

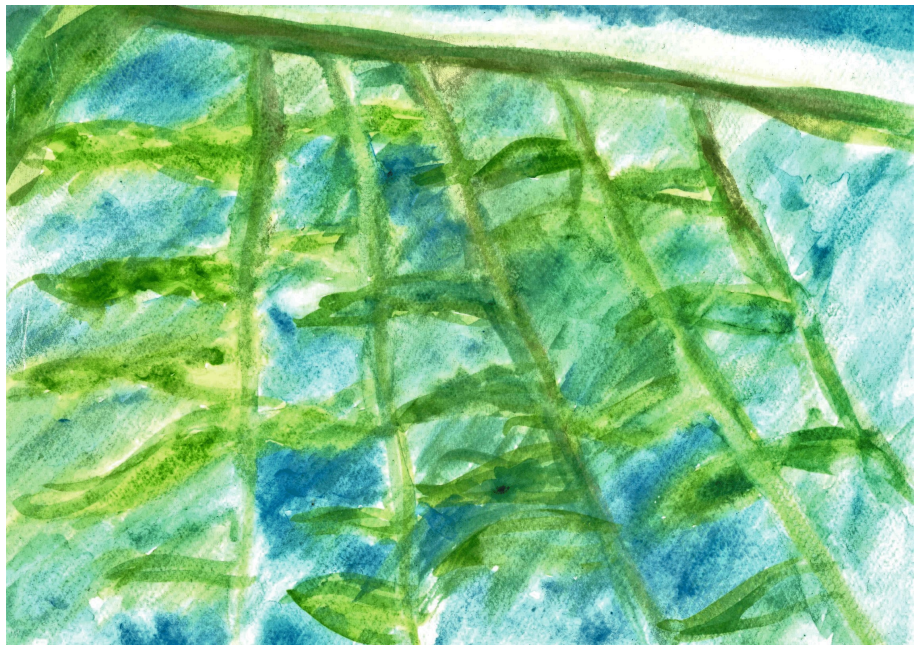
Sunniva Kristine Bauck Dahl

## Vegetarian soy burgers - effect of alginate, brown seaweed and methyl cellulose on texture

A rheological study on the effect of implementing alternative binders in a food matrix based on soy protein isolate.

Master's thesis in Chemical Engineering and Biotechnology  
Supervisor: Turid Rustad

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Norwegian University of Science and Technology  
Faculty of Natural Sciences  
Department of Biotechnology and Food Science



# Preface

This thesis is part of the master's program *Chemical Engineering and Biotechnology* at the Department of Biotechnology and Food Science, at NTNU – Norwegian University of Science and Technology. The thesis was connected to a part of the Norwegian Seaweed Biorefinery Platform, through NTNU.

First and foremost, I would like to express my gratitude to my supervisor Turid Rustad. Thank you for your invaluable feedback, for answering all my questions when needed, and for great company throughout the master year of 2021-2022. I feel very fortunate to have had you as my supervisor.

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And finally, thank you to my adorable boyfriend, Fredrik, for believing in me and for listening to me when I was stressed out. I feel lucky to have you in my life.



# Abstract

Due to the environmental impact of red meat production and the health issues regarding its consumption, meat analogues are becoming increasingly popular. Many meat analogues based on soy protein isolate (SPI) mimic meat texture by using methyl cellulose (MC). This thesis aimed to see if complete or partial substitution of MC with different binders could lead to the same texture as MC does in meat analogue burgers today.

The thesis consisted of six experiments and two sub-experiments. The initial experiment consisted of formulating the burger recipe. Each burger weighed approximately 40 g and was made of 30.0% SPI, 3.8% coconut oil, 3.8% canola oil, and 62.5% distilled water. The amount of binder was added as a percentage of the total weight of the burger. The binders investigated were 0-2% MC, 0-1% alginate – one with a high fraction of G-blocks (G) and one with a low fraction (M) – and 0-2% seaweed from *Saccharina latissima* (SL) pre-treated with either enzymes or water with pH 8. A mixture of alginate (G and M) and MC, at different ratios, were also tested as binders. Sub-experiment *a* and *b* investigated the viscosity of the alginates and pre-treated seaweed, respectively.

The burgers were mixed, pressed, and rested for a chosen amount of time, at a chosen temperature, depending on the experiment. The burgers were fried at a chosen temperature until the core temperature was 75°C. The hardness, cohesiveness, springiness, and chewiness of the burgers were obtained with a shearing test and texture profile analysis. Experiments 2 and 3 optimised the method of the experiments. Experiment 3 concluded that the burgers should be dry-fried at 250 W in a raw state instead of frozen. A resting time was included before frying to allow the binders to set in the food matrix, varying from 2 hours in room temperature to 2 hours (experiment 4) and 24 hours (experiments 5 and 6) in a cold room.

C-0% (burger with no binder) fell apart in nearly every experiment, proving the importance of a binder. 2% MC was not significantly different from 1% at 5% level of significance. 1% G and 1% M were not significantly different and yielded significantly lower textural parameters than 1% MC. 0.5% MC, G, and M resulted in lower textural parameters than 1% MC. The burger with 1% of the SL pre-treated with high pH (H) was the only burger with seaweed as a binder not resulting in significantly different textural properties to 1% MC. The enzyme pre-treated SL gave lower results than 1% MC, regardless of concentration. The results may suggest that the high pH treatment led to higher availability of alginate than the enzyme pre-treatment. 2% H did not yield the same results as 1% H, but this may be due to the low water availability in the food matrix. The mixture of alginate and MC gave the most promising results. There were no significantly different textural parameters between 1% MC and the burgers with alginate and MC, regardless of the ratio. This result suggested that the polymers interacted with each other, and coincided well with previous studies reporting a synergistic effect between MC and alginate. No reliable conclusion was drawn on the optimal ratio between the two polymers. The partial substitution of MC with alginate should be investigated further.





# Sammendrag

Grunnet miljøpåvirkninger fra storfeproduksjon og helseproblemer knyttet til konsum av rødt kjøtt, har det oppstått en økt interesse for kjøttanaloger de siste årene. Mange kjøttanaloger basert på soyaproteinisolat (SPI) bruker metylcellulose (MC) for å oppnå samme tekstur som kjøtt. Målet for denne hovedoppgaven var å undersøke om full eller delvis substitusjon av MC med forskjellige bindemidler kunne føre til samme tekstur som MC gjør i dagens kjøttanalogburgere.

Avhandlingen besto av seks eksperimenter, i tillegg til to deleksperimenter. Starteksperimentet ble brukt til å formulere burgeroppskriften. Hver burger veide ca. 40 g og var laget av 30.0% SPI, 3.8% kokosolje, 3.8% rapsolje, og 62.5% destillert vann. Mengden bindemiddel ble tilsatt i prosent av totalvekten av en burger. Bindemidlene som ble undersøkt var 0-2% MC, 0-1% alginat – en med høy G-blokk fraksjon (G) og en med lav fraksjon (M) – 0-2% tare fra *Saccharina latissima* (SL) forbehandlet med enten enzymer eller vann med pH 8. En blanding av alginat (G og M) og MC i forskjellige forhold ble også testet som bindemiddel. Deleksperiment *a* og *b* undersøkte henholdsvis alginatene og tarene.

Burgerne ble blandet, presset, og lagt til hvile for en bestemt tid, ved en bestemt temperatur avhengig av eksperimentet. Deretter ble de stekt ved en bestemt temperatur til kjernetemperaturen var 75°C. Hardheten, kohesiteten, spenstigheten og seigheten til burgerne ble gitt med en skjæretest og teksturprofilanalyse. Eksperiment 2 og 3 optimaliserte metoden brukt i eksperimentene. Eksperiment 3 konkluderte med at burgerne burde tørrstekes ved 250 W fra rå tilstand istedenfor frossen. Hviletiden ble inkludert i oppskriften slik at bindemiddelet kunne få tid til å sette seg i matrisen. Denne endret seg fra to timer i romtemperatur til to timer (eksperiment 4) og 24 timer i kjølerom (eksperiment 5 og 6).

C-0% (burger uten bindemiddel) falt sammen under nesten hvert eksperiment. Dette viste viktigheten av et bindemiddel. 2% MC var ikke signifikant forskjellig fra 1% med 5% signifikansnivå. 1% G og 1% M var heller ikke signifikant forskjellig fra hverandre og ga signifikant lavere teksturegenskaper enn 1% MC. 0.5% MC, G, og M resulterte i lavere teksturparametre enn 1% MC. 1% SL behandlet med høy pH (H) var det eneste bindemiddelet fra tare som ikke ga signifikant forskjellig teksturparametre fra 1% MC. Enzymbehandlet SL ga signifikant lavere verdier, uavhengig av konsentrasjon. Disse resultatene kan tyde på at forbehandling med høy pH førte til en høyere grad av alginatiltgjengelighet enn enzymbehandling. 2% H resulterte ikke i de samme resultatene som 1% H. Dette kan være på grunn av den lave vanntilgjengeligheten i matrisen. Blandingen mellom alginat og MC ga de mest lovende resultatene. Det var ingen signifikant forskjellige teksturparametre mellom noen av burgerne med en blanding mellom 1% MC og blandingen av MC og alginat, uavhengig av blandingsforhold. Dette kan tyde på at polymerene interagerer med hverandre. Dette samsvarer med tidligere funn av en synergieffekt som oppstår mellom alginat og MC. Det ble imidlertid ikke trukket noen pålitelig konklusjon angående det optimale forholdet mellom alginat og MC. En delvis substitusjon av MC med alginat bør undersøkes nærmere.

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# Introduction

## 1.1 Background

Today, food production stands for approximately 26% of the greenhouse gases (GHG) emitted into the atmosphere. Meat production, especially red meat, is one of the most polluting productions that exist today (Ritchie & Roser, 2020). The average European diet includes 56% meat and egg (Sandström et al., 2018). Red meat has been part of the Western diet for many centuries. It has a good nutritional profile, including vitamins and minerals, and has a chemical score of 57% (Coultate, 2016). Red meat is made of approximately 21% protein, including all the essential amino acids (Damodaran & Parkin, 2017). However, due to the high cholesterol and saturated fatty acids levels in meat, a rich red meat diet may lead to increased incidences of cardiovascular diseases (Vang et al., 2008, Wang and Beydoun, 2009). In addition, the production of red meat emits a substantial amount of CO<sub>2</sub> equivalents. Beef has a carbon footprint on average of 71 kilograms of CO<sub>2</sub> equivalents per kilogram (including the methane emissions) (Ritchie, 2020). With the increasing demographic and the development of underdeveloped countries, the demand for meat is increasing to a point where it is impossible to meet these new demands (Kurek et al., 2022, Ritchie and Roser, 2017, IPCC, 2019).

With the increasing awareness of the impact of red meat production and consumption, as well as animal welfare, alternative proteins have become increasingly popular (Boukid, 2021). The Intergovernmental Panel on Climate Change has reported that changing to healthy and sustainable diets will have a great impact on reducing GHG emissions (IPCC, 2019). The production of plant-based protein alternatives has a much lower carbon footprint than meat. Tofu, peas and root vegetables have a carbon footprint on average of 3, 1, and 0.4 kilograms of CO<sub>2</sub> equivalents per kilogram, respectively (Ritchie, 2020). Alternative proteins are more cost-effective and less polluting than meat (Boukid, 2021). In addition, they may also reduce the risk of cardiovascular disease, diabetes, obesity, and total mortality (Singh et al., 2021). The nutritional profile of plant proteins often lacks a complete profile of the essential amino acids (Singh et al., 2021). However, most plant proteins still provide a high chemical score: peanuts have 65%, and wheat 49% (Coultate, 2016). Plant-based protein foods rich in protein include legumes, quinoa, lentils, and hemp protein. Other alternative proteins with promising futures

are fungi, insects, and algae. Legumes include soybeans, green peas, lentils and fava beans (Singh et al., 2021). Soybeans are produced in vast quantities in the world today, although most have traditionally been used as animal feed in Western countries (Ritchie & Roser, 2021). With the success of Impossible Foods, Beyond Meat, and Gardein, plant-based meat analogues (PBMA) have become part of today's global food market (Sha & Xiong, 2020). Many PBMA are based on soy, such as Orkla's Naturli' and the Impossible burger. Soybeans include all essential amino acids, along with many minerals and a lot of fibre (Coultate, 2016, Kurek et al., 2022, Singh et al., 2021).

Despite these facts, there are many barriers for consumers to eat PBMA. Some are based on practicality, such as cost and convenience. The lack of knowledge on how to prepare meals with soybeans, and not having tasted any soy products are common. Other barriers are psychological. These include unawareness of the impact on climate change from eating red meat compared to eating plant-based proteins, as well as habits and routines of everyday life (Niva et al., 2017). To make PBMA more suitable for meat-lovers, they need to mimic meat in look, taste, mouthfeel, texture, and smell. The texture and rheological properties of the food matrix are essential to investigate further, to produce a product consumers are willing to buy (Kurek et al., 2022).

This thesis investigates the influence of alginate and seaweed on the texture of vegetarian soy burgers. The following sections cover soy protein, including soybean production, composition, and specific properties utilised in PBMA, the use of the hydrocolloids methyl cellulose and alginate in food products, challenges surrounding PBMA, a quick introduction to texture analysis, and a description of the objective of the thesis.

## 1.2 Soy protein

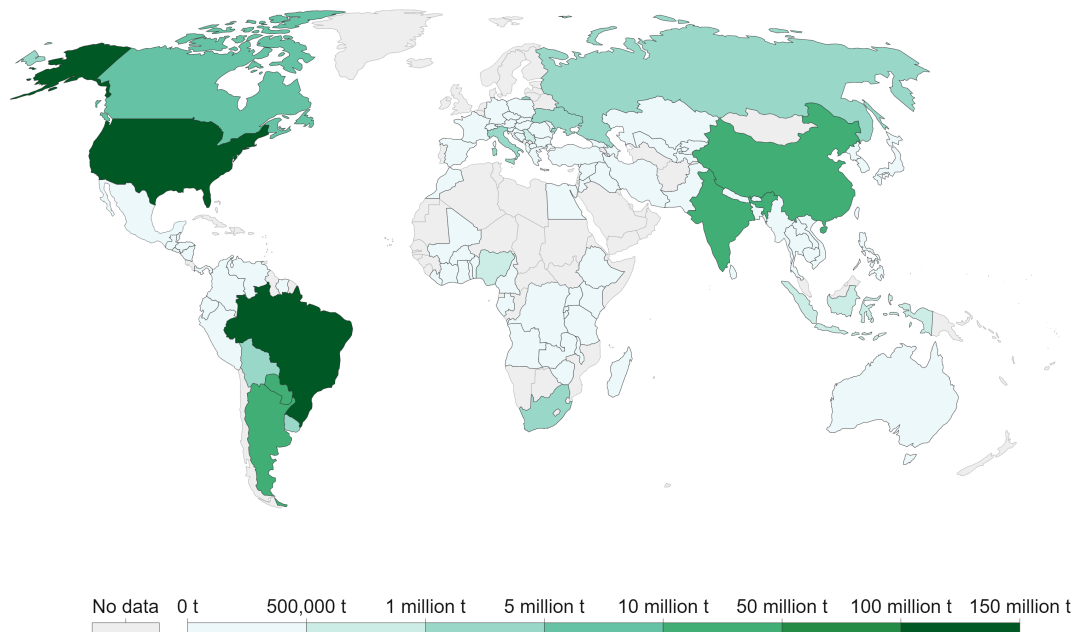
The base of many PBMA is soy, in the form of soy protein isolate (SPI). This section focuses on the production and composition of soybeans, and the extraction and use of SPI in PBMA.

### 1.2.1 Soybean production

Soybeans have been cultivated in Asian countries for thousands of years, and are still today a common part of the Asian diet. In Western countries, soy is mostly consumed as part of a vegetarian diet (Coultate, 2016). Despite the fact that most soy produced worldwide goes to animal feed (77%), a significant portion (19%) goes directly to human food. Nowadays, the main producers are USA (~ 125 million tonnes per year) and Brazil (~ 118 million tonnes per year) (Figure 1.2.1). Argentina comes in third (~ 40 million tonnes per year), followed by China and India (both ~ 14 million tonnes per year). Soy is also grown in a few European countries. However, the leading soy producer in Europe (Ukraine) cultivates only up to 4% of the soy grown in the USA (Ritchie & Roser, 2021).

#### Soybean production, 2018

Soybean production is measured in tonnes.



Source: UN Food and Agriculture Organization (FAO)

OurWorldInData.org/agricultural-production • CC BY

Figure 1.2.1: Production of soy in the world in 2018 (Ritchie & Roser, 2021).

### 1.2.2 Soybean composition

A raw soybean consists mainly of storage proteins and lipids, 40% and 20%, respectively (Figure 1.2.2). The remaining part consists of soluble carbohydrates, dietary fibre, water and other substances such as minerals. The storage proteins can be classified into four types: 7S ( $\beta$ -conglycinin), 11S (glycinin), 2S and 15S. 7S globulins are trimers consisting of three subunits: the hydrophilic polypeptides  $\alpha$  and  $\alpha'$ , and the hydrophobic polypeptide  $\beta$ . 11S globulins are hexamers composed of three subunits linked by disulfide bonds. Each subunit is made of an acidic hydrophilic peptide and a basic hydrophobic peptide (Nishinari et al., 2014, Sha and Xiong, 2020).

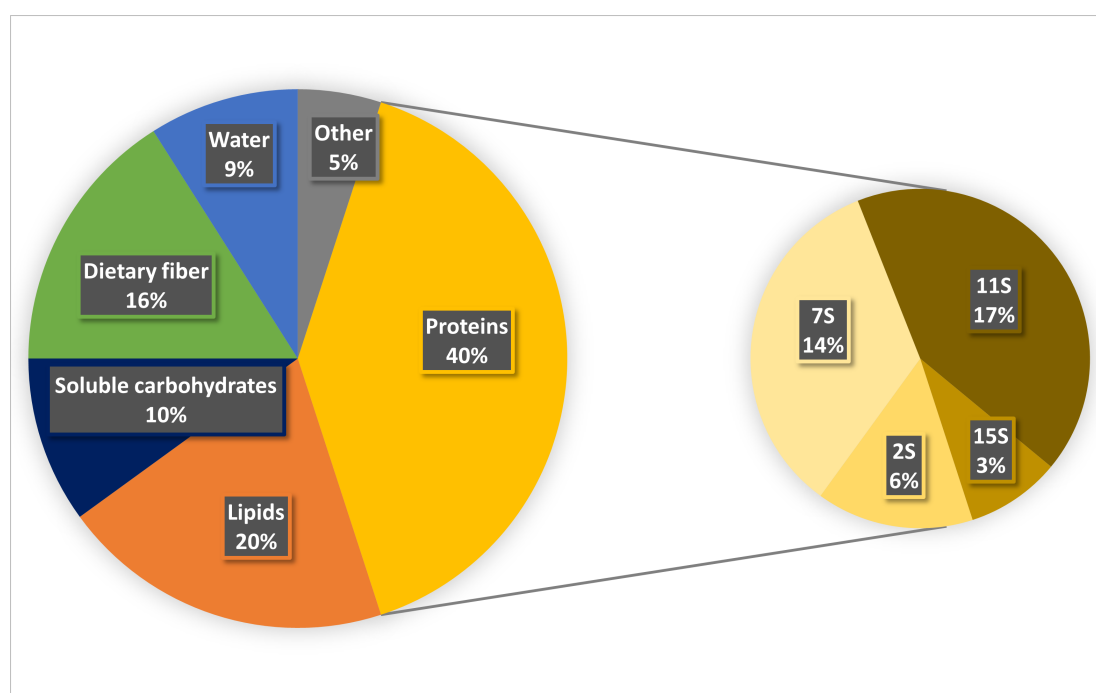


Figure 1.2.2: Composition of a soybean (Norwegian Food Safety Authority, n.d., U.S. Department of Agriculture - Agricultural Research Service, n.d., Guan et al., 2021, Medic et al., 2014, Nishinari et al., 2014).



### 1.2.3 SPI and its gelling properties

Three major products are obtained from soybeans: soy protein isolates (SPI), soy protein concentrates (SPC), and defatted soy flours (Ma, 2014). Despite being low in cysteine and the essential amino acid methionine, SPI has a high lysine content and a PDCAAS<sup>1</sup> of approximately 100% (Boye et al., 2012). There already exists some soy protein products in the market today like tofu, miso and tempeh. Although these products are fermented, they are similar to SPI as they are packed with proteins that form a strong gel network. Many steps are required to produce both SPI and tofu. Each step may have an impact on the soy protein and the protein network (Guan et al., 2021).

SPI is obtained by defatting soybeans. This process can be done in several ways: by using organic solvents, aqueous or mechanical extraction, supercritical extraction, or enzymatic treatments. The aqueous extraction technique includes defatting the soy flakes by using dilute alkali (pH 8-9) (Figure 1.2.3). This is followed by a centrifugation step and acidification (pH 4.5). By reducing the pH, the protein forms a curd allowing other substances to be easily removed. Finally, the curd is neutralised (pH 7) and dried. The SPI obtained has a protein content of approximately 90% protein on dry weight basis (Ma, 2014).

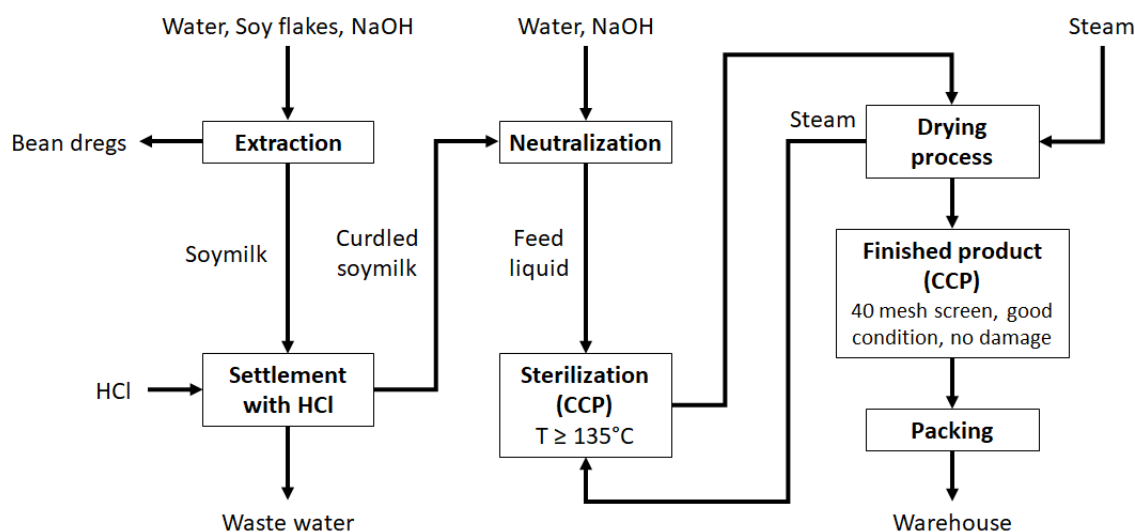


Figure 1.2.3: Flow chart of soy protein isolate production on an industrial scale with permission from Star Nutrition, Norway.

SPI is used in alternative protein products such as plant-based meat analogues (PBMA). In these products, SPI provides strong gel-forming abilities. This ensures moisture in the food product by trapping water and immobilising fat in the food matrix. The gel-formation originates from the 7S and 11S globulins dissociating, unfolding and reaggregating under heating condi-

<sup>1</sup>Protein Digestibility Corrected Amino Acid Score. The PDCAAS refers to how well a food product's amino acid profile is compared to the standard profile. A high PDCAAS means the product covers all the essential amino acids required for humans.

tions (Sha and Xiong, 2020, Fukushima, 2011). Stanley (1987) reported that the hardness of the gel network formed by SPI increased with rising temperatures up to 80°C. The hardness of the gel weakened above this temperature. The study suggested this was due to the disulphide bonds between the 11S globulins, and the hydrogen bondings and hydrophobic interactions between the soy proteins (Stanley, 1987).

The gelation mechanism is influenced by pH and salt. The pI of SPI is around 4. Nishinari et al. (2014) reported that the gel network becomes stronger at acidic pH. The study suggested this was most likely due to the increasing  $\beta$ -conglycinin denaturation with decreasing pH. At pH 7, the gelling was not reported to be very prominent. Adding salt, such as sodium chloride, has also proved to delay gelation. The protein molecules are stabilised and less prone to heat denaturation (Nishinari et al., 2014).

The technological abilities of SPI alone are not able to mimic the texture of meat. To get the best result, plant proteins are often used together as textured vegetable protein (TVP). TVP varies in composition according to the producer, but is usually based on blends of SPI, wheat gluten, and pea protein isolate (PPI). Gluten is used in many PBMA due to its viscoelastic properties, and ability to form a strong protein network. These provide the right amount of chewiness in the PBMA. However, due to celiac disease and gluten intolerance, PBMA with gluten are not desired by many producers (Kurek et al., 2022).

Another way to achieve the proper texture with SPI, without using TVP, is to mix the isolate with hydrocolloids. Hydrocolloids are used as binding and thickening agents in many food products. In plant-based burgers, methyl cellulose is common to find due to its ability to gel when heated (Cash & Caputo, 2009). Alginate is another common hydrocolloid in food products and non-edible products. To the best of the author's knowledge, alginate is not used in PBMA. However, alginate gels chemically and is not influenced by temperature changes (Helgerud et al., 2009). It should be possible to implement it in a PBMA. The next section covers the general properties of methyl cellulose and alginate.

## 1.3 Hydrocolloids

Hydrocolloids are used as thickening and binding agents in food products. Depending on the hydrocolloid, they provide emulsion and foam stability, increase viscosity, adhesiveness, and swelling. Adding hydrocolloids to a food product, will add nutritional value and may also prolong its shelf life (Sworn, 2004, de Vries, 2004). This section focuses on two hydrocolloids: Methyl cellulose (MC) and alginate. MC is common to find in plant-based meat analogues (PBMA). To the best of the author's knowledge, alginate is not used in PBMA. This thesis investigates alginate as a potential substitution for MC.

### 1.3.1 Methyl cellulose: E461

Methyl cellulose (MC) is a derivative of cellulose. Cellulose is a linear polysaccharide polymer made of  $\beta$ -D-glucopyranosyl units linked with  $\beta$ (1,4)-glycosidic linkages. It is found abundantly in nature as the main component in the cell walls of many plants. To form MC, cellulose is etherified. Cellulose reacts with alkali, which disrupts the rigid crystalline regions of the polymer. This is subsequently etherified through Williamson etherification or alkoxylation. Methyl chloride is used to form MC (Cash and Caputo, 2009, Nasatto et al., 2015). MC has eight possible structures (Figure 1.3.1). The configuration depends on the degree of substitution (DS) of the methylated cellulose. The DS varies from 0 (unsubstituted cellulose) to 3 (fully substituted cellulose). Commercial MC has a DS usually between 1.7 to 2.2. This creates a semi-flexible polymer (Coughlin et al., 2021).

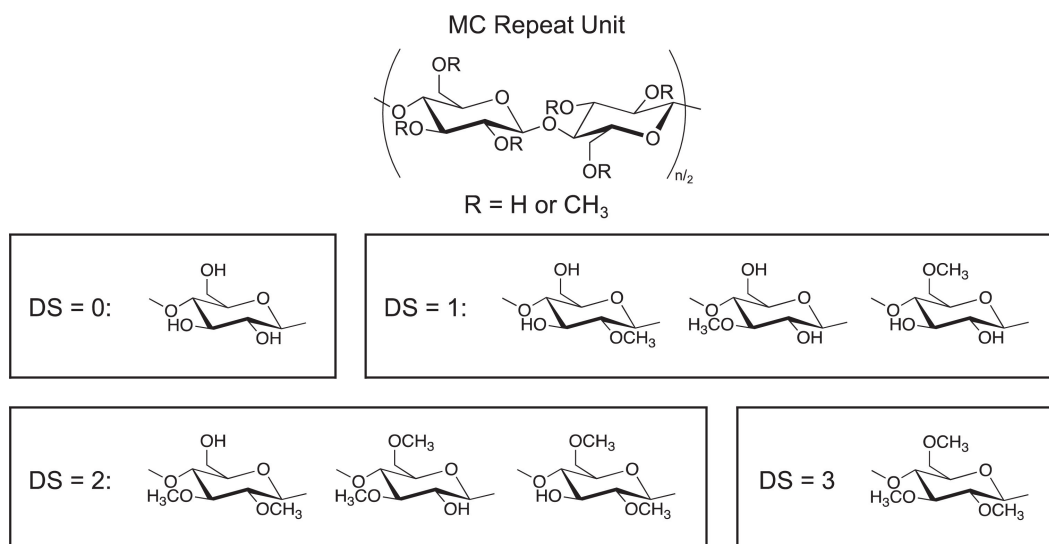


Figure 1.3.1: The eight possible structures of methyl cellulose. The degree of substitution ranges from 0 (unsubstituted cellulose) to 3 (fully substituted cellulose) (Coughlin et al., 2021).

MC is used in food products with the E-number 461. Due to its gelling ability during heating, MC is used in food products that require heating, such as soups, sausages, burgers, and PBMA. In plant-based food, MC is a good substitute to use instead of wheat gluten (Coultrate,

2016, Sworn, 2004). Despite MC being a non-ionic amphiphilic polymer and soluble in cold water, lumps will form if the solution is not highly agitated. Adding MC with dry ingredients is advised as a good way to avoid lumping and getting the MC uniformly distributed in the food product (Cash & Caputo, 2009).

Coughlin et al. (2021) suggest that the MC hydrogel is formed due to cross-links between fibrils. The fibrils are self-assembled MC polymer chains which are formed upon heating and dissolve upon cooling (Figure 1.3.2). The formation of fibrils, as well as their structure and diameter, seem to be independent of both the concentration and molecular weight of MC. The variation in temperature of gelation and the methyl moieties' regiochemical distribution does not seem to influence the formation of fibrils (Coughlin et al., 2021).

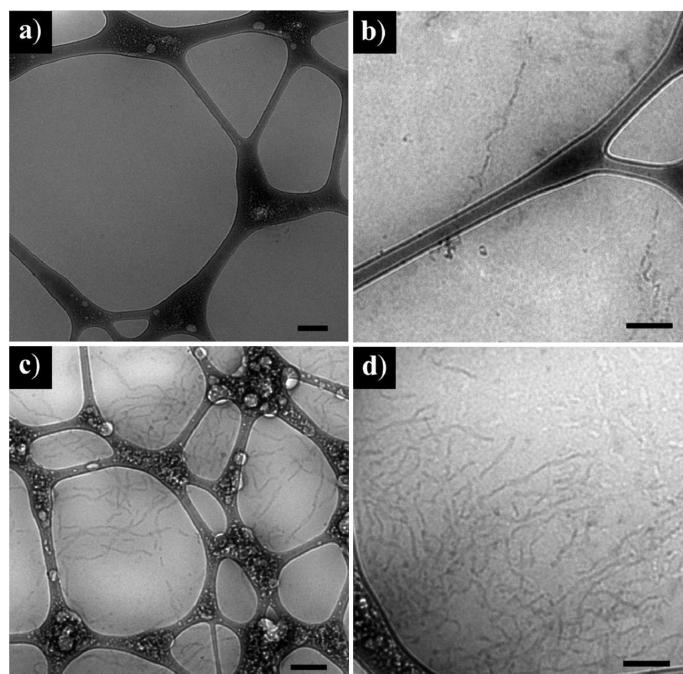


Figure 1.3.2: Cryo-TEM images taken upon heating of 0.2wt% methyl cellulose (MC) solutions. The images show the formation of MC fibrils at a) 50°C, b) 55°C, c) 60°C, and d) 65°C. The scale bar is 200 nm (Coughlin et al., 2021).

