experimental design and results will be presented with coded factors to protect trade secrets. In a CCC experiment, each factor is present at five levels. In coded units, these levels are ±α, ±1 and 0 [64]. The value of α, which is the distance of the axial experiment from the centre point 0 in coded units, is calculated with the following formula [62]:

$$\alpha = (2^N)^{1/4} \quad (5)$$

N is the number of factors

With three factors, α equals 1.682. On the basis of preliminary experiments, the real values of all three factors corresponding to -α and α were set at 0 m-% and 1 m-% respectively. Using these settings, corresponding real values were calculated for coded values 0 and ±1 (Table 6, Appendix 4).

Table 6. Coded and real values of each factor.

Coded units	**X_1 [m-%]**	**X_2 [m-%]**	**X_3 [m-%]**
-1.682	0.00	0.00	0.00
-1	0.20	0.20	0.20
0	0.50	0.50	0.50
1	0.80	0.80	0.80
1.682	1.00	1.00	1.00

A CCC design was created with 8 factorial experiments, 6 axial experiments and 6 centre point experiments (Table 7). The recipes for each mass can be found in Appendix 5. In the factorial portion, an experiment is performed with every combination of levels at values ±1. The axial portion is generated by assigning one factor at a time the value ±α and the rest 0. [65; 66, p. 84.] Each experiment consisted of preparing the FFB mass, making 3 patties and performing the measurements. The order of execution was randomized by assigning a random number to each experiment and ordering them from smallest to largest. The result of each experiment was defined as the average of three patties. Five experiments were performed in a day for 4 days. The centre point experiments were randomized, but it was ensured that each day at least one centre point experiment was performed to control variability between days [67].

4.6 Statistical analysis

Statistical analysis of the results was done in Microsoft Excel. Independence and normal distribution of the centre point experiments were evaluated graphically by drawing scatter and normal probability plots. A scatter plot is created by plotting the observations in order of execution. If the values do not show a clear decreasing or rising trend, the results can be said to be independent of the order of execution. [68, p. 55–56.] A normal probability plot can be used to detect outliers and is created by ordering the observations from smallest to largest and assigning each a running number. The corresponding

values of the x axis are calculated by taking the inverse cumulative distribution function of a number calculated with the following formula [68; 69, p. 57–59.]:

$$R_i - \frac{0,5}{N} \tag{6}$$

where R_i is the running number of the observation, $i = 1 \dots 6$, and N is the number of observations, $N = 6$.

The table made for normal probability plots can be found in Appendix 6. For the purposes of regression analysis, a table including main effects, two and three-factor interactions and quadratic effects in coded units was created (Appendix 7). Regression analysis was performed with Excel's built-in analysis tool. The full model to be fitted to each response variable is as follows:

$$y_i = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{123}X_1X_2X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2$$

where y_i is the response variable, $i = 1, 2, 3$, X_i is the mass percentage of the hydrocolloid, $i = 1, 2, 3$, and b is the coefficient of the term.

The models created by regression analysis were evaluated for fitness by comparing the standard error of residuals and experimental error calculated from the centre point experiments. If the errors are similar, the model can be said to be fitting. [66, p. 91.] The coefficient of determination, R^2, was used to evaluate how well the model can be used to predict values of the response variables. After the first regression analyses, the statistically significant coefficients ($P \leq 0.05$) were used to iterate the analyses. Statistically significant coefficients were picked for the final model.

5 Results

The cooking loss, hardness, and toughness of three replicates from each mass were analysed. The full results of cooking loss, hardness, and toughness

analyses of the CCC experiments can be found in Appendix 8. In Table 7 the results are presented as averages of three patties. Standard deviations were calculated from the averages of centre point experiments.

Table 7. Results of CCC experiments in systematic order expressed as average of three patties (average ± SD).

Experiment no.	**X_1**	**X_2**	**X_3**	**Cooking loss (y_1) [%]**	**Hardness (y_2) [kg]**	**Toughness (y_3) [kg*s]**
1	-1	-1	-1	8.8 ± 0.6	0.47 ± 0.05	1.18 ± 0.16
2	1	-1	-1	9.2 ± 0.6	0.51 ± 0.05	1.44 ± 0.16
3	-1	1	-1	10.4 ± 0.6	0.79 ± 0.05	2.15 ± 0.16
4	1	1	-1	7.7 ± 0.6	0.70 ± 0.05	2.13 ± 0.16
5	-1	-1	1	7.9 ± 0.6	0.53 ± 0.05	1.29 ± 0.16
6	1	-1	1	9.5 ± 0.6	0.62 ± 0.05	1.48 ± 0.16
7	-1	1	1	7.9 ± 0.6	0.68 ± 0.05	1.78 ± 0.16
8	1	1	1	8.4 ± 0.6	0.85 ± 0.05	2.30 ± 0.16
9	1.682	0	0	7.6 ± 0.6	0.71 ± 0.05	1.89 ± 0.16
10	-1.682	0	0	8.6 ± 0.6	0.61 ± 0.05	1.44 ± 0.16
11	0	1.682	0	8.6 ± 0.6	0.79 ± 0.05	2.18 ± 0.16
12	0	-1.682	0	9.2 ± 0.6	0.42 ± 0.05	1.07 ± 0.16
13	0	0	1.682	8.8 ± 0.6	0.70 ± 0.05	1.75 ± 0.16

14	0	0	-1.682	8.8 ± 0.6	0.64 ± 0.05	1.76 ± 0.16
15	0	0	0	8.6 ± 0,6	0.61 ± 0,05	1.48 ± 0.16
16	0	0	0	8.3 ± 0.6	0.71 ± 0.05	1.64 ± 0.16
17	0	0	0	8.5 ± 0.6	0.57 ± 0.05	1.43 ± 0.16
18	0	0	0	8.2 ± 0.6	0.61 ± 0.05	1.55 ± 0.16
19	0	0	0	7.9 ± 0.6	0.60 ± 0.05	1.56 ± 0.16
20	0	0	0	9.5 ± 0.6	0.65 ± 0.05	1.88 ± 0.16

Centre point experiments were evaluated to be approximately normally distributed and independent of order of execution for all response variables. Normal probability and scatter plots for toughness are presented as examples of said plots in Figures 6 and 7.

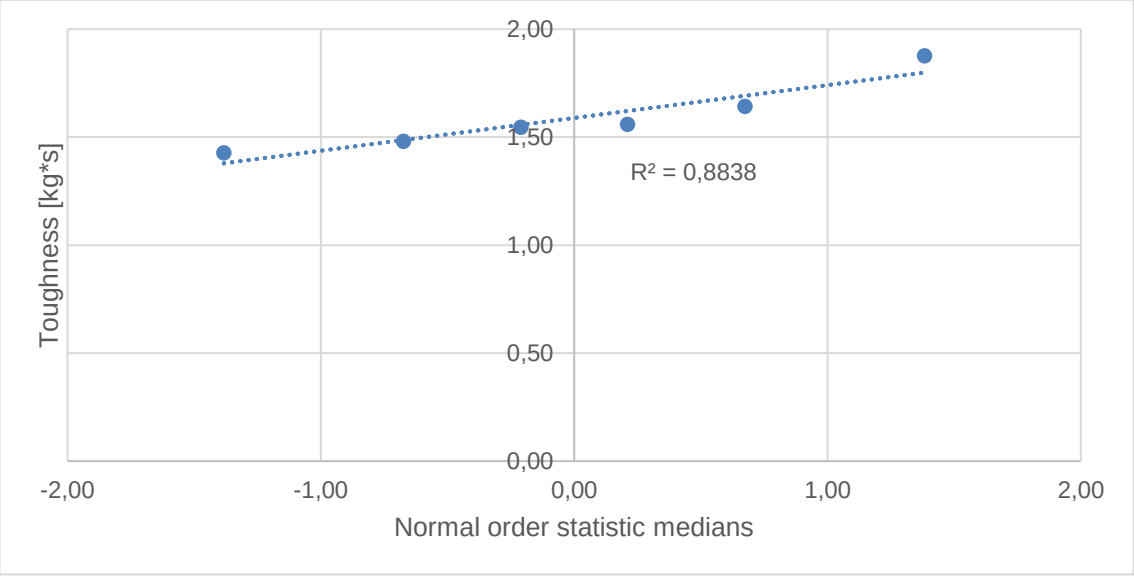

Figure 6. Normal probability plot of centre points experiments for toughness. The X axis indicates normal order statistic medians calculated with Equation 6., and the Y axis represents toughness [kg*s].

