LUND UNIVERSITY

Effects of Microwave and Enzyme Treatments on the Softness of Barley Kernels

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Kan man med mikrovågor och enzymer göra kornkärnor mindre hårda ?

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ABSTRACT

The present master thesis was performed in cooperation with the company “Aventure AB”. The company’s R&D department envisioned a new yoghurt-based product able to decrease the blood glucose levels after a meal. The innovative characteristic of the product is due to the diet fibers of the barley grain, which is going to be incorporated inside the yoghurt.

The preliminary experiments which were carried by the Aventure R & D team were very promising as they revealed that the decrease of the blood glucose levels could last up to 10 to 12 hours from the consumption of the product. The most important problem with barley, is that its kernels are very hard and thus, if not softened, they cannot be used in food formulation.

The aim of this present study was to determine if microwave or enzymatic treatments (xylanase, cellulase) could have any effect on the barley kernel’s hardness. The results were promising where microwave heating was related. Unfortunately, the present study is unable to claim the same about the enzymatic treatment.

The better results were obtained by soaking the barley kernels for 2 minutes in distilled water and then treating them with microwave radiation for 1 minute followed by 4 hours of drying in the convection oven (2m/s velocity of air and 50^{o}C air temperature). Acceptable results were also obtained after 8 hours of drying. The hardness of the kernels was evaluated using the texture analyzer in Lund University. Additionally, microwave treatment resulted in a very pleasant roasted taste, after tasting them, which is desirable in food products.

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1. Introduction

The cost of treating diabetes is increasing every year and at the same time is a huge public health problem. The pathological process of diabetes is the autoimmune destruction of the pancreatic β-cells resulting in insulin deficiency. Diabetes is defined as the metabolic syndrome characterized by a disorder of carbohydrates, proteins and fats metabolism due to complete or partial deficiency of insulin or some disorder of its biological activity. The main characteristic of diabetes is the increased levels of blood glucose. The main consequence is an increased risk of developing neuropathic and vascular complications. Diabetes therefore, is considered as a chronic disease that has serious implications for both the sufferers and their families with enormous social and economic impact (Kassahun, et al. 2016).

It is important to educate the population on the prevention, management and control of diabetes, with the aim of both delaying complications and improving the cost of the disease. Data from the World Health Organization show that its prevalence reached 7.04%, 2014 (WHO, 2016). That is why companies in the food industry, have focused on producing products which prevent high blood sugar spikes.

The present master thesis was performed in cooperation with the company “Aventure AB”. In the frame of the development of a new yoghurt-based product, the company’s goal was to prevent the blood sugar spikes after a meal. The first experiments conducted by the Aventure R & D team, were very promising as they reveal that this effect could last up to 10 to 12 hours from the consumption of the product. The yoghurt is already developed, and the next step was to incorporate muesli to give taste and to take advantage of the fibers present in the cereals that are responsible for the prebiotic effect, which is the result of the dietary fibers inside the muesli. The fibers are not absorbed or hydrolyzed by the stomach or the small intestine and reach the colon where they stimulate the growth of health beneficial bacteria.

The problem with barley, is that its kernels are very hard and thus, if not softened, they cannot be used in food formulation. Therefore, the objective of the present master thesis was to test two different methods, microwave and enzyme treatments, in order to soften barley kernel, but also to preserve barley’s fibers. So far, some processes are tested without the desired results.

2. Background

To be able to understand the problems and find a solution, the botanical structure of barley kernels needs to be firstly understood. Furthermore, it is essential to understand and describe the hydrothermal effects on starch, because they play a key role in softening the barley kernels. In conclusion, the different treatment methods, will be described, in order to introduce the hypothesis of the present study as of which properties of the barley kernel were altered after those particular methods.

2.1 Barley in Food Industry

As far as barley applications in the food industry are concerned, barley grains can be further processed into grains, flakes and flour (Chatterjee et al 1977). In most western countries, barley can be found in breakfast cereals, flat bread and porridge (Bhatty, 1993). Trogh et al. (2005) managed to produce acceptable quality bread with an increased content of soluble fiber (70% wheat and 30% barley flour) using endo-xylanase as the improver. Compared to bread baked only from wheat flour, the bread from the wheat flour and barley mix was similar to the bread volume and maintained softness for baking for a longer period of time. Ereifej et al. (2006) found in their research that wheat based bread with 15-30% barley flour was acceptable, but it became harder and darker, properties attributed to the incorporation of barley into bread.

During the last decades, the focus of research has been directed towards recognizing the biologically active components of foods that have the potential both to improve physical and mental wellbeing and which may reduce the possibility of developing a disease (Baik et al 2016).

Barley (*Hordeum vulgare*) grain based food products are recently in the spotlight for their health benefits by all participants in the food supply chain. Ames and Rhymer (2008), highlighted the protective effect of the barley regarding cardiovascular diseases. Barley grain is an important source of various bioactive phytochemicals, minerals and tocols which are responsible for inducing low glycemic response and cholesterol and/or high antioxidant activity (Baik et al 2016). In addition, whole grain barley is also rich in dietary fiber, vitamins which make it an ideal ingredient for aiding in the effective control of the abovementioned health issues (Baik et al 2016).