The addition of salt in the food matrix will lower the gelling temperature of MC. 2% sodium chloride lowers the temperature of a 2% MC solution by 10-15°C (Cash & Caputo, 2009). The salt will induce fibril formation at room temperature, resulting in elastic regions in the food matrix (Coughlin et al., 2021). However, if the salt concentration increases more than 5%, the MC will start to precipitate. This is due to the "salting-out" effect: the higher affinity towards water will let the sodium chloride remove water from MC. Sucrose will also lower the gelling temperature. Adding 10% sucrose will lower the gelling temperature by up to 10°C, while 40% may lower the temperature up to 30°C (Cash & Caputo, 2009). To the best of the author's knowledge, no interactions have been reported between calcium and MC, nor between SPI and MC.

### 1.3.2 Alginate: E400-405

Alginate is found in the cell wall and intercellular space of brown algae as the structural polysaccharide calcium alginate. The alginate level varies according to seaweed species, the season, the harvest location, and the storage method (Indergaard, 2010). Previous research has reported a maximal alginate level of  $28.5 \pm 3.9\%$  and  $37.4 \pm 4.0\%$  in dry weight of *Saccharina latissima* (SL) and *Alaria esculenta* (AE), respectively (Schiener et al., 2015). The biopolymer consists of two monomers:  $\beta$ -D-mannuronic acid (M) and its C-5 epimer,  $\alpha$ -L-guluronic acid (G) (Figure 1.3.3). These two building blocks are linked together by (1,4)-glycosidic bonds. The sequence of M and G varies according to seaweed species and harvest season, affecting the gelling properties of the biopolymer. The gel formation is attributed to the reaction between divalent ions and the G-blocks. The model is often referred to as the “egg-box” model: the G-blocks and divalent ions being the egg box and eggs, respectively (Figure 1.3.4). The G residues cross-link with divalent ions forming junction zones and, finally, a strong heat-stable gel (Helgerud et al., 2009, Indergaard, 2010).

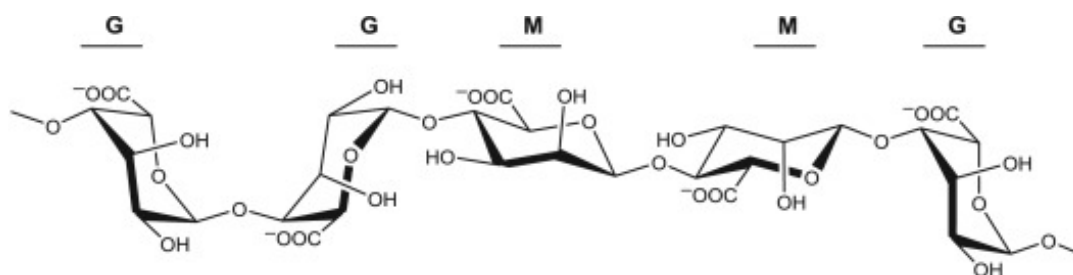


Figure 1.3.3: Alginate is composed of an alternating sequence of two building blocks:  $\beta$ -D-mannuronic acid (M) and  $\alpha$ -L-guluronic acid (G). These are linked with (1,4)-glycosidic bonds. The length and amount of M and G may vary according to species, season, and harvest location (Rioux & Turgeon, 2015).

The junction zone can be viewed as an ion-exchange process. The negative charge on the alginate’s carboxyl group reacts with a divalent cation. The binding affinity of alginate for the divalent cations varies, from lowest to highest affinity: potassium < sodium < calcium (Peteiro, 2018). The firmness of the gel is also attributed to the number and length of G-blocks (-GGGG-). More G-blocks will form a stronger gel. Blocks of M (-MMMM-) will form weak cross-links, and therefore a weak gel. However, alternating blocks of M and G (-MGMG-) will interfere with the intermolecular cross-linking, and therefore not form a gel at all (Helgerud et al., 2009).

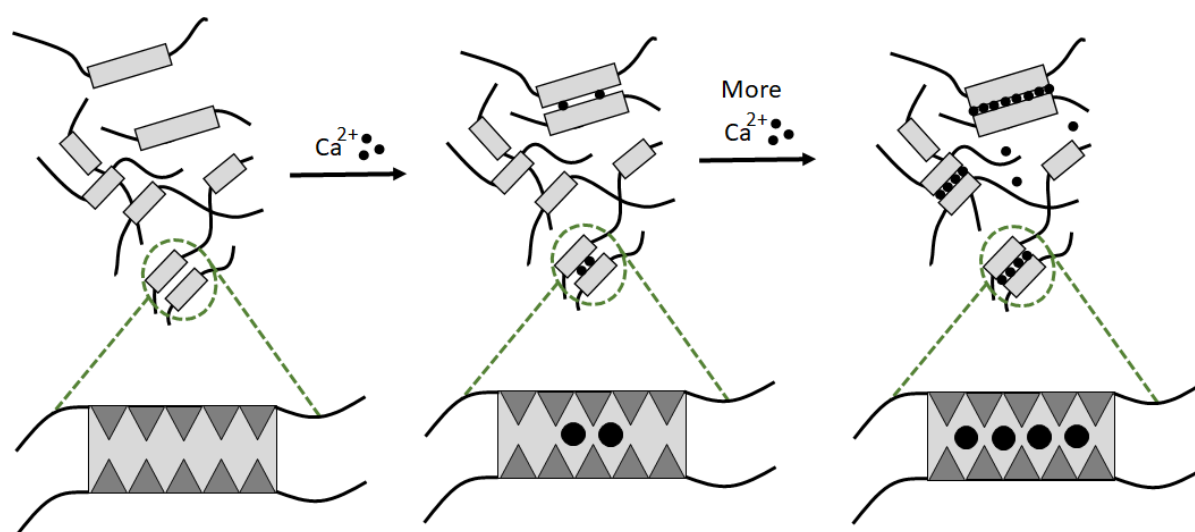


Figure 1.3.4: Alginate gel formation viewed as the "egg-box" model. Blocks of  $\alpha$ -L-guluronic acid (G) react with divalent ions shown as  $\text{Ca}^{2+}$ . G-blocks are shown as grey boxes.  $\text{Ca}^{2+}$  are shown as black circles. The ions can be seen as eggs, and the G-blocks as the top and bottom of the egg box.

Since calcium alginate is insoluble within the seaweed, it needs to be extracted. Although the extraction process is theoretically simple, the process is long and requires a large amount of dilute acid and alkaline solution (Figure 1.3.5). The extraction of alginate can be seen as three steps: a pre-extraction step, a neutralisation step, and a precipitation step. During the pre-extraction step, the seaweed reacts with a large amount of acid (0.1-0.2 M HCl). This sets the counterion free, and the free acid form of alginate is formed: alginic acid with E-number E400. Since alginic acid is insoluble, the acid water is drained away, containing mannitols, fucoidan, laminaran, and salts that exist in the algae and algae cell wall. In the neutralisation step, the alginic acid reacts with a large amount of alkali (0.2 M NaOH/ $\text{NaCO}_3$ , pH 7-8). The sodium reacts with the alginic acid and forms sodium alginate with E-number E401. After centrifugation, the soluble sodium alginate is separated from the solution. The last step is the precipitation of sodium alginate. This is either done by lowering the pH with acid, or reacting the sodium alginate with calcium first, then acid. The precipitated alginic acid is separated from the solution, the pH is increased, and the sodium alginate can now be dried and stored for use (Draget, 2009). In the neutralisation step, the alginic acid can react with other divalent ions, such as potassium, ammonium, calcium, or propylene glycol, and form potassium alginate (E402), ammonium alginate (E403), calcium alginate (E404), or propylene glycol alginate (E405), respectively. Sodium alginate is used as an emulsifier, stabiliser and thickening agent in food products. If calcium is added, due to the difference in affinity as previously mentioned, calcium will react with the carboxyl groups on alginate, and start forming a strong or weak gel according to the amounts of G-blocks (Helgerud et al., 2009).

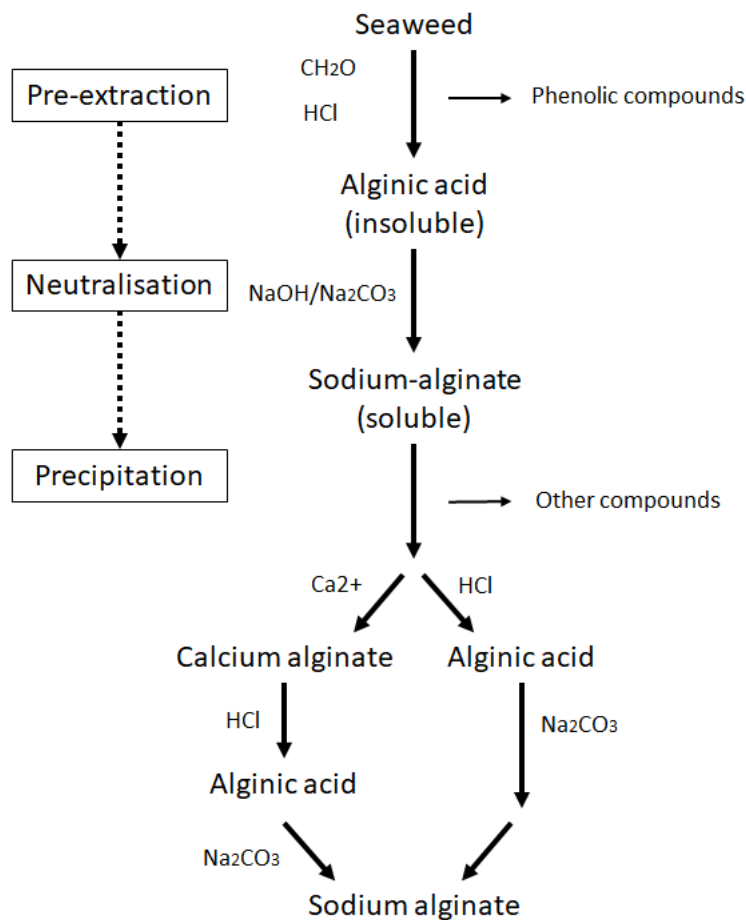


Figure 1.3.5: Flow chart illustrating the extraction process of alginate on an industrial scale (Draget, 2009, Draget et al., 2016).

Sodium alginate is bought as a powder with a chosen particle size. When water is added, it easily lumps, and needs high shear mixing or a long time before it dissolves completely. To avoid lumping, ethanol can be added to the sodium alginate before dissolving it in water. Sodium alginate is not soluble in ethanol. When ethanol is added, this causes the sodium alginate on the surface of the particles to precipitate. When water is added, the alginate will not be able to unfold and make entanglements with the surface of other alginate grains. After a while, the ethanol will be diluted, and the alginate can dissolve as usual. However, the alginate particles will be so distanced from each other that they will not be able to lump together. The same effect is observed when alginate is added dry with sugar (Draget and Nordgård, 2021).

To add calcium,  $\text{CaSO}_4$  may be used. However, the solubility of  $\text{CaSO}_4$  is very high, resulting in spontaneous gelation. Complexing agents such as polyphosphates are used to control the gelling kinetics and slow down the process. Tetrasodium pyrophosphate (TSPP), for example, can be added prior to calcium in a sodium alginate solution. When calcium is added, TSPP will sequester calcium and slow down the gelation process. The alginate can thus be distributed uniformly in the food product prior to setting completely (Melvik et al., 2004, Draget

and Nordgård, 2021). SPI has been reported to have a similar binding effect to calcium, slowing down the gelation effect. However, these mechanisms are not yet fully understood (Bao et al., 2008). TSPP will also increase the pH in the solution, and work as a dispersing agent, emulsifier, and protein modifier. The addition of TSPP will also reduce syneresis and the cook loss of the food product (Lampila & Godber, 2002).

Many hydrocolloids used in food products today are thermoreversible. A unique property of sodium alginate is its ability to form a thermoirreversible gel. The junction zones formed with the divalent ions will not change conformation upon gelling. A drastic change in pH is required to change the conformation (Peteiro, 2018). In a food matrix such as a PBMA, alginate could be a possible alternative to methyl cellulose, which is currently used for this type of vegan food. An important factor to be aware of is that the gel will not set if the pH is below the pKa of alginate (pKa = 3.5). However, many food products have a neutral or slightly acidic pH, so this is relatively easy to achieve (Coulter, 2016). Due to its negatively charged groups, alginate may also interact electrostatically with positively charged amino acids on proteins (Draget & Nordgård, 2021).



## 1.4 Challenges with meat analogues

There are several challenges when making meat analogues. To compete with a meat product, the PBMA needs to have a comparable nutritional value, the right sensory experience when consumed, and be safe to eat (Boukid, 2021). This thesis investigates the implementation of seaweed into burgers based on SPI. This section focuses therefore on challenges related to texture and the difficulties of mimicking meat, as well as the food safety around the burger analogue.

### 1.4.1 Texture

One of the most important technological hurdles for PBMA is mimicking the texture of meat. This is one of the barriers which prevents consumers from choosing to eat more plant-based protein (Niva et al., 2017). This section focuses on the components responsible for the texture of meat.

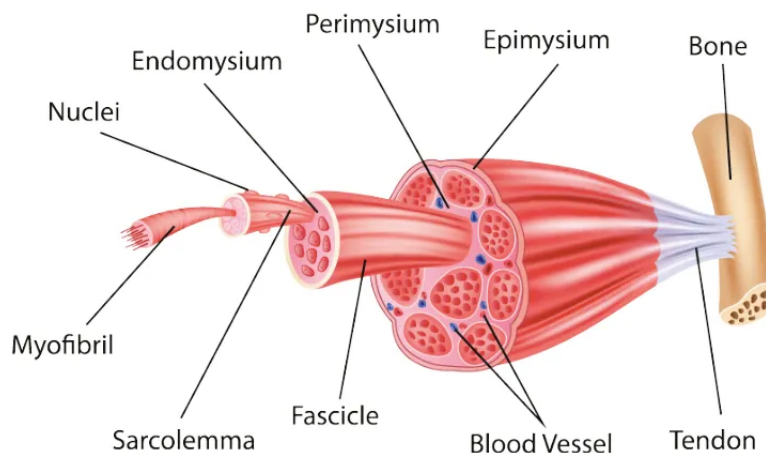


Figure 1.4.1: Illustration of a skeletal muscle structure (Parker, 2021).

Skeletal muscles are composed of approximately 70% water, 21% protein, 7% fat, 1% carbohydrate and 1% ash, including minerals (Damodaran & Parkin, 2017). The skeletal muscle is made up of muscle bundles known as fascicles (Figure 1.4.1). These are composed of muscle fibres which are the contractile units of the muscle. Each muscle fibre is approximately 10-100  $\mu\text{m}$  in diameter and up to 30 cm long (Coultate, 2016). Myofibrils, which are made of myosin and actin filaments, make up the muscle fibres. Myofibrils play an important role in the organoleptic properties of processed meat products. They form a protein gel matrix that traps water and immobilises fat. This is possible due to the high length-to-diameter ratio of myosin, in addition to its bipolar properties (Damodaran and Parkin, 2017, Sha and Xiong, 2020). The difficulty with mimicking meat with plant proteins lies in engineering the interstitial space, which naturally exists between myofilaments (Sha & Xiong, 2020).

From a nutritional perspective, SPI is comparable to meat. However, the structure of meat is difficult to replicate. At a microscopic level, plant proteins will never be the exact replicate of muscle proteins. This being said, SPI is still a good alternative as it provides gelling abilities, fibre formation, and aggregation when heated and during extrusion (Sha & Xiong, 2020). Extrusion is a common processing technique where plant proteins are transformed into structured aggregates and fibrils. This is a low-cost, versatile process which is common for whole-muscle meat analogues. A dry mix of plant protein mass is added to a drag flow device with a forward pumping action called an extruder. The protein undergoes several processes through the extruder, such as hydration, compression, mixing, kneading, and shredding. The extruder uses high, intermediate or low moisture, as well as varying the temperature to form different types of fibrils. The resulting fibrils formed are used as meat analogues. Textured vegetable protein (TVP) is common to use in combination with extrusion. TVP is composed of different types of plant proteins, such as soy protein, wheat gluten, and pea protein (Zhang et al., 2019, Sha and Xiong, 2020).

Despite the gelling abilities of SPI, without extrusion, it does not succeed in forming a replicate of meat on its own. Binders, such as hydrocolloids, are necessary for the proper texture and mouthfeel. Methyl cellulose (MC) and alginate improve consistency, bind water and reduce syneresis. The negatively charged carboxyl groups of alginate bind strongly to water through hydrogen-bonding and ion-dipole interactions. This improves the thickness of the product, as well as its consistency, and reduces cook loss. As previously mentioned (Section 1.3.2), alginate has a strong affinity to calcium. When calcium interacts with the G-blocks in the biopolymer, a cold-setting gel is formed. The gel formation may bind protein particles and provide the right mouthfeel for the meat analogue. Also, the addition of minerals such as sodium pyrophosphates increases the nutritional value of the product, and binds water which contributes to the texture and reduces syneresis (Sha & Xiong, 2020). Tetrasodium pyrophosphate (TSPP) will also slow down the gelation of sodium alginate, and reduce the cook loss of the food product (Lampila & Godber, 2002).

Lipids are added in PBMA to improve the texture. In meat, fat is trapped in a gel matrix. During heating, the fat melts and ensures flavour, as well as juiciness and tenderness in the final product. The flavour is discussed in the next section. In soy burgers, fat is added during the cold-mixing of the ingredients. By adding the fat in a cold stage, it can remain solid until heat is added, creating the same textural sensation as with meat (Kyriakopoulou et al., 2021).

Furthermore, from a nutritional perspective, fat in PBMA is usually considered healthier than fat found in meat products. However, this depends on the type of fat used in the PBMA. Both a raw beef burger and a plant-based burger may be composed of up to 20% fat, but there are differences in the fatty acid composition. Beef fat consists mainly of long saturated and mono-saturated fatty acid chains, approximately 28 mol% 16:0, 20% 18:0, and 34% 18:1 fatty acids. Coconut oil used in PBMA, includes mainly short saturated fatty acid chains, approximately 12 mol% 8:0, 49% 12:0, 16 mol% 14:0, and 7 mol% 16:0 fatty acids. Depending on the length, degree of saturation, and placement of the double bonds (including cis and trans

conformation), the fatty acids can be deemed healthier than other fatty acids. The fatty acids regarded as most unhealthy today, are palmitic, myristic and lauric acids. Palmitic acids have a typical configuration of 16:0, myristic of 14:0, and lauric of 12:0. Looking at these acids, beef fat and coconut oil share a quite unhealthy amount of fatty acid chains. However, PBMA also include other plant oils, such as canola oil, which have healthier fatty acids, such as linolenic acids (18:3), and oleic acids (18:1). The different fatty acids lead to the production of different cholesterol when consumed. The unhealthy fatty acids lead to high levels of LDL cholesterol (i.e. the "bad" cholesterol), while healthy fatty acids lead to high levels of HDL (i.e. the "good" cholesterol). Cholesterol is important for the human body. It is either consumed or synthesised by the liver, and is an important building block in the membrane of cells as a pathway for fatty acids. However, LDL circulates in the bloodstream, while HDL absorbs LDL from the blood and transports it to the liver, which removes it. High levels of LDL can cause plaque formation on the artery walls, which may lead to atherosclerosis, blood clots and cardiac arrest. The fat in PBMA is therefore healthier than the animal fat in meat products (Coulter, 2016, Sha and Xiong, 2020, Vang et al., 2008, Wang and Beydoun, 2009).

Water is an element often forgotten in a food matrix, but with very important features. Water hydrates dry ingredients, such as SPI and dried seaweed. Water availability improves a product's textural properties by interacting with different compounds in the food matrix, such as biopolymers. Finally, water can retain sensory properties such as juiciness and mouthfeel, which is important for meat analogues. As mentioned above, skeletal muscle includes up to 70% water. This water is trapped in between the myofibrils during heating, resulting in a food product with high moisture. For meat analogues to succeed in mimicking meat, both the water amount and keeping it trapped in the food matrix are very important (Kyriakopoulou et al., 2021).

### 1.4.2 Flavour and aroma

Another important factor for a successful PBMA is the desired flavours and aromas (Niva et al., 2017). To mimic meat, the complex pathways to the resulting flavour and volatile compounds must be understood.

When a meat burger is heated, it undergoes several reactions. Thiamines undergo thermal degradation, resulting in flavour compounds, such as furans. Sugars undergo caramelisation, resulting in flavours, volatile compounds, and brown pigments. Sulfur compounds undergo thermal degradation. Lipids undergo lipid oxidation and interact with intermediates in the Maillard reaction, resulting in flavour and volatile compounds. Sugars, amino acids and peptides, and sulfur compounds also undergo the Maillard reaction. This is a series of reactions essential for releasing the desired flavours and volatile compounds. At high temperatures, where meat is typically cooked, glucose reacts with free amino acids and forms the Amadori product. This in turn reacts to form several different compounds: furanone derivatives, furfurals, dicarbonyl compounds, and hydroxy ketones. These compounds react further through the different pathways (Schiff base pathways and Amadori rearrangement) with a number of other

compounds, such as amino acids, amines, hydrogen sulfides, ammonia, and aldehydes. All of these compounds provide flavour to the meat product.  $\alpha$ -dicarbonyl compound reacts with  $\alpha$ -amino acids to form volatile aldehydes through the Stecker degradation. Finally, after several reactions, brown pigments (melanoidins) are formed (Coultate, 2016, Arshad et al., 2018, Sun et al., 2022).

Although PBMA consists of lipids and proteins, the raw material of PBMA is not the same as meat. The Maillard reaction takes place when PBMA is fried. However, many fat-soluble volatiles contributing to the flavour of meat will not arise during heating of meat analogues (Sha & Xiong, 2020). Despite this, soy contributes to the umami taste, which is associated with meat. Otherwise, SPI is relatively flavourless, making it easier to mimic meat flavours and aromas by adding different ingredients and spices into the food matrix (Sun et al., 2022).

### 1.4.3 Food safety

There are a few undesirables when working with soybeans and seaweed. This section focuses on lectins, phytoestrogens, iodine, and heavy metals.

Soy protein can include up to 3% lectins. These are the plant's protection against insect pests. They inhibit the digestive enzyme of insects. Lectins are not directly lethal for humans. However, if too much lectins are consumed, humans may experience some symptoms of lectin poisoning. This includes vomiting, diarrhoea, and reduced uptake of nutrients from the digestive tract. The latter is suggested to be due to damage to the intestinal mucosa. Therefore, a high level of lectins is not desired in food products (Coultate, 2016). Fortunately, most lectins are inactivated when heated at high enough temperatures (Norwegian Food Safety Authority, 2010). Shi et al. (2018) reported that 93.77–99.81% of the lectins were gone from the legumes after boiling them for 1 hour at 95°C.

Soybeans also include phytoestrogens, classed as isoflavones. There are both potential health benefits and deleterious health effects from phytoestrogens. The benefits include a lower risk of breast and prostate cancer, heart disease and osteoporosis, while negative health effects include disruption of the endocrine system. These negative effects originate from the substance's affinity to oestrogen receptors. However, the affinity is very low (between one-thousandth and one ten-thousandth of that of oestradiol). Also, Asian diets have traditionally consisted of many fermented soy products. The daily intake of phytoestrogens of Chinese and Japanese adults is reported to be between 20 to 200 mg. Hence, eating soybeans is not seen as unsafe today (Coultate, 2016).

Seaweed is a good source of many macro- and micronutrients such as vitamin B<sub>12</sub>, and omega-3 and omega-6 fatty acids. They are rich in dietary fibre and minerals, such as sodium, potassium, zinc, iodine, and trace minerals. Iodine is an essential micronutrient for humans. It is necessary for the synthesis of thyroid glands which are vital for growth and development. A deficiency in iodine leads to dysfunction of the thyroid glands. At the early ages of development,

this can result in severe iodine deficiency syndrome characterised by an enlargement of the thyroid glands (goitre). However, too high levels of iodine can also lead to dysfunction of the thyroid glands (Coultate, 2016, National Food Institute et al., 2019). The Norwegian public authorities recommend a daily intake of iodine for children over 10 years and adults of 150  $\mu\text{g}$ . However, an intake above 600  $\mu\text{g}$  is not advised (Helsedirektoratet, 2012). The level of iodine should therefore be analysed when food includes dried seaweed. Seaweed may also contain heavy metals, such as cadmium (Cd), lead (Pb), mercury (Hg) and inorganic arsenic (iAs). High exposure to these metals is harmful to humans. Therefore, the levels of these heavy metals also need to be analysed (National Food Institute et al., 2019).

## 1.5 Texture analysis

Rheology is the study of flow and deformation of matter. Food rheology focuses on the textural properties of a food product. By studying these properties, food products can be altered to satisfy the consumer's demands (Bourne, 2002). Depending on the food product, different set-ups are required for texture analysis. For a burger, the shearing test and TPA are most relevant. This section focuses therefore on these two analysis methods.

### 1.5.1 Shearing test

Shearing tests are used for many food products. The test imitates the slicing movement done by the incisors when food is introduced into the mouth. The properties measured by a shearing test are bite strength, firmness (or hardness), tenderness and toughness. The set-up for a shearing test requires a knife blade (Figure 1.5.1a). The texture analyser measures the force needed to cut through the sample with the blade (Figure 1.5.1b). The maximum shear force is a value for the hardness of the food sample (García-Segovia et al., 2014).

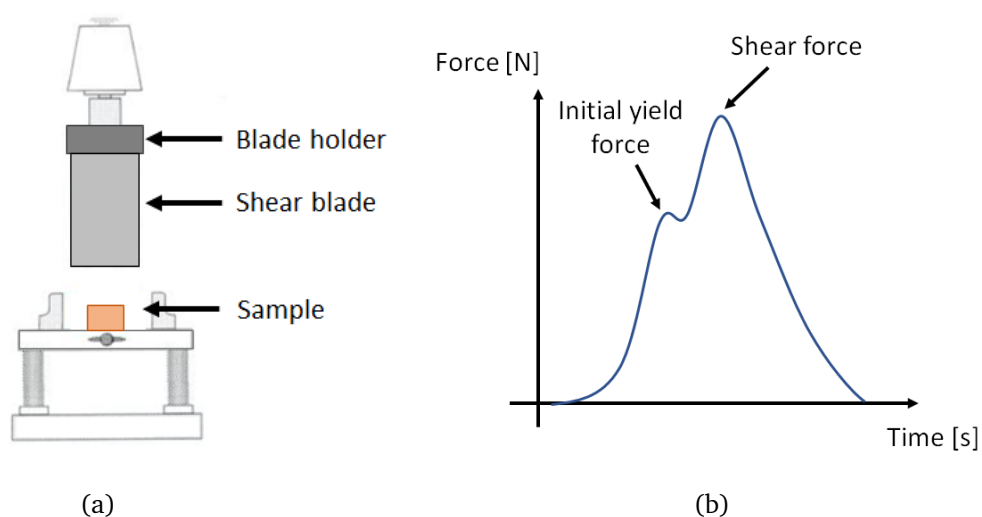


Figure 1.5.1: a) Set-up of a shearing test, from Belović et al., 2014 with some modifications. The slicing of the food sample imitates the movement done by the incisors when food is introduced into the mouth. b) A typical curve obtained after a successful shearing test. The peak shear force gives the hardness of the sample (García-Segovia et al., 2014).

### 1.5.2 TPA

Texture Profile Analysis (TPA) is used frequently in the food industry. The analysis imitates the movement of the first and second bite when food is introduced into the mouth. The sample is compressed by a cylinder probe twice with a chosen strain (Figure 1.5.2). The probe pauses for a chosen number of seconds between the two compressions. From the resulting TPA curve, different rheological parameters are obtained. Some of these are fracturability, hardness, stringiness (or adhesiveness), springiness, cohesiveness, chewiness, gumminess and resilience (Figure 1.5.3 and Table 1.5.1) (Bourne, 2002).

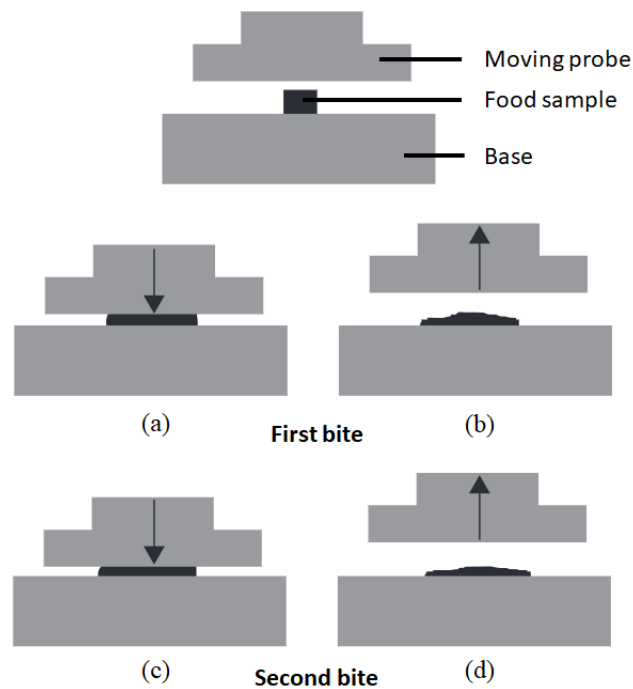


Figure 1.5.2: Set-up of Texture Profile Analysis. The first compression imitates the first bite when food is introduced into the mouth (a, b). The second compression imitates the second bite (c, d) (Bourne, 2002).

### 1.5.2.1 Challenges with TPA

There are several challenges when using TPA. The analysis is easily misunderstood and often wrongly applied. Many do not use a high enough compression strain. However, TPA is a way to imitate the mastication that happens in the mouth. The strain should therefore be similar to this destructive process. For gelled systems, a strain of 70-80% will break the sample entirely and is not advised. On the other hand, a strain of 20-50% is not advised either since it may not break the sample at all. Testing out different strains is a good way to find the right strain for the chosen product. This also applies to the speed of the compression probe and the time elapsed between the compressions (TTC, n.d., Smewing, 2014).

Another challenge is the size of the sample and probe. TPA should have bite-sized samples (Bourne, 2002). The probe should be a cylinder probe or a compression platen, and its surface should be the same size or larger than the sample surface (TTC, n.d., Systems, n.d., Smewing, 2014).

Finally, all parameters are not relevant to use for all food products. Chewiness, for example, is only applied to solid samples, while gumminess is applied to semi-solids. Researchers using both chewiness and gumminess to describe a food product most likely do not understand the use of these words correctly (Smewing, 2014, Systems, n.d.). The TPA analysis should not be forced onto a food product. When in need of a specific parameter, it is better to use a test

designed specifically for this parameter. For example, for hardness, the shearing test can be used. The result from the shearing test, designed to give the hardness value for a sample, is more trustworthy than the value obtained from TPA (TTC, n.d.).

Table 1.5.1: Definition of hardness, cohesiveness, springiness and chewiness, and how they are obtained from a TPA curve. The areas and distances refer to Figure 1.5.3 (TTC, n.d., Systems, n.d.).