Linear trendline fitted to the observations shows an R^2 value of 0.884 indicating a decent fit to standard probability.

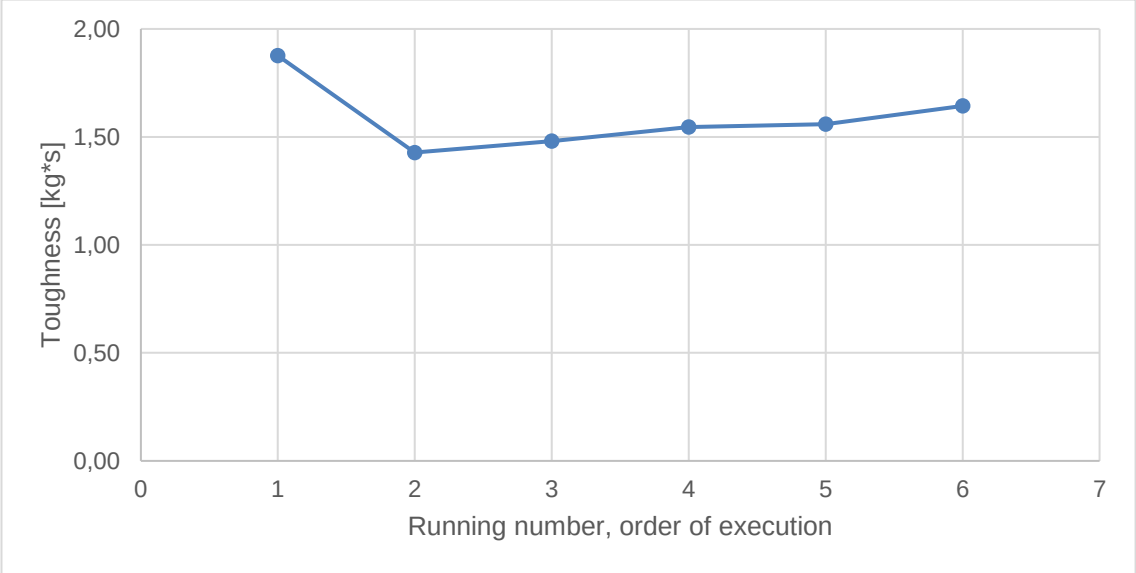

Figure 7. Scatter plot of centre point experiments for toughness. X axis indicates running number of experiments. Y axis indicates toughness [kg].

No clear trend is seen in toughness, indicating independence of order of execution. Corresponding plots for cooking loss and hardness can be found in Appendix 9.

5.1 Cooking loss

Results of regression analyses for cooking loss can be found in Appendix 10. The first analysis with all terms resulted in a model with three statistically significant coefficients ($P \leq 0.05$): b_0, $b_1 * b_2$ and $b_1 * b_3$. However, the model had a significance F value of 0.06, meaning the model was not statistically significantly better than a model where cooking loss is constant. Significance F is the P-value of a statistical test, where the response variable is assumed to be constant. Therefore, P-values higher than 0.05 do not allow the null hypothesis to be rejected at a 95 % confidence level. The analysis was nonetheless iterated with the significant coefficients. The second model had the same significant coefficients as the first one and a significance F value of 0.004.

However, R^2 expectedly dropped from 0.76 to 0.49. R^2 describes how well the model predicts the observed values. An R^2 value of 1 would predict the observations perfectly – 0.49 indicates a poor fit of the model to the observations. The results indicate that in this experimental setup cooking loss is not influenced by the concentrations of KG, CA, and SA. Predicted cooking loss is therefore the average cooking loss of all experiments: 8.6 % ± 0.7 %.

5.2 Hardness and toughness

Results of regression analyses for hardness and toughness can be found in appendices 11 and 12, respectively. Statistically significant, fitting models were obtained for both hardness and toughness. Important parameters of the iterated regression analyses are presented in Table 8.

Table 8. Parameters for final models of hardness and toughness.

Parameter	**Hardness**	**Toughness**
R^2	0.91	0.89
Significance F	$9.0*10^{-7}$	$7.1*10^{-8}$
SD from centre points	0.05	0.16
SD of residuals	0.04	0.12

Standard residuals for all observations of both models were smaller than 3 (appendices 11 and 12). Standard residuals exceeding 3 would suggest a mistake in the experiment in question [66, p. 75]. The models predict the observations quite well and are clearly a better fit to the data, than a model where hardness is constant as shown by the value of significance F. Standard deviations calculated from centre points are approximately equal to the standard deviations of residuals, indicating again that the models fit the data well (Table 8). The following model was obtained for hardness:

$$Hardness\ [kg] = 0.64 + 0.03 * X_1 + 0.11 * X_2 + 0.02 * \mathrm{X}_3 + 0.04 * X_1 * X_3 \qquad (7)$$

The individual effects of X_1, X_2 and the combined effect of X_1 and X_3 were statistically significant at a 95 % confidence level. The individual effect of X_3 was barely not statistically significant (P=0.058). However, sensory observations during the experiments support the idea, that X_3 does have an effect on hardness. For this reason, and X_3 having an interaction effect, the individual effect of X_3 was included in the model. X_2 had by far the highest individual effect on hardness.

For toughness, the following model was obtained:

$$Toughness\ [kg * s] = 1.63 + 0.12 * X_1 + 0.35 * X_2 \quad (8)$$

The individual effects of X_1 and X_2 were statistically significant at a 95 % confidence level. As with hardness, X_2 had the clearly strongest effect. No quadratic or combined effects or individual effect for X_3 were observed. The highest values for hardness (0.85 kg ± 0.05 kg) and toughness (2.30 kg*s ± 0.16 kg*s) were observed in Experiment 8 (Figure 8) when the concentrations of X_1, X_2 and X_3 were at 0.80 m-%. Similar hardness (0.79 kg ± 0.05 kg) and toughness (2.18 kg*s ± 0.16 kg*s) were observed in Experiment 11 (Figure 8) with X_1 and X_3 concentrations of 0.20 m-% and X_1 concentration of 1 m-%.

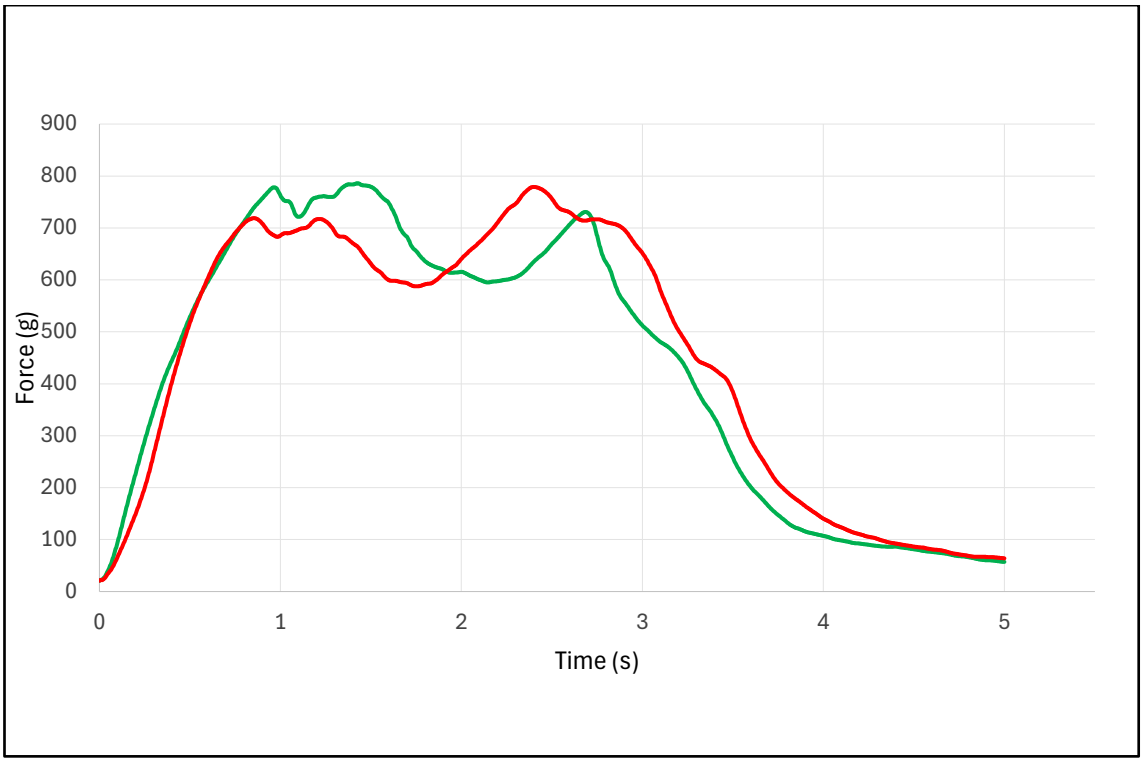

Figure 8. Typical texture analysis graphs of experiments 8 (green) and 11 (red). X axis indicates time [s]. Y axis indicates force [g]. Experiment 8: X_1: 1, X_2: 1, X_3: 1. Experiment 11: X_1: 0, X_2: 1.682, X_3: 0.