The prebiotic effect of barley fibers is crucial for the colonic health as β-glucans are degraded and form mainly butyric acid which is very important for the cells of the mucosa (Wang, 2009). Prebiotics in comparison with probiotics are not living organisms, but substances that in a way feed on the probiotics and enhance their concentration. Prebiotic or prebiotic fibers are fructo-oligosaccharides and inulin. Prebiotics also include lactic oligosaccharides (Laurell, 2017).

Barley is very rich in β-glucans which makes it a perfect source of prebiotics. A recent study in rats has proven that the use of malt together with Lactobacilus rhamnosus increased the levels of butyric acid in the colon (Y.Zhong et al., 2014). Moreover, another study by Mattia P. Arrena et al., in 2014 showed that β-glucans coming from barley were able to stimulate the growth of tested probiotic bacteria and especially Lactobacillus rhamnosus. Another important result of this study was that besides from stimulating the growth of probiotic strains β-glucans also helped the bacteria resisting the stress from the harsh conditions (acid, bile salts etc.) that bacteria meet when passing through the gastrointestinal tract (M.P. Arrena et al., 2014). Additionally dietary fibers found in barley as well as in other cereal grains largely contribute to improving the proportion of beneficial bacteria in the gut but also to the lowering of plasma cholesterol (P.Cominno et al., 2016). Finally these dietary fibers are proven to have a positive effect on the relief of constipation and to prevent colorectal cancer (A.Lazaridou et al., 2007).

It is widely accepted, as it was noticed above, that the nutrition value of β-glucans as dietary fiber , is based on the fact that they are indigestible in the small intestine but are completely or partially fermented by the colonic microflora, producing low molecular weight fatty acids, such as propionic and butyric acid, elements which can guarantee colon's healthy function, because they are an important source of energy for the cells in the intestinal epithelium (Halifax et al., 2003). In recent studies, indications of pre-bionic activity of β-glycans have been found and even selective for some lactobacilli species. It is known that diets rich in dietary fiber, such as beta-glucans, may have protective action against colorectal cancer. Diets which are known to have incorporate beta-glucans are known for their ability to reduce the proportion of bile salt derivatives, which are precursors to carcinogenesis. In particular, it is believed that the "hydrated" dietary fibers that have not biodegraded "dilute" bile salts and thus they forbid them any interaction with the intestinal epithelium (Izydorczyk, 2008).

The increased interest in the use of β-glucan in the food supply chain is not only linked to their beneficial nutritional properties but also to the optimization of the processing of these diet products. One of the greatest examples regarding the use of beta-glucans is the one in dairy products. Recent studies have focused on the use of soluble dietary fiber, especially beta-glucans, in the production of low-fat and yoghurts. The incorporation of β-glucans together with other soluble dietary fiber into dairy products can alter mouthfeel and organoleptic properties to resemble those of fat-full products (Brennan & Cleary, 2005). Similarly, incorporation of β-glycans in

cheese curds with low fat content showed beneficial effects on gel formation and rheological properties (Tudorica et al., 2004). These effects appear to be related to the β-glucan gel formation capacity and their ability to form a sufficiently flexible casein-protein-glycan matrix. A very interesting study is the one by Tudorica et al. (2004) who investigated the possible use of barley-to-cheese β-glucan, and in particular how it affects milk coagulation, yield, texture and structure of the product. The results show that rising β-glucan levels significantly reduce the coagulation time.

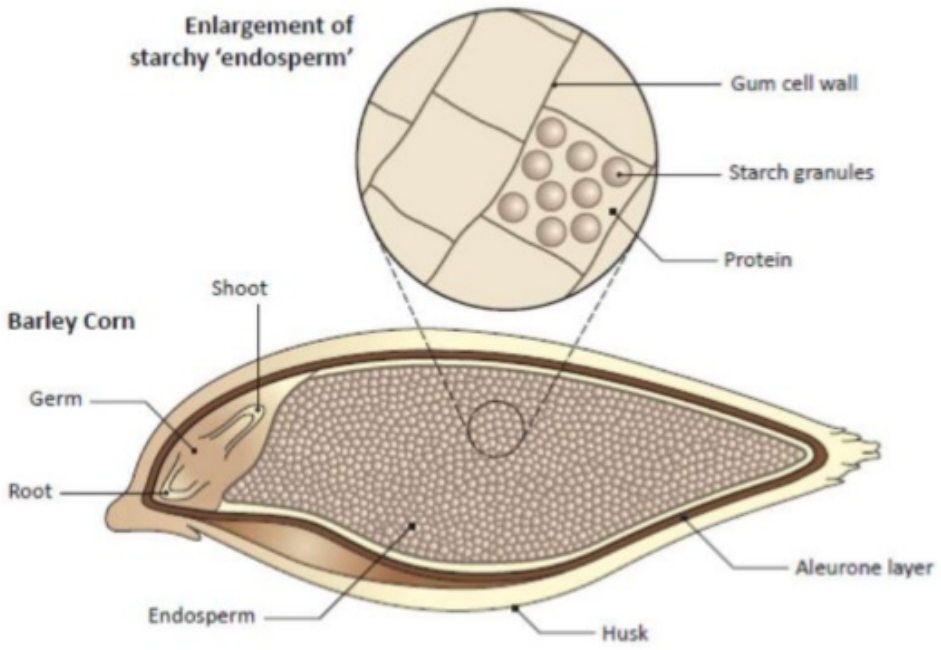
The authors also noted that the addition of β-glucan reduces the loss of soluble protein (the loss of protein during the serum separation phase leads to a reduction in the yield of the curd and to weak agglomerates formed during the addition of rennet.) Additionally, it was highlighted that increasing levels of β-glucans will be responsible for the increased pore size and the formation of a more open protein net. Such a matrix appears to trap a larger amount of serum as the amount of β-glucan increases (Thiaboma et al., 2004). Lee (1997) stated that soluble β-glucan content was greater for oat than barley.