Parameter	From curve	Definition
Hardness [N]	Maximum peak force from the first compression	The force required to break through the sample for the first time.
Cohesiveness [-]	Area 2/Area 1	Ratio of how well the sample resists deformation from a second compression compared to the first.
Springiness [%]	Distance 2/Distance 1	The measure of how well the sample springs back to its original starting point.
Chewiness [N]	Hardness x Cohesiveness x Springiness	The work required to chew the sample.

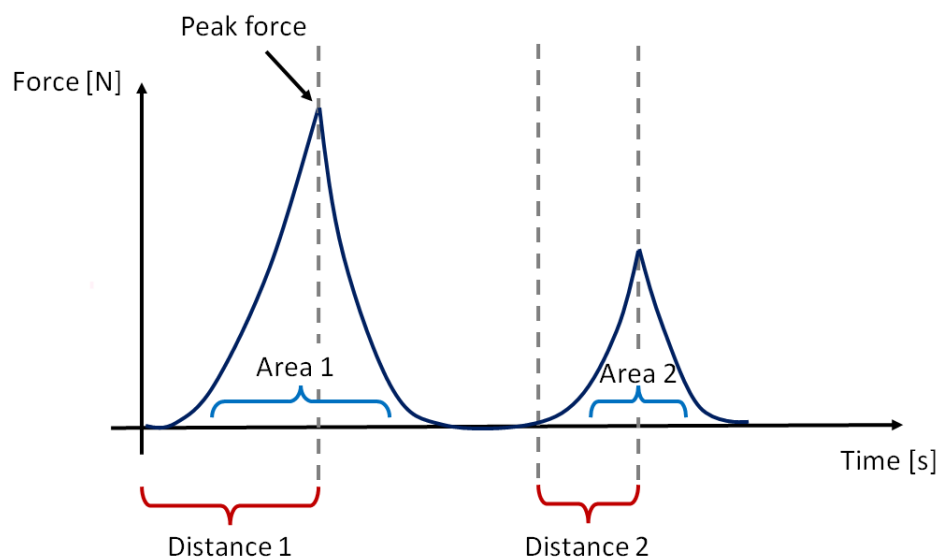


Figure 1.5.3: Typical curve from a successful TPA analysis. The first and second compression imitate the first and second bite when food is introduced into the mouth, respectively. Different parameters are obtained from the curve, such as hardness, cohesiveness, chewiness, and cohesiveness (Bourne, 2002).



## 1.6 Objective

As mentioned in the sections above, PBMA's are becoming increasingly popular. Despite this, there are still many barriers to overcome to satisfy consumers. One of these is texture. Many meat burger analogues are based on SPI, and MC is often used as a binder due to its unique ability to gel during heating. However, to the best of the author's knowledge, sodium alginate has not been tested as a binder in soy burgers. Sodium alginate is a unique hydrocolloid as it gels chemically when calcium is introduced. It is thermally irreversible compared to MC, which is thermally reversible.

The objectives of this thesis were therefore to:

- Form a meat analogue burger inspired by the food matrix in previous papers (Bakhsh, Lee, Lee, Hwang, et al., 2021, Bakhsh, Lee, Lee, Sabikun, et al., 2021, Wi et al., 2020) and the Orkla's Naturli' burger based on SPI.
- Investigate the influence of different binders on the textural properties of the soy burger, such as hardness, cohesiveness, springiness, and chewiness, by performing a shearing test and TPA.
- The binders analysed were: MC, alginate with a high and low fraction of G-blocks, pre-treated seaweed from *Saccharina latissima* and *Alaria esculenta*, and a mix of MC and alginate.



# Chapter 2

## Materials and methods

This section focuses on the materials used for the formulation of the PBMA, as well as the recipe and procedure for the burger preparation. Six experiments, in addition to two sub-experiments, were performed. An overview and detailed description of each experiment is given below. A proximate, physicochemical, and texture analysis was performed on the burgers. The texture analysis included both a shearing test and TPA. A statistical analysis was carried out on all the results.

## 2.1 Materials

All materials used with relevant information are shown in Table 2.1.1. Soy protein isolate (SPI) was used as the base for the burger. The SPI contained 84.1% protein. Each 40 g burger was made of 12.0 g SPI (30.0%), 1.5 g coconut oil (3.8%), 1.5 g canola oil (3.8%), and 25.0 g (62.5%) distilled water. Methyl cellulose (MC), alginate from *Laminaria hyperborea* (LH) and seaweed from *Saccharina latissima* (SL) were tested as binders. The binder amount tested varied from 0.5% to 2% for most binders. Alginate and seaweed had corresponding amounts added of calcium sulphate dihydrate ( $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ ) and tetrasodium pyrophosphate (TSPP). All binders,  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ , and TSPP amounts are given in percentage of the total wet weight of the burger prior to adding the binder. The water, SPI, and oil amounts were therefore the same for all burgers. An overview of the soy burgers analysed in this thesis, with the abbreviations explained, is shown in Table 2.1.2. C-0%, with no binder added, and MC-1%, with 1% of MC added, were used as control samples for all the experiments. The behaviour of dissolved alginate from LH (two alginates were tested: one with a high G-block fraction, and one with low), as well as seaweed from SL and *Alaria esculenta* (AE), were observed in two sub-experiments. SL and AE had gone through different pre-treatments. The pre-treatments are described in detail in section 2.2.6.

Table 2.1.1: Overview of the material used in the thesis. The relevant information for each material is shown, as well as the name of the deliverer.

Material	Relevant information	Deliverer
Soy protein isolate	Neutral flavour, 1 kg	Star Nutrition, Norway
Coconut oil	Ecological	Greenchoice, Meny, Norway
Canola oil	-	Eldorado, Meny, Norway
Methyl cellulose	Product Number: M0512, viscosity 4000 cP, MW 88 kDa, DS $\in$ [1.5-1.9], Methoxy substitution $\in$ [27.5%-31.5%] (weight)	Sigma-Aldrich
Alginate from <i>Laminaria hyperborea</i>	stem (G-block fraction, $F_G = 0.684$ ) and leaf ( $F_G = 0.460$ )	DuPont
Seaweed from <i>Saccharina latissima</i>	washed and pre-treated	Orkla Foods Norge AS, SINTEF Industry
Seaweed from <i>Alaria esculenta</i>	washed and pre-treated	Orkla Foods Norge AS, SINTEF Industry

Table 2.1.2: Overview of soy burgers tested in this thesis, with abbreviations explained. The type and amount of each binder added, as well as the corresponding amounts of calcium sulphate dihydrate ( $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ ) and tetrasodium pyrophosphate (TSPP) are shown. All amounts are given in % of the total wet weight of the burger prior to binder addition.

Abbreviation	Type of binder	Amount of: Binder	$\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$	TSPP
C-0%	None	0	0	0
MC-0.5%	MC <sup>a</sup>	0.5%	0	0
MC-1%	MC	1%	0	0
MC-2%	MC	2%	0	0
G-0.5%	G <sup>b</sup>	0.5%	0.13%	0.02%
G-1%	G	1%	0.25%	0.04%
M-0.5%	M <sup>c</sup>	0.5%	0.13%	0.02%
M-1%	M	1%	0.25%	0.04%
E-1%	E <sup>d</sup>	1%	0.13%	0.02%
E-2%	E	2%	0.25%	0.04%
H-1%	H <sup>e</sup>	1%	0.13%	0.02%
H-2%	H	2%	0.25%	0.04%
MC-G(0.25%-0.75%)	MC, G	0.25% MC, 0.75% G	0.19%	0.03%
MC-G(0.5%-0.5%)	MC, G	0.5% MC, 0.5% G	0.13%	0.02%
MC-G(0.75%-0.25%)	MC, G	0.75% MC, 0.25% G	0.06%	0.01%
MC-M(0.25%-0.75%)	MC, M	0.25% MC, 0.75% M	0.19%	0.03%
MC-M(0.5%-0.5%)	MC, M	0.5% MC, 0.5% M	0.13%	0.02%
MC-M(0.75%-0.25%)	MC, M	0.75% MC, 0.25% M	0.06%	0.01%

<sup>a</sup>MC = Methyl cellulose

<sup>b</sup>G = Alginate from stem *Laminaria hyperborea* (high level of G-block fraction,  $F_G$ )

<sup>c</sup>M = Alginate from leaf *Laminaria hyperborea* (low level of G-block fraction,  $F_G$ )

<sup>d</sup>E = Seaweed from enzyme treated *Saccharina latissima*

<sup>e</sup>H = Seaweed from *Saccharina latissima* treated with high pH

## 2.2 Method

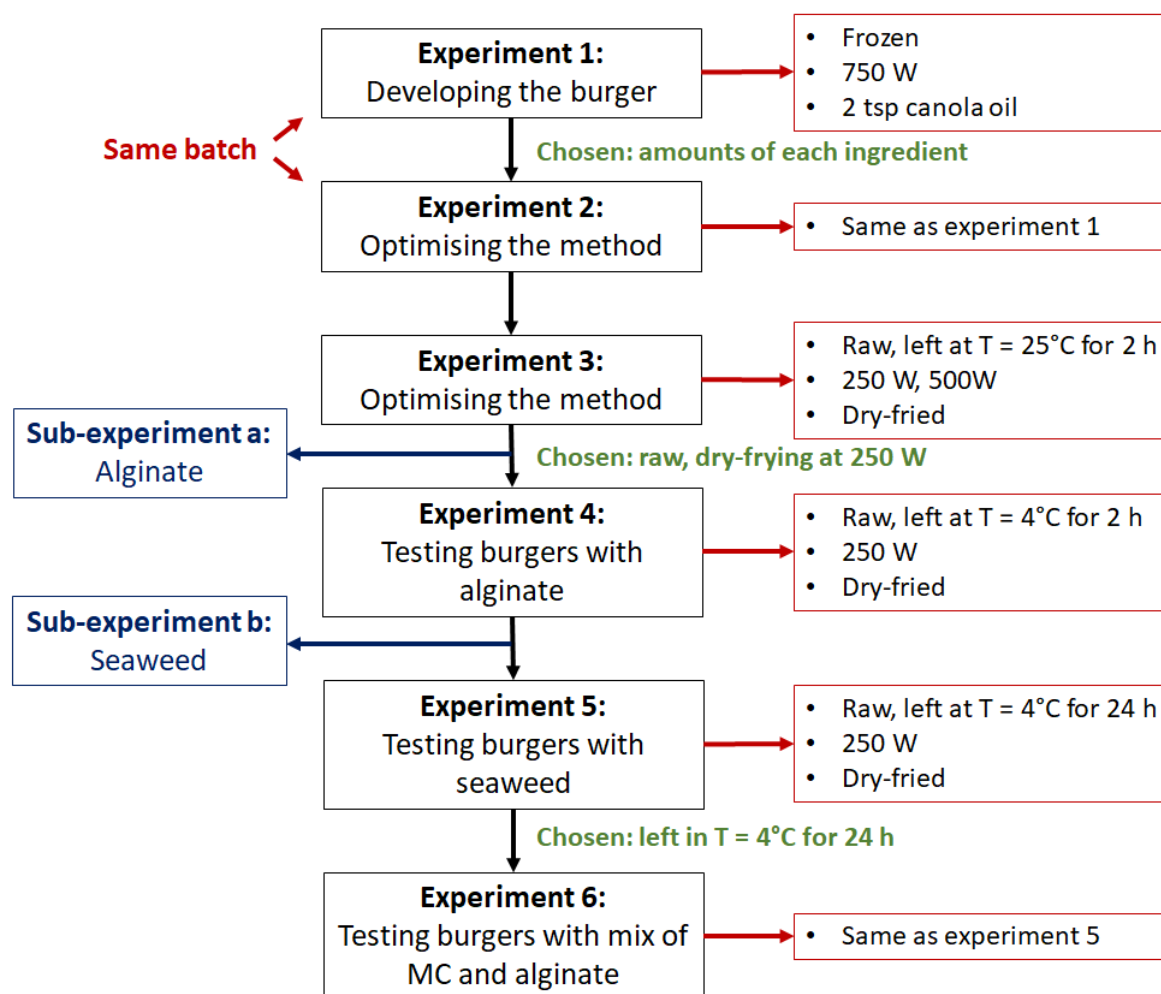


Figure 2.2.1: Flow chart showing an overview of the experiments performed in the thesis. The writing in green between some boxes are the parameters chosen to continue with in the following experiments. The boxes on the right are the parameters for the respective experiment. In addition to the six main experiments of the thesis, two sub-experiments were carried out (boxes on the left). A texture analysis (shearing test and TPA) was performed on the burgers.

Six experiments were carried out, each designed for a specific goal (Figure 2.2.1). The purpose of experiment 1 was to develop the food matrix with correct amounts of water, SPI, lipids and binder. Experiments 2 and 3 consisted of optimising the following steps: frying temperatures (250 W, 500 W, or 750 W), frying with or without canola oil (dry-frying), and state of the burger before frying (frozen, raw, left at room temperature or cold room, and amount of time prior to frying). Experiments 4, 5 and 6 consisted of testing the burgers with different binders, respectively alginate, seaweed and a mix of MC and alginate. In addition, two sub-experiments were carried out. Sub-experiment *a* was conducted prior to experiment 4. It consisted of observing the behaviour of the alginate given by Dupont. Sub-experiment *b* was carried out prior to experiment 5. It consisted of observing the behaviour of the seaweeds given by Orkla and

SINTEF Industry, and choosing two types to implement in the food matrix in the following experiment.

The burgers were made by rehydrating SPI for 1 hour in a cold room ( $T = 4^{\circ}\text{C}$ ), mixing all the ingredients and pressing them into patties (Figure 2.2.2). Afterwards, the burgers rested for a chosen amount of time and were fried at a chosen temperature. A texture analysis (shearing test and TPA) was performed on the burgers. Each burger weighed approximately 40 g. The detailed procedure for experiments 1-6 is shown in Table 2.2.1. More information for each experiment is provided in the sections below.

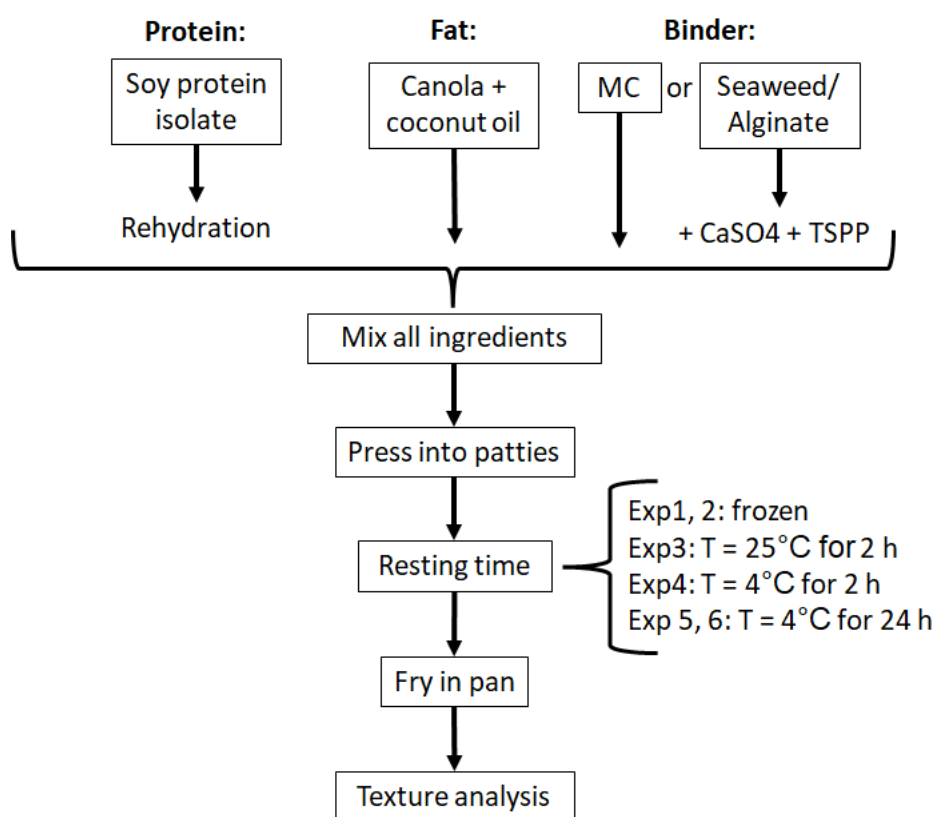


Figure 2.2.2: Flow chart showing an overview of the burger preparation.

Table 2.2.1: Recipe for making the soy burgers for experiments 1-6. The procedure is divided into parts: preparing the SPI, preparing the different binders (MC, alginate, seaweed, mix of MC and alginate), pressing and frying, and preparing for the texture analysis.

	Step	Procedure
<b>Preparing the SPI:</b> (Experiment 1-6)	1	Weigh SPI and distilled water, and add them into a suitable container. <b>NB!</b> Spare 25 g of the water if alginate or seaweed is used as a binder.
	2	Mix gently by pulsing a food mixer for 1 min, or until everything is mixed. <sup>a</sup>
	3	Let rehydrate for 1 hour in a cold room (T = 4°C).
	4	Weigh coconut and canola oil separately into two 50 mL beakers.
	5	Melt the coconut oil by warming up the beaker gently.
	6	Add both oils into the container with SPI, and gently mix.
<b>Preparing MC:</b> (Experiment 1-2, 4-6)	7a	Weigh MC.
<b>Preparing alginate or seaweed:</b> (Experiment 4-5)	7b	Weigh alginate or seaweed, and add it in a 50 mL beaker.
	8b	Stir while adding drops of 96% EtOH. Add drops until alginate (10-30 drops) or seaweed (20-40 drops) is "dissolved".
	9b	Slowly add the spared water into the beaker with the binder. Let stir at maximum speed until binder is dissolved.
	10b	Weigh CaSO <sub>4</sub> · 2 H <sub>2</sub> O and TSPP.
	11b	Add TSPP into the beaker with the binder.
	12b	Dissolve CaSO <sub>4</sub> · 2 H <sub>2</sub> O in some droplets of distilled water.
	13b	Remove stirrer and add CaSO <sub>4</sub> · 2 H <sub>2</sub> O into the beaker with the binder.
	14b	Mix quickly with a spatula.
<b>Preparing mix of MC and alginate:</b> (Experiment 6)	7c	Follow 7a and 7b-14b for the chosen amounts of MC and alginate, respectively.
<b>Pressing and frying:</b> (Experiment 1-6)	15	Gently mix the binder with the rehydrated SPI and fat.
	16	Weigh ~40 g of the raw burger mix in a burger press. <sup>b</sup>
	17	Press burger.
	18	Turn on the oven-top to the right temperature and wait for 10 min. <sup>c</sup>
	19	Fry the burgers. Flip the burger every 2 min for 4 min or until the core temperature reaches 75°C. <sup>d</sup>
	20	Let cool on baking paper for 30 min.
<b>Preparing for the texture analysis:</b> (Experiment 1-6)	21a	Cut burgers in rectangle pieces 1x2 cm for shear force analysis.
	21b	Cut burgers in square pieces 1x1 cm for TPA analysis.
	22	Analyse the pieces.

<sup>a</sup>A handheld electric mixer from Philips was used to mix the components.

<sup>b</sup>A non-stick Kitchen Craft mini hamburger press in aluminium was used, 3 burgers per use. Size: 3 x 6 cm.

<sup>c</sup>The following power levels of the oven-top were studied: 250 W, 500 W and 750 W.

<sup>d</sup>Depending on the experiment, the frying time was 4-8 min, and 2 tbsp canola oil were used.



### 2.2.1 Experiment 1: Developing a food product

Experiment 1 consisted of developing the food matrix and performing a shearing test to measure the hardness of the product. A soy burger was formulated by following the ingredient list on Orkla's Naturli' burger, and stripping it down to the main components: SPI, water, canola oil, coconut oil, and a binder. The food matrix obtained was composed of 5 components making it easier to analyse afterwards, instead of the original formulation with 13 components. The complete list of ingredients of the Orkla Naturli' burger compared with the thesis burger is found in the Appendix Section A.1. The effect of MC and comparisons of rheological properties in soy burgers have been reported before (Bakhsh, Lee, Lee, Hwang, et al., 2021, Bakhsh, Lee, Lee, Sabikun, et al., 2021, Wi et al., 2020). The formulations from these articles were used to find the ratio between the components in the soy burger.

One batch of each of the following burgers was made: C-0%, MC-1%, and MC-2%. After pressing the burgers, three burgers of each batch were packed in baking paper and frozen ( $T = -20^{\circ}\text{C}$ ). One burger of each batch was fried with 2 tsp of canola oil. The burgers were flipped every 2 minutes, until the core temperature reached  $75^{\circ}\text{C}$ . To ensure an even temperature in the frying pan, the pan was placed on the oven-top, adjusted to 750 W, approximately 10 minutes prior to frying. A shearing test was performed on the burgers to measure the hardness of the burgers.

### 2.2.2 Experiment 2: Optimising the method, part 1

In experiment 2, the flipping technique during the frying process was optimised. The remaining two burgers from the batches made in experiment 1 (C-0%, MC-1%, and MC-2%) were used in experiment 2. The burgers were fried with 2 tsp of canola oil at 750 W. The frying technique was optimised, and the frying time was more exact (flipped after 2 min for 6 min). A shearing test and TPA were performed on the burgers to measure the burger's textural properties (hardness, cohesiveness, springiness, and chewiness).

### 2.2.3 Experiment 3: Optimising the method, part 2

Experiment 3 consisted of optimising the frying method and analysing a raw C-0% burger. In this experiment, one batch of C-0% was made. After pressing three burgers, one was analysed directly by performing a shearing test and TPA. The remaining two burgers were placed on baking paper, unpacked, and left on the lab bench for 2 hours while the raw burger was analysed. Afterwards, one burger was fried at 250 W, and the other at 500 W. The burger fried at 250 W was flipped every 3 min for 8 min. The burger fried at 500 W was flipped every 2 min for 4 min. A shearing test and TPA were performed on the burgers to measure the texture.

### 2.2.4 Sub-experiment *a*: Alginate

Sub-experiment *a* consisted of observing how alginate behaved in water, and during the addition of TSPP and  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ . The amounts of  $\text{CaSO}_4$  and TSPP for an alginate slurry were given by researcher Kurt Ingar Draget from the Department of Biotechnology and Food Science at NTNU. The amount of  $\text{CaSO}_4$  was then adjusted to give an amount of  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ . The calculations behind this are found in the Appendix Section B.1. Since the recipe had a limited amount of water, which was supposed to mainly rehydrate SPI, the experiment was necessary to find the minimal amount of water needed for alginate to dissolve.

Alginate from *Laminaria hyperborea* (LH) stem and leaf were supplied by DuPont. The alginate had been industrially produced and extracted as sodium alginate. The industrial extraction of sodium alginate is briefly explained in Section 1.3.2. The alginate from stem and leaf LH had a G-block fraction,  $F_G$ , of 0.684, and 0.460, respectively. The detailed procedure for this experiment is shown in Table 2.2.2. The amount needed for 1% alginate (0.400 g) was weighed for the stem alginate (G) and the leaf alginate (M), and added into two separate beakers. A few drops of ethanol were added to each beaker. Water was added slowly into the beakers, and left for several minutes before adding more. The amount of water needed before the alginate dissolved was the required amount chosen to spare for the rest of the experiment. The alginate was deemed dissolved when there were no visible lumps.

Table 2.2.2: Detailed procedure for sub-experiment *a*.

	Step	Procedure
Parallel 1	1a	Weigh 0.400 g of alginate, and add it to a 50 mL beaker.
	2a	While stirring, add water 25 g of distilled water slowly.
Parallel 2	1b	Weigh the 0.400 g of alginate, and add it to a 50 mL beaker.
	2b	While stirring, add a few drops of EtOH until the alginate "dissolves".
	3b	While stirring, add 25 g of distilled water slowly.
Parallel 1-2	4	Stir until the alginate is dissolved.
	5	Weigh 0.1012 g of $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ .
	6	Weigh 0.0160 g of TSPP.
	7	Add TSPP directly in the dissolved alginate.
	8	Dissolve $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ in some droplets of distilled water, and add it to the dissolved alginate.
	9	Mix with a spatula, and observe.

### 2.2.5 Experiment 4: Testing burgers with alginate

Experiment 4 consisted of testing alginate as a binder in the food matrix instead of MC. The resting time was also changed to 2 hours in the cold room instead of room temperature as in experiment 3. The following four batches were made: C-0%, MC-1%, G-1%, and M-1%.  $\text{CaSO}_4$  and TSPP were added in the right amounts (Table 2.1.2). The burgers were placed on baking paper, unpacked, and left in a cold room ( $T = 4^\circ\text{C}$ ) for 2 hours. The burgers were dry-fried

at 250 W, flipped every 2 min for 4 min. C-0% was flipped after 4 min and fried for 8 min. The longer frying time for C-0% was a consequence of difficulties frying the burger without it tearing up. Without a binder, the burger easily fell apart when flipped. Both a shearing test and TPA was performed on all burgers to measure the texture.

### 2.2.6 Sub-experiment *b*: Seaweed

Sub-experiment *b* consisted of observing how dried seaweed behaved in water, and when TSPP and  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$  were added. The purpose of the experiment was also to choose two appropriate seaweed samples of the six possible samples for further study in the following experiment.

Six different types of dried seaweed from *Alaria esculenta* (AE) and *Saccharina latissima* (SL) were supplied by Orkla and SINTEF Industry. The AE and SL seaweed powders were the same as previously analysed during the fall of 2021 as a substitution for carrageenan in pork liver pâté (Dahl, 2021). All seaweeds had been washed with a water-seaweed ratio (5:4), gone through a pre-treatment, and had been dried and milled. The pre-treatments of all the seaweeds are shown in Table 2.2.3.

Table 2.2.3: Overview of the pre-treatments of the seaweeds used in sub-experiment *b*. The six different types of dried seaweed from *Alaria esculenta* (AE) and *Saccharina latissima* (SL) were supplied by Orkla and SINTEF Industry. They were all washed in the water-seaweed ratio (5:4) before the pre-treatment, and dried and milled afterwards.

Sample	Source	Pre-treatment
1	AE	washed <sup>a</sup> , enzyme <sup>b</sup>
2	AE	enzyme <sup>b</sup>
3	AE	washed, high pH <sup>c</sup>
4	AE	high pH <sup>c</sup>
5	SL	enzyme <sup>b</sup>
6	SL	high pH <sup>c</sup>

<sup>a</sup> The seaweed was washed with doubly-distilled water at room temperature. After removal of the supernatant, the same amount in water was used to wash the seaweed again.

<sup>b</sup> The seaweed was treated with Neutrase and Flavourzyme under incubation.

<sup>c</sup> The seaweed was treated with water with pH 8.

The detailed procedure for this experiment is shown in Table 2.2.4. Schiener et al. (2015) reported a maximal alginate level of  $28.5 \pm 3.9\%$  and  $37.4 \pm 4.0\%$  in dry weight of SL and AE, respectively. Based on this, to ensure that the seaweed consisted of the same amount of alginate as in sub-experiment *a* (0.400 g), the amount of seaweed was doubled ( $\sim 1.0$  g). The amount needed for 2% seaweed ( $\sim 1.0$  g) was weighed for all six seaweeds and added into two separate beakers. The amount of water found in sub-experiment *a* ( $\sim 25$  g) was added to each beaker. The respective amount of TSPP and  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$  were added (Table 2.1.2). Droplets of each seaweed solution were placed on the lab bench to visually observe whether the seaweed had dissolved, started to create a gel network, or separated from the water.

Table 2.2.4: Detailed procedure for sub-experiment *b*.

Step	Procedure
1	Weigh 1.00 g of alginate, and add it to a 50 mL beaker.
2	While stirring, add a few drops of EtOH until the seaweed "dissolves".
3	While stirring, add 25 g of distilled water slowly.
4	Stir until the seaweed is dissolved.
5	Weigh 0.1012 g of $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ .
6	Weigh 0.0160 g of TSPP.
7	Add TSPP directly into the beaker.
8	Dissolve $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ in some droplets of distilled water, and add it to the beaker.
9	Mix with a spatula, and observe.

### 2.2.7 Experiment 5: Testing burgers with seaweed

Experiment 5 consisted of testing dried seaweed from SL, chosen from sub-experiment *b*, as a binder in the food matrix instead of MC. The experiment also tested a reduced amount of MC and alginate as binders (50% reduction). The reduction in alginate was tested to reduce the water amount required to dissolve the binder, and use more for rehydration of the SPI. The following batches were made: C-0%, MC-0.5%, MC-1%, G-0.5%, M-0.5%, E-1%, E-2%, H-1%, and H-2%.

Two samples from SL were tested as binders. Both samples had first been washed with doubly-distilled water at room temperature (water-seaweed ratio (5:4)). One seaweed type had been pre-treated with enzymes (Flavorzyme and Neutrase) under incubation. The other seaweed type had been treated with alkaline water (pH 8). Both seaweed samples were dried and milled, and added in the chosen amounts (Table 2.1.2).  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$  and TSPP were added in the respective amounts.

Three burgers were pressed for each batch. Two burgers from each batch were dry-fried at 250 W, flipped every 2 min for 4 min. Due to difficulties with frying the burgers without tearing apart, C-0% and MC-0.5% were fried for slightly longer than the rest (6 min, i.e. flipped three times). Both a shearing test and TPA was performed on the fried burgers. The remaining burgers were used for a cook loss analysis the following day. This is explained further in Section 2.4.

### 2.2.8 Experiment 6: Testing burgers with a mix of MC and alginate

Experiment 6 consisted of testing a mix of MC and alginate as a binder in the food matrix instead of MC. The following batches were made: C-0%, MC-1%, G-1%, MC-G(0.25%-0.75%), MC-G(0.5%-0.5%), MC-G(0.75%-0.25%), M-1%, MC-M(0.25%-0.75%), MC-M(0.5%-0.5%), and MC-M(0.75%-0.25%).

The amount of water found in sub-experiment *a* (~ 25 g) was used to dissolve the alginate amounts in separate beakers. A mix of MC and alginate was tested at different ratios as a binder in the burgers (Table 2.1.2).  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$  and TSPP were added in the respective amounts. To measure the texture, a shearing test and TPA were performed on all burgers.

## 2.3 Proximate analysis

Two batches of C-0% and MC-1% were made for the proximate analysis. Both batches were pressed and left in the cold room ( $T = 4^\circ\text{C}$ ) overnight. One batch was cut into arbitrary pieces and put in a suitable container. The other batch was dry-fried at 250 W, flipping every 2 minutes for 4 minutes. After resting for 30 minutes, the fried burgers were cut into arbitrary pieces and put in a suitable container. The protein, fat, and dry matter content of C-0% and MC-1%, both raw and fried, were determined. Three parallels were performed for each batch.

The protein content was determined following the Kjeldahl procedure. This procedure is based on the assumption that food materials contain an insignificant proportion of non-protein organic nitrogen (Coultate, 2016). The analysis followed the protocol from the producer BUTCHI (KjelDigester K-149, Scrubber K-415 and KjelMaster K-375). The reference substance used was tryptophan, and the conversion factor used was 5.71 (BUTCHI (Switzerland), 2013)). The fat content was determined following the Bligh and Dyer method (Bligh & Dyer, 1959). The dry matter content was determined by drying the samples in a heating cabinet ( $T = 105^\circ\text{C}$ ) for 24 hours. The dry weight content was measured by subtracting the weight before drying from the weight after drying.

## 2.4 Physicochemical analysis

The Cook Loss (CL) was determined for 17 burgers with different binders (Table 2.4.1). All batches were left in the cold room ( $T = 4^\circ\text{C}$ ) overnight prior to the analysis.