Results of experiments 8 and 11 highlight the strong effect of X_2 to hardness and toughness. The lowest hardness (0.42 kg ± 0.05 kg) and toughness (1.07 kg*s ± 0.16 kg*s) were observed in Experiment 12 (Figure 9) with X_1 and X_3 concentrations of 0.20 m-% and X_2 concentration of 0 m-%.

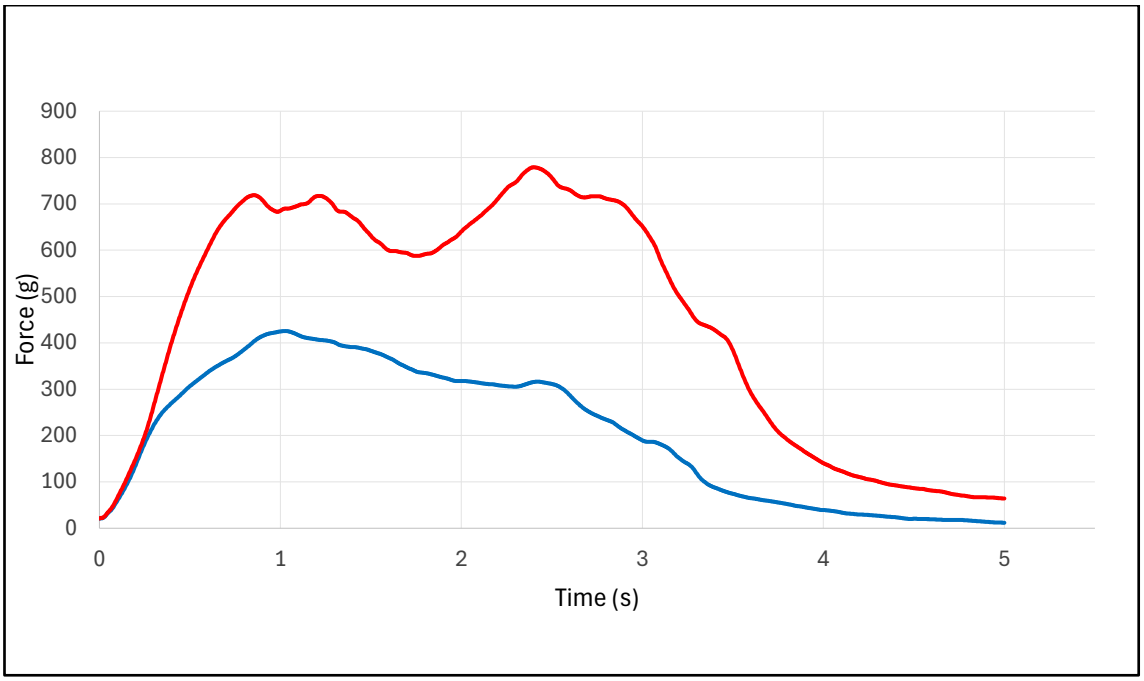

Figure 9. Typical texture analysis graphs of experiments 11 (red) and 12 (blue). X axis indicates time [s]. Y axis indicates force [g]. Experiment 11: X_1: 0, X_2: 1.682, X_3: 0. Experiment 12: X_1: 0, X_2: -1.682, X_3: 0.

Again, Experiment 12 shows the effect of X_2 clearly: when X_2 concentration drops from 1 m-% to 0 m-%, hardness drops by 47 % and toughness by 51 %. The shape of graphs in experiments 8 and 11 (Figure 8) show peaks on both fried surfaces and a valley in the middle where the blade goes through the inside of the patty. This effect is much less pronounced, though still visible, in Experiment 12. A contour plot was created to demonstrate the interaction of X_1 and X_3 in regard to hardness (Figure 10). X_2 was set constant to 2.1 coded units (corresponding to 1.1 m-%) due to it having a linear effect with no interactions. The lower limit for coded units remained -1.682 because this corresponds to a concentration of 0 m-%.

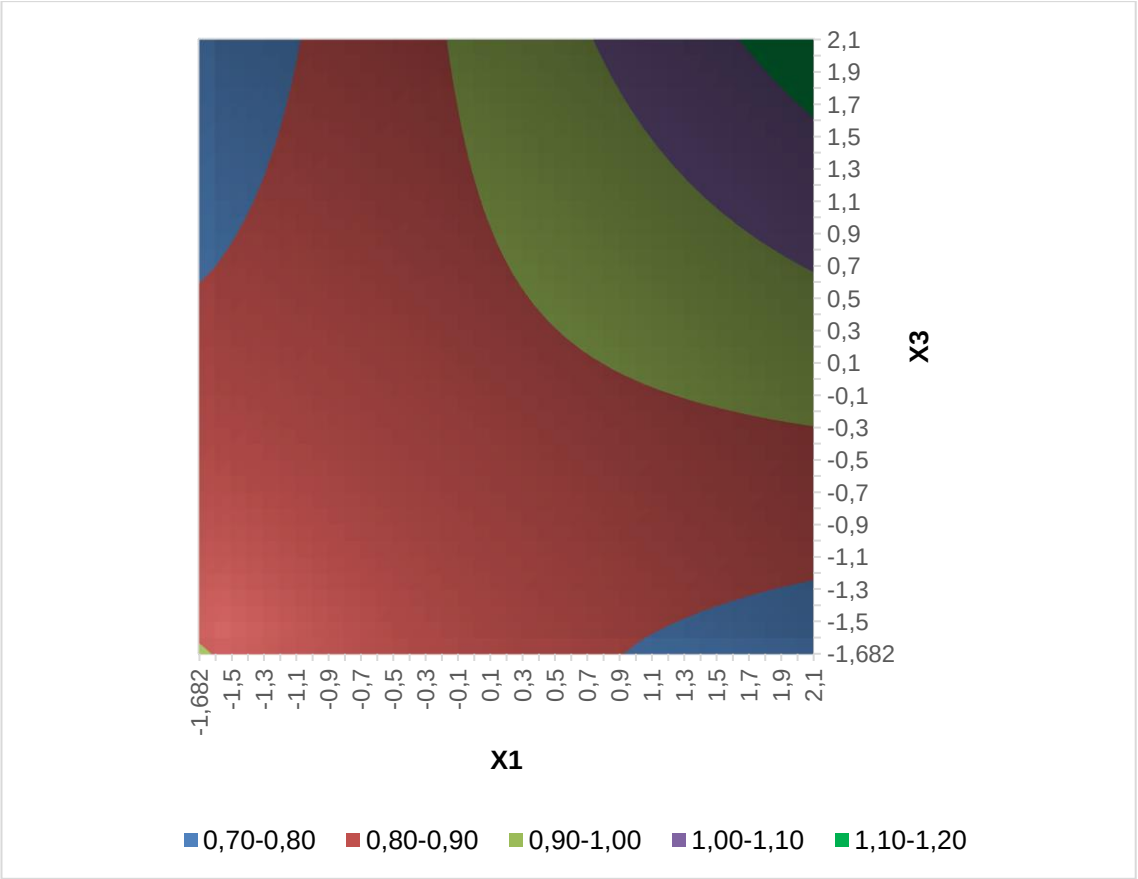

Figure 10. Contour plot of hardness. Colours indicate hardness in kilograms. X_2 is constant at coded value 2.1. X axis indicates the coded value of X_1. Y axis indicates the coded value of X_3.