2.2 Barley's Botanical Structure

The barley kernel is comprised of the lemma and palea, the caryopsis, and the rachilla.

The endosperm is consisted of starch granules within dead cells (picture 1). These structures (lemma and the palea) will eventually consist the hulls of the mature kernel. There can be observed four types of cells inside lemma and palea (i) elongated epidermal cells, (ii) blast cells, (iii) a cell layer of parenchyma tissue, and (iv) an inner epidermis cell layer (Newman 2008).

The caryopsis can be divided in five parts the pericarp, the seed coat, the epidermis nucellus, the endosperm, and the embryo . Inside the pericarp, the epicarp, the hypodermic cells, the cross cells, and the tube cells, can be found (Newman 2008). The endosperm, which is also the largest section of the kernel is consisted of starch granules within dead cells. These dead cells are covered by cell walls that are consisted of 70% β-glucans and 30% arabinoxylan, the first being very important for the prebiotic effect of the product. In the literature there are numerous studies that prove that consumption of β-glucans lowers the postprandial glucose response and insulin improves insulin resistance (Brockman et al 2013).



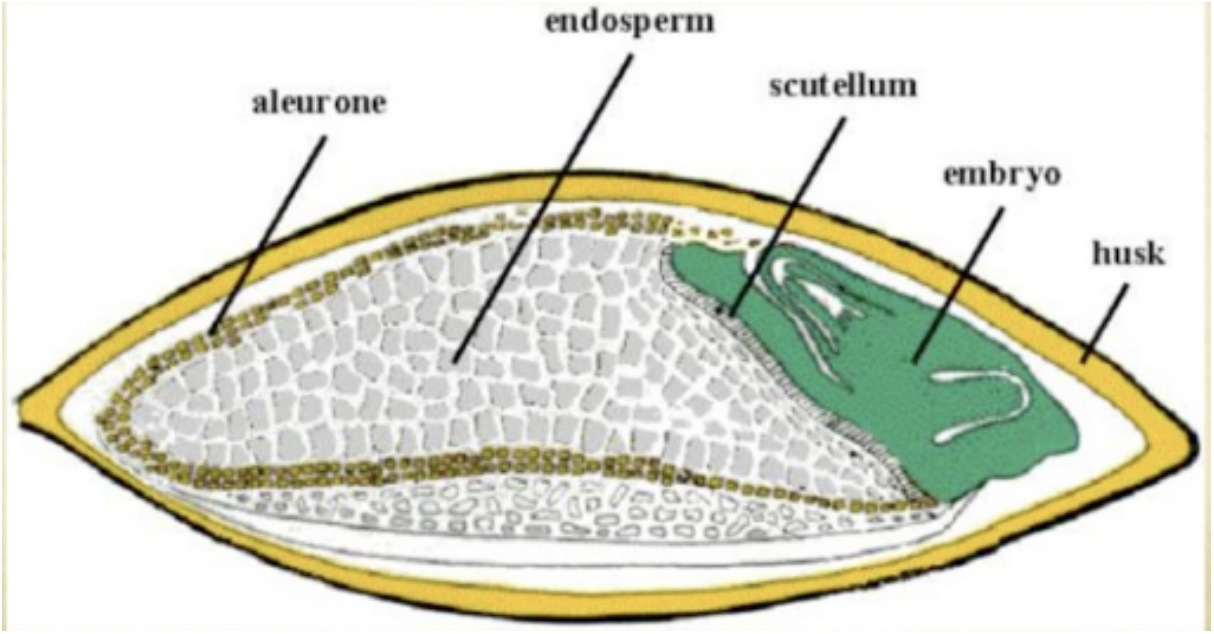
Picture 1: *Malted barley grain (source: Crisp Malting group accessed on 1st Feb.2020)*

The endosperm (picture 1) account for 85% of the fruit, and therefore the composition of all dietary fiber is affected by it. Since the whole grain varieties is removed at harvest, the fibers isolated from the corresponding varieties are quantitatively less and have a different composition (less cellulose and arabinoxylans but more β-glucans). Indicatively, hull-less varieties have 19-26% less dietary fiber, namely 40-50% less cellulose, 46-55% less lignin, and 30% -50% less arabinoxylans than whole grain varieties. In contrast, the β-glucan content is 15-18% more because the removal of the hull reduces cellulose and arabinoxylans but not β-glucans (Musatto et al 2006).

The embryo, which is the most complex part of the kernel, can be found on the dorsal side of the caryopsis (picture 2). The germ consists of the embryonic axis, the plumule, and the radicle, which are characterized to have protective properties for these tissues. A bud primordial is also located within the coleoptile. The scutellum is

a flat protective tissue positioned between the embryo and the endosperm (Briggs 1978).

B-glucans and arabinoxylans are the main ingredients of soluble fiber in barley. In particular for β-glucans, their structure studies have focused on the molecular weight distribution and enzyme treatment products. The amylose: amylopectin ratio in barley starch, protein content of the embryo, β-glucan activity, and environmental factors have a significant effect on the amount of β-glucans (Fastnaught et al 2009).