The CL gives a value of how much water that has been lost during heating and centrifuging of a food product. It is related to the water-holding capacity (WHC) of a food product. However, the difference between WHC and CL, is that WHC focuses mainly on the water lost during the centrifugation step, while CL also takes into account the heating step. Therefore, analysing the CL instead of the WHC is more relevant for burgers. To get the right texture in a PBMA, low CL is desired.

After weighing 2 g of each sample, the samples were warmed up to 75°C for 15 minutes in a heating cabinet. This temperature was chosen as the burgers were fried until the core temperature reached 75°C in the experiments of the thesis. After cooling for 15 minutes at room temperature in a desiccator, the samples were centrifuged in a cooling centrifuge ( $T = 4^\circ\text{C}$ ) at 3600 rpm for 5 minutes. The samples were weighed, and the CL was calculated by using the following equation:

$$CL = \frac{\Delta p_2}{p_1} \cdot 100\%, \quad (2.4.1)$$

where  $CL$  is the cook loss,  $\Delta p_2$  is the total weight reduction of the sample, after warming and centrifugation, and  $p_1$  is the initial weight of the sample prior to the analysis. The CL is expressed in % weight loss during the analysis.

Table 2.4.1: Overview of the batches made for the cook loss analysis. Some batches were made in experiment 5. All batches were analysed after resting in the cold room for 24 hours.

Sample	Source
C-0%	Experiment 5
MC-0.5%	Experiment 5
MC-1%	Experiment 5
G-0.5%	Experiment 5
G-1%	New batch
MC-G(0.25%-0.75%)	New batch
MC-G(0.5%-0.5%)	New batch
MC-G(0.75%-0.25%)	New batch
M-0.5%	Experiment 5
M-1%	New batch
MC-M(0.25%-0.75%)	New batch
MC-M(0.5%-0.5%)	New batch
MC-M(0.75%-0.25%)	New batch
E-1%	Experiment 5
E-2%	Experiment 5
H-1%	Experiment 5
H-2%	Experiment 5

The pH was determined by using a digital pH meter (MP220 Basic pH Meter, Mettler Toledo). The pH was measured for C-0% for experiments 3, 5 and 6. In experiment 5, the pH was also measured for MC-0.5%, MC-1%, G-0.5%, M-0.5%, E-1%, E-2%, H-1%, and H-2%. In experiment 6, the pH was also determined for MC-1%, G-1%, MC-G(0.25%-0.75%), MC-M(0.25%-0.75%), MC-M(0.5%-0.5%), MC-G(0.75%-0.25%), and MC-M(0.75%-0.25%).

## 2.5 Texture analysis

The texture analysis consisted of a shearing test and a texture profile analysis (TPA). Both tests were done on two separate Stable Micro System Texture Analysers TA.XTplusC. The loading cell used was 5 kg.

A shearing test and TPA was performed on all burgers from experiments 2-6. For experiment 1 only a shearing test was performed. For the shearing test and TPA, the burgers were cut into pieces of 1 cm x 2 cm x 1 cm and 1 cm x 1 cm x 1 cm, respectively (Figure 2.5.1). The burgers had a diameter of approximately 6.5 cm.

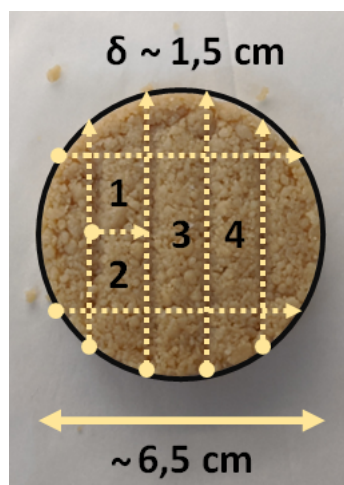


Figure 2.5.1: Each burger (6.5 cm in diameter) was cut into four pieces. For the TPA, the pieces had the following dimensions: 1 cm x 1 cm x 1 cm (1, 2). For the shearing test, the pieces had the following dimensions: 1 cm x 2 cm x 1 cm (3, 4). The dimensions are given in height x length x width. The burgers were cut manually with a scalpel.

The shearing test used a standard knife blade set-up (Figure 2.5.2). The sample was placed in the middle of the sample holder. The knife was placed slightly above the sample so that it was not in contact with the sample. The set-up was not calibrated before experiment 6. Despite this error, the results from the previous experiments are assumed to be valid. The results may have more noise, but are assumed to be trustworthy. For all shearing tests, the pre-test speed, test speed, and post-test speed were: 2.00 mm/sec, 5.00 mm/sec, and 10.00 mm/sec, respectively. The trigger force was 50.0 g and, the strain was set to 10.0%. The values for the parameters were recommended by the producer Stable Micro System.

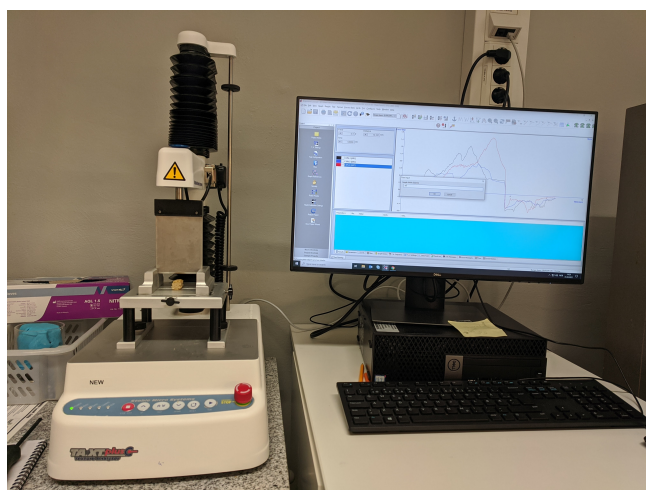


Figure 2.5.2: Set-up for the shearing test on the Stable Micro System Texture Analyser TA.XTplusC. A standard knife blade set-up was used with a loading cell of 5 kg. The pre-test speed, test speed, and post-test speed were: 2.00 mm/sec, 5.00 mm/sec, and 10.00 mm/sec, respectively. The trigger force and strain were 50.0 mg and 10.0%, respectively.

The TPA used a standard TPA set-up with a cylinder probe with a 25 mm diameter (Figure 2.5.3). The sample was placed in the middle of the sample holder. The probe was placed slightly above the sample so that it was not in contact with the sample. No calibration was required for this set-up. For all TPA, the pre-test speed, test speed, and post-test speed were all 1.00 mm/sec. The trigger force was 5.0 g and, the strain was set to 60.0%. The values for the parameters were recommended by the producer Stable Micro System.

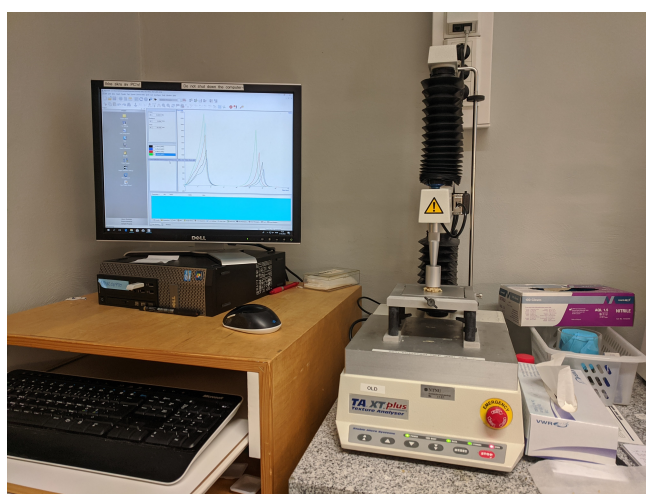


Figure 2.5.3: Set-up for the Texture profile analysis (TPA) on the Stable Micro System Texture Analyser TA.XTplusC. A standard TPA set-up with a cylinder probe with 25 mm diameter was used, with a loading cell of 5 kg. The pre-test speed, test speed, and post-test speed were all 1.00 mm/sec. The trigger force and strain were 5.0 g and 60.0%, respectively.



The texture analysis was performed on all burgers that held their shape during frying and cutting of the pieces. As many burgers fell apart, the number of pieces for the analysis of each burger may vary. The built-in software of the system, *Exponent Connect*, was used to acquire rheological parameters from the shearing test and TPA. From the shearing test, hardness was obtained. The following parameters were obtained from the TPA: hardness, cohesiveness, springiness and chewiness. The raw data from *Exponent Connect* was imported and plotted in Python.

## 2.6 Statistical analysis

The standard deviation (SD) was given or calculated for all values, except for the pH results. The SD for the textural parameters were obtained with the software *Exponent Connect*, from Stable Micro Systems. The SD for the proximate analysis (protein, fat, and dry matter content), as well as the cook loss (CL) were calculated with Excel and the statistical software Minitab. The pH was measured only once for each sample and did therefore not have any calculated SD. No statistical analysis was performed on these results.

An unpaired t-test with a significance level of 5% was performed on the values from the proximate analysis. A one-way analysis of variance (One-way ANOVA), with a significance level set at 5%, was performed on the textural parameters from the shearing tests and TPA, as well as the CL. The t-test and ANOVA were done in the statistical software Minitab. Both tests are based on data which is randomly sampled and normally distributed. A Shapiro-Wilk test was performed on the data before the t-test and ANOVA to ensure that the data followed a normal distribution. Any outliers found were eliminated.



## Results and discussion

The results from the proximate, physicochemical, and texture analysis are presented and discussed in the sections below. All raw data are given in Appendix Sections A.2 and A.3. The results from the unpaired t-tests, with an explanation regarding the null and alternative hypotheses and the relevant p-values, are found in the Appendix Section B.2.

### 3.1 Proximate analysis

The protein, fat and moisture content in C-0% and MC-1%, both raw and fried, were very close to the expected values of a raw 40 g soy burger (Table 3.1.1). The expected values were calculated based on the composition of the raw materials. The protein content in the SPI used was composed of 84.1% protein. The 40.0 g burger was composed of 12.0 g SPI, i.e. 10.1 g proteins, which equals 25.2% of the total weight. The SPI also contained 1.5% fat, i.e. 0.2 g of the 12.0 g SPI added was fat. Together with the 3.0 g from the coconut and canola oil, this equalled 3.2 g, i.e. 8.0% of the total weight. The expected moisture content of 62.5% was calculated from the water added (25.0 g in the 40.0 g burger). The remaining part of the burger contained salt, carbohydrates, fibre, and ash from the SPI, and made up 4.3% of the total weight.

The protein content of the raw C-0% burger was not significantly different from the expected value, at 5% level of significance. In comparison, the fat and moisture content were significantly higher than the expected values (+0.8% and +1.2%, respectively). On the other hand, the protein content of the raw MC-1% burger was significantly lower than the expected value (-1.1%). In contrast, the fat and moisture content were not significantly different from the expected values. The increase in fat and moisture content for C-0%, and decrease in protein content for MC-1%, were not expected. For both raw burgers, water was expected to evaporate during the resting phase in the cold room overnight, leading to a decrease in moisture and higher values of both protein and fat. The deviations from the expected values may suggest that some raw material was lost during the burger making, or the components were not mixed properly. Consequently, this would have varied the ratios between the different components. Another reason for such results could be an uptake of moisture. Despite the continuous ventilation, the cold room door was often opened during the daytime, as many students used this cold

room for their experiments. The burgers were placed on baking paper openly without any form of sealing. This was done to let this water evaporate freely during the resting time. However, if the door was opened sufficiently long enough to let moisture into the cold room, the food product could have absorbed the moisture instead of releasing it. This could have increased the moisture percentage and decreased the percentage of the other components, such as fat and protein. To avoid this possibility in future investigations, the burgers should be put in a sealed container, letting the water evaporate but not letting any water vapour inside the container.

The protein content of the fried C-0% was significantly higher than the raw burger (+1.3%), while the moisture content was significantly lower (-1.2%). The fat content was not significantly different from the raw burger. These results coincided well with the expectations. Since water evaporated during the resting and frying process, the fat and protein content of the fried burgers was expected to be higher, and the moisture content lower.

However, for the fried MC-1%, the protein and moisture content were not significantly different from the raw burger. The fat content, on the other hand, was significantly higher (+1.3%). These results can be explained the gelling ability of MC during heating. During the frying process, MC would have formed fibrils, which would have trapped the surrounding water in the burger, keeping the moisture in the burger (Coughlin et al., 2021). This coincided well with the results from Bakhsh, Lee, Lee, Sabikun, et al. (2021) where plant-based meat analogues burgers were compared with beef burgers at different MC concentrations. The results suggested that the moisture content increased along with an increase in the MC concentration in both burgers.

Table 3.1.1: The expected protein, fat and moisture composition of a raw 40 g soy burger without binder, as well as the composition of the control burgers of the experiments in the thesis: a burger without a binder (C-0%), and a burger with 1% methyl cellulose as a binder (MC-1%). The expected values were calculated based on the composition of the raw materials. C-0% and MC-1% were analysed, both raw and fried (250 W for 4 minutes, flipped once after 2 minutes). The data is given in percentage of the total wet weight of the sample. An unpaired t-test was used to compare the experimental values with the expected values.

Sample	% protein	% fat	% moisture
Expected values of a raw burger	25.2	8.0	62.5
Raw C-0%	24.3 ± 0.4	8.90 ± 0.04 <sup>a</sup>	63.7 ± 0.4 <sup>a</sup>
Raw MC-1%	24.08 ± 0.06 <sup>a</sup>	11 ± 1.5	61.8 ± 0.4
Fried C-0%	26.5 ± 0.12 <sup>a</sup>	9.2 ± 0.7	60.53 ± 0.02 <sup>a</sup>
Fried MC-1%	24 ± 1.0	9.4 ± 0.11 <sup>a</sup>	61 ± 1.5

<sup>a</sup> p-value < 0.05, the value is significantly different from the expected raw burger value, at 5% level of significance.

### 3.2 Physicochemical analysis

The cook loss was analysed for 17 different burgers (Figure 3.2.1). MC alone did not seem to affect the cook loss (CL) of the burger. C-0%, MC-0.5%, and MC-1% were not significantly different at 5% level of significance. This result was different from the findings of Bakhsh, Lee, Lee, Sabikun, et al. (2021). This study compared beef burgers with plant-based analogues made of either TVP or SPI. All the burgers were added MC at different concentrations. The study found that the CL was reduced with an increasing MC concentration for all burgers. However, the concentration of MC tested in the study was between 1.5% and 4%, while this thesis investigated the CL in burgers with MC from 0.5% to 1%. The low impact of MC in the burger may therefore have been due to the small amount added.

The CL of the burgers with G did not vary much. None of the burgers with G, except MC-G(0.25%-0.75%), were significantly different. G-0.5%, G-1% and MC-G(0.25%-0.75%) had significantly lower CL than C-0% and MC-1%. The CL in the burgers with only G was approximately 2% lower than C-0% and MC-1%. This effect could be attributed to the G-blocks of alginate, trapping the water in a strong gel network.

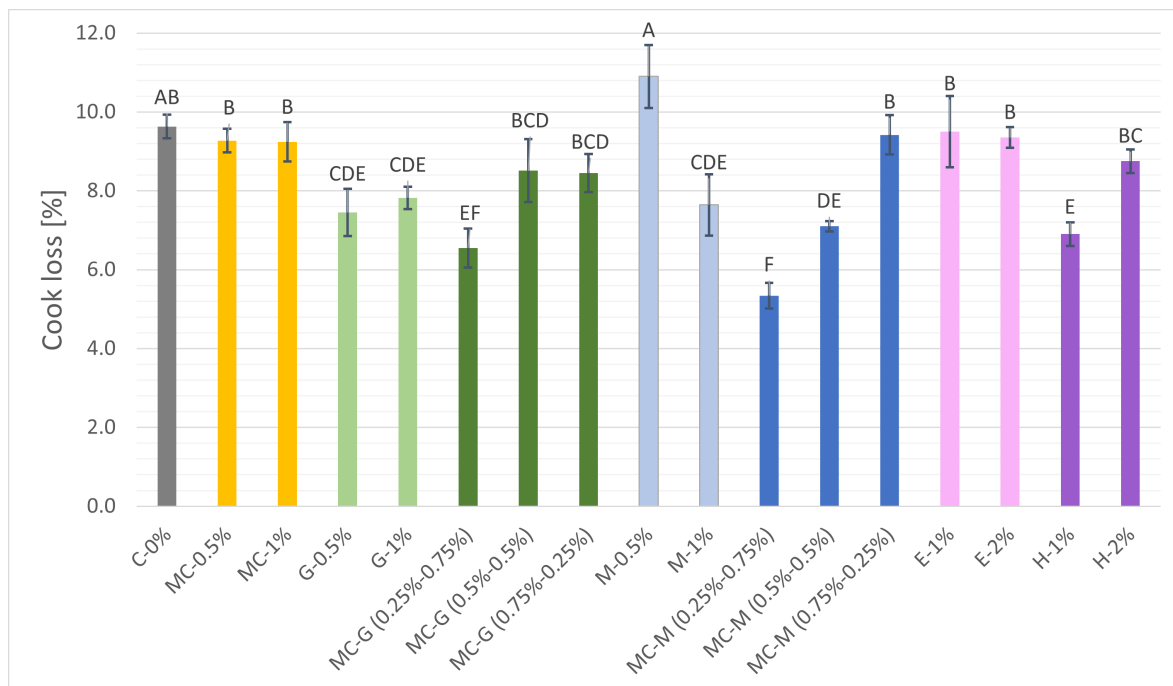


Figure 3.2.1: Results of the cook loss analysis. 17 burgers were analysed, from left to right: C-0%, MC-0.5%, MC-1%, G-0.5%, G-1%, MC-G(0.25%-0.75%), MC-G(0.5%-0.5%), MC-G(0.75%-0.25%), M-0.5%, M-1%, MC-M(0.25%-0.75%), MC-M(0.5%-0.5%), MC-M(0.75%-0.25%), E-1%, E-2%, H-1%, and H-2%. All burgers were raw and had rested in a cold room for 24 hours before the analysis. Burgers with different letters at the top of the column (A-F) are significantly different, at 5% level of significance.

The burgers with M varied over a wide range. M-0.5% had the highest CL of all the burgers, significantly different from all burgers except C-0%. MC-M(0.25%-0.75%) had the lowest CL of all the burgers and was significantly different from all burgers, except from MC-G(0.25%-0.75%). The difference between the highest and lowest CL was approximately 5%. At a level of 0.5%, M did not seem to affect the burger. In comparison, at the same concentration, G already reduced the CL. At 1%, M was not significantly different from G-0.5% and G-1%. This result suggested that 1% of M was needed to affect the burger and form a strong enough gel to trap water, while G only required 0.5% for the same effect. This finding coincided well with the gelling ability of M and G. G, with a high fraction of G-blocks, provides a stronger gel network than M, with a low fraction of G-blocks. As explained by the egg-box model (Section 1.3.2), a higher amount of G-blocks will provide greater interaction with calcium and a firmer gel network, trapping moisture in a food product (Helgerud et al., 2009, Indergaard, 2010).

Mixing MC with G and M gave different results. Of the burgers with MC and G, only MC-G(0.25%-0.75%) had a significantly lower CL than MC-1% and C-0%. The other burgers with MC and G were not significantly different from MC-1%, but significantly lower than C-0%, regardless of the ratio. With M, MC seemed to have a varying impact according to the concentration. Increasing the MC concentration and lowering the M concentration resulted in higher CL. As mentioned above, it seemed that the MC concentration was too small to have an impact by itself on the CL at these concentrations. However, in presence of MC, it seemed that the M concentration had a threshold of 0.5% before the CL was significantly lower than C-0% and MC-1%. For G, this threshold was 0.75%. For both alginates, the lowest CL was with 0.25% MC and 0.75% alginate. This was also the lowest CL for all burgers. The significantly lower CL for the burgers with a mixture of alginate and MC than MC-1% suggests that the two polymers interacted with each other. The gel formed with the polymers in the same matrix trapped more water than MC as an individual binder. This finding coincided well with previous studies reporting a synergistic effect between MC and alginate (Liang et al., 2004, Eskens et al., 2020). These studies are discussed further in experiment 6.

H-1% was the only SL burger significantly different from C-0% and MC-1%. H-1% was not significantly different from the burgers with alginate, G-0.5%, G-1%, and M-1%, as well as MC-G(0.25%-0.75%) and MC-M(0.5%-0.5%). The impact of 1% H, compared to 1% and 2% E, could suggest that the pre-treatment with high pH made the alginate, and thus the G-blocks, more accessible to interact with calcium and gel than the pre-treatment with enzymes. Despite this, at 2%, H did not seem to have the same impact. However, this result could be due to the lack of water, and thus a low mobility of the components in the food matrix. The seaweeds (E and H) swelled more than alginate when water was added. At 1%, the magnet stirring the beaker with the binder could still move around, but at 2% the stirrer stood still, even at maximum speed. For future investigations of seaweed at different concentrations, the water content in the food matrix could be changed to accommodate for possible interactions between alginate in the seaweed and the calcium added.

The pH of the food matrix varied between 6.85 and 7.31 (Table 3.2.1). The SPI did most likely not contribute much to gel formation due to the neutral pH. Nishinari et al. (2014) reported that a decrease in pH increased the gel formation in soy, and at pH 7, gel formation was not prominent. However, Nagano et al. (1994) also reported that the storage modulus  $G'$  increased in a 15% (w/v) 7S globulin solution at 80°C for 30 minutes. The temperature was kept constant, and the pH was reported to be 7.6. Upon cooling to 20°C, a slight decrease in  $G'$  was observed. The results were explained by the hydrophobic interactions, and the contribution of hydrogen bonding to the protein network formation between water and 7S globulins. This could suggest that a protein network was formed in the food matrix due to SPI, despite a neutral pH, increasing during heating and slightly decreasing when resting afterwards.

None of the binders seem to have had any impact on the pH of the food matrix. However, the pH was only measured once for each sample. Therefore, it was difficult to draw a reliable conclusion for the slight decrease measured in the burgers with E and H. Any interaction between SPI and alginate was most likely quite weak, as the pH was above the pI of SPI. The protein had more negatively charged groups than positive. Due to the alginate's negatively charged groups, the alginate could have had electrostatic interactions with the few positively charged groups on SPI.

Table 3.2.1: The pH results of the raw burgers mass for experiments 3, 5 and 6. The pH was not determined for the burgers made in experiments 1, 2, and 4.

Burgers	Experiment 3	Experiment 5	Experiment 6
C-0%	7.24	7.20	7.22
MC-0.5%	- <sup>a</sup>	7.22	- <sup>a</sup>
MC-1%	- <sup>a</sup>	7.19	7.21
G-0.5%	- <sup>a</sup>	7.31	- <sup>a</sup>
M-0.5%	- <sup>a</sup>	7.24	- <sup>a</sup>
G-1%	- <sup>a</sup>	- <sup>a</sup>	7.23
E-1%	- <sup>a</sup>	7.02	- <sup>a</sup>
E-2%	- <sup>a</sup>	6.85	- <sup>a</sup>
H-1%	- <sup>a</sup>	6.99	- <sup>a</sup>
H-2%	- <sup>a</sup>	6.88	- <sup>a</sup>
MC-G(0.25%-0.75%)	- <sup>a</sup>	- <sup>a</sup>	7.24
MC-M(0.25%-0.75%)	- <sup>a</sup>	- <sup>a</sup>	7.20
MC-M(0.5-0.5%)	- <sup>a</sup>	- <sup>a</sup>	7.19
MC-G(0.75%-0.25%)	- <sup>a</sup>	- <sup>a</sup>	7.19
MC-M(0.75%-0.25%)	- <sup>a</sup>	- <sup>a</sup>	7.20

<sup>a</sup> The pH was not measured.

### 3.3 Texture analysis

#### 3.3.1 General remarks

C-0% and MC-1% were used as reference burgers. The choice of two burgers as a control parameter was due to the fragility of the C-0%. Without any binder, the burger fell apart quite easily. To prevent this, C-0% was often fried for a longer time than the other burgers (2-4 min longer). As a result, a thicker crust was formed for the C-0% burgers. Consequently, all the textural properties (hardness, cohesiveness, springiness, and chewiness) were higher for C-0% than the rest of the burger samples. Comparing the burgers with C-0% alone would therefore have been misleading. By also using MC-1% as a reference, the comparison between the burgers was more reliable. The MC-1% burger was also the burger closest to the commercial Orkla Naturlig burger. The results from the C-0% burger were still chosen to be included in the discussion, as well as in the graphs along with the rest of the samples, as it was regarded as interesting to compare all burgers with each other, despite different frying time. Consequently, all statistical t-tests compared each burger type with both C-0% and MC-1%.

Another important remark concerns the hardness parameter. This parameter was obtained from both the shearing test and TPA. As previously mentioned (Section 1.5), due to its design, the shearing test results in a more reliable hardness value than TPA. Despite this, TPA was prioritised in the cases where there were not enough samples to perform both tests. The reason behind this choice was the requirement of larger sample pieces for the shearing test than for the TPA, and the amount of information provided by TPA (four textural parameters instead of one). As the pieces for TPA were smaller than for the shearing test, it was easier to cut more than one piece when TPA was prioritised. In some cases, only one parallel was provided for the shearing test. As a consequence, some tests had too few parallels to give a trustworthy conclusion. In these cases, the statistical t-test was not performed, as this would not have given a reliable result.



### 3.3.2 Experiment 1

Experiment 1 was an initial experiment. The goal was to press and fry three different types of burgers, as well as to perform a successful shearing test. The knife set-up was used for the first time, and only two pieces were successfully analysed for each burger type. Due to the small sample size and lack of experience with the shearing test and TPA set-up, the hardness values obtained from the texture analysis were not reliable (Figure 3.3.1b). However, there are a few remarks which made this experiment worth mentioning.

The burgers were fried for 8-9 minutes with canola oil, resulting in a very thick crust (Figure 3.3.1a). The crust was so thick that it was visible as two waves in the shearing test graph, especially for MC-1% (Figure 3.3.2). The crust was deemed to be too thick for the desired burger. Consequently, the frying time was reduced to 6 minutes for the following experiments.

Another interesting observation concerns the MC burgers. The two burgers with MC held their shape much better than the C-0% burger during the frying process. This could suggest an effect from the MC as a binder. As the temperature increased, the MC may have started to form fibrils and strengthened the food matrix network (Coughlin et al., 2021). The hardness of MC-1% was more than three times the hardness of MC-2%. This value is quite strange as 2% MC should form more fibrils than 1% MC, resulting in a firmer gel. However, as stated above, the sample size was small, and the burgers were fried for a long time, resulting in a very thick uneven crust. The hardness values were not reliable. All three burger types were replicated in the following experiment with a shorter frying time.

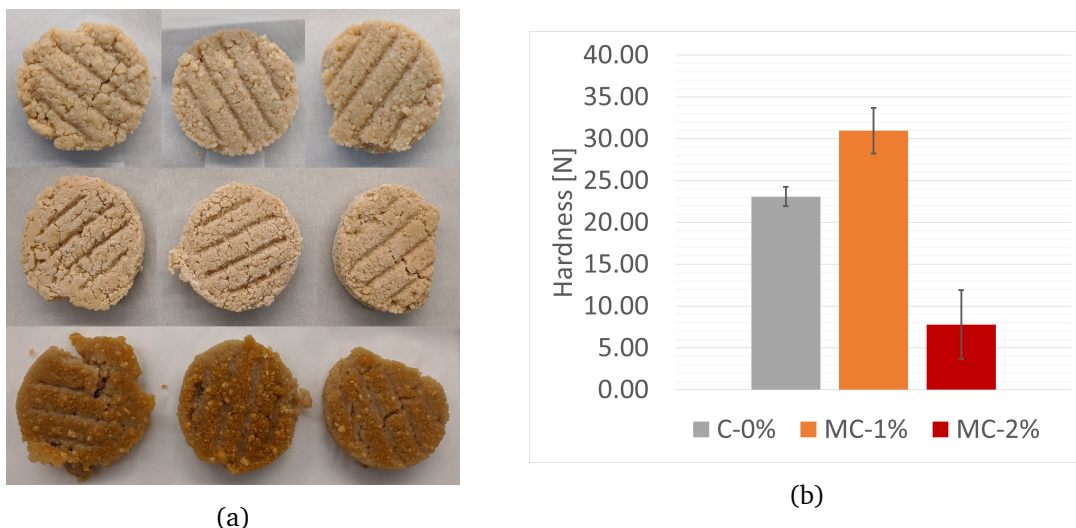


Figure 3.3.1: (a) Photos of the burgers obtained from experiment 1, from left to right: C-0%, MC-1% and MC-2%; from top to bottom: raw right after pressing, frozen, and fried at 750 W for 8 minutes. (b) The average hardness (in Newton) for each burger, from left to right: C-0% (grey), MC-1% (orange), and MC-2% (red).

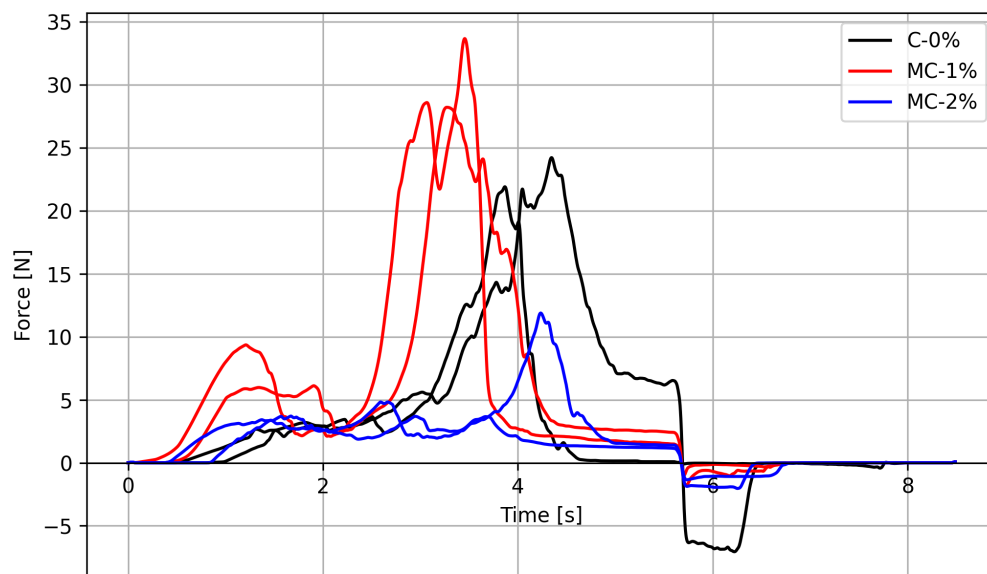


Figure 3.3.2: Graph obtained from performing a shearing test on the burgers from experiment 1. The force (in Newton) as a function of time (in seconds) is shown for C-0% (black), MC-1% (red), and MC-2% (blue).

### 3.3.3 Experiment 2

The goal of experiment 2 was to optimise the methods and techniques used. The shearing test and TPA were both performed successfully on all burgers. The sample size for the shearing test was three pieces for each burger. For the TPA, two pieces were analysed for C-0%, and three were analysed for both MC-1% and MC-2%. The small sample size was due to difficulties when cutting the pieces because of the thick crust. During cutting, the two outer crust layers squeezed out the softer middle layer of the burger. Many pieces were therefore not possible to analyse. The small sample size resulted in high standard deviations for some results.