As expected, the highest hardness values are predicted when both X_1 and X_3 concentrations are highest. Areas of lower hardness are seen when either X1 or X_3 concentration is low, and the other is high. Surprisingly, a tiny area of high hardness is seen when both concentrations are at their lowest.

6 Discussion

The effects of konjac gum, κ-carrageenan, and sodium alginate to cooking loss and texture of patties made from fermented fava bean were studied. To this end, methods to measure cooking loss, hardness, and toughness were developed and shown to be repeatable. Through preliminary experiments, a suitable recipe for the FFB mass was developed.

Previously, the cooking loss during deep frying of meat-free sausages, made with soy protein isolate and texturized soy protein, has been reduced from 8.27 % to 5.96 % using 1 m-% of konjac and to 6.91 using 1 m-% of κ-carrageenan. [3, p. 1273.] Lee et al. [70, p. 780] showed that alginate reduced cooking loss of soy protein patties from 7.27 % to 2.72 % at a concentration of 0.5 m-%. Surprisingly, in this thesis no hydrocolloid was observed to have a significant effect on cooking loss. Cooking loss was found to be on average 8.6 % regardless of the concentrations of hydrocolloids. It is possible that another cooking method, such as deep frying or oven baking might bring out the effects of the hydrocolloids.

Han et al. [71 p. 12] observed a 28 % increase in hardness in pea protein patties with 1 m-% of κ-carrageenan. 2 m-% of konjac did not have a statistically significant effect on the hardness of fermented soybean patties in a study by Yuliarti et al. [72, p.4]. Alginate has even been observed to reduce hardness of soy patties by 27 % [70, p. 780]. In this thesis, all hydrocolloids had a positive, statistically significant effect on hardness, X_2 having by far the largest individual effect and X_1 and X_3 having an interaction effect in addition to their moderate individual effects. As expected, the highest hardness is both observed (Table 7) and predicted (Figure 10) when the concentrations of all hydrocolloids are the highest. Due to the interaction between X_1 and X_3, lower hardness is predicted when either concentration is low, and the other is high assuming X_2 is constant. Hardness is predicted to peak again when both concentrations are low. Toughness was affected individually by X_1 and X_2. The model obtained was linear with no interactions. Toughness and hardness were closely related: the highest and lowest values of toughness were observed in the same experiments as the corresponding hardness values.

7 Conclusions

Texture analysis data indicates that hydrocolloids increase the hardness of the fried surface. This is seen for example in Figures 8 and 9 as clear peaks in experiments 8 and 11 on both surfaces and a lack of said peaks in Experiment

12 where the concentrations of hydrocolloids were lower. This might be related to the hydrocolloids directly or to the lower amount of unbound water in the patties which could lead to enhanced frying. Texture profile analysis might reveal more about this effect as well as the effects of KG, CA, and SA on texture in general and should be employed in future studies. Texture profile analysis is a double compression method, which can be used to determine texture parameters such as hardness, cohesiveness, springiness, and chewiness [73].

Due to the interaction between X_1 and X_3, hardness is predicted to drop when either concentration is low, and the other is high assuming a constant concentration of X_2. There is no apparent reason for either of these phenomena and they might indicate problems with the model in these situations. The predictions should be confirmed or refuted by additional experiments.

The methods developed for this study can be used in related R&D tasks. The results of this study can be used as a reference in product development within MeEat Food Tech when considering the optimal concentrations of KG, CA, and SA and their applicability in products containing FFB.

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Calculations to determine the amounts of $CaCO_3$ and lactic acid

Draget et al. [Homogenous alginate gels, p. 163] used 15 mM of $CaCO_3$ and 30 mM of lactic acid in 1 % (w/v) alginate solution. In this study, these concentrations were applied by converting the concentration of SA to 1 m-% and the concentrations of $CaCO_3$ and LA as follows:

$$c(CaCO_3) = 15\,mM = 0.015\frac{mol}{l}$$

$$c(LA) = 30\,mM = 0.030\frac{mol}{l}$$

For simplicity, mol/l will be converted to mol/$g_{FFB\text{-}mass}$.

$$=> c(CaCO_3) = \frac{0.015\frac{mol}{l}}{\frac{1000\,g_{FFB\_mass}}{l}} = 1.5 * 10^{-5}\frac{mol}{g_{FFB-mass}}$$

$$=> c(LA) = \frac{0.030\frac{mol}{l}}{\frac{1000\,g_{FFB\_mass}}{l}} = 3.0 * 10^{-5}\frac{mol}{g_{FFB-mass}}$$

Next, mol/$g_{FFB\text{-}mass}$ will be converted to $g_{CaCO3/LA}$/$g_{FFB\text{-}mass}$.

$$w(CaCO_3) = c(CaCO_3) * M(CaCO_3) = 1.5 * 10^{-5}\frac{mol}{g_{FFB-mass}} * 100.1\frac{g_{CaCO_3}}{mol}$$
$$= 1.50 * 10^{-3}\frac{g_{CaCO_3}}{g_{FFB-mass}}$$

$$w(LA) = c(LA) * M(LA) = 3.0 * 10^{-5}\frac{mol}{g_{FFB-mass}} * 90.1\frac{g_{LA}}{mol} = 2.7 * 10^{-3}\frac{g_{LA}}{g_{FFB-mass}}$$

where $w(CaCO_3)$ is the mass fraction of CaCO3 in FFB mass containing 1 m-% SA, and $w(LA)$ is the mass fraction of pure lactic acid in FFB mass containing 1 m-% SA.

Finally, the masses of $CaCO_3$ and LA in all recipes can be calculated. All FFB recipes have a total mass of 310 g. A lactic acid solution of 80 m-% was used.

$$m(CaCO_3)_i = w(CaCO_3) * 310\ g * m - \%(SA)_i$$

$$m(LA)_i = \frac{w(LA)}{m - \% \frac{GAL}{100\ \%}} * 310\ g * m - \%(SA)_i$$

$$m(CaCO_3)_{17} = 1.50 * 10^{-3} * 310\ g * 0.50 \cong 0.23\ g$$

$$m(LA)_{17} = 2.7 * \frac{10^{-3}}{0.80} * 310\ g * 0.50 \cong 0.52\ g$$

where $m(CaCO_3)_i$ is the mass of $CaCO_3$ in recipe $i, i = 1 \dots 20$, $m(LA)_i$is the mass of lactic acid in recipe $i, i = 1 \dots 20$, $m - \%(SA)_i$ is the mass percentage of sodium alginate in recipe $i, i = 1 \dots 20, and$ m-% GAL is the mass percentage of lactic acid in Galacid Excel 80.

Results of preliminary experiments with higher concentrations of $CaCO_3$ and lactic acid

Table 2.9 Results of preliminary experiments with higher concentrations of CaCO3 and lactic acid.

Mass (m-% of $CaCO_3$)	Repetition	Mass of patty, uncooked (g)	Mass of patty, cooked (g)	Cooking loss (g)	Cooking loss (%)
I (0 %)	1	32.55	27.97	4.58	14.1
	2	32.07	27.17	4.90	15.3
	3	32.56	27.24	5.32	16.3
	4	30.45	26.11	4.34	14.3
	5	32.54	27.46	5.08	15.6
II (0.5 %)	1	30.39	25.34	5.05	16.6
	2	30.51	25.59	4.92	16.1
	3	30.46	25.76	4.70	15.4
	4	33.32	28.37	4.95	14.9
	5	31.00	25.81	5.19	16.7
III (1 %)	1	30.94	26.17	4.77	15.4
	2	32.01	27.00	5.01	15.7
	3	30.91	25.87	5.04	16.3
	4	30.16	25.01	5.15	17.1
	5	30.46	25.25	5.21	17.1

Table 10.2 Averages and standard deviations of five patties from each mass

Mass (m-% of $CaCO_3$)	**Cooking loss (%)**	**Hardness (kg)**	**Toughness (kg*s)**
I (0 %)	15.1 ± 0.9	0.60 ± 0.10	1.30 ± 0.11
II (0.5 %)	16.0 ± 0.8	0.86 ± 0.10	1.80 ± 0.11
III (1 %)	16.3 ± 0.8	0.75 ± 0.15	1.43 ± 0.30

Results of preliminary experiments with maximum amounts of hydrocolloids

Table 3.1 Results of preliminary experiments with maximum amounts of hydrocolloids.