Picture 2. *Barley grain morphology and anatomy (source: www.researchgate.net accessed on 5th May 2019).*

2.3 Hydrothermal Effects on the Starch

The barley grain is usually subjected to a variety of hydrothermal treatments (of crystallizing from high-temperature solutions at high vapor pressures) in order to inactivate hydrolytic enzymes, which are responsible for many of the quality failures on a new developed product. Hydrothermal treatments quite often alter some of the functional properties of barley starch (Bertolini et al 2009).

Starch is a material that is abundant in nature, so it is a low-cost material, and is completely degradable in soil and water. However, natural starch has limitations in its applications, because it has disadvantages such as: loss of viscosity at low pH, its hydrophilic nature, difficult processing and brittleness. Starch is sometimes modified in order to obtain desired properties. It is also plasticized and mixed with other materials (Bertolini et al 2009).

Starch is the basic element of the barley kernel and its percentage exceeds 70% of its dry weight. The final quality of barley-containing products is greatly influenced by starch properties such as gelatinization and retrogradation.

Starch is gelatinized by heating combined with high water content, which are its degradation agents. When the starch is heated in water, with the energy provided, hydrogen bonding occurs between crystalline micelles. The grain begins to hydrate and swell, and eventually bursts. Amylose in hot water forms a colloidal dispersion (which helps to create gel) and viscosity is increased. In contrast, amylopectin is insoluble (does not readily form gels) (Eliasson 2014).

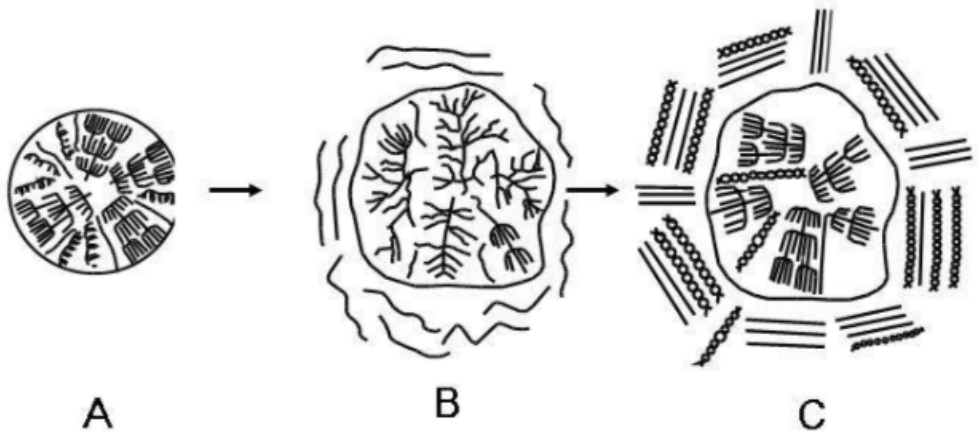
Starch due to hydrogen bonds is insoluble in cold water, while in hot water it swells to form a thick paste (gelatinization occurs). Gelatinization is defined as the expansion of starch granules with water when heated above a critical temperature. This occurs in a temperature range, which is affected by many factors. Peak gelatinization temperature of 14 hull less genotypes ranged from 55.6 to 61.8°C. In comparison with maize starch, barley starch has lower gelatinization temperature (Zhu 2017).

The gelatinization process causes significant changes in both the chemical nature and the physical form of the starch granules due to the rearrangement of the intramolecular and intermolecular hydrogen bonds between the water and the starch resulting in the breakdown of the molecular structures within the starch granules. This leads to irreversible changes in starch properties, that is, degradation of the starch. Thus, most of the hydrogen bonds are destroyed and a decrease in the melting temperature (Tm) and the glass transition temperature (Tg) is observed. The process of gelatinization depends on many factors. The temperature at which

gelatinization begins and its changes during the course are dependent on the variety of starch (Karlsson et al 2007).

A heat-treated starch when cooled may, with a high cooling rate, form a gel, while with slow cooling its linear components may form a spherical crystal precipitate. The phenomenon of starch digestion to transform the starch chains into crystalline form is called retrogradation. The degree of retrogradation depends on the type of starch, the amylose content, the size of the linear molecules as well as on the conditions for processing and preserving the material. Retrogradation of amylose and amylopectin differs significantly. Retrogradation of amylose is completed within 48 hours, and of amylopectin can be continued for weeks (Karlsson et al 2007).

Annealing is happening by treating the starch in excess water or an intermediate water content (40-50% w / w) and heating it below the gel initiation temperature. The aim of annealing is to approach the glass transition temperature, which enhances molecular mobility without initiating gelatinization. Shi et al., (2008) in their study showed that the annealing process increased the peak gelatinization temperature from 58.1 up to 71.4°C.



Picture 3. *Gelatinization and retrogradation of starch. A : native starch, B Gelatinized starch, C : Retrrogradated starch (Source :http://www.food-info.net/uk/carbs/starch.htm., Accessed on 11/05/2019)*

Native barley kernels are very hard due to the composition of the endosperm that contains high amounts of β-glucans and arabinoxylans as discussed in the introduction. There is a positive correlation between the amount of these compounds and the hardness of the kernels (J.Gamlath et al., 2007). The barley used was malted which means that there has been an incomplete germination and breakup of these molecules, but the barley was still too hard for food applications.