The frying time of 6 minutes and the use of canola oil led to a very thick crust on all the fried burgers (Figure 3.3.3). This resulted in a high hardness value for all burgers (Figure 3.3.5). The crust was still deemed too thick for the desired burger, with the same reasoning as experiment 1. Consequently, the frying time was reduced to 4 minutes, and the canola oil was removed from the procedure for the following experiments.



Figure 3.3.3: Photos of the burgers obtained from experiment 2, (a) raw right after pressing, and (b) fried at 750 W for 6 minutes, from top to bottom: C-0%, MC-1%, and MC-2%.

The hardness measured from the shearing test of C-0%, MC-1%, and MC-2% were not significantly different at 5% level of significance. However, the hardness determined by the TPA, indicated a significant difference between C-0% and both MC burgers, regardless of the concentration. The hardness of MC-1% and MC-2%, obtained from the TPA, were not significantly different. The difference between the shearing test and TPA results was most likely due to high standard deviations of the shearing test, especially for MC-2% (+/- 50% of the value). The irregularities are shown in the shearing test graph (Figure 3.3.4). The food matrix could explain the uneven curves from the shearing test graph and the high standard deviation. The matrix was mainly composed of rehydrated SPI, which was observed to have a consistency similar to grains like cooked couscous. However, some grains were observed to be larger than others.

When all the ingredients were mixed, the raw burger was not a smooth mass, but rather a large amount of grains held together quite poorly. When fried, the grains stuck better together depending on the presence of a binder. The MC burgers held their shape much better than C-0%. The irregularity of the rehydrated SPI grains in the matrix may explain why the curves from the shearing test were uneven. This observation was also made in all the following experiments, even after the calibration of the knife set-up in experiment 6. The calibration is discussed further in experiment 6.

MC-1% and MC-2% were not significantly different from each other for any of the textural parameters. This could suggest that the increase in MC in the food matrix did not increase the number of MC fibrils formed during the frying time. Increasing the MC amount did not seem to affect the network. Since adding unnecessary amounts of binder was not regarded as desired for a burger, a higher amount than 1% MC was not investigated further in any of the following experiments.

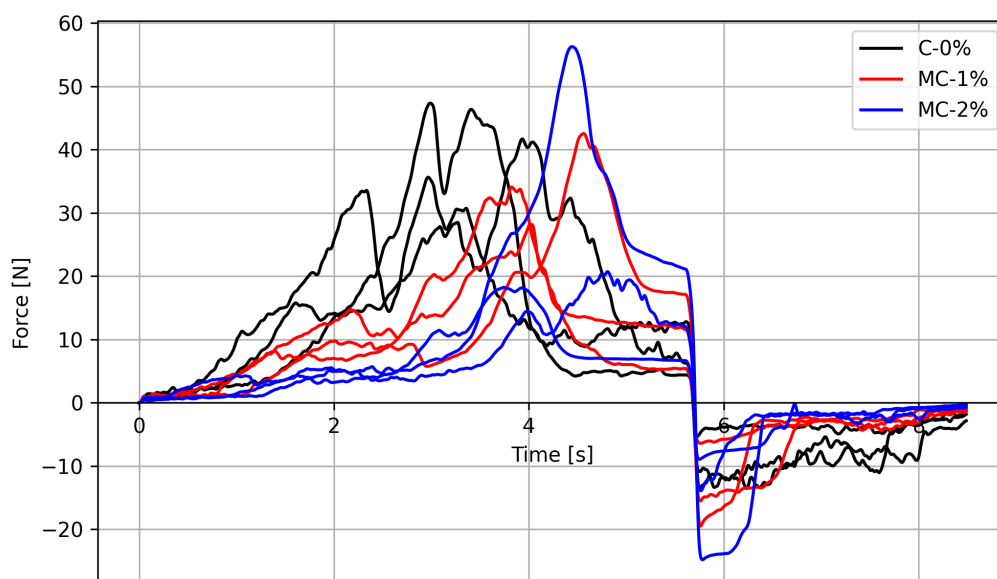


Figure 3.3.4: Graph obtained from performing a shearing test on the burgers from experiment 2. The force (in Newton) as a function of time (in seconds) is shown for C-0% (black), MC-1% (red), and MC-2% (blue).

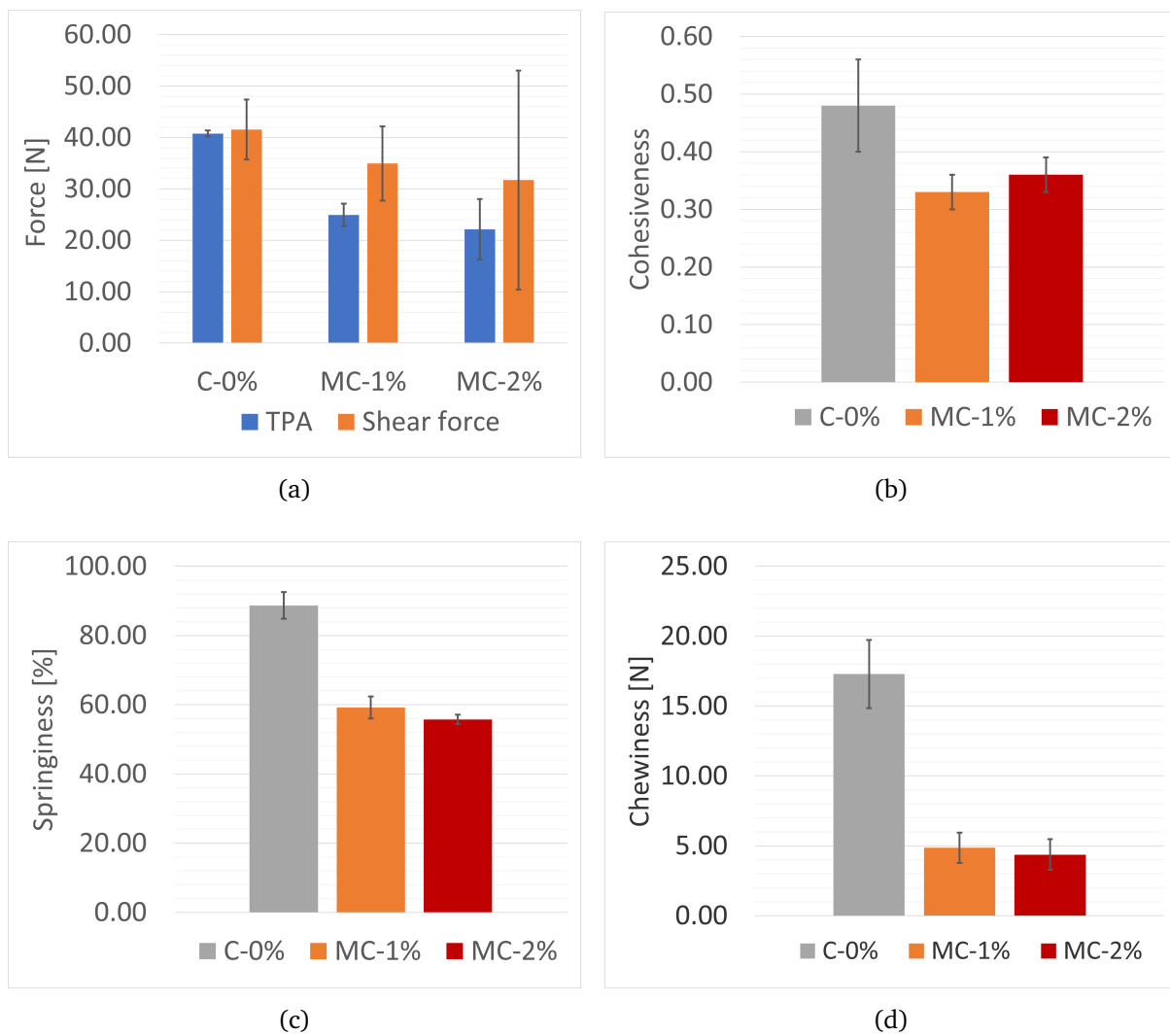


Figure 3.3.5: The textural parameters obtained from the shearing test and TPA on the burgers from experiment 2. The average hardness (in Newton) for each burger (a), from left to right: C-0%, MC-1%, and MC-2%. The hardness obtained from the TPA and shearing test is shown in blue to the left and orange to the right, respectively. The cohesiveness (b), springiness (c, in % of wet weight of the sample), and chewiness (d, in Newton) obtained from the TPA are shown, from left to right: C-0% (grey), MC-1% (orange), and MC-2% (red).

### 3.3.4 Experiment 3

The goal of experiment 3 was to compare burgers fried at 250 W and 500 W to find an optimal frying temperature. In addition, a raw burger without a binder was analysed to see if it was interesting to investigate raw burgers further.

The TPA graph obtained from the raw C-0% burger is shown in the Appendix Section A.3.2. The graph showed a smaller peak prior to the peak force. This illustrated that a crust had already started to form during the 2 hours of resting time at room temperature. As no binder was added, this was most likely due to the evaporation of water on the surface of the burger. However, as stated in the proximate analysis (Section 3.1), the burger may also have absorbed water during the resting time in the cold room. If this was the case, the formation of a slight crust is difficult to explain. Comparing the hardness of C-0% and the fried burgers, there were significant differences due to the formation of a crust when fried (Figure 3.3.7). The fried burgers obtained a hardness 15-20 N higher than C-0% with the TPA, and 5-10 N higher with the shearing test. Since commercial plant-based burgers, such as the Orkla Naturli' burger, are fried, a decision was made to investigate fried burgers rather than raw burgers in this thesis.

However, it would have been interesting to look into the textural properties of a raw alginate burger, as alginate reacts chemically, and not thermally like MC. Interacting with calcium, it gels regardless of the temperature (Helgerud et al., 2009, Indergaard, 2010). For this reason, it would have been interesting to perform a shearing test and TPA on raw burgers with alginate with different G-block fractions. Moreover, it would have been interesting to vary the amount of calcium and TSPP added to the food matrix to see the effect of these compounds on the gelling properties of the different alginates. Raw burgers with MC would not have been interesting to investigate since MC does not provide an effect before the temperature rises.

Furthermore, the resting time of 2 hours at room temperature made it easier to fry the burgers at 250 W and 500 W. The burgers were more firm than in the previous experiments. The burgers were fried until the core temperature was approximately 75°C. The 500 W burger was fried for a shorter time (4 minutes) than the one at 250 W (8 minutes). Both burgers were much easier to cut into suitable pieces for the textural analysis than in the previous experiments (Figure 3.3.6). Hence, the procedure for the following experiments was changed to include a resting time and frying the burgers from a raw state instead of frozen.



Figure 3.3.6: Photos of the C-0% burgers obtained from experiment 3, from left to right: in raw state after pressing, fried at 250 W for 8 minutes, fried at 500 W for 4 minutes. The fried burgers were cut into pieces for the textural analysis.

The hardness obtained from the TPA was significantly different between C-0% fried at 250 W and fried at 500 W, at 5% level of significance (Figure 3.3.7). Nevertheless, the hardness determined by the shearing test was not significantly different between the same burgers. The different results were most likely due to the high standard deviations of the shearing test. Despite this inaccuracy, the shearing test was designed to give a hardness value of a food product. As a result, the shearing test result was judged to be more reliable than the TPA. Besides a similar hardness, the chewiness was significantly different between the two burgers. In comparison, the cohesiveness and springiness were not significantly different. The chewiness was higher for the burger fried at 250 W than 500 W. Since the burgers were not compared to a typically chewy product, it was difficult to know if this parameter was especially high or not. However, a plant-based burger requires a certain amount of chewiness to compete with meat burgers. A common problem with PBMA is the softness of plant proteins. When Bakhsh, Lee, Lee, Sabikun, et al. (2021) compared PBMA with beef, both PBMA made of TVP and SPI had a significantly lower hardness and chewiness than the meat burger, at 5% level of significance. Similar results were found by Bakhsh, Lee, Lee, Hwang, et al. (2021) when PBMA made of TVP was compared with beef and pork. These studies suggested that the difference in texture was related to the toughness of the myofibrillar network when muscle protein denatured during heating. Additionally, C-0% fried at 250 W was fried for a longer time, which may have affected the chewiness of the burger. In any case, 250 W was chosen as the power level on the oven-top for the following experiments since it was easier to control the burgers during frying. By heating the pan for at least 10 minutes before frying, the core temperature of 75°C could be attained after 4-5 minutes, instead of 8 minutes. Therefore, the procedure was changed to frying at 250 W for 4-5 minutes for the subsequent experiments.

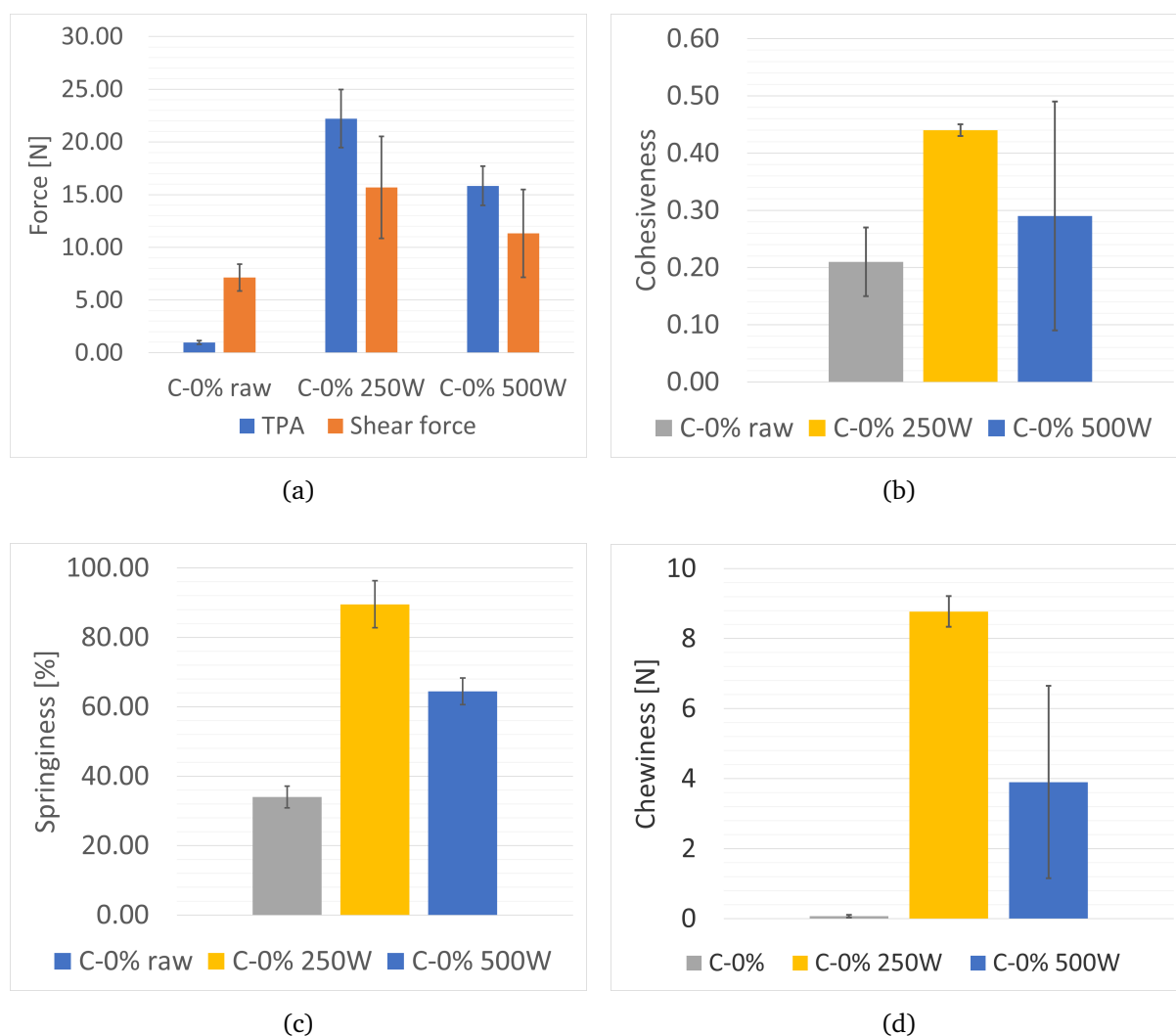


Figure 3.3.7: The textural parameters obtained from the shearing test and TPA on the burgers from experiment 3. The average hardness (in Newton) for each burger (a), from left to right: C-0% in the raw state, C-0% fried at 250 W, and C-0% fried at 500 W. The hardness obtained from the TPA and shearing test is shown in blue to the left and orange to the right, respectively. The cohesiveness (b), springiness (c, in % of wet weight of the sample), and chewiness (d, in Newton) obtained from the TPA are shown, from left to right: C-0% in raw state (grey), C-0% fried at 250 W (yellow), and C-0% fried at 500 W (blue).



### 3.3.5 Sub-experiment a: Alginate

The goal of the sub-experiment *a* was to observe alginate during dissolution with (parallel 1) or without (parallel 2) addition of ethanol.

Parallel 1 was aborted due to using more than 1 hour to dissolve. The alginates in parallel 2 used between 30 to 50 minutes to dissolve completely. This proved that the effect of ethanol was significant, reducing the time needed by approximately 50%. The ethanol caused the alginate of the surface of the particles to precipitate, forcing the alginate to separate from each other. When water was added, the alginate particles were so distanced from each other that they could not unfold and create entanglements (Helgerud et al., 2009). Adding ethanol before dissolving the alginate in water was therefore added to the procedure for the subsequent experiments.

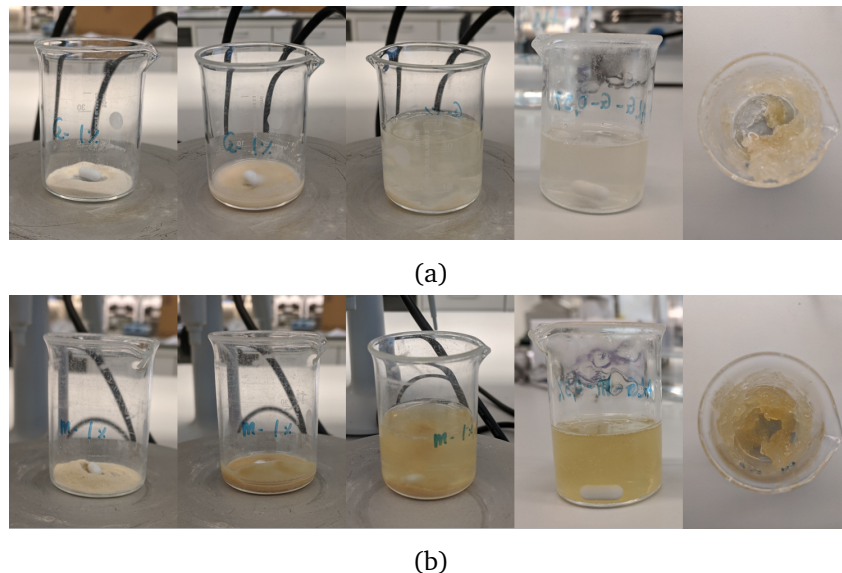


Figure 3.3.8: Photos of the steps for dissolving (a) alginate from stem *Laminaria hyperborea*, and (b) alginate from leaf *Laminaria hyperborea*. Drops of ethanol were added in step 2 (second photo from the left).

The alginate from stem LH (G) resulted in a nearly transparent liquid with a slightly higher viscosity than water, but was still quite runny (Figure 3.3.8a). The alginate from leaf LH (M) resulted in a brown liquid with a higher viscosity than G. It was also runny, but closer to thin honey than G (Figure 3.3.8b). When TSPP was added, no new observations were made for either G or M. When  $\text{CaSO}_4 \cdot \text{H}_2\text{O}$  was added, both alginates started to gel momentarily. As mentioned previously (Section 1.3.2),  $\text{CaSO}_4$  has a very high solubility when added to a sodium alginate solution. The role of TSPP was to slow down the spontaneous gelling of alginate (Draget and Nordgård, 2021). It seemed to have worked better with G than M as G resulted in a smoother gel than M, which had a more marmalade-looking structure. The differences observed when dissolving and gelling, were most likely due to the difference in G-block fractions in the

two alginates. G had a higher fraction of G-blocks than M. More G-blocks interacted with the calcium and formed a strong network without lumps. As a result, G dissolved easier in the distilled water and formed a smoother gel. M had fewer G-blocks than G, and more M-blocks and alternating M and G blocks (-MGMG-). Therefore, M had fewer parts in the polymer which could interact with calcium, resulting in a marmelade-like structure and not a smooth gel. Future investigations could experiment with varying the amount of TSPP to slow the gelling kinetics even more.

### 3.3.6 Experiment 4

This experiment aimed to observe the behaviour of implementing a binder made of alginate with a high (G) and low (M) G-block fraction compared to MC. The hardness of C-0%, obtained from the shearing test, was significantly different from MC-1% and M-1%, at 5% level of significance (Figure 3.3.10). The data obtained from G-1% were not normally distributed according to the Shapiro-Wilk test. The t-test is based on the assumption that all data is randomly sampled and that the variables follow a normal distribution. Since the latter assumption was not valid for the shearing test data obtained from the G-1% samples, the t-test was not performed on the data. Therefore, the hardness for G-1% obtained from the shearing test was deemed unreliable. From the TPA, the hardness obtained from analysing C-0% was significantly different from all burgers. From both tests, C-0% resulted in a higher hardness than the other burgers. This is most likely due to the thick crust formed by frying C-0% for a longer time than the other burgers (Figure 3.3.9). The crust also resulted in significantly higher values for cohesiveness, springiness, and chewiness than the other burgers.

The hardness of MC-1%, obtained from the shearing test, was significantly different from M-1%, but not G-1%. In comparison, the hardness of MC-1%, obtained from the TPA, was not significantly different from both M-1% or G-1%. Likewise, the hardness for G-1% and M-1%, obtained from TPA, were not significantly different. Since the standard deviations were larger for TPA than the shearing test for M-1% and quite similar for MC-1%, the hardness results from the shearing test were deemed more reliable than from the TPA. The other parameters, cohesiveness and chewiness, were not significantly different between MC-1%, G-1%, or M-1%. Inversely, springiness was significantly different between all burgers. The differences in hardness and springiness could originate from the difference in binder used: MC gels during heating and forms fibrils, while alginate sets chemically when interacting with calcium. The different gels seem to have had a different impact on the hardness and springiness of the burgers, with MC-1% having a higher result than the alginate burgers.

M-1% and G-1% did not seem to have significant differences for any textural parameter. This result was not expected since the high G-block fraction of G-1% would, in theory, have interacted with more calcium and formed a firmer gel than M-1%, resulting in a harder burger than M-1%. The similarity between the alginate burgers could suggest there was no difference between using alginate with high or low G-block fractions in the food matrix. This being said, the burgers were observed to be wet before frying, and during frying, only a thin crust was

formed on the alginate burgers. The thin crust may be due to the water on the surface of the burgers that did not allow the Maillard reactions to take place. During the short time in the pan, the water on the surface of the burgers evaporated, but the browning reaction, which happens during the Maillard reactions, did not happen. The burgers were boiled rather than fried. In addition, the G-1% data from the shearing test was unavailable (due to not following a normal distribution). For these reasons, a conclusion would not have been entirely reliable. G-1% and M-1% were therefore analysed further in the following experiments. As the resting time in the cold room gave firmer but relatively wet alginate burgers, the resting time was changed in the procedure to be overnight (approximately 24 hours) in the cold room for the subsequent experiments.

Moreover, due to its negatively charged carboxyl groups, alginate may have interacted electrostatically with the positively charged amino acids on surrounding proteins in a food matrix (Draget & Nordgård, 2021). However, the pI of SPI is around 4, and the food matrix was around 7. The SPI in the food matrix would most likely therefore have had more negatively charged groups than positively charged. If there were any interactions between alginate and the SPI, they must have been few.

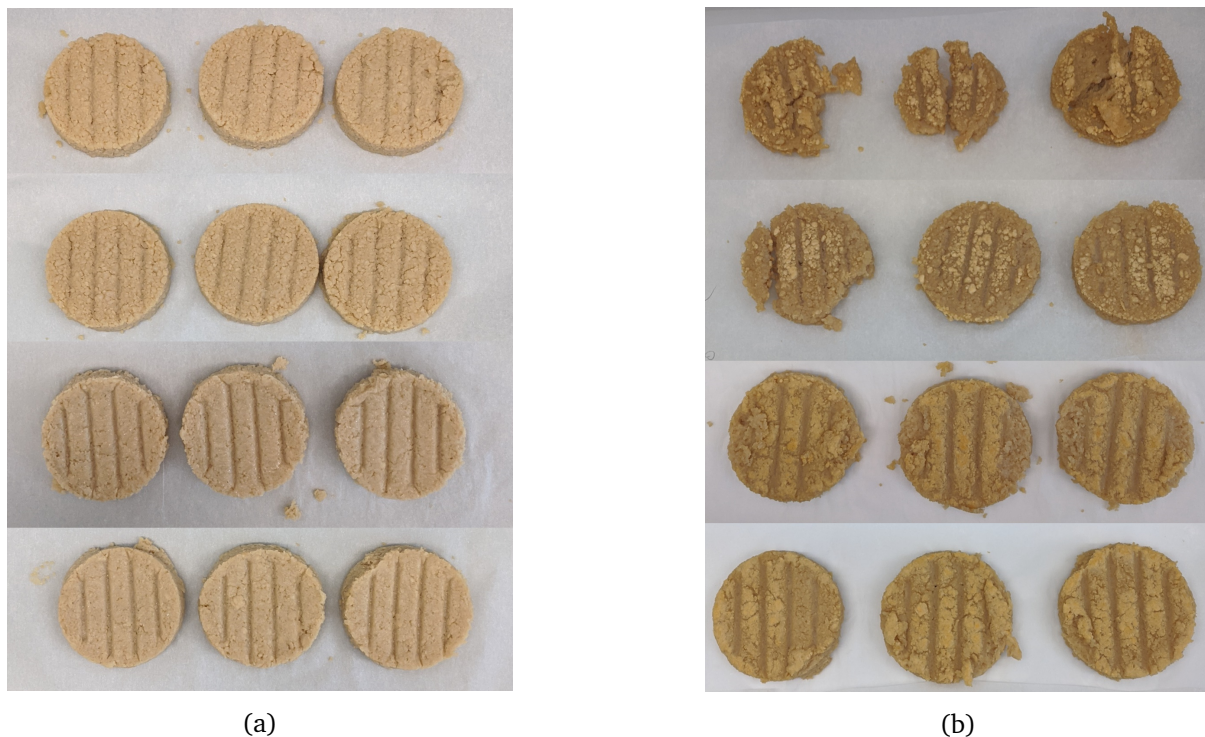


Figure 3.3.9: Photos of burgers obtained from experiment 4 (a) raw, right after pressing, and (b) fried at 250 W after resting in a cold room for 2 hours. From top to bottom: C-0%, MC-1%, G-1%, and M-1%. The burgers were fried for 4 minutes, and flipped after 2 minutes. C-0% was fried for 8 minutes and flipped after 4 minutes.

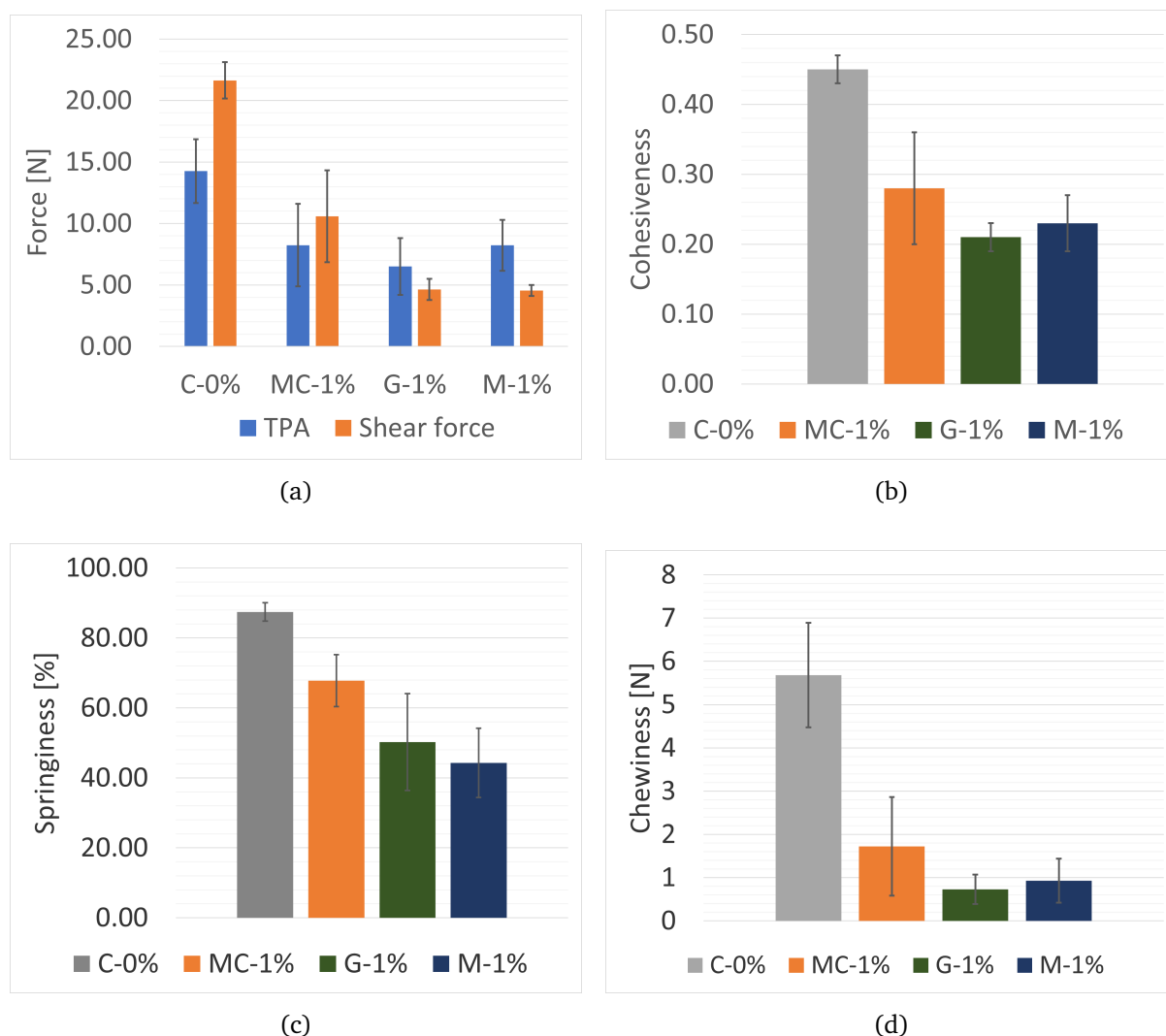


Figure 3.3.10: The textural parameters obtained from the shearing test and TPA on the burgers from experiment 4. The average hardness (in Newton) for each burger (a), from left to right: C-0%, MC-1%, G-1%, and M-1%. The hardness obtained from the TPA and shearing test is shown in blue to the left and orange to the right, respectively. The cohesiveness (b), springiness (c, in % of wet weight of the sample), and chewiness (d, in Newton) obtained from the TPA are shown, from left to right: C-0% (grey), MC-1% (orange), G-1% (dark green), and M-1% (dark blue).

### 3.3.7 Sub-experiment *b*: Seaweed

Sub-experiment *b* aimed to observe the behaviour of the six seaweed samples, pre-treated differently, dissolved in water and after the addition of  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$  and TSPP. From these observations, two seaweed samples were selected for further investigation in the following experiment as a replacement for MC in the food matrix.