Mass	Repetition	Mass of patty, uncooked (g)	Mass of patty, cooked (g)	Cooking loss (g)	Cooking loss (%)
I	1	30.11	27.40	2.71	9.0
	2	31.60	28.83	2.77	8.8
	3	31.52	28.48	3.04	9.6
II	1	30.91	27.03	3.88	12.6
	2	30.22	26.93	3.29	10.9
	3	30.66	27.33	3.33	10.9
III	1	30.14	26.94	3.2	10.6
	2	30.30	26.94	3.36	11.1
	3	30.04	26.77	3.27	10.9

Table 3.2 Averages and standard deviations of three patties from each mass and combined averages and standard deviations.

Mass	**Cooking loss (%)**	**Hardness (kg)**	**Toughness (kg*s)**
I	9.1 ± 0.5	1.19 ± 0.06	3.13 ± 0.12
II	11.4 ± 1.0	1.29 ± 0.15	3.49 ± 0.11
III	10.9 ± 0.2	1.41 ± 0.10	3.51 ± 0.14
Total	10.5 ± 1.2	1.30 ± 0.11	3.38 ± 0.21

Determining the real and coded values of X_1, X_2, and X_3 in main experiments

Real values of $|\alpha| = 8^{1/4} = 1{,}682$ are set at 1 m-% (1,682) and 0 m-% (-1.682) for all factors based on preliminary experiments. Real values of levels 0 and ±1 are scaled accordingly. The relationship between the real values R and coded values X is linear.

$$R_i = \frac{R_{max} - R_{min}}{X_{max} - X_{min}} * X_i + \frac{R_{max} + R_{min}}{2}$$

$$R_{-1} = \frac{1\ m\% - 0\ m\%}{1.682 - (-1.682)} * (-1) + \frac{1\ m\% + 0\ m\%}{2} = 0.2027 \ldots\ m\% \cong 0.20\ m\%$$

where R_i is real value at coded value i, R_{max} is the real value (1 m-%) at coded value 1.682, R_{min} is the real value (0 m-%) at coded value -1.682, X_i is the coded value at $i, i = \pm 1.682,\ \pm 1,\ 0$, X_{max} is coded value α = 1.682, and X_{min} is coded value -α = -1.682.

Conversely, real values are converted to coded values with the following formula:

$$X_i = \frac{R_i - \frac{R_{max} - R_{min}}{2}}{\frac{R_{max} - R_{min}}{X_{max} - X_{min}}}$$

$$X_{0.20} = \frac{0.2027 m\% - \frac{1 m\% - 0 m\%}{2}}{\frac{1.682 - (-1.682)}{1 m\% - 0 m\%}} = -1.000 \ldots \cong -1$$

Recipes of FFB masses in main experiments

Table 5.1. Recipes of FFB masses in order of execution (g).

	Experiment no									
Ingredient	3	8	6	2	20	17	15	13	12	11
FFB+water+oil	304.0	299.4	301.2	304.0	302.6	302.6	302.6	300.3	304.1	301.0
FFB	245.0	241.3	242.8	245.0	243.9	243.9	243.9	242.0	245.1	242.6
Rapeseed oil	29.5	29.0	29.2	29.5	29.4	29.4	29.4	29.1	29.5	29.2
Water, total	29.5	29.0	29.2	29.5	29.4	29.4	29.4	29.1	29.5	29.2
Water (SA)	19.5	19.0	19.2	19.5	19.4	19.4	19.4	19.1	19.5	19.2
Water (LA)	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Mineral salt	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
X_1	0.63	2.47	2.47	2.47	1.55	1.55	1.55	1.55	1.55	1.55
X_2	2.47	2.47	0.63	0.63	1.55	1.55	1.55	1.55	0.00	3.10
X_3	0.63	2.47	2.47	0.63	1.55	1.55	1.55	3.10	1.55	1.55
Lactic acid	0.21	0.83	0.83	0.21	0.52	0.52	0.52	1.05	0.52	0.52
CaCO3	0.09	0.37	0.37	0.09	0.23	0.23	0.23	0.46	0.23	0.23
Total mass	310	310	310	310	310	310	310	310	310	310

Table 5.2. Recipes of FFB masses in order of execution continued (g).

	Experiment no									
Ingredient	1	18	10	7	14	5	19	9	4	16
FFB+water+oil	305.8	302.6	304.1	301.2	304.9	303.1	302.6	301.0	302.1	302.6
FFB	246.5	243.9	245.1	242.8	245.7	244.3	243.9	242.6	243.5	243.9
Rapeseed oil	29.7	29.4	29.5	29.2	29.6	29.4	29.4	29.2	29.3	29.4
Water, total	29.7	29.4	29.5	29.2	29.6	29.4	29.4	29.2	29.3	29.4
Water (SA)	19.7	19.4	19.5	19.2	0.0	19.4	19.4	19.2	19.3	19.4
Water (LA)	10.0	10.0	10.0	10.0	0.0	10.0	10.0	10.0	10.0	10.0
Mineral salt	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
X_1	0.63	1.55	0.00	0.63	1.55	0.63	1.55	3.10	2.47	1.55
X_2	0.63	1.55	1.55	2.47	1.55	0.63	1.55	1.55	2.47	1.55
X_3	0.63	1.55	1.55	2.47	0.00	2.47	1.55	1.55	0.63	1.55
Lactic acid	0.21	0.52	0.52	0.83	0.00	0.83	0.52	0.52	0.21	0.52
CaCO3	0.09	0.23	0.23	0.37	0.00	0.37	0.23	0.23	0.09	0.23
Total mass	310	310	310	310	310	310	310	310	310	310

Table made for normal probability plots

Table 6.1. Calculations for normal probability plots.

Running number, smallest to largest	=(running number-0.5)/6	Cooking loss [%]	Hardness [kg]	Toughness [kg*s]	=NORM.S.INV((running number-0.5)/6))
1	0.08	7.9	0.57	1.43	-1.383
2	0.25	8.2	0.60	1.48	-0.674
3	0.42	8.3	0.61	1.55	-0.210
4	0.58	8.5	0.61	1.56	0.210
5	0.75	8.6	0.65	1.64	0.674
6	0.92	9.5	0.71	1.88	1.383

Regression analysis table with all terms and corresponding coded values

Table 7.1. Regression analysis table.

Experiment no	X1	X2	X3	X_1*X_2	X_1*X_3	X_2*X_3	X_1*X_2*X_3	X_1*X_1	X_2*X_2	X_3*X_3
1	-1	-1	-1	1	1	1	-1	1	1	1
2	1	-1	-1	-1	-1	1	1	1	1	1
3	-1	1	-1	-1	1	-1	1	1	1	1
4	1	1	-1	1	-1	-1	-1	1	1	1
5	-1	-1	1	1	-1	-1	1	1	1	1
6	1	-1	1	-1	1	-1	-1	1	1	1
7	-1	1	1	-1	-1	1	-1	1	1	1
8	1	1	1	1	1	1	1	1	1	1
9	1.682	0	0	0	0	0	0	2.828	0	0
10	-1.682	0	0	0	0	0	0	2.828	0	0
11	0	1.682	0	0	0	0	0	0	2.828	0
12	0	-1.682	0	0	0	0	0	0	2.828	0
13	0	0	1.682	0	0	0	0	0	0	2.828
14	0	0	-1.682	0	0	0	0	0	0	2.828
15	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0

Full results of CCC experiments in systematic order

Table 8.1. Full results of CCC experiments.