2.4 Treatment Techniques

2.4.1 Microwave treatment

Microwave energy is used in foods primarily for its heating properties. Microwaves are electromagnetic waves and differ from electromagnetic radioactivity of light waves and radio waves only in frequency. The frequency of microwaves is between radio waves and infrared radiation, with wavelengths ranging from 25 million to 0.75 billion nanometers, i.e. 0.025-0.75m. Microwave wavelengths range from 0.025 to 0.75m corresponding to the frequency of 20000-400MHz (lHz = lcyc / sec). Since the frequency of microwaves is relatively close to the frequencies of radio waves and radar, microwaves can interfere with communications, which is why only specific microwave frequencies are used.

Microwave heating uses electromagnetic radiation with a frequency of between 3000 and 30000 MHz. For domestic use, the frequency of 2450 MHz has been allocated, corresponding to a wavelength of 1224 cm. In contrast, for industrial use in Europe the frequency is 895 MHz and in the US the frequency is 915 MHz.

Microwaves, such as light, move in a straight line and are reflected by metals, as well as by some types of glass, paper and plastic materials, while being absorbed by various food ingredients including water. Most foods vary in their ingredients and the physical distribution of their ingredients, resulting in different microwave heating conditions (Scott 1993).

Other heat treatment techniques were also used in studies on barley and other cereals such as wheat in order to evaluate changes in the physicochemical properties. In a recent study in 2015 the water retention capacity of barley was evaluated after being heat treated for different times in a forced air oven. The conclusion was that heat treated barley fibers had higher water retention capacity than untreated barley (R.Caprita et al., 2015). This is a very important result as water retention capacity has various positive effects on the physiological function pf the upper and lower intestine (R.M Kay et al., 1982). Furthermore, as barley is used primarily for food applications that include baking, fermentation and various other processes it is important that fibers are preserved. According to a recent study arabinoxylan and β-glucan amounts were not affected by the above processes even with baking at 200°C (P.Cominno et al., 2016).

The use of microwave for food processing and research has been introduced during the 1970s (M.Koubaa et al., 2016). Microwave ovens use electromagnetic radiation in the range of microwaves. In it has been used for various applications among which are drying, pasteurization, tempering and sterilization as well as softening (M.Zhang et al., 2006). In all these applications the concept is to create heat in the food (G.

Spigno, 2016). This can be possible by converting the electromagnetic energy into heat by dielectric heating (G. Spigno, 2016).

The use of microwave radiation has several benefits which are that we need lower processing times and we observe higher nutrient retention according to recent studies (Krokida et al., 2000). On the other hand, microwave treatment has also its disadvantages. One drawback is that it is very difficult to predict and control the temperature inside the food product. Additionally, the limit that microwaves can penetrate in the material is low and thus, the heating is not uniform (Li-Zhen Deng et al., 2017). The heating time in these studies varied from 60s to 110s and that is why similar times where chosen for experiments on the barley kernels. According to another study microwave treatment gave promising results on softening palm fruits for similar treatment times (M.Chin Chow., 2005).

The purpose of this study, as mentioned before, is to investigate the effect of microwave and enzyme treatment on softening of barley kernels in order that in the future barley kernels could be used as a supplement for a yoghurt that is intended to lower the blood glucose after each meal due to the fibers in the kernels that have a prebiotic effect. Specifically, in order for the finished product to be pleasant and commercially acceptable the barley kernel must be softened as even the malted barley kernels are too hard for human consumption. However recent studies have shown that microwave treatment, no matter the intensity and time, increased starch digestibility and lowered the levels of resistant and slowly digestible starch in three different barley types (S.Emami et al., 2012). In addition, cereals tend to have high amounts of rapidly digestible starch which can lead to type 2 diabetes and metabolic syndrome (Åkerberg et al., 1998).

López-Perea (2008) at their study proved that not malted barley kernels showed higher kernel hardness than the malting barleys tested in microwave irradiation for 4 sec Microwave irradiation for 4 sec. improved the quality of the feed and malting barleys, which reached the values specified by the brewing industry.

2.4.2 Enzymatic treatment

Enzyme treatment involves the enzymatic modification of dietary fiber as well as the use of enzymes to increase the content of dietary fiber or to prepare dietary fiber concentrates. Enzyme modification of dietary fiber uses enzymes (mainly endoxylanases) that hydrolyze cell wall of polysaccharides. In general, enzyme treatments lead to a small reduction in total dietary fiber in combination with changes in insoluble fiber, as well as a change in molecular weight distribution. Enzymatic treatment of dietary fiber can improve its functionality, by changing the hydration and viscosity properties and the organoleptic properties of the foods to which they are added (Arrigoni et al 2001).

The enzymes used in this study were xylanase and cellulase. Lamsal et al., (2008) used also these enzymes in order to determine the physical and milling characteristics of wheat kernels. The optimum temperature for the xylanase was 60°C and for the cellulase, it was 40°C.