The six seaweed samples were studied in the fall of 2021 in pork liver pâté as a possible substitution for carrageenan (Dahl, 2021). Of the two seaweeds, AE and SL, AE had the most similarities with carrageenan. Triple amounts of AE and pre-treated AE were reported to give similar results as carrageenan. However, no specific conclusion was drawn due to a small sample size and inconsistent results.

The AE separated when dissolved in water, and seaweed particles were visible in the drops placed on the lab bench (Figure 3.3.11). Also, no gelling was observed when TSPP and subsequently  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$  were added. There seemed to be a slightly higher surface tension in sample 4 (high pH treated AE) than with the other AE samples. This could suggest an increase in viscosity and that the high pH treatment made the alginate more available than the other pre-treatments. However, sample 3 (high pH, washed AE) did not seem to have a similar viscosity change. It was impossible to draw a reliable conclusion with only observations by the naked eye.

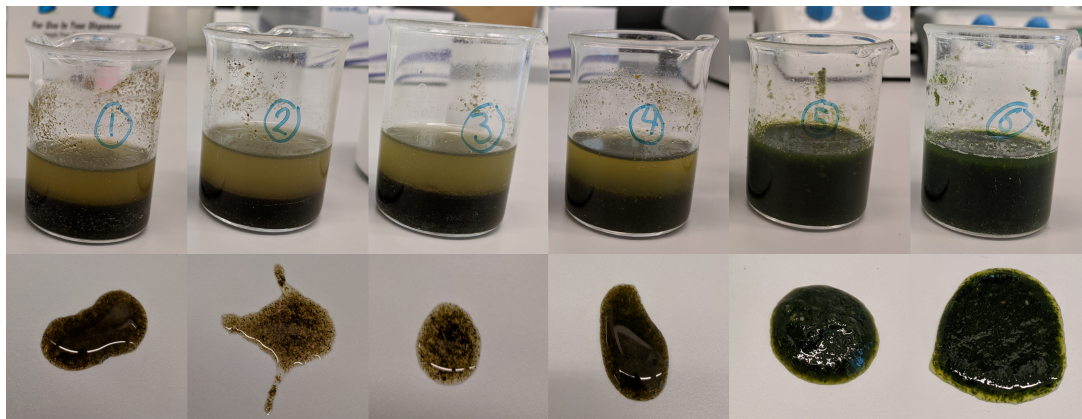


Figure 3.3.11: Photos of the six seaweeds dissolved in distilled water. Drops of ethanol were added prior to the addition of water. TSPP and  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$  were added after the samples were dissolved. From left to right: (1) AE pre-treated with washing and enzymes, (2) AE pre-treated with enzymes, (3) AE pre-treated with washing and high pH, (4) AE pre-treated with high pH, (5) SL pre-treated with enzymes, and SL pre-treated with high pH.

SL behaved similarly: no visible gelling occurred when TSPP and  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$  were added. However, SL swelled much more than AE. Although it was possible to see some separation between the seaweed particles and SL, it was not as visible as for AE. After adding  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ , the beakers with SL were gently turned upside down. Nothing fell out of the beakers. Moreover, after the addition of calcium, sample 5 (enzyme-treated SL) reminded of porridge consistency, while sample 6 (high pH treated SL) was a bit smoother, more like a watery pesto. These observations may suggest that the alginate in the SL was more available than the alginate in AE.

In conclusion, SL became a thicker paste than AE. AE separated from the water, and no increase in viscosity was observed. However, it is difficult to know if SL increased in viscosity or only swelled by absorbing the surrounding water. Despite this, samples 5 and 6 were selected for further investigation in the following experiment.

### 3.3.8 Experiment 5

The goal of this experiment was to observe the behaviour of the food matrix when the amount of MC and alginate were reduced. The experiment also aimed to see the effect of implementing seaweed into the matrix. The burgers were fried after approximately 24 hours in the cold room ( $T = 4^\circ\text{C}$ ). The resting time in the cold room resulted in firm burgers that had set relatively well. The burgers were easier to fry than the burgers in the previous experiments. Hence, the resting time was changed to 24 hours in the cold room for the next experiment.

Despite this, the experiment was characterised by fragile burgers and many falling apart (Figure 3.3.13). As previously mentioned (Section 3.3.1), TPA was prioritised when there were not enough sample pieces for both analyses. TPA gave more results and a higher sample size due to smaller pieces than required by the shearing test. Due to the burgers falling apart, the shearing test was not performed on C-0% and M-0.5%, and only one sample was analysed of E-1% and H-2%. The hardness obtained from the shearing test resulted in high standard deviations (Figure 3.3.14). None of the burgers was consequently significantly different at 5% level of significance with the ANOVA test. Due to the small sample size and high standard deviations, the results from the shearing test were deemed unreliable and are not discussed further in this thesis.

The hardness determined by the TPA of C-0%, MC-1%, and H-1% was not significantly different from any of the burgers, except E-2%. E-2% had a lower hardness than the three burgers. All the burgers with seaweed as a binder were difficult to fry. E-1% and H-2% fell apart. Due to very gentle handling, E-2% and H-1% did not fall apart. The cohesiveness of the seaweed burgers was significantly lower than C-0% and MC-1%, but not significantly different from each other (Figure 3.3.15). Of the seaweed burgers, only the springiness of H-1% was not significantly lower than C-0% and MC-1%. The chewiness of E-1% and H-1% were not significantly different from any of the other burgers. The chewiness of MC-1% showed a very large standard deviation ( $\pm 90\%$  of the average value) and was therefore difficult to rely on. However, H-1% was overall significantly different from the rest of the seaweed burgers. These results may sug-

gest that SL pre-treated with high pH made the alginate more available for interactions with calcium and SPI. This would have formed a stronger gel network and thus a firmer burger than the enzyme pre-treatment. The lack of similar results obtained from H-2% may also show that the ratio of seaweed affected the burger's texture differently. H-2% did not have a significantly different hardness, and the springiness and chewiness were significantly lower than C-0%, MC-1%, E-1%, and H-1%. However, these results may also have been due to the amount of water available in the food matrix. When dissolved, the binder with 2% seaweed had absorbed and trapped all of the water added. It seemed like a thick dry paste, while the binder with 1% seaweed reminded more of a wet pesto (Figure 3.3.12). When this was added with the SPI, more water was available for interactions for burgers with 1% seaweed, than with 2%. Future work should investigate the different ratios of high pH pre-treated seaweed and mixtures between high pH pre-treated seaweed and MC. Varying the amount of water in the food matrix could also be interesting to investigate.

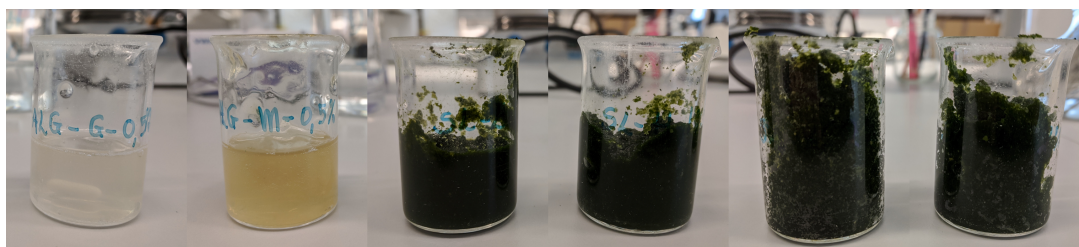


Figure 3.3.12: Photos of the six binders dissolved in distilled water. Drops of ethanol were added prior to the addition of water. TSPP and  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$  were added after the samples were dissolved. From left to right: G-0.5%, M-0.5%, E-1%, E-2%, H-1%, and H-2%.

To prevent MC-0.5% from falling apart, it was fried for a slightly longer time than the rest of the burgers (for 6 minutes instead of 4 minutes). Despite this, MC-0.5% was not significantly different from MC-1% for any textural parameters except for cohesiveness. Based on these observations and results, a reduction of MC amount seemed to weaken the gel network in the burger and not form enough fibrils to make a firm gel. An MC amount lower than 1% was therefore not investigated further.

The cohesiveness and chewiness of G-0.5% and the seaweed burgers were significantly lower than C-0% and MC-1%. The springiness of G-0.5% was significantly lower than C-0%, but not from any of the other burgers. In addition, both M-0.5% fell completely apart<sup>1</sup>, and one of the G-0.5% fell apart. Based on these results and observations, a reduction in alginate did not seem to have a positive impact on strengthening the gel alginate network in the food matrix. A lower concentration of alginate, regardless of the fraction of G-blocks, was therefore deemed undesirable and not investigated in the subsequent experiment.

<sup>1</sup>An error was made during the preparation of M-0.5%: the amount of SPI and water were not added in the correct ratio. As a result, the burgers were relatively thin. With the water evaporating from the burgers over the 24 hours in the cold room, the burgers easily fell apart. M-0.5% was therefore not analysed further.

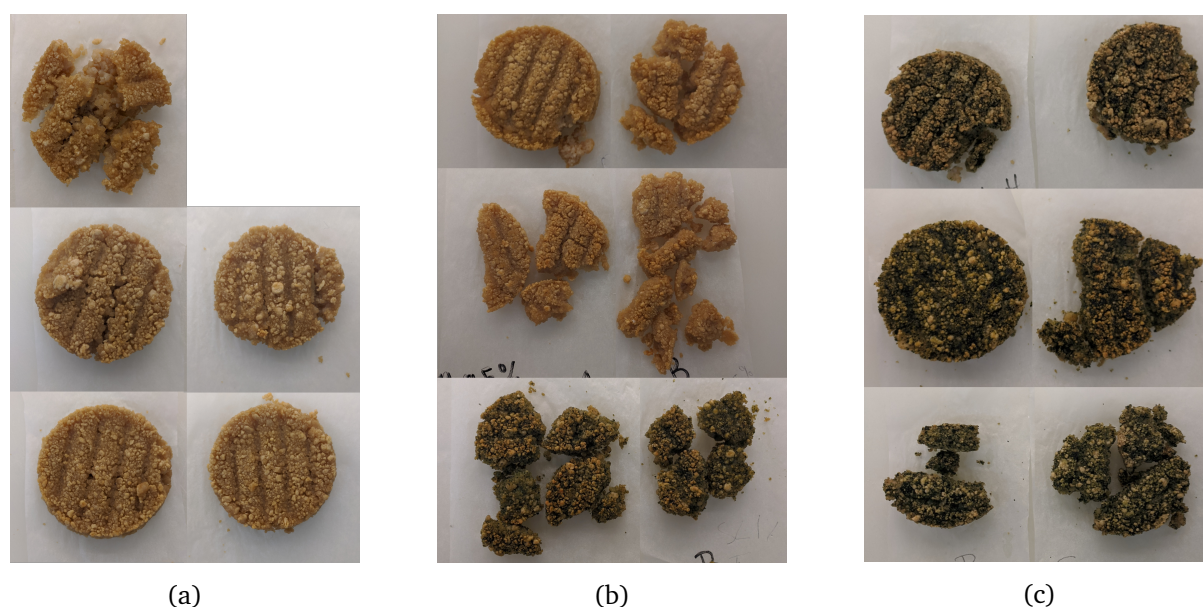


Figure 3.3.13: Photos of burgers obtained from experiment 5, fried at 250 W after 24 hours in the cold room. From top to bottom: (a) C-0%, MC-0.5%, MC-1%, (b) G-0.5%, M-0.5%, E-1%, (c) H-1%, E-2%, and H-2%. The burgers were fried for 4 minutes and flipped after 2 minutes. C-0% and MC-0.5% were fried for 6 minutes and flipped every 2 minutes.

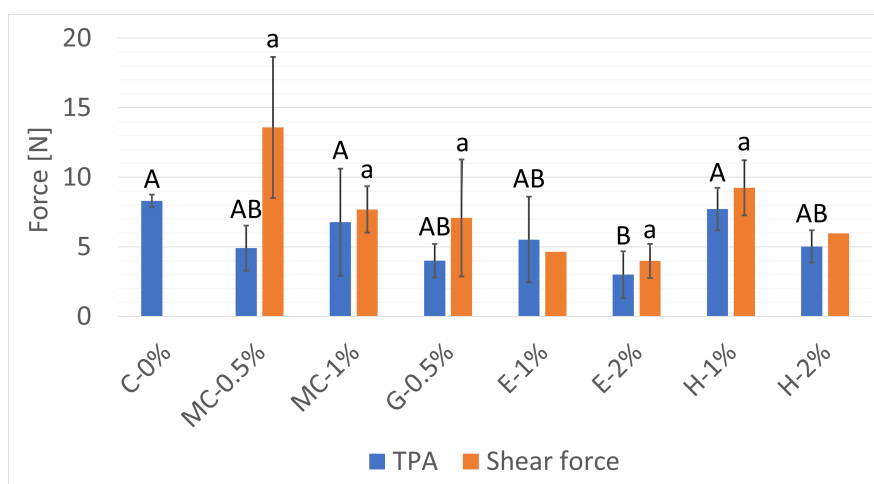
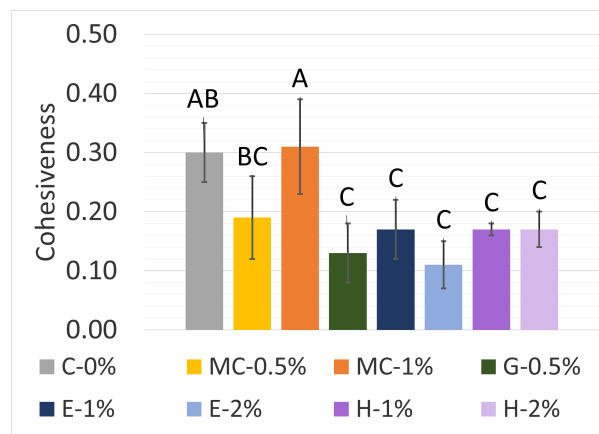
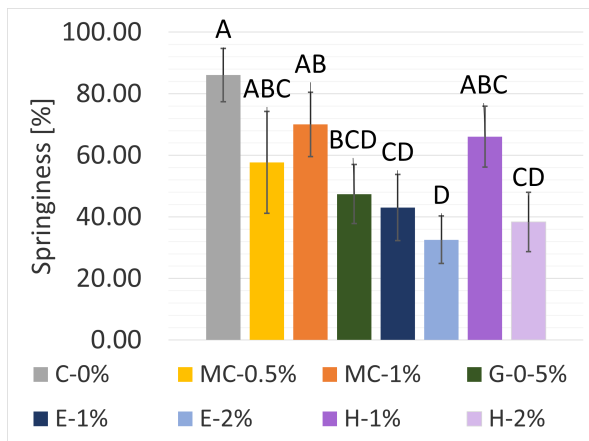


Figure 3.3.14: The average hardness (in Newton) obtained from the shearing test and TPA on the burgers from experiment 5, from left to right: C-0%, MC-0.5%, MC-1%, G-0.5%, E-1%, E-2%, H-1%, and H-2%. The hardness obtained from the TPA and shearing test is shown in blue to the left and orange to the right, respectively. Burgers with different letters on top of the column are significantly different at 5% level of significance (A and B are used for the values obtained from TPA, and a is used for the shearing test).

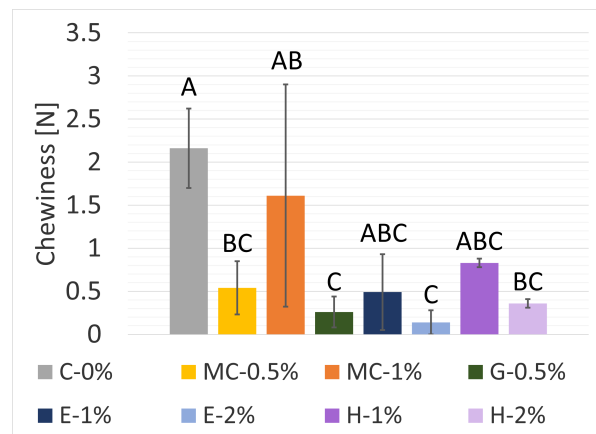




(a)



(b)



(c)

Figure 3.3.15: The textural parameters obtained from the TPA on the burgers from experiment 5. The cohesiveness (b), springiness (c, in % of wet weight of the sample), and chewiness (d, in Newton) obtained from the TPA are shown, from left to right: C-0% (grey), MC-0.5% (yellow), MC-1% (orange), G-0.5% (dark green), E-1% (dark blue), E-2% (light blue), H-1% (purple), and H-2% (light pink). Burgers with different letters on the top of the column (A-C) are significantly different at 5% level of significance.

### 3.3.9 Experiment 6

This experiment aimed to observe the behaviour of implementing a binder made of alginate and MC in different ratios. These burgers were the easiest to fry of all the experiments. This was most likely due to the overnight resting time, the accumulated experience with flipping the burgers in the pan, and the binder's interaction in the food matrix. All burgers, including C-0%, held together and were not fried longer than 4 minutes. It was therefore possible to make a trustworthy comparison between all of the burgers. This being said, an error occurred during the burger preparation of G-1%.  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$  was added directly into the alginate solution without being dissolved in some droplets of distilled water. This resulted in burgers where the calcium most likely did not interact with the alginate to form a gel network, and white spots were observed on the burger both raw and fried. G-1% can therefore be considered as a burger with only alginate as a binder, without the interaction of calcium. The alginate alone acted as a thickening agent, but did not gel without the interaction of calcium (Helgerud et al., 2009).

The texture analyser knife set-up was calibrated prior to the shearing test. The calibration permitted the system to cancel out noise and disturbances in the surroundings. Therefore, the curves obtained from the shearing test were smoother than in the other experiments. The graphs of the shearing tests are found in the Appendix Section A.3.5. Despite the lack of calibration, the prior results from experiments 1 to 5 were deemed reliable. The calibration was not a requirement and did not seem to change the irregularities of the curves, even though it cancelled some of the noise disturbances. The results from the previous shearing tests may however, have had slightly more noise disturbances which led to higher standard deviations.

The hardness obtained from the shearing tests was not significantly different between any of the burgers, at 5% level of significance (Figure 3.3.19). The hardness obtained from the TPA gave the same result, except for C-0%, which was significantly higher than all the other burgers. Since the shearing test is designed for finding the hardness of a food product, and the standard deviations were low in this experiment, the significance of the C-0% hardness obtained by the TPA was not regarded as a reliable result. In conclusion, the mixture of alginate and MC seemed to have given the same textural effect on the hardness of the burgers as 1% MC.

C-0% also had a significantly higher cohesiveness, springiness, and chewiness than all the other burgers (Figure 3.3.20). However, the chewiness result had a very high standard deviation ( $\pm 50\%$  of the average value) and was therefore deemed unreliable. The springiness was not significantly different between any of the burgers, except for C-0%, which was significantly higher than all the other burgers. The higher cohesiveness and springiness of C-0% may suggest that the implementation of any binder, MC or alginate, decreases the textural properties of the burger. However, it is worth mentioning that this was the first of all the C-0% burgers in this thesis that did not need to be fried for more than 4 minutes to prevent it from falling apart. The burger was still fragile and had to be handled very carefully. The binder was seen as essential for keeping the burger together, and removing it from the food matrix is not a possibility for a commercial burger, which should withstand being moved several times without falling apart.

As mentioned, all burgers with a mixture of alginate and MC burgers had a similar hardness, springiness, and chewiness (Figure 3.3.20). The only parameter with significantly different results was cohesiveness. MC-1% was not significantly different from MC-G(0.5%-0.5%) and MC-G(0.75%-0.25%), MC-M(0.25%-0.75%), and MC-M(0.75%-0.25%). In comparison, MC-1% was significantly higher than G-1%, MC-G(0.25%-0.75%), M-1%, and MC-M(0.5%-0.5%). The mixture of alginate and MC seemed to have affected the textural properties of the food matrix: 0.5% G and 0.5% MC, 0.75% M and 0.25% MC, and 0.25% G and 0.75% MC gave similar results as 1% MC alone. Also, the burgers with only alginate as a binder fell apart, while the burgers with a mixture of alginate and MC did not (Figures 3.3.16, 3.3.17, and 3.3.18). This may suggest that some interaction between MC and the alginates may have occurred. Liang et al. (2004) reported, when making alginate/MC hydrogels for drug research, that sodium alginate added with MC formed an entangled network with hydrophobic interactions, hydrogen bonds, and cross-linking. The hydrogen bonds were formed between the carboxyl and hydroxyl groups of the two polymers. The study investigated the hydrogels with the addition of different salts. The gelation temperature was reported to decrease with the addition of calcium chloride. Similarly, Eskens et al. (2020) reported a synergistic gel system when adding alginate, MC and epidermal growth factors (EPF) to a solution. The study investigated the impact of EPF in the gel network formed with the two polymers. The mixture of alginate and MC without EPF was reported to give a higher viscosity than solutions with the individual component (alginate or MC). Both studies worked with matrices around the neutral pH. The promising results from the mixtures of MC and alginate from this thesis may therefore have been due to the synergistic network between alginate and MC, as well as the decrease in gelation temperature with calcium. However, based on the obtained results, it is difficult to point out the specific ratio that worked best for the food matrix investigated in this thesis. Future investigations are needed to find the optimal ratio between MC and alginate.

G-1% was not significantly different from any of the other burgers, except C-0%, and the cohesiveness of MC-1%. As a reminder, G-1% did most likely not have any interactions with calcium and can be looked upon as a burger with only alginate as a binder. The results of G-1% may suggest that the alginate itself also could be used as a binder. It would have been interesting to make burgers with a mixture of alginate and MC without any calcium interaction. Since MC and alginate have a synergistic effect together, this would be interesting to investigate further. Hence, future work should investigate the texture of a burger with alginate alone as a binder, and a mixture of alginate and MC as a binder, without the addition of calcium or TSPP.

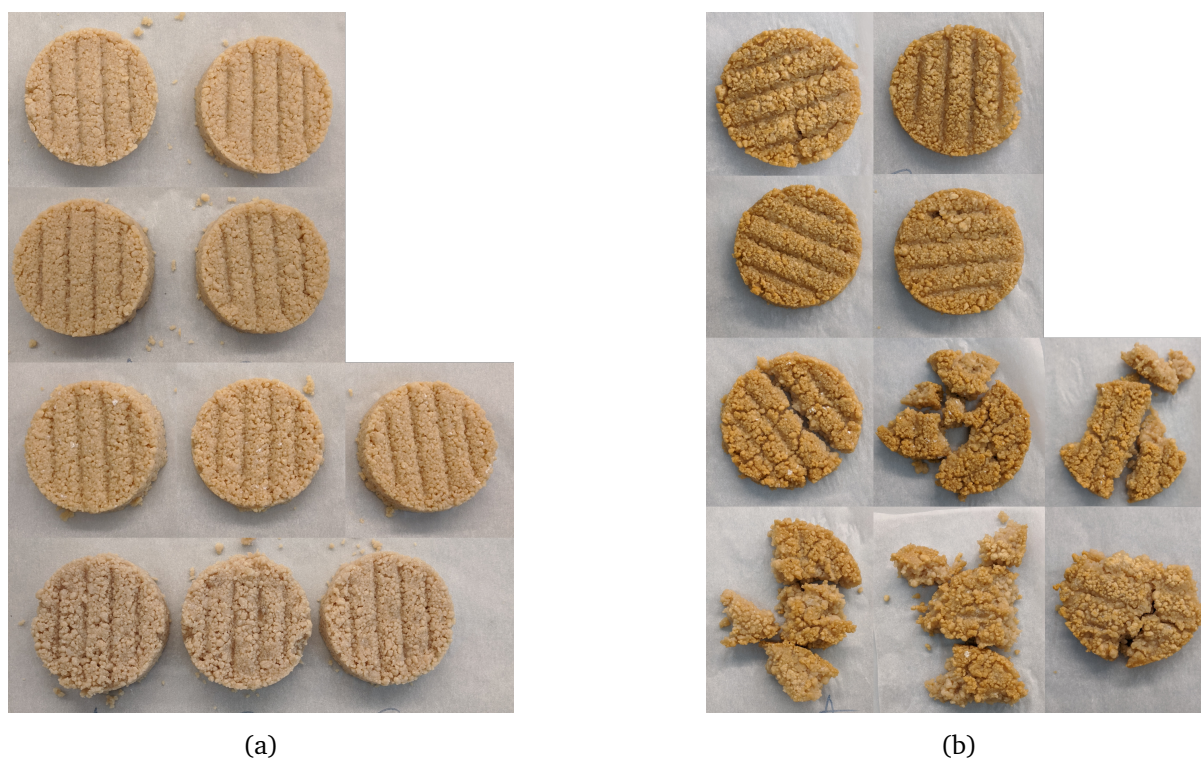


Figure 3.3.16: Photos of burgers obtained from experiment 6, (a) raw, right after pressing, and (b) fried at 250 W for 4 minutes. From top to bottom: C-0%, MC-1%, G-1%, and M-1%.



Figure 3.3.17: Photos of burgers obtained from experiment 6, (a) raw, right after pressing, and (b) fried at 250 W for 4 minutes. From top to bottom: MC-G(0.25%-0.75%), MC-G(0.5%-0.5%), and MC-G(0.75%-0.25%).



Figure 3.3.18: Photos of burgers obtained from experiment 6, (a) raw, right after pressing, and (b) fried at 250 W for 4 minutes. From top to bottom: MC-M(0.25%-0.75%), MC-M(0.5%-0.5%), and MC-M(0.75%-0.25%).

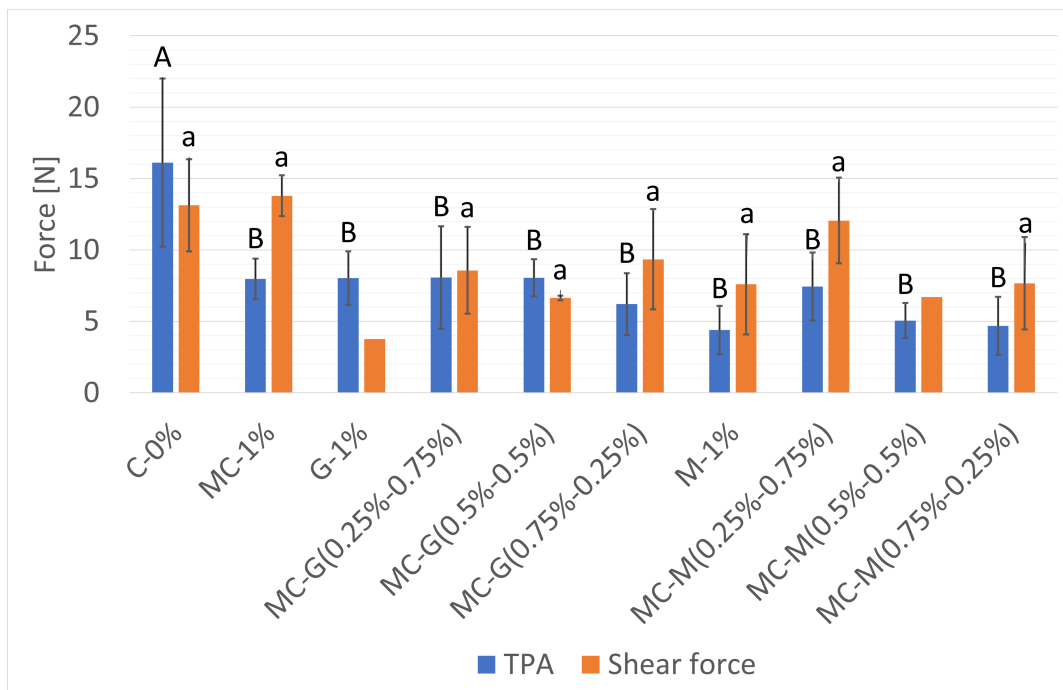
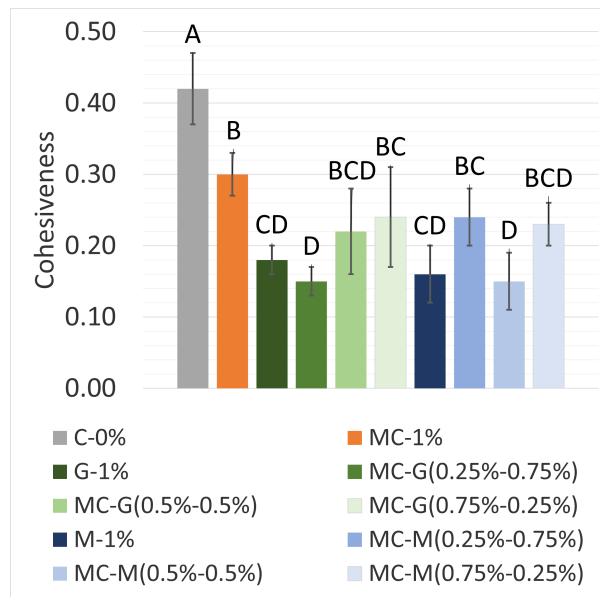
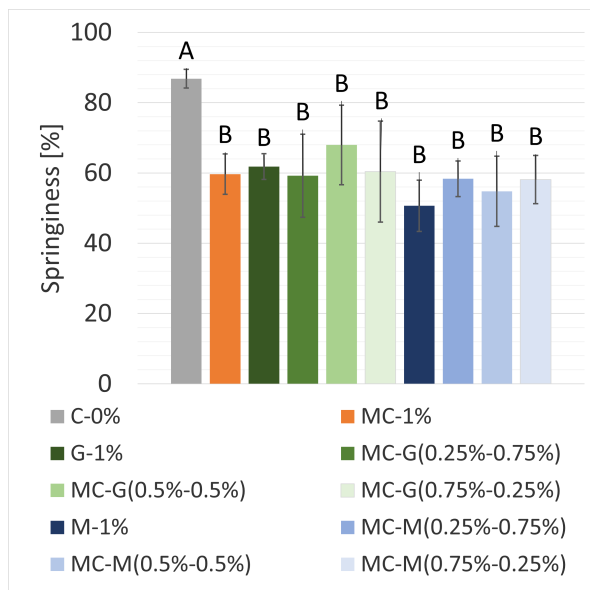


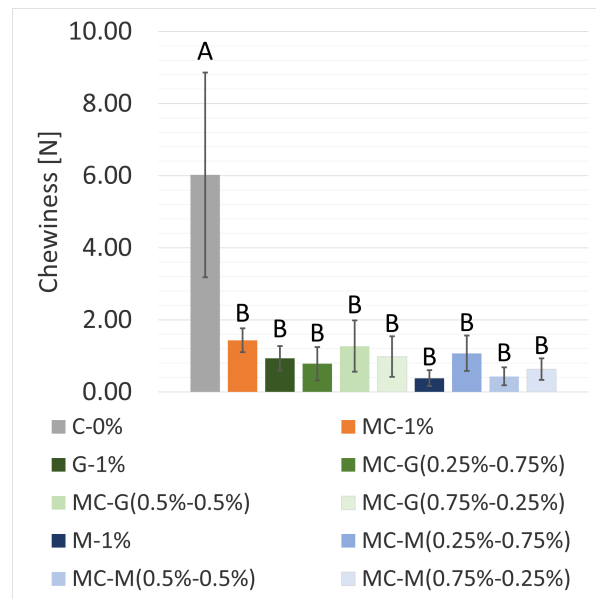
Figure 3.3.19: The average hardness (in Newton) obtained from the shearing test and TPA on the burgers from experiment 6, from left to right: C-0%, MC-1%, G-1%, MC-G(0.25%-0.75%), MC-G(0.5%-0.5%), MC-G(0.75%-0.25%), M-1%, MC-M(0.25%-0.75%), MC-M(0.5%-0.5%), and MC-M(0.75%-0.25%). The hardness obtained from the TPA and shearing test is shown in blue to the left and orange to the right, respectively. Burgers with different letters on top of the column are significantly different at 5% level of significance (A and B are used for the values obtained from TPA, and a is used for the values obtained from the shearing test).