Experiment no	Order of execution	Repetition	Raw weight [g]	Cooked weight [g]	Cooking loss [g]	Cooking loss [%]	Hardness [kg]	Toughness [kg*s]
1	11	1	30.46	27.84	2.62	8.6	0.43	1.28
1	11	2	30.49	27.79	2.70	8.9	0.48	1.15
1	11	3	30.04	27.36	2.68	8.9	0.50	1.11
2	4	1	30.15	27.47	2.68	8.9	0.47	1.30
2	4	2	30.35	27.51	2.84	9.4	0.54	1.59
2	4	3	30.49	27.64	2.85	9.3	0.51	1.42
3	1	1	30.50	27.08	3.42	11.2	0.80	2.30
3	1	2	30.00	26.86	3.14	10.5	0.81	2.16
3	1	3	30.12	27.25	2.87	9.5	0.77	2.01
4	19	1	30.04	27.57	2.47	8.2	0.74	2.23
4	19	2	30.21	27.97	2.24	7.4	0.71	2.11
4	19	3	30.17	27.88	2.29	7.6	0.66	2.06

5	16	1	30.81	28.54	2.27	7.4	0.52	1.29
5	16	2	30.51	28.05	2.46	8.1	0.52	1.33
5	16	3	30.27	27.73	2.54	8.4	0.55	1.26
6	3	1	30.19	27.57	2.62	8.7	0.66	1.60
6	3	2	30.69	27.59	3.10	10.1	0.62	1.44
6	3	3	30.53	27.55	2.98	9.8	0.57	1.41
7	14	1	30.44	28.13	2.31	7.6	0.67	1.71
7	14	2	30.06	27.51	2.55	8.5	0.72	1.83
7	14	3	30.05	27.72	2.33	7.8	0.63	1.82
8	2	1	30.39	27.87	2.52	8.3	0.79	2.19
8	2	2	30.17	27.61	2.56	8.5	0.93	2.50
8	2	3	30.38	27.78	2.60	8.6	0.84	2.20
9	18	1	30.55	28.33	2.22	7.3	0.64	1.80
9	18	2	30.54	28.29	2.25	7.4	0.72	1.89
9	18	3	30.27	27.84	2.43	8.0	0.77	1.99
10	13	1	30.20	27.62	2.58	8.5	0.54	1.45
10	13	2	30.22	27.49	2.73	9.0	0.67	1.42

10	13	3	30.42	27.88	2.54	8.3	0.61	1.47
11	10	1	30.15	27.55	2.60	8.6	0.78	2.26
11	10	2	30.24	27.53	2.71	9.0	0.85	2.19
11	10	3	30.46	27.92	2.54	8.3	0.75	2.10
12	9	1	30.30	27.22	3.08	10.2	0.43	1.04
12	9	2	30.42	27.77	2.65	8.7	0.42	1.02
12	9	3	30.63	27.95	2.68	8.7	0.41	1.16
13	8	1	30.37	27.54	2.83	9.3	0.74	1.77
13	8	2	30.39	27.90	2.49	8.2	0.65	1.69
13	8	3	30.43	27.74	2.69	8.8	0.71	1.78
14	15	1	30.53	27.72	2.81	9.2	0.65	1.82
14	15	2	30.27	27.67	2.60	8.6	0.63	1.70
14	15	3	30.03	27.45	2.58	8.6	0.66	1.75
15	7	1	30.26	27.53	2.73	9.0	0.57	1.39
15	7	2	30.56	27.96	2.60	8.5	0.62	1.58
15	7	3	30.06	27.53	2.53	8.4	0.63	1.47
16	20	1	30.66	28.13	2.53	8.3	0.68	1.70

16	20	2	30.54	28.11	2.43	8.0	0.68	1.57
16	20	3	30.34	27.74	2.60	8.6	0.76	1.66
17	6	1	30.17	27.63	2.54	8.4	0.53	1.48
17	6	2	30.14	27.50	2.64	8.8	0.57	1.37
17	6	3	30.49	27.91	2.58	8.5	0.61	1.43
18	12	1	30.30	27.96	2.34	7.7	0.57	1.37
18	12	2	30.43	27.89	2.54	8.3	0.59	1.64
18	12	3	30.28	27.69	2.59	8.6	0.67	1.63
19	17	1	30.33	27.96	2.37	7.8	0.59	1.50
19	17	2	30.64	28.24	2.40	7.8	0.58	1.56
19	17	3	30.10	27.67	2.43	8.1	0.62	1.62
20	5	1	30.08	27.27	2.81	9.3	0.74	1.95
20	5	2	30.18	27.33	2.85	9.4	0.63	1.93
20	5	3	30.55	27.56	2.99	9.8	0.58	1.75

Scatter and normal probability plots for cooking loss and hardness

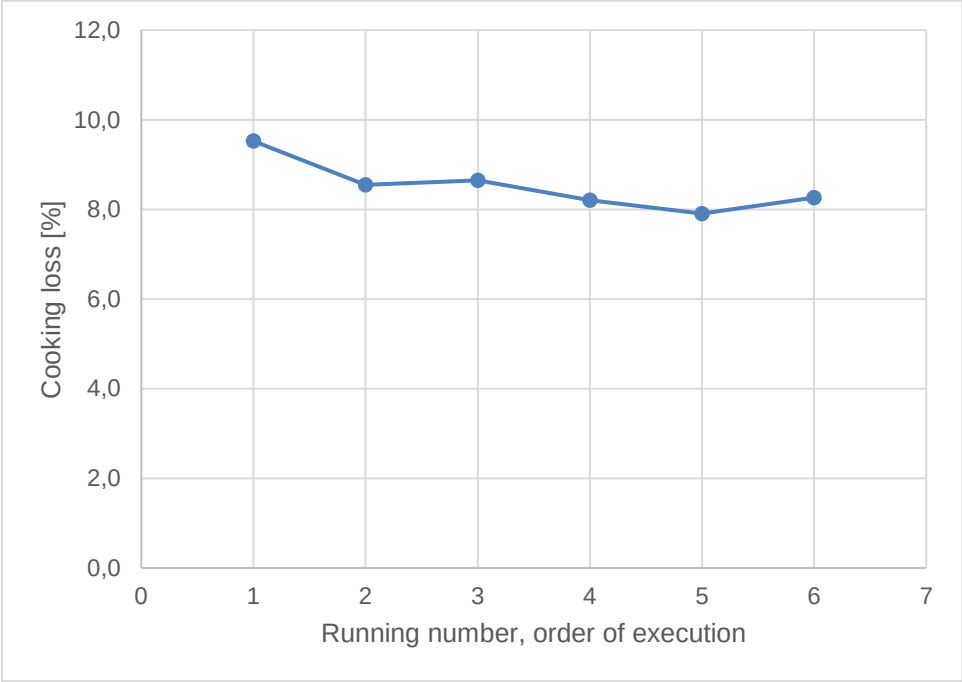

Figure 9.1. Scatter plot for cooking loss.

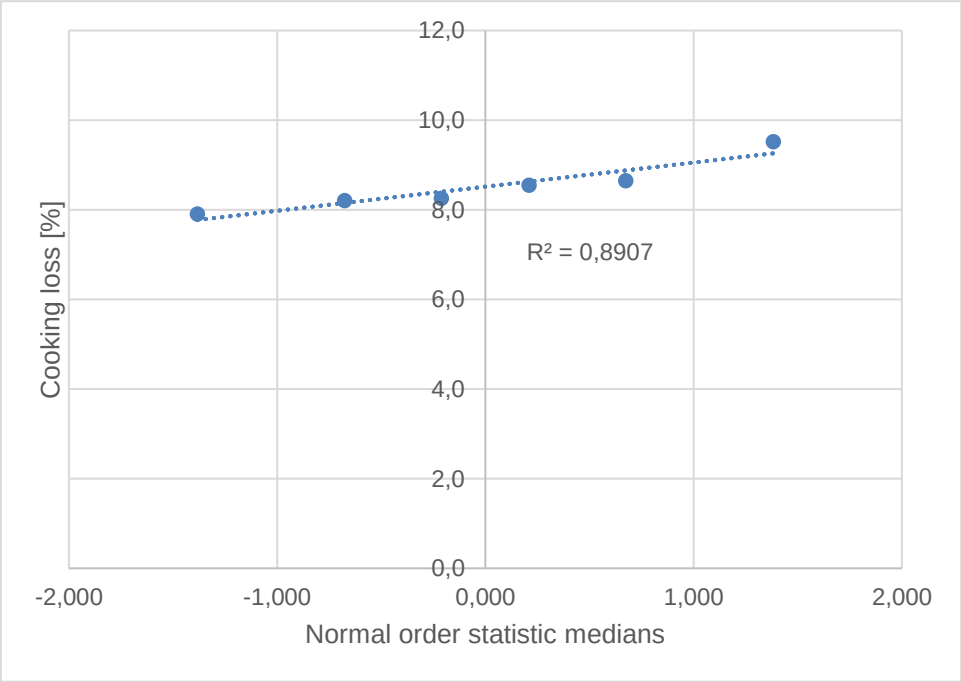

Figure 9.2. Normal probability plot for cooking loss.