2.5 Hypothesis and Aim

The present study's hypothesis is based on two levels. Firstly, on the hydrolytic properties of xylanase, which are majorly effective when hydrolyzing the glucosodic linkages in the backbone of arabinoxylans between xylopyranosyl units. Secondly, on the disruptive behavior of cellulase on the crystalline structure of cellulose. Therefore, there is a possibility of water transportation inside the endosperm, because of the microchannels, which are created either because of the enzymes or by the microwaves in the non-starch polysaccharides of barley

The aims of the present study are to:

- Examine the effect of microwave treatment on barley's kernels hardness
- Examine the effect of enzymatic treatment on barley's kernels hardness

3. Experiments Overview

Below we demonstrate the resume of all methods and techniques which were used during the experimental process

Microwaves					
Materials	Soaking Method	Microwave Treatments (600 Watt)	Drying Process	Texture Analyses	Statistical Analysis
10 grams Malted barley	2 minutes in water at Room Temperature	30 sec 1 min 1,5 min 2 min	(1) Drying for 4h with 50°C and 2m/s air velocity (2) Drying for 8h with 50°C and 2m/s air velocity	(1) TA-TXT2 texture analyser before drying (2) TA-TXT2 texture analyser immediately after drying (3) TA-TXT2 texture analyser 7 days after drying	ANOVA one way

Enzymes					
Materials	Enzymes Treatments	Soaking Method	Drying Process	Texture Analyses	Statistical Analysis
20 grams Malted barley	2,5 gr of cellulase and xylanase	Water bath at 50˚C for 12h hours	(1) Drying for 4h with 50°C and 2m/s air velocity (2) Drying for 8h with 50°C and 2m/s air velocity	(1) TA-TXT2 texture analyser immediately after drying (2) TA-TXT2 texture analyser 7 days after drying	ANOVA one way

4. Materials and Methods

4.1 Materials

Malted barley kernels were received in 1kg bags (picture 4) from the company Viking Malt in Halmstad, Sweden. The specific product received was Viking Dextrin Malt with product number 8452 and was produced on the 11 of October 2018. This product was used for all treatments and the untreated barley was used for reference. Also the enzymes used in this study were cellulase and xylanase.

Picture 4*: Malted barley used for the experiments in this thesis.*

4.2 Treatments

4.2.1 Microwave Treatment

The microwave treatment was performed in a Whirlpool microwave oven at 600W. At first 10gr of barley kernels are weighted and soaked in 250ml of water at a temperature of 20˚C for 2 minutes and then were filtered. After that the kernels were treated with the microwave stated above for 30 seconds,1 minute 1,5 minutes and 2 minutes. Immediately after the microwave treatment, the kernels where transferred to the convection oven in the pilot plant of the Food Technology department in Lund University for drying and then the moisture content was measured. Below a figure is showing an overview of the procedure (figure 1). The texture was measured both before and after drying.