(a)



(b)



(c)

Figure 3.3.20: The textural parameters obtained from the TPA on the burgers from experiment 6. The cohesiveness (b), springiness (c, in % of wet weight of the sample), and chewiness (d, in Newton) obtained from the TPA are shown, from left to right: C-0% (grey), MC-1% (orange), G-1% (dark green), MC-G(0.25%-0.75%) (green), MC-G(0.5%-0.5%) (light green), MC-G(0.75%-0.25%) (very light green), M-1% (dark blue), MC-M(0.25%-0.75%) (blue), MC-M(0.5%-0.5%) (light blue), and MC-M(0.75%-0.25%) (very light blue). Burgers with different letters on the top of the column (A-B) are significantly different at 5% level of significance.

### 3.4 Limitations of the study

This thesis investigated the influence of different binders in burgers, resulting in many burgers falling apart. To prevent the burgers from tearing, they were handled very gently when moved, fried, and cut for the texture analyses. The burgers obtained in this thesis are therefore far from any commercial burger. They would not withstand to be packed and moved around in a warehouse. Further research of a food matrix, which can withstand everything a food product undergoes from the warehouse to the store, is necessary.

The results can be criticised for being unreliable because of a small sample size overall. However, the statistical analyses took the sample size into account. The consequence of smaller sample sizes is a higher standard deviation, and not being able to reject the null hypothesis (that the means of the data group 1 and 2 are equal) at 5% level of significance. By doing more experiments, obtaining more results, and increasing the sample size, the null hypothesis may be rejected.

The burger preparation, frying, and cutting was done manually. Human errors are therefore another limitation of this study. This may include loss of raw material and slight differences in all the burgers. The burgers were pressed as similarly as possible, but all were pressed manually. This may have resulted in burgers pressed with different forces. A difference in the force used when pressing the burger could have led to quite different textural properties. When pressing the burger, the air was squeezed out of the food matrix. If less force was used on some samples, this could have resulted in more loose burgers with more air, inhibiting the binder's gelling ability, for example.





## Conclusion

This thesis aimed to see if complete or partial substitution of sodium alginate or seaweed with methyl cellulose could lead to the same texture as burgers with only methyl cellulose. The alginates gelled as expected in sub-experiment *a*. G yielded a smoother gel than M, most likely due to its higher G-block fractions interacting better with the added calcium and TSPP. The seaweeds analysed in sub-experiment *b* were from *Saccharina latissima* (SL) and *Alaria esculenta* (AE), which had been through different pre-treatments. The two SL seaweeds – one enzyme pre-treated (E) and one treated with water at high pH (H) – were chosen to investigate in this thesis. This choice was based on the observed higher viscosity.

The food matrix was formulated in experiment 1. The burger was made of 30.0% SPI, 3.8% coconut oil, 3.8% canola oil, and 62.5% distilled water. A binder was added as a percentage of the total weight of the burger. A binder was essential to keep the burger from falling apart. All C-0% burgers had to be fried longer than the rest of the burgers to prevent them from falling apart. This resulted in a thicker crust and higher textural properties than the other burgers. The frying time was reduced from 8 to 6 minutes.

Experiments 2 and 3 optimised the method. Experiment 2 concluded that 1% of MC and 2% of MC in the food matrix did not give significantly different textural results from each other, at 5% level of significance. Experiment 3 concluded that the burgers should be dry-fried at 250 W, and fried in a raw state instead of frozen, as this was easier to handle in the pan. A raw C-0% burger was also analysed. However, since the commercial plant-based burgers were fried, only fried burgers were investigated for the remainder of the thesis. The frying time was reduced to 4 minutes.

Experiment 4 investigated the influence of sodium alginate in the soy burgers compared to MC. Alginate did not work as well as MC, regardless of the fraction of G-blocks. The burgers were also quite wet, which most likely inhibited the Maillard reaction by lowering the temperature. The hardness and springiness were higher for 1% MC than 1% alginate, regardless of the G-fraction. G and M were not significantly different.

Experiment 5 concluded that a reduction in MC and alginate concentration resulted in a fragile burger that easily tore. H resulted in slightly better textural properties than E. 1% H had textural parameters similar to 1% MC. This may suggest that the high pH treatment led to higher availability of alginate than the enzyme pre-treatment.

Experiment 6 investigated the influence of a mixture of sodium alginate and MC at different ratios in the soy burgers compared to MC. Regardless of the ratio, none of the burgers with a mixture resulted in significantly different textural parameters from 1% MC. This suggested that two polymers interacted with each other. The addition of calcium may also have resulted in a decrease in gelation temperature. Furthermore, the burgers with the mixture of alginate and MC were the easiest burgers to fry of all the burgers in the thesis. However, it was difficult to draw a reliable conclusion for the optimal ratio between these two polymers, due to no significant difference between the burgers with different ratio. Nevertheless, the partial substitution of MC gave promising results and should be investigated further.

## Future work

The study was characterised by fragile burgers. Most of the burgers had to be handled very gently to prevent them from tearing. The methods used were mostly manual, resulting in slight differences in each burger. Additional experiments should be performed to make the results more reliable.

Burgers with alginate without the interaction from TSPP and calcium were not investigated thoroughly in this thesis. It would be interesting to investigate alginate as a binder alone due to its thickening ability. A suggested approach could be to vary the calcium and TSPP amounts to investigate their impact on gel formation. This could easily have been investigated in raw burgers. Raw burgers took less time to prepare overall and were easier to cut than fried burgers. Future investigations could also include more experiments on the pre-treated seaweeds and other differently pre-treated seaweed. Furthermore, to ensure complete dispersion of alginate in the burger, more TSPP could be added after dissolution of the alginate.

There were no significant differences between the alginates at 5% level of significance. This questions the water availability in the food matrix. Water is essential for compounds to interact with each other. Experimenting with adding more water to the food matrix could help improve the interactions between the compounds.

Leaving the burgers with the binder for 24 hours in the cold room resulted in firmer burgers that were easier to fry. However, the burgers could have been placed in a container, so as not to let the burgers absorb additional moisture or completely dry out.

In addition, studying different cylinder probes for the TPA would be interesting, as well as varying the strain on the food product. More experiments with both TPA and the shearing test are required to get more reliable results with lower standard deviations.

Moreover, the burgers could be fried in an oven instead of a pan. This could lead to a more controlled environment and fewer variations in the burger preparation, frying, and texture analysis.



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# Ingredients and raw data

## A.1 Ingredients

The ingredients in Orkla’s Naturli’ burger are compared with the chosen ingredients in the thesis’ food matrix in Table A.1.1. The original ingredient list was stripped down to the main components: soy protein, vegetable oil and water, as well as a binder. The obtained food matrix was therefore made of five components instead of Orkla’s thirteen. This made it easier to analyse the interactions between the different components afterwards.

Table A.1.1: Comparison between Orkla’s Naturli’ burger with the burger formulated in this thesis. The ingredients, number of components and nutritional content of each burger are shown.

Burger	Ingredient	Nutritional content per 100 g/mL
Orkla’s Naturli’ burger (13 components)	water, soy protein, vegetable oil (coconut, canola), onion, aroma, spices, tomato, stabilisator (methyl cellulose), colouring agent (beetroot, caramel), mushroom, garlic	fat: 16% (of which 7% are saturated), carbohydrates: 2.80% (of which 0.90% contain sugars), protein: 12%, salt: 1.20%
Thesis burger (5-6 components) <sup>a</sup>	water, soy protein, vegetable oil (coconut, canola), binder (methyl cellulose, alginate or seaweed)	fat: 8.0%, protein: 25.2%

<sup>a</sup>The burgers with one binder had a total number of 5 components. One burger type was however, prepared with two binders (methyl cellulose and alginate), i.e. 6 components.

## A.2 Proximate analysis

The proximate analysis were performed on C-0% and MC-1%, both raw and fried. The proximate analysis results are shown in section A.2.1, A.2.2, and A.2.3.

### A.2.1 Kjeldahl analysis

The protein content was analysed using the Kjeldahl method. The results are shown in Table A.2.1. Tryptophan was used as the reference substance. The analysis followed the protocol from the producer BUTCHI (KjelDigester K-149, Scrubber K-415 and KjelMaster K-375). The conversion factor used was 5.71 (BUTCHI (Switzerland), 2013)). Three parallels were performed. However, one sample for C-0% fried and MC-1% raw was aborted due to an error during the use of the KjelMaster.

Table A.2.1: Results from the Kjeldahl analysis of C-0% and MC-1%, raw (R) and fried (F). Two samples were aborted due to an error when using the KjelMaster.

Tube	Sample type	Mass [g]	Titration volume [mL]	%N	%Pr
1	Blank	-	0.302	-	-
2	Blank	-	0.298	-	-
3	Blank	-	0.302	-	-
4	Reference substance (tryptophan)	0.1612	8.299	13.900	n/a
5	Reference substance (tryptophan)	0.1645	8.256	13.548	n/a
6	C-0%R	1.6926	26.095	4.269	24.377
7	C-0%R	1.6558	25.099	4.195	23.956
8	C-0%R	1.7635	27.544	4.328	24.711
9	C-0%F	1.3379	22.507	4.650	26.550
10	C-0%F	1.1200	18.775	4.621	26.385
11	C-0%F	1.2975	aborted	-	-
12	MC-1%R	1.8000	aborted	-	-
13	MC-1%R	1.6620	25.277	4.210	24.038
14	MC-1%R	1.7600	26.837	4.224	24.117
15	MC-1%F	1.1515	18.567	4.444	25.374
16	MC-1%F	1.1838	19.028	4.432	25.305
17	MC-1%F	1.2388	18.564	4.130	23.582
18	Reference substance (tryptophan)	0.1589	8.328	14.152	n/a
19	Blank	-	0.319	-	-
20	Blank	-	0.274	-	-

### A.2.2 Bligh and Dyer analysis

The lipid content was analysed using the Bligh and Dyer method (Bligh & Dyer, 1959). The results are shown in Table A.2.2. Three parallels were performed.

Table A.2.2: Results from the Bligh and Dyer analysis of C-0% and MC-1%, raw (R) and fried (F).

Tube	Sample type	Mass [g]	Fraction 19 hours after evaporation [g]	% Fat content in sample
1	C-0%R	10.68	0.048	8.96
2	C-0%R	10.09	0.045	8.92
3	C-0%R	10.45	0.046	8.83
4	C-0%F	10.89	0.047	8.69
5	C-0%F	10.14	0.051	10.02
6	C-0%F	10.38	0.046	8.90
7	MC-1%R	10.19	0.055	10.88
8	MC-1%R	10.19	0.066	12.89
9	MC-1%R	10.49	0.052	9.85
4	MC-1%F	10.80	0.052	9.54
5	MC-1%F	10.25	0.048	9.40
6	MC-1%F	10.43	0.049	9.32

### A.2.3 Dry matter analysis

The dry matter content was analysed by heating the samples in a heating cabinet (over 100°C) overnight, and subtracting the weight prior heating with the weight after heating. The results are shown in Table A.2.3. Three parallels were performed. However, one sample of fried C-0% was aborted due to insufficient raw material.

Table A.2.3: Results from the dry matter analysis of C-0% and MC-1%, raw (R) and fried (F). Three parallels were performed for all samples, except C-0%F, with only two due to insufficient raw material.

Tube	Sample type	Mass of sample [g]	Dry matter after 24 hours [g]	Dry matter % after 24 hours
1	C-0%R	4.20	3.34	35.94
2	C-0%R	4.15	3.36	36.69
3	C-0%R	4.16	3.35	36.35
4	C-0%F	4.40	3.86	37.18
5	C-0%F	4.10	3.86	39.46
6	MC-1%R	4.17	3.80	37.88
7	MC-1%R	4.12	3.81	38.24
8	MC-1%R	4.12	3.82	38.58
9	MC-1%F	4.81	4.13	39.49
10	MC-1%F	4.09	3.53	39.59
11	MC-1%F	4.26	3.62	39.92

### A.3 Texture analysis

Graphs obtained from the shearing test and TPA of experiments 1 to 6 are shown below in the following sections.

#### A.3.1 Experiment 2

A TPA was performed on C-0%, MC-1%, and MC-2% made in experiment 2 (Figure A.3.1).

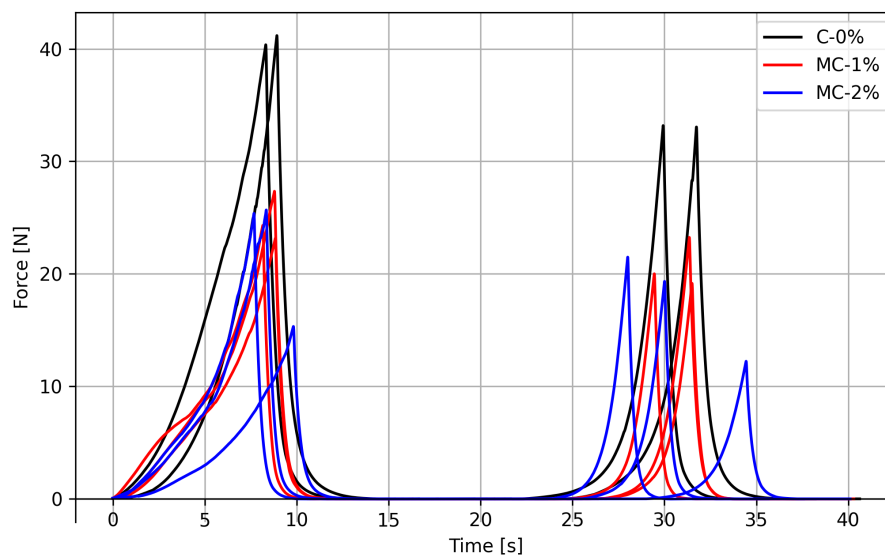


Figure A.3.1: Graph obtained from performing a TPA on the burgers from experiment 2. The force (in Newton) as a function of time (in seconds) is shown for C-0% (black), MC-1% (red), and MC-2% (blue).

### A.3.2 Experiment 3

Three C-0% burgers were made in experiment 3. One was kept in a raw state, one was fried at 250 W, and the last was fried at 500 W. A shearing test was performed on all three burgers (Figure A.3.2) and a TPA (Figure A.3.3 and A.3.4).

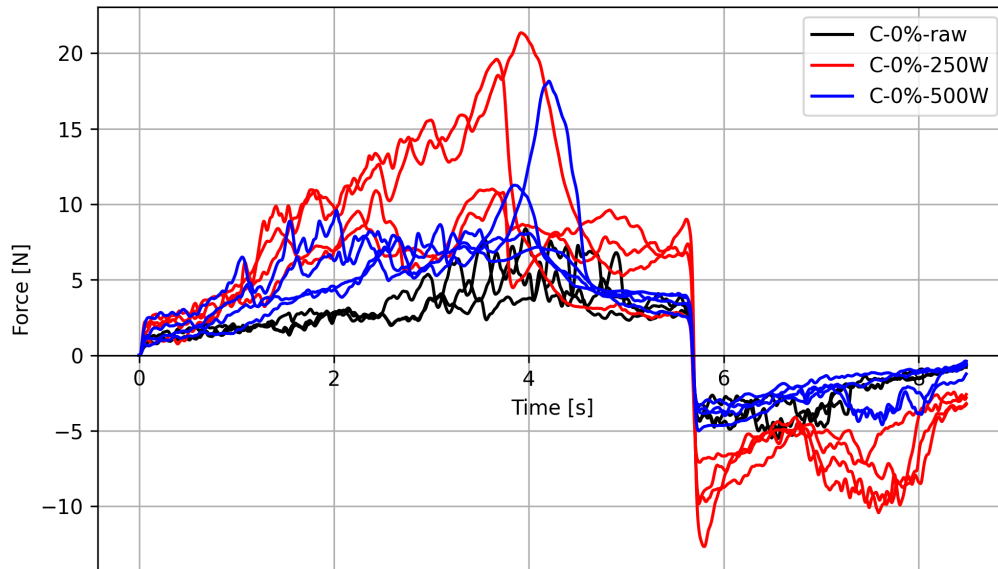


Figure A.3.2: Graph obtained from performing a shearing test on the burgers from experiment 3. The force (in Newton) as a function of time (in seconds) is shown for C-0% in raw state (black), C-0% fried at 250 W (red), and C-0% fried at 500 W (blue).

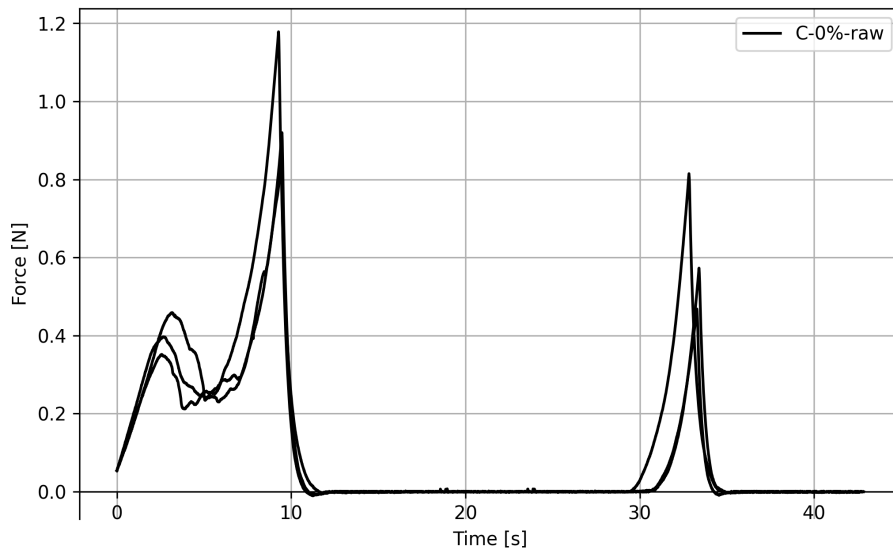


Figure A.3.3: Graph obtained from performing a TPA on the raw C-0% burger from experiment 3. The force (in Newton) is shown as a function of time (in seconds).

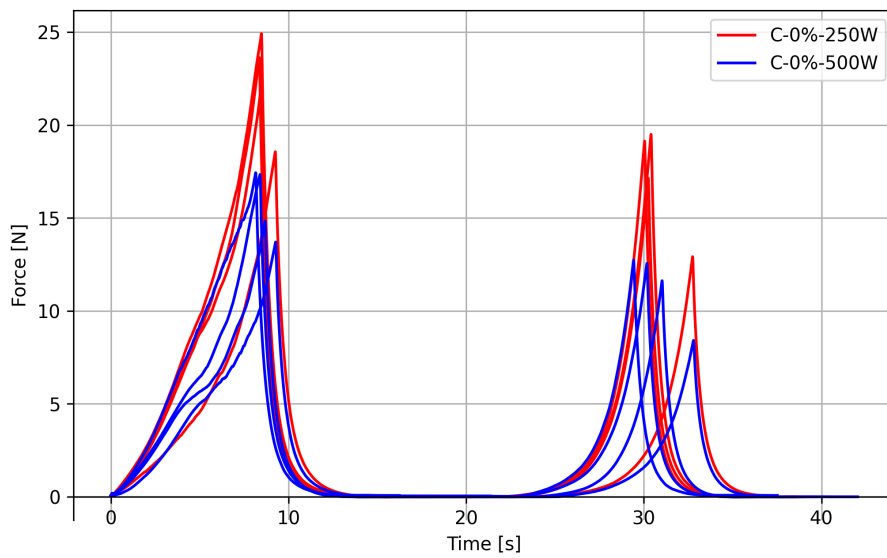


Figure A.3.4: Graph obtained from performing a TPA on the burgers from experiment 3. The force (in Newton) as a function of time (in seconds) is shown for C-0% fried at 250 W (red) and C-0% fried at 500 W (blue).



### A.3.3 Experiment 4

Four different burgers were made in experiment 4: C-0%, MC-1%, G-1%, and M-1%. All were pressed, dry-fried at 250 W and cut into pieces for a texture analysis. An overview of the sample size for the shearing test and TPA of the burgers from experiment 4 is shown in Table A.3.1. C-0% had the smallest sample size due to difficulties when cutting the pieces for the analyses. A shearing test and a TPA was performed on all burgers (Figure A.3.5 and A.3.6).

Table A.3.1: Overview of sample size for the shearing test and TPA of the burgers from experiment 4.

Sample	Shearing test	TPA
C-0%	3	4
MC-1%	4	5
G-1%	7	6
M-1%	7	6

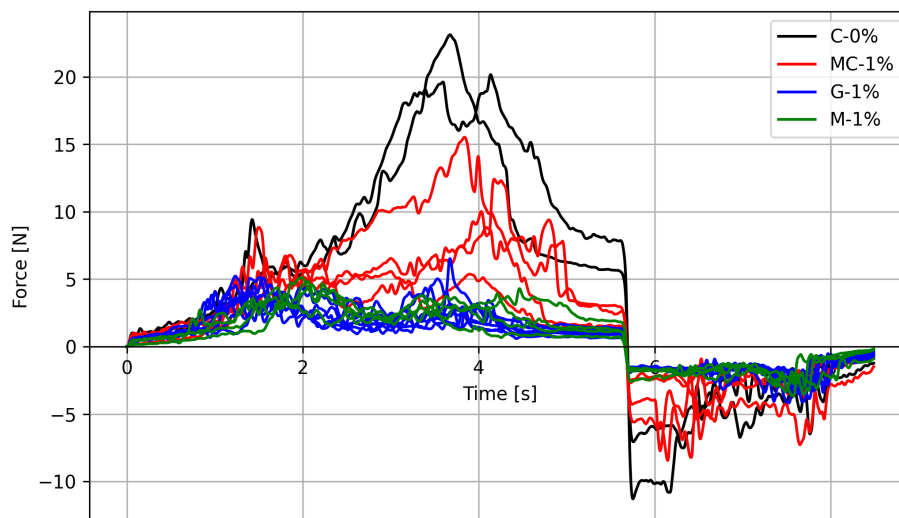


Figure A.3.5: Graph obtained from performing a shearing test on the burgers from experiment 4. The force (in Newton) as a function of time (in seconds) is shown for C-0% (black), MC-1% (red), G-1% (blue), and M-1% (green).

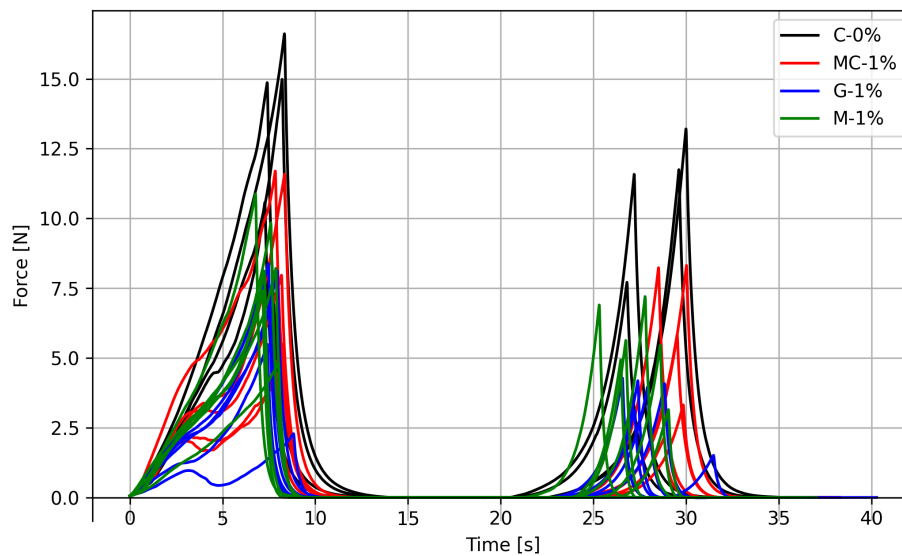


Figure A.3.6: Graph obtained from performing a TPA on the burgers from experiment 4. The force (in Newton) as a function of time (in seconds) is shown for C-0% (black), MC-1% (red), G-1% (blue), and M-1% (green).

#### A.3.4 Experiment 5

Nine different burgers were made in experiment 5: C-0%, MC-0.5%, MC-1%, G-0.5%, M-0.5%, E-1%, H-1%, E-2%, and H-2%. All were pressed (Figure A.3.7), rested in the cold room for 24 hours (Figure A.3.8), dry-fried at 250 W, and cut into pieces for a texture analysis. An overview of the sample size for the shearing test and TPA of the burgers from experiment 5 is shown in Table A.3.2. C-0% had a small sample size due to difficulties when cutting the pieces for the analyses. M-0.5% was aborted due to falling apart. While preparing the burger, an error was made with the ratio of the ingredients. A shearing test was performed on all burgers (Figures A.3.9, A.3.10, and A.3.11). A TPA was also performed on all burgers (Figures A.3.12, A.3.13, and A.3.14).

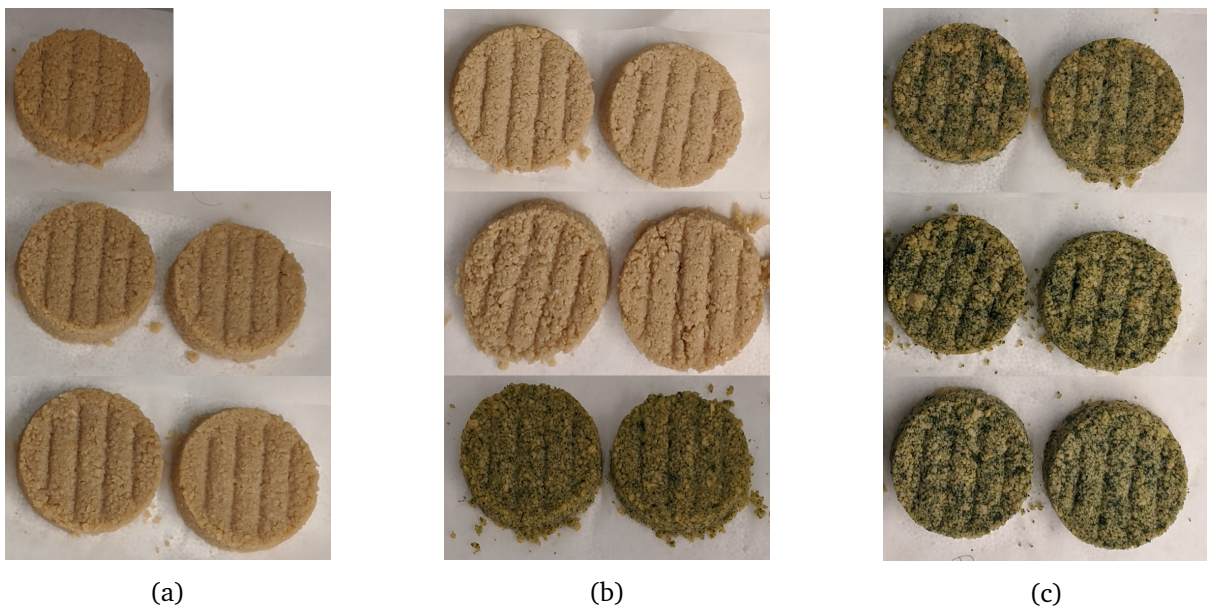


Figure A.3.7: Photos of the burgers obtained from experiment 5, in raw state, right after pressing. From top to bottom: (a) C-0%, MC-0.5%, MC-1%, (b) G-0.5%, M-0.5%, E-1%, (c) H-1%, E-2%, and H-2%.

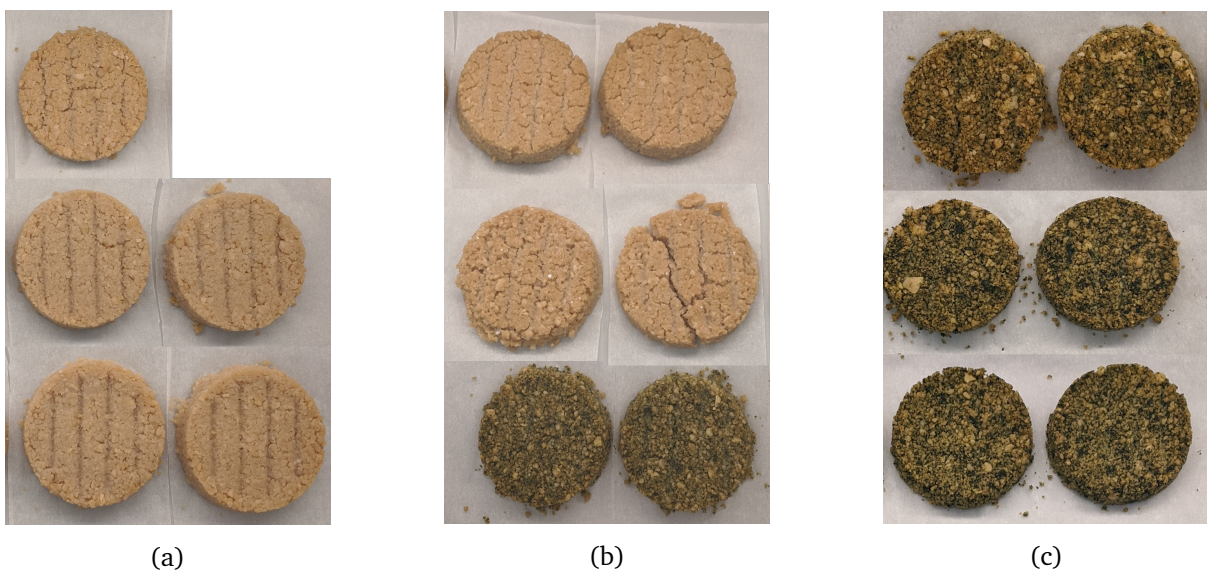


Figure A.3.8: Photos of the burgers obtained from experiment 5, in raw state, after resting for 24 hours in the cold room. From top to bottom: (a) C-0%, MC-0.5%, MC-1%, (b) G-0.5%, M-0.5%, E-1%, (c) H-1%, E-2%, and H-2%.

Table A.3.2: Overview of sample size for the shearing test and TPA of the burgers from experiment 5.

Sample	Shearing test	TPA
C-0%	0	2
MC-0.5%	3	5
MC-1%	4	5
G-0.5%	3	3
M-0.5%	0	0
E-1%	1	4
E-2%	2	5
H-1%	2	3
H-2%	1	4

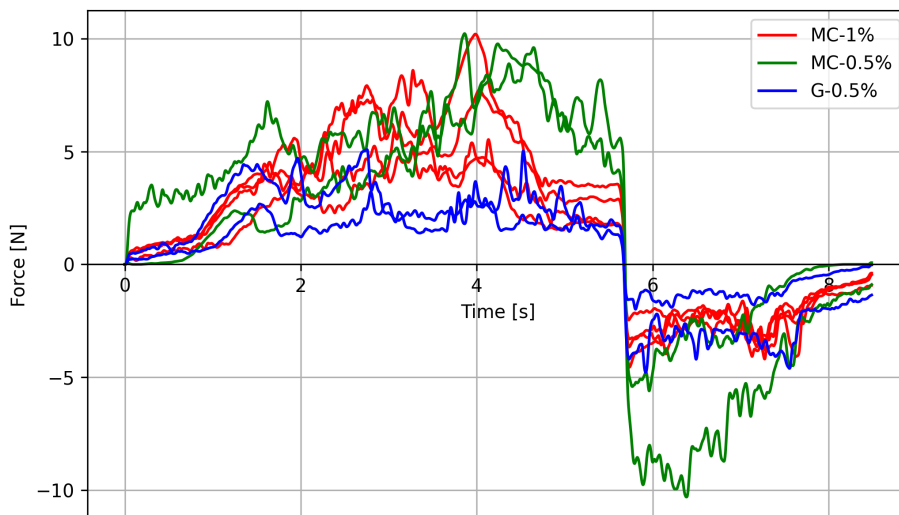


Figure A.3.9: Graph obtained from performing a shearing test on the burgers from experiment 5. The force (in Newton) as a function of time (in seconds) is shown for MC-1% (red), MC-0.5% (green), and G-0.5% (blue).