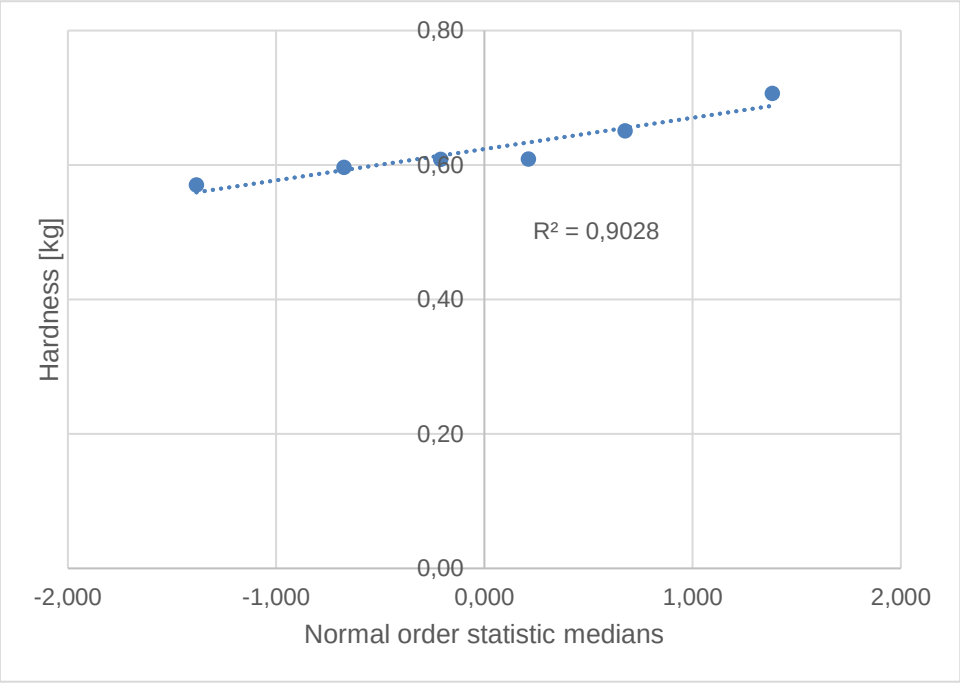

Figure 9.3. Normal probability plot for hardness.

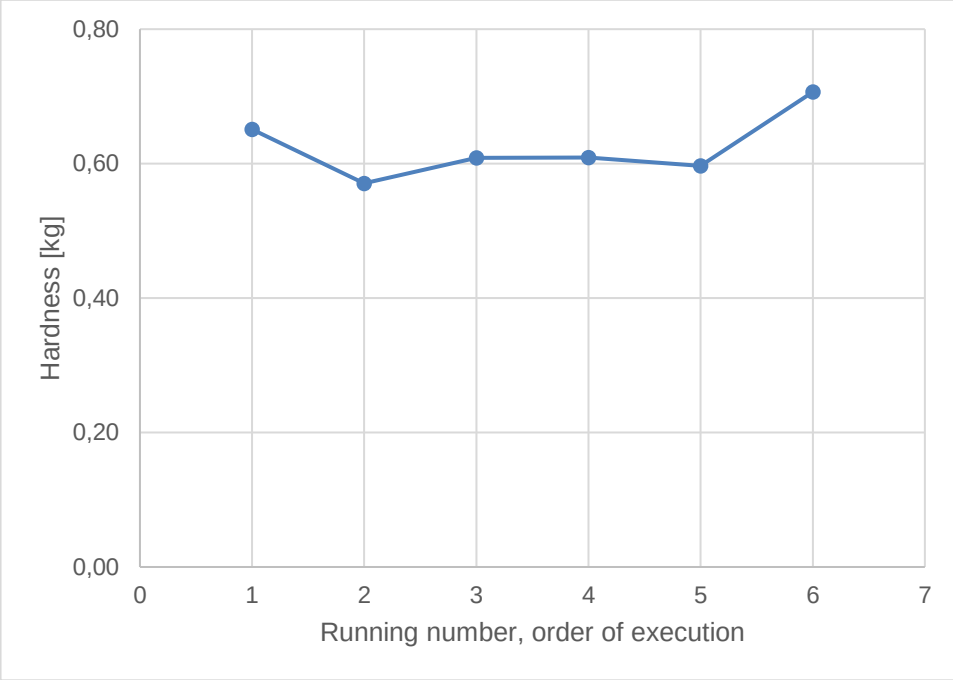

Figure 9.4. Scatter plot for hardness.

Results of regression analyses for cooking loss

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.872
R Square	0.761
Adjusted R Square	0.496
Standard Error of residuals	0.495
Observations	20

Experimental error from centre point experiments 0.56

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	10	7.027	0.702748638	2.869198992	0.064020017
Residual	9	2.204	0.244928512		
Total	19	9.232			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95,0%*	*Upper 95,0%*
b0	8.5	0.2	42.2	0.000	8.1	9.0	8.1	9.0

RESIDUAL OUTPUT

Observation	*Predicted average Cooking loss) (%)*	*Residuals*	*Standard Residuals*
1	8.8	0.0	0.1
2	8.9	0.3	0.8
3	10.3	0.1	0.2
4	7.4	0.3	0.9
5	8.2	-0.2	-0.6
6	9.5	0.0	0.1
7	8.1	-0.2	-0.5
8	8.4	0.1	0.2
9	7.9	-0.4	-1.1
10	8.4	0.2	0.6

b1	-0.1	0.1	-1.1	0.301	-0.5	0.2	-0.5	0.2	11	8.8	-0.1	-0.4
b2	-0.1	0.1	-1.0	0.334	-0.4	0.2	-0.4	0.2	12	9.2	0.0	-0.1
b3	-0.2	0.1	-1.3	0.238	-0.5	0.1	-0.5	0.1	13	8.6	0.2	0.6
b1*b2	-0.5	0.2	-3.0	0.016	-0.9	-0.1	-0.9	-0.1	14	9.2	-0.4	-1.0
b1*b3	0.5	0.2	3.1	0.013	0.1	0.9	0.1	0.9	15	8.5	0.1	0.4
b2*b3	-0.2	0.2	-0.9	0.406	-0.5	0.2	-0.5	0.2	16	8.5	-0.3	-0.7
b1*b2*b3	0.2	0.2	1.4	0.188	-0.1	0.6	-0.1	0.6	17	8.5	0.0	0.1
b1*b1	-0.1	0.1	-0.9	0.387	-0.4	0.2	-0.4	0.2	18	8.5	-0.3	-0.9
b2*b2	0.2	0.1	1.3	0.215	-0.1	0.5	-0.1	0.5	19	8.5	-0.6	-1.8
b3*b3	0.1	0.1	1.0	0.360	-0.2	0.4	-0.2	0.4	20	8.5	1.0	3.0

Iteration with statistically significant terms

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.70
R Square	0.49
Adjusted R Square	0.42
Standard Error of residuals	0.53
Observations	20

Experimental error from centre point experiments: 0.56

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	2	4.482	2.241	8.021	0.004
Residual	17	4.750	0.279		
Total	19	9.232			

RESIDUAL OUTPUT

Observation	*Predicted average Cooking loss (%)*	*Residuals*	*Standard Residuals*
1	8.7	0.1	0.3
2	8.6	0.6	1.2
3	9.7	0.7	1.4
4	7.6	0.2	0.3
5	7.6	0.4	0.7
6	9.7	-0.2	-0.4
7	8.6	-0.7	-1.3
8	8.7	-0.2	-0.4
9	8.6	-1.1	-2.2
10	8.6	0.0	0.0
11	8.6	0.0	0.0
12	8.6	0.6	1.1
13	8.6	0.1	0.3
14	8.6	0.2	0.3
15	8.6	0.0	0.0
16	8.6	-0.4	-0.8