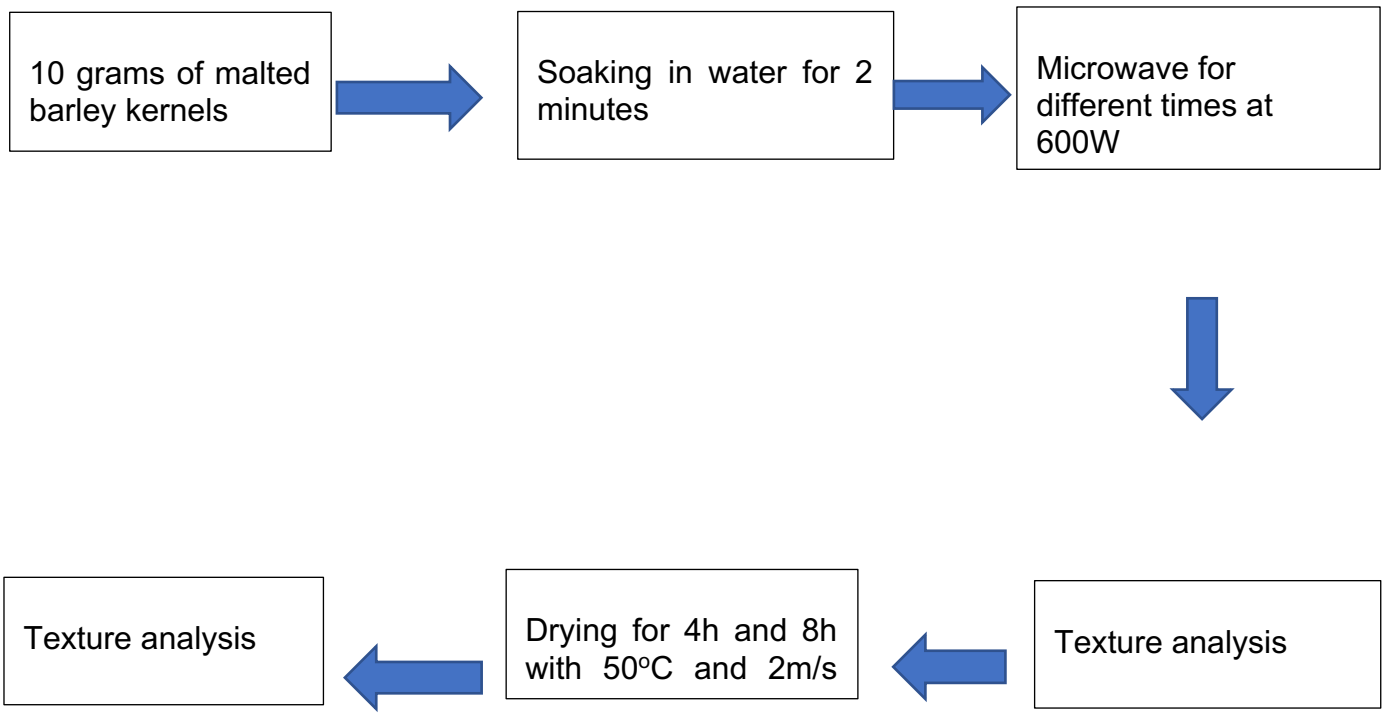


Figure 1*: Microwave treatment.*

4.2.2 Enzyme Treatment (cellulase and xylanase)

The enzymes used were cellulase and xylanase used together in a homogenized mixture. The cellulase was cellulase from *Aspergillus niger* bought from Sigma-Alldrich company in 2,5 gr bottles (product number C1184). The xylanase was xylanase from *Trichoderma viride* bought also from Sigma-Alldrich company in 5gr bottles (product number X3876). At first 2,5 gr of each enzyme were weighted and transferred in an Erlenmeyer flask along with 250ml of distilled water heated to 50˚C. Then the mixture is homogenized using a magnetic stirrer for 30 minutes. After that 50gr of barley kernels are weighted and added to the mixture and transferred to a water bath at a temperature of 50˚C. The temperature of 50˚C was chosen because it is the temperature between the optimal temperature of cellulase and xylanase which are 40˚C and 60˚C respectively. The duration of the experiment was 12 hours and finally the kernels were transferred to the texture analyser. Below figure 2 is showing an overview of the procedure.

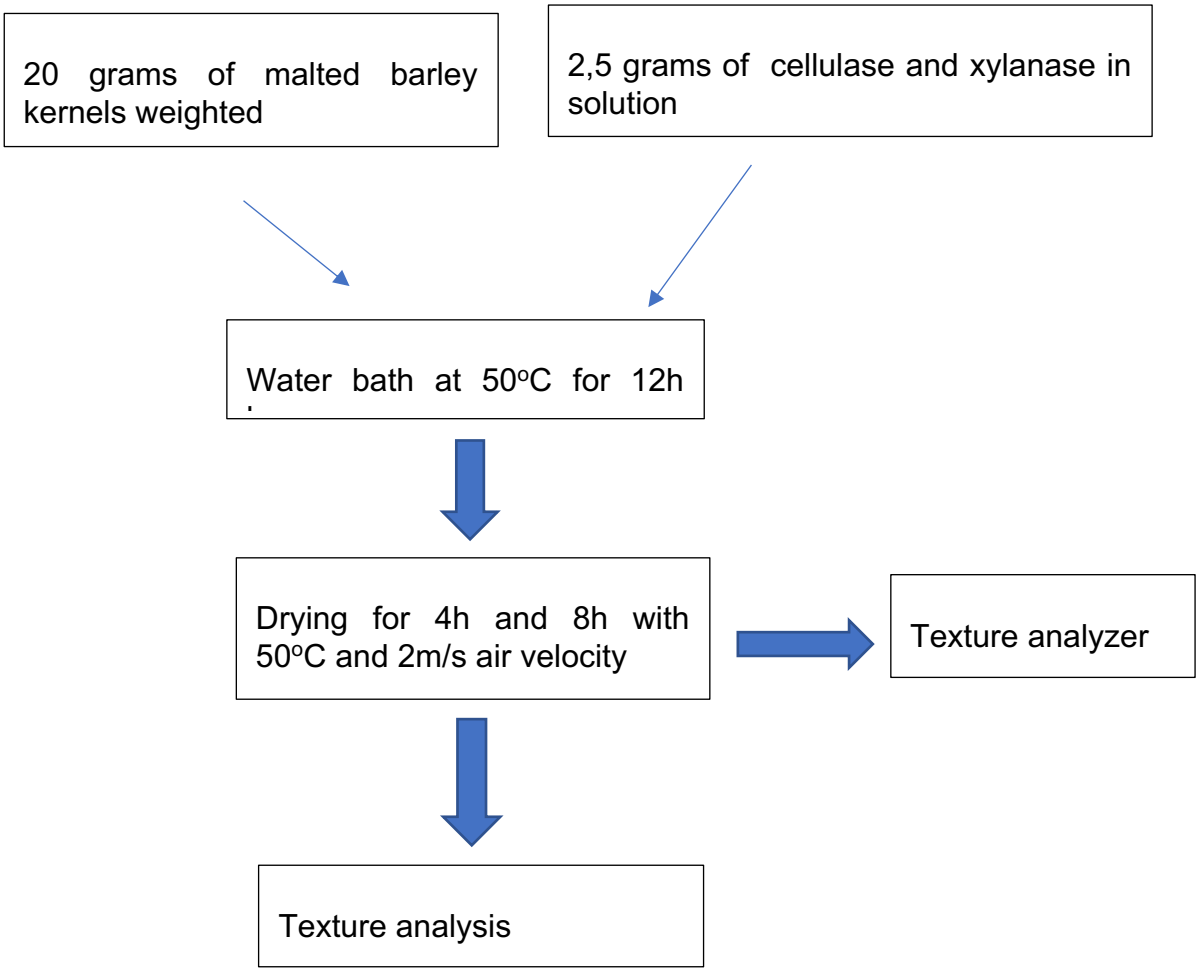


Figure 2:*Enzyme treatment.*

4.3 Texture analysis

The texture analysis was done by using the TA-TXT2 texture analyser manufactured by Stable Microsystems company in Surrey, UK (picture 6, p.23). The texture analysis was performed immediately after drying and after one week of each treatment. The probe that was used for the texture analysis was developed in Lund University especially for measuring cereal kernels. The instrument was firstly calibrated for height and force according to the instructions. Each time kernels with similar size were chosen and putted in the probe with the suture facing the back of the probe. During each repetition 3 kernels where measured and the instrument was giving the average value of the 3. Each compression was performed 3 times. The positive force that is required to reach a strain of 20% and the gradient of the force was measured each time. The force was measured in grams and the gradient in grams per second.

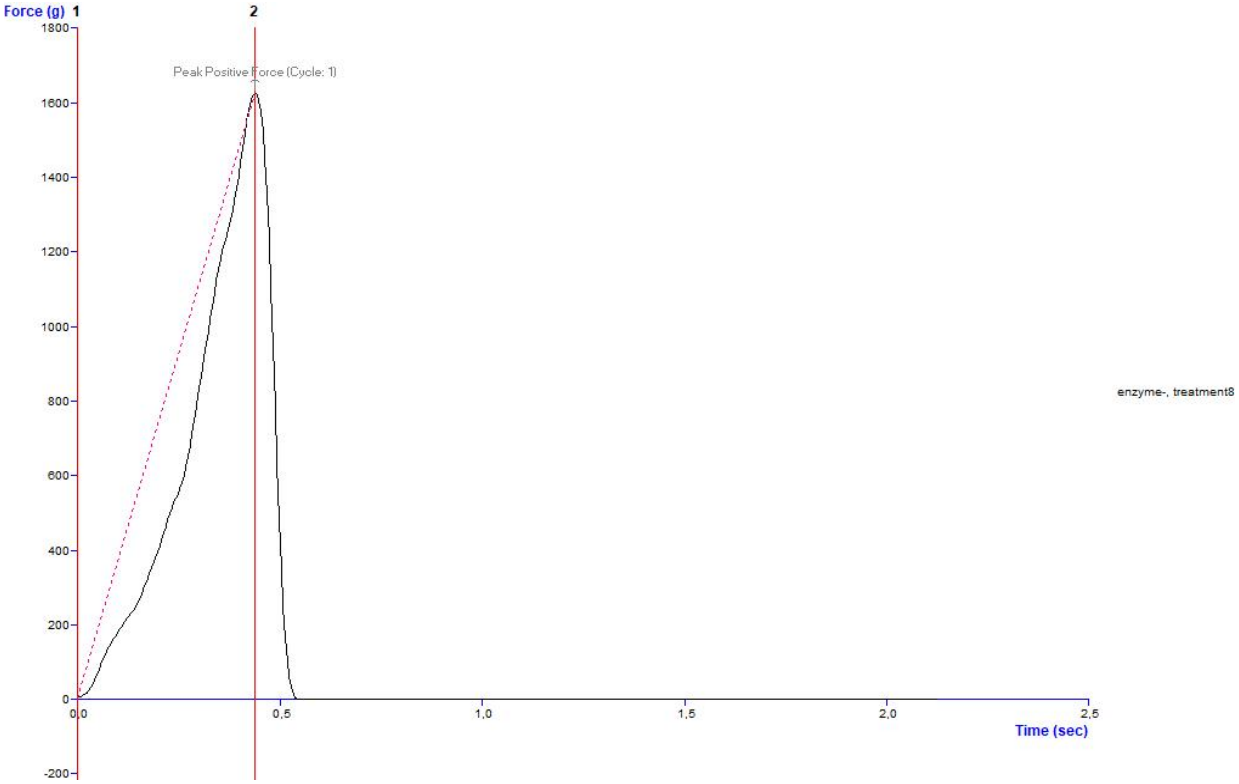


Figure 3*: Example of positive force peak and gradient graph*

The above graph in figure 3 is showing an example of the positive force peak and the gradient in the case of enzyme treated kernels dried for 4 hours. Exponent software was used to generate the graph from data given by the texture analyzer.

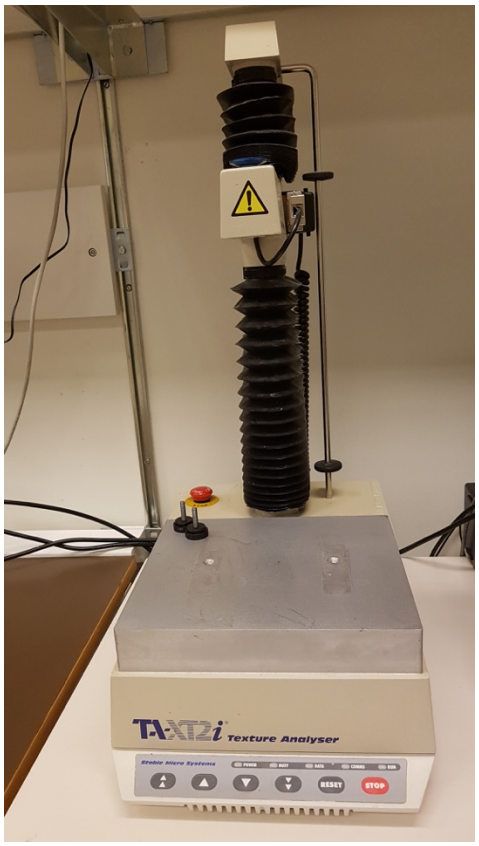
4.4 Drying

The convection oven that was used was found in the pilot plant at the Food Technology department in Lund University and was built in 1973. The convection oven generates