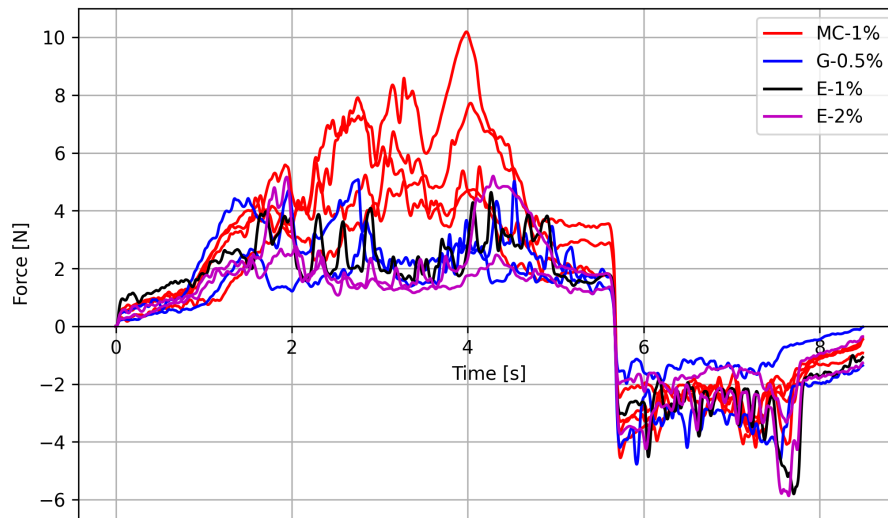


Figure A.3.10: Graph obtained from performing a shearing test on the burgers from experiment 5. The force (in Newton) as a function of time (in seconds) is shown for MC-1% (red), G-0.5% (blue), E-1% (black), and E-2% (purple).

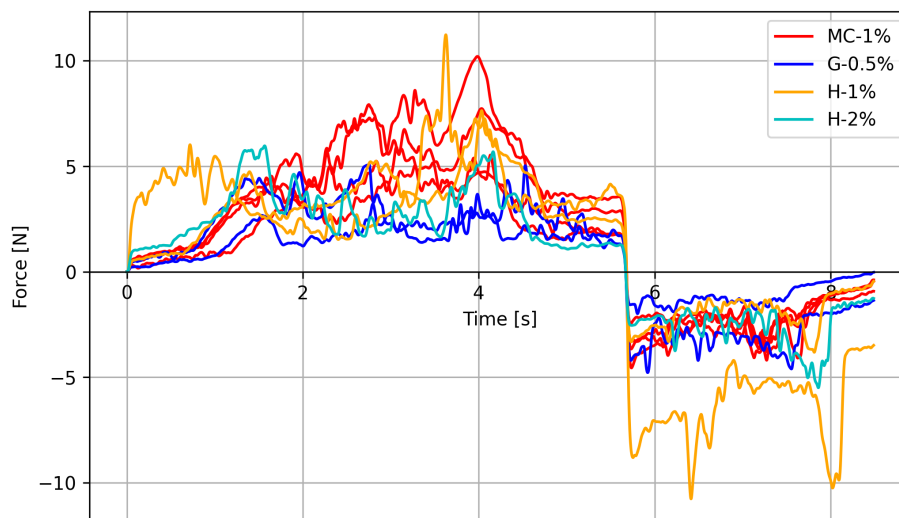


Figure A.3.11: Graph obtained from performing a shearing test on the burgers from experiment 5. The force (in Newton) as a function of time (in seconds) is shown for MC-1% (red), G-0.5% (blue), H-1% (orange), and H-2% (light blue).

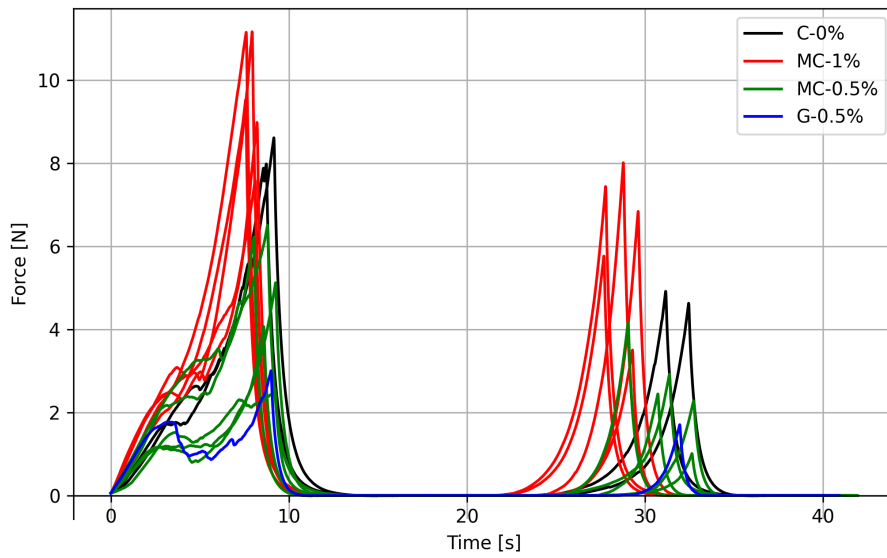


Figure A.3.12: Graph obtained from performing a TPA on the burgers from experiment 5. The force (in Newton) as a function of time (in seconds) is shown for MC-1% (red), MC-0.5% (green), and G-0.5% (blue).

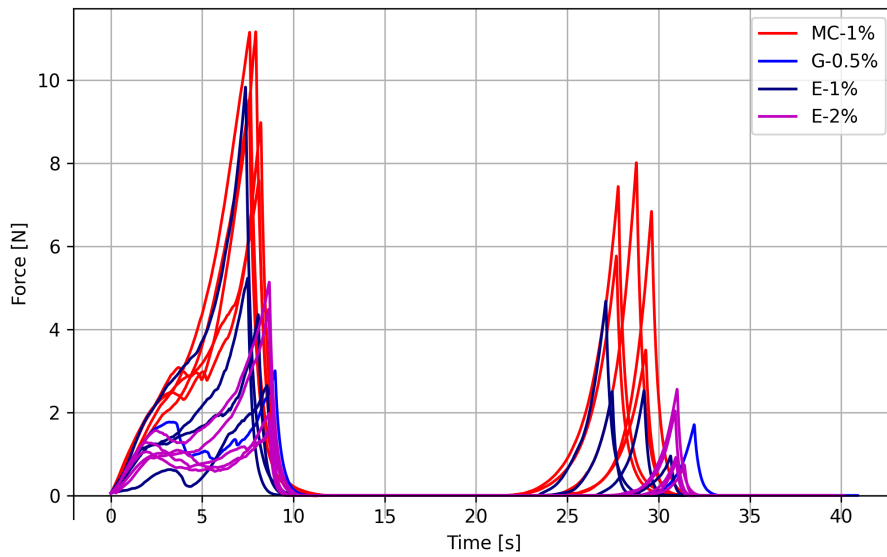


Figure A.3.13: Graph obtained from performing a TPA on the burgers from experiment 5. The force (in Newton) as a function of time (in seconds) is shown for MC-1% (red), G-0.5% (blue), E-1% (black), and E-2% (purple).

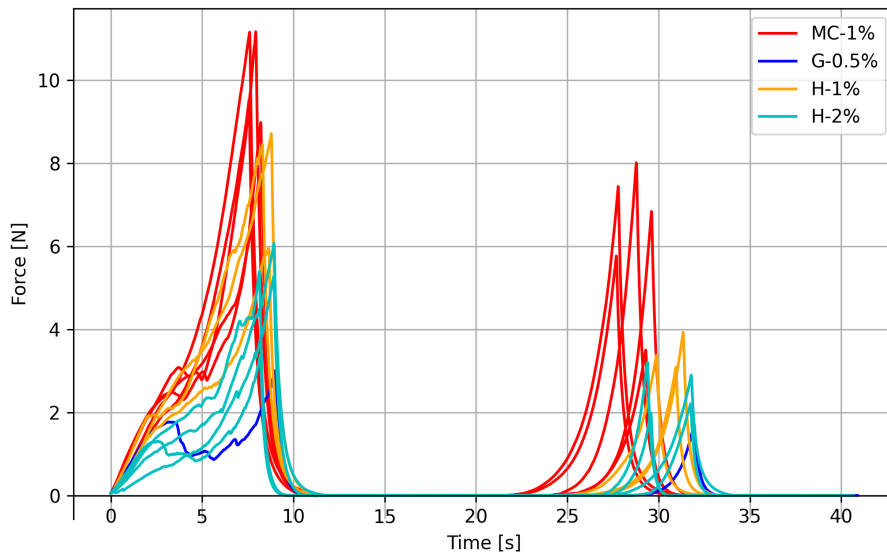


Figure A.3.14: Graph obtained from performing a TPA on the burgers from experiment 5. The force (in Newton) as a function of time (in seconds) is shown for MC-1% (red), G-0.5% (blue), H-1% (orange), and H-2% (light blue).

### A.3.5 Experiment 6

10 different burgers were made in experiment 5: C-0%, MC-1%, G-1%, M-1%, MC-G(0.25%-0.75%), MC-G(0.5%-0.5%), MC-G(0.75%-0.25%), MC-M(0.25%-0.75%), MC-M(0.5%-0.5%), and MC-M(0.75%-0.25%). All were pressed, rested in the cold room for 24 hours, dry-fried at 250 W, and cut into pieces for a texture analysis. An overview of the sample size for the shearing test and TPA of the burgers from experiment 6 is shown in Table A.3.3. A shearing test was performed on all the burgers (Figures A.3.15, A.3.16, and A.3.17). A TPA was also performed on all the burgers (Figures A.3.18, A.3.18, A.3.19, and A.3.20).

Table A.3.3: Overview of sample size for the shearing test and TPA of the burgers from experiment 6.

Sample	Shearing test	TPA
C-0%	4	5
MC-1%	4	4
G-1%	1	4
M-1%	2	3
MC-G(0.25%-0.75%)	6	5
MC-G(0.5%-0.5%)	2	4
MC-G(0.75%-0.25%)	6	6
MC-M(0.25%-0.75%)	3	6
MC-M(0.5%-0.5%)	1	6
MC-M(0.75%-0.25%)	4	5

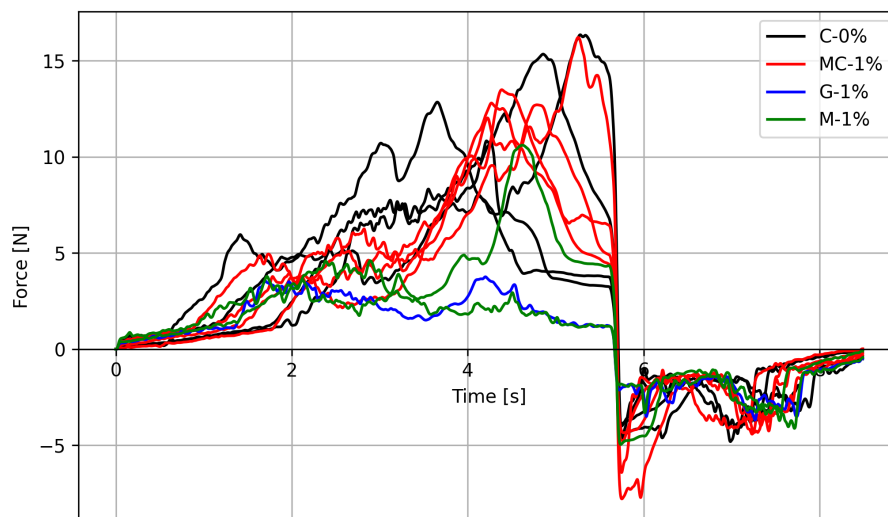


Figure A.3.15: Graph obtained from performing a shearing test on the burgers from experiment 6. The force (in Newton) as a function of time (in seconds) is shown for C-0% (black), MC-1% (red), G-1% (blue), and M-1% (green).



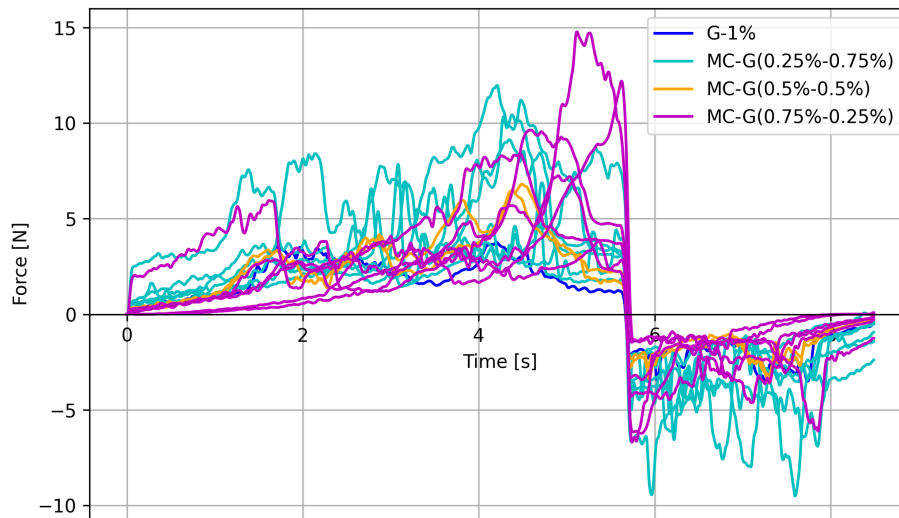


Figure A.3.16: Graph obtained from performing a shearing test on the burgers from experiment 6. The force (in Newton) as a function of time (in seconds) is shown for G-1% (blue), MC-G(0.25%-0.75%) (light blue), MC-G(0.5%-0.5%) (orange), and MC-G(0.75%-0.25%) (purple).

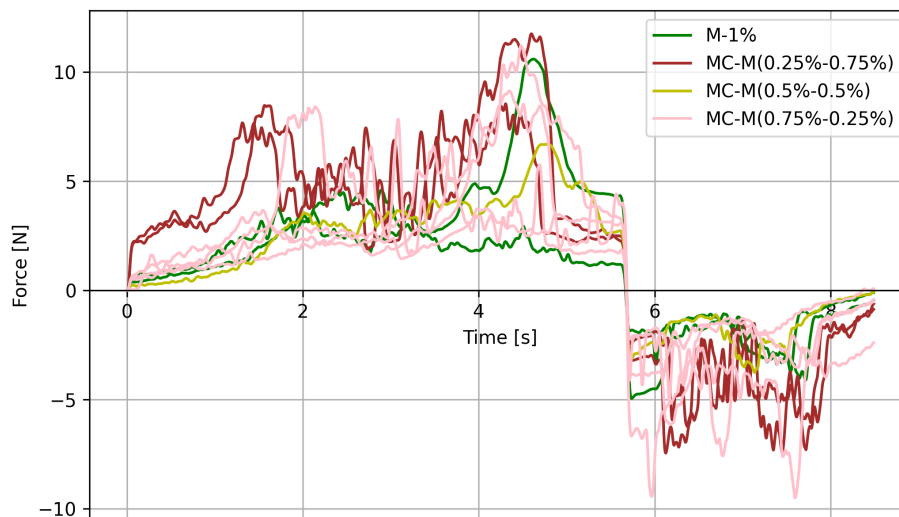


Figure A.3.17: Graph obtained from performing a shearing test on the burgers from experiment 6. The force (in Newton) as a function of time (in seconds) is shown for M-1% (green), MC-M(0.25%-0.75%) (brown), MC-M(0.5%-0.5%) (light green), and MC-M(0.75%-0.25%) (light pink).

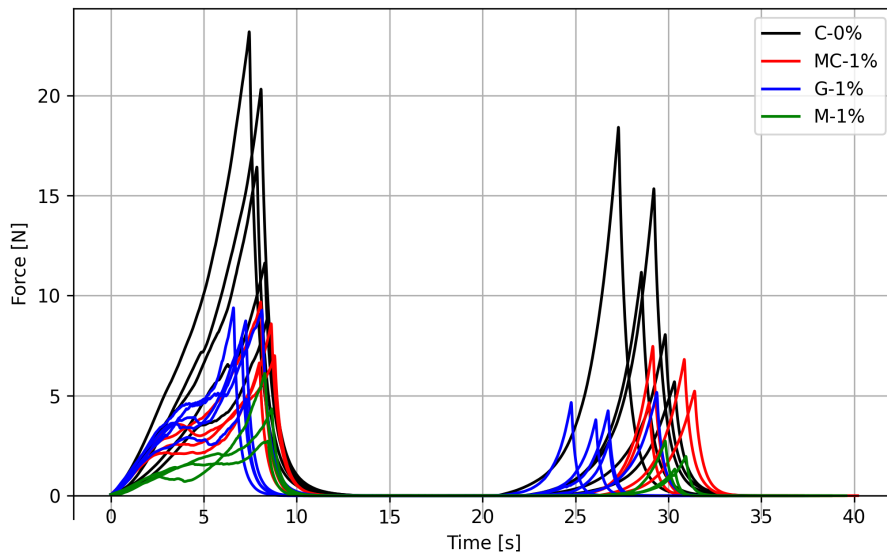


Figure A.3.18: Graph obtained from performing a TPA on the burgers from experiment 6. The force (in Newton) as a function of time (in seconds) is shown for C-0% (black), MC-1% (red), G-1% (blue), and M-1% (green).

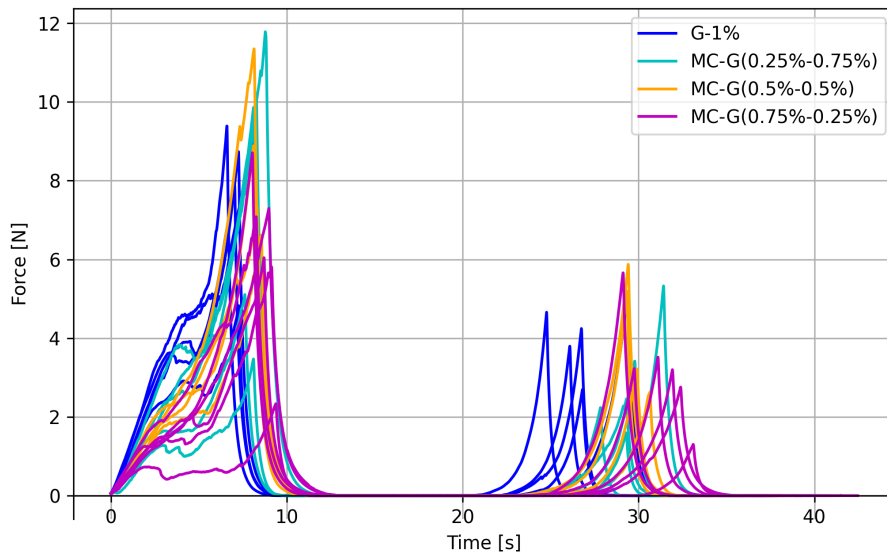


Figure A.3.19: Graph obtained from performing a TPA on the burgers from experiment 6. The force (in Newton) as a function of time (in seconds) is shown for G-1% (blue), MC-G(0.25%-0.75%) (light blue), MC-G(0.5%-0.5%) (orange), and MC-G(0.75%-0.25%) (purple).

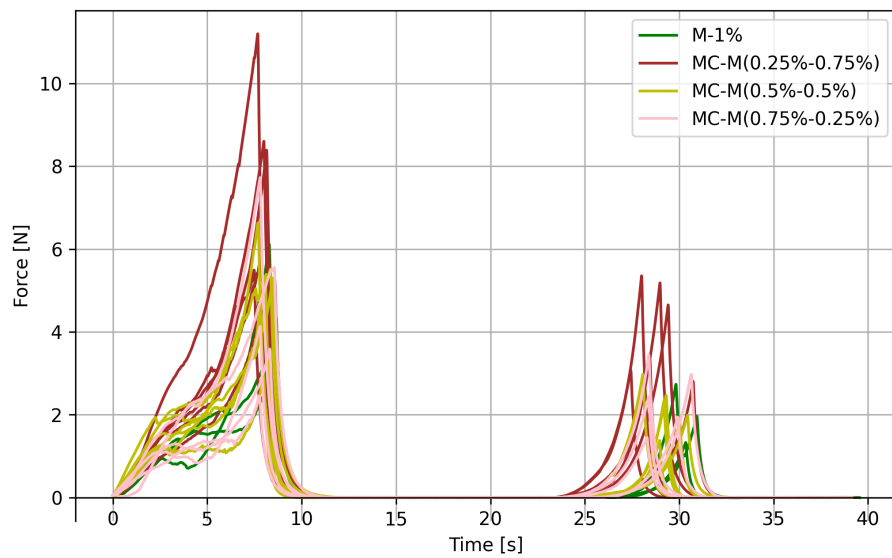


Figure A.3.20: Graph obtained from performing a TPA on the burgers from experiment 6. The force (in Newton) as a function of time (in seconds) is shown for M-1% (green), MC-M(0.25%-0.75%) (brown), MC-M(0.5%-0.5%) (light green), and MC-M(0.75%-0.25%) (light pink).



## Calculations

### B.1 Alginate slurry

The procedure to make an alginate slurry was given by a researcher, Kurt Ingar Draget, from the Department of Biotechnology and Food Science, at NTNU. To make an alginate slurry, tetrasodium pyrophosphate (TSPP) and  $\text{CaSO}_4$  were added to the dissolved sodium alginate. The amount of  $\text{CaSO}_4$  and TSPP were 20% and 4% of the alginate amount, respectively. As  $\text{CaSO}_4$  was only available in the form of  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$  at the laboratory, the amount had to be adjusted for  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ . The calculations for adjusting the amount of  $\text{CaSO}_4$  to are shown below.

$$m_{\text{CaSO}_4} = 0.20 * m_{\text{Alginate}} [\text{g}] \quad (\text{B.1.1})$$

where  $m_{\text{CaSO}_4}$  and  $m_{\text{Alginate}}$  are the mass of calcium sulphate and alginate, respectively.

$$n_{\text{CaSO}_4} = \frac{m_{\text{CaSO}_4}}{M_{\text{CaSO}_4}} [\text{mol}] \quad (\text{B.1.2})$$

where  $n_{\text{CaSO}_4}$  and  $M_{\text{CaSO}_4}$  are the number of mole and molar mass of calcium sulphate, respectively.

Assuming the amount of water in  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$  is negligible,

$$m_{\text{CaSO}_4 \cdot 2\text{H}_2\text{O}} = n_{\text{CaSO}_4} * M_{\text{CaSO}_4 \cdot 2\text{H}_2\text{O}} [\text{g}] \quad (\text{B.1.3})$$

where  $m_{\text{CaSO}_4 \cdot 2\text{H}_2\text{O}}$  and  $M_{\text{CaSO}_4 \cdot 2\text{H}_2\text{O}}$  are the mass and molar mass of calcium sulphate dihydrate, respectively.

## B.2 Statistical analysis

A statistical analysis was performed on all reliable results. The sections below show the unpaired t-test results on the proximate analysis values and the experiments 2, 3 and 4 of the texture analysis. A One-way ANOVA was performed on the results from experiments 5 and 6 of the texture analysis. Both the t-test and ANOVA are based on normally distributed data. Before the statistical analyses, all data were therefore checked to see if they followed a normal distribution by performing a Shapiro-Wilk test. Any outliers were eliminated. All statistical analyses used a 5% significance level and were performed with Minitab Statistical Software. No statistical analysis was performed on the results from experiment 1 of the texture analysis since the results were unreliable.

### B.2.1 Proximate analysis

The results from the proximate analysis were compared to the expected values for a 40 g raw burger. The t-test used the following hypothesis:

$$H_0 : \mu = \mu_0 \quad (\text{B.2.1})$$

$$H_1 : \mu \neq \mu_0 \quad (\text{B.2.2})$$

where  $\mu$  and  $\mu_0$  are the mean value and the mean expected value, respectively. The null hypothesis,  $H_0$ , states that both means are equal, while the alternative hypothesis,  $H_1$ , states that the means of the two groups are not equal. The resulting p-values are two-tailed. If the p-value is larger than 0.05,  $H_0$  is rejected at 5% level of significance. The p-values from the unpaired t-test are shown in Table B.2.1.

Table B.2.1: Resulting two-tailed p-values from performing an unpaired t-test on the proximate analysis results, at 5% level of significance. The mean values that are compared against each other are shown as  $\mu$  and  $\mu_0$ , where  $\mu$  and  $\mu_0$  are the mean value and the mean expected value, respectively. The statistical analysis was performed with Minitab Statistical Software. All data was tested with the Shapiro-Wilk test prior to the t-test. Any outliers were eliminated.

		Protein	Fat	Moisture
Expected value for raw burger	$\mu_0$	25.2	8.0	62.5
Raw C-0%	$\mu$	24.3 ± 0.4	8.90 ± 0.04	63.7 ± 0.4
	p-value	0.060	0.002 <sup>a</sup>	0.032 <sup>a</sup>
Raw MC-1%	$\mu$	24.08 ± 0.06	11 ± 1.5	61.8 ± 0.4
	p-value	0.022 <sup>a</sup>	0.069	0.068
Fried C-0%	$\mu$	26.5 ± 0.12	9.2 ± 0.7	60.53 ± 0.02
	p-value	0.041 <sup>a</sup>	0.101	0.005 <sup>a</sup>
Fried MC-1%	$\mu$	24 ± 1.0	9.4 ± 0.11	61 ± 1.5
	p-value	0.526	0.002 <sup>a</sup>	0.247

<sup>a</sup> p-value < 0.05,  $\mu$  is significantly different from  $\mu_0$  at 5% level of significance.

### B.2.2 Texture analysis

The results from the texture analysis were compared to each other. The t-test used the following hypothesis:

$$H_0 : \mu_1 = \mu_2 \quad (\text{B.2.3})$$

$$H_1 : \mu_1 \neq \mu_2 \quad (\text{B.2.4})$$

where  $\mu_1$  and  $\mu_2$  are the mean values for groups 1 and 2, respectively. The null hypothesis,  $H_0$ , states that both means are equal, while the alternative hypothesis,  $H_1$ , states that the means of the two groups are not equal. For p-values  $< 0.05$ ,  $H_0$  is rejected at 5% level of significance.

#### B.2.2.1 Experiment 2

In experiment 2, C-0%, MC-1%, and MC-2% are compared with each other. The p-values from the unpaired t-test are shown in Table B.2.2.

Table B.2.2: Resulting two-tailed p-values from performing an unpaired t-test on the results from the texture analysis of experiment 2, at 5% level of significance. The mean values that are compared against each other are shown as  $\mu_1$  and  $\mu_2$ , where  $\mu_1$  and  $\mu_2$  are the mean values for groups 1 and 2, respectively. The statistical analysis was performed with Minitab Statistical Software. All data was tested with the Shapiro-Wilk test before the t-test. Any outliers were eliminated.

$\mu_1$	$\mu_2$	Shear: Hardness	TPA: Hardness	Cohesiveness	Springiness	Chewiness
C-0%	MC-1%	0.285	0.002 <sup>a</sup>	0.050 <sup>a</sup>	0.003 <sup>a</sup>	0.004 <sup>a</sup>
C-0%	MC-2%	0.484	0.024 <sup>a</sup>	0.091	0.001 <sup>a</sup>	0.003 <sup>a</sup>
MC-1%	MC-2%	0.817	0.481	0.294	0.159	0.609

<sup>a</sup> p-value  $< 0.05$ ,  $\mu_1$  is significantly different from  $\mu_2$  at 5% level of significance.

#### B.2.2.2 Experiment 3

In experiment 3, C-0% fried at 250 W and C-0% fried at 500 W are compared with each other. The p-values from the unpaired t-test are shown in Table B.2.3.

Table B.2.3: Resulting two-tailed p-values from performing an unpaired t-test on the results from the texture analysis of experiment 3, at 5% level of significance. The mean values that are compared against each other are shown as  $\mu_1$  and  $\mu_2$ , where  $\mu_1$  and  $\mu_2$  the mean values for groups 1 and 2, respectively. The statistical analysis was performed with Minitab Statistical Software. All data was tested with the Shapiro-Wilk test prior to the t-test. Any outliers were eliminated.

$\mu_1$	$\mu_2$	Shear: Hardness	TPA: Hardness	Cohesiveness	Springiness	Chewiness
C-0% 250 W	C-0% 500 W	0.281	0.009 <sup>a</sup>	0.053	0.455	0.025 <sup>a</sup>

<sup>a</sup> p-value  $< 0.05$ ,  $\mu_1$  is significantly different from  $\mu_2$  at 5% level of significance.

**B.2.2.3 Experiment 4**

In experiment 4, C-0% fried at 250 W and C-0% fried at 500 W are compared with each other. The p-values from the unpaired t-test are shown in Table B.2.4. The data from the shearing test performed on G-1% was not normally distributed according to the Shapiro-Wilk test. A t-test was therefore not performed on the data.

Table B.2.4: Resulting two-tailed p-values from performing an unpaired t-test on the results from the texture analysis of experiment 4, at 5% level of significance. The mean values that are compared against each other are shown as  $\mu_1$  and  $\mu_2$ , where  $\mu_1$  and  $\mu_2$  are the mean values for groups 1 and 2, respectively. The statistical analysis was performed with Minitab Statistical Software. All data was tested with the Shapiro-Wilk test prior to the t-test. Any outliers were eliminated. The data from the shearing test performed on G-1% was not normally distributed according to the Shapiro-Wilk test. The t-test analysis was therefore not performed on these results.

$\mu_1$	$\mu_2$	Shear: Hardness	TPA: Hardness	Cohesiveness	Springiness	Chewiness
C-0%	MC-1%	0.030 <sup>a</sup>	0.032 <sup>a</sup>	0.005 <sup>a</sup>	0.002 <sup>a</sup>	0.002 <sup>a</sup>
C-0%	G-1%	- <sup>b</sup>	0.0010 <sup>a</sup>	0.000 <sup>ac</sup>	0.000 <sup>ac</sup>	0.000 <sup>ac</sup>
C-0%	M-1%	0.000 <sup>ac</sup>	0.003 <sup>a</sup>	0.000 <sup>ac</sup>	0.000 <sup>ac</sup>	0.000 <sup>ac</sup>
MC-1%	G-1%	- <sup>b</sup>	0.910	0.125	0.021 <sup>a</sup>	0.273
MC-1%	M-1%	0.016 <sup>a</sup>	0.614	0.284	0.002 <sup>a</sup>	0.306
G-1%	M-1%	- <sup>b</sup>	0.430	0.225	0.057	0.782

<sup>a</sup> p-value < 0.05,  $\mu_1$  is significantly different from  $\mu_2$  at 5% level of significance.

<sup>b</sup> The data of G-1% was not normal according to the Shapiro-Wilk test.

<sup>c</sup> The p-value is very small (<0.0005)