		Standard Error	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95,0%*	*Upper 95,0%*
b0	8.6	0.1	73.1	0.00	8.4	8.9	8.4	8.9
b1*b2	-0.5	0.2	-2.8	0.01	-0.9	-0.1	-0.9	-0.1
b1*b3	0.5	0.2	2.9	0.01	0.1	0.9	0.1	0.9

17	8.6	-0.1	-0.2
18	8.6	-0.4	-0.9
19	8.6	-0.7	-1.5
20	8.6	0.9	1.8

Results of regression analyses for hardness

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.974
R Square	0.948
Adjusted R Square	0.890
Standard Error of residuals	0.036
Observations	20

Experimental error from centre point experiments	0.048

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	10	0.215	0.022	16.320	1,4E-04
Residual	9	0.012	0.001		
Total	19	0.227			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95,0%*	*Upper 95,0%*
b0	0.62	0.01	42.14	0.000	0.59	0.66	0.59	0.66

RESIDUAL OUTPUT			
Observation	*Predicted average Hardness (kg)*	*Residuals*	*Standard Residuals*
1	0.47	0.00	-0.11
2	0.51	-0.01	-0.25
3	0.80	0.00	-0.11
4	0.71	-0.01	-0.25
5	0.53	0.00	0.15
6	0.62	0.00	0.01
7	0.67	0.00	0.15
8	0.85	0.00	0.01
9	0.70	0.01	0.23
10	0.61	0.00	-0.09

b1	0.03	0.01	2.79	0.021	0.01	0.05	0.01	0.05	11	0.79	0.00	0.07
b1	0.11	0.01	11.39	0.000	0.09	0.13	0.09	0.13	12	0.42	0.00	0.07
b3	0.02	0.01	2.21	0.055	0.00	0.04	0.00	0.04	13	0.71	-0.01	-0.24
b1*b2	0.00	0.01	-0.38	0.711	-0.03	0.02	-0.03	0.02	14	0.63	0.01	0.38
b1*b3	0.04	0.01	3.09	0.013	0.01	0.07	0.01	0.07	15	0.62	-0.02	-0.62
b2*b3	-0.02	0.01	-1.36	0.208	-0.05	0.01	-0.05	0.01	16	0.62	0.08	3.31
b1*b2*b3	0.03	0.01	2.07	0.068	0.00	0.06	0.00	0.06	17	0.62	-0.05	-2.13
b1*b1	0.01	0.01	1.15	0.280	-0.01	0.03	-0.01	0.03	18	0.62	-0.01	-0.59
b2*b2	-0.01	0.01	-0.73	0.485	-0.03	0.01	-0.03	0.01	19	0.62	-0.03	-1.09
b3*b3	0.02	0.01	1.75	0.114	0.00	0.04	0.00	0.04	20	0.62	0.03	1.09

Iteration with statistically significant terms

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.95
R Square	0.91
Adjusted R Square	0.87
Standard Error of residuals	0.04
Observations	20

Experimental error from centre point experiments	0.05

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	5	0.206	0.041	27.310	9.43E-07
Residual	14	0.021	0.002		
Total	19	0.227			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95,0%*	*Upper 95,0%*
b0	0.64	0.009	73.499	0.000	0.619	0.657	0.619	0.657

RESIDUAL OUTPUT

Observation	*Predicted average Hardness (kg)*	*Residuals*	*Standard Residuals*
1	0.49	-0.02	-0.56
2	0.52	-0.01	-0.36
3	0.77	0.03	0.78
4	0.69	0.01	0.39
5	0.51	0.02	0.68
6	0.59	0.03	0.88
7	0.68	0.00	-0.07
8	0.87	-0.02	-0.46
9	0.68	0.02	0.68
10	0.59	0.01	0.44
11	0.83	-0.03	-0.96
12	0.45	-0.03	-0.96
13	0.67	0.03	0.81
14	0.60	0.04	1.28
15	0.64	-0.03	-0.89

b1	0.03	0.011	2.612	0.020	0.005	0.050	0.005	0.050	16	0.64	0.07	2.05
b2	0.11	0.011	10.646	0.000	0.089	0.134	0.089	0.134	17	0.64	-0.07	-2.02
b3	0.02	0.011	2.062	0.058	-0.001	0.044	-0.001	0.044	18	0.64	-0.03	-0.87
b1*b3	0.04	0.014	2.893	0.012	0.010	0.069	0.010	0.069	19	0.64	-0.04	-1.24
b1*b2*b3	0.03	0.014	1.938	0.073	-0.003	0.056	-0.003	0.056	20	0.64	0.01	0.39

Results of regression analyses for toughness

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0,97
R Square	0,94
Adjusted R Square	0,87
Standard Error of residuals	0,12
Observations	20

Experimental error from centre point experiments	0,16

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	10	2,09	0,21	13,84	2,7E-04
Residual	9	0,14	0,02		
Total	19	2,22			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95,0%*	*Upper 95,0%*
b0	1,59	0,05	31,706	0,000	1,47	1,70	1,47	1,70

RESIDUAL OUTPUT

Observation	*Predicted average Toughness (kg*s)*	*Residuals*	*Standard Residuals*
1	1,18	0,00	0,04
2	1,45	-0,01	-0,12
3	2,12	0,04	0,45
4	2,11	0,02	0,30
5	1,29	0,00	0,00
6	1,49	-0,01	-0,16
7	1,75	0,03	0,41
8	2,27	0,02	0,25
9	1,90	0,00	-0,02
10	1,48	-0,03	-0,39

b1	0,12	0,03	3,73	0,005	0,05	0,20	0,05	0,20	11	2,24	-0,06	-0,70
b2	0,35	0,03	10,67	0,000	0,28	0,43	0,28	0,43	12	1,05	0,02	0,28
b3	-0,01	0,03	-0,15	0,882	-0,08	0,07	-0,08	0,07	13	1,76	-0,01	-0,15
b1*b2	0,00	0,04	0,11	0,915	-0,09	0,10	-0,09	0,10	14	1,78	-0,02	-0,26
b1*b3	0,06	0,04	1,33	0,215	-0,04	0,16	-0,04	0,16	15	1,59	-0,11	-1,26
b2*b3	-0,04	0,04	-1,01	0,337	-0,14	0,05	-0,14	0,05	16	1,59	0,06	0,65
b1*b2*b3	0,08	0,04	1,74	0,116	-0,02	0,17	-0,02	0,17	17	1,59	-0,16	-1,90
b1*b1	0,03	0,03	1,08	0,309	-0,04	0,11	-0,04	0,11	18	1,59	-0,04	-0,50
b2*b2	0,02	0,03	0,63	0,547	-0,05	0,09	-0,05	0,09	19	1,59	-0,03	-0,34
b3*b3	0,06	0,03	1,98	0,079	-0,01	0,14	-0,01	0,14	20	1,59	0,29	3,41

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0,94
R Square	0,89
Adjusted R Square	0,87
Standard Error of residuals	0,12
Observations	20

Experimental error from centre point experiments	0,16

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	3	1,98	0,66	42,91	7,1E-08
Residual	16	0,25	0,02		
Total	19	2,22			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95,0%*	*Upper 95,0%*
b0	1,63	0,04	45,97	2,0E-18	1,55	1,70	1,55	1,70

RESIDUAL OUTPUT

Observation	*Predicted average Toughness (kg*s)*	*Residuals*	*Standard Residuals*
1	1,21	-0,03	-0,26
2	1,46	-0,02	-0,15
3	1,92	0,24	2,07
4	2,17	-0,04	-0,31
5	1,21	0,08	0,70
6	1,46	0,02	0,19
7	1,92	-0,13	-1,18
8	2,17	0,13	1,14
9	1,84	0,06	0,49
10	1,42	0,02	0,21
11	2,23	-0,04	-0,37
12	1,03	0,04	0,35
13	1,80	-0,05	-0,43
14	1,80	-0,04	-0,35
15	1,63	-0,15	-1,30
16	1,63	0,01	0,12
17	1,63	-0,20	-1,77

b1	0,12	0,03	3,70	0,002	0,05	0,20	0,05	0,20	18	1,63	-0,08	-0,73
b2	0,35	0,03	10,57	1,3E-08	0,28	0,43	0,28	0,43	19	1,63	-0,07	-0,61
b3*b3	0,06	0,03	1,83	0,086	-0,01	0,13	-0,01	0,13	20	1,63	0,25	2,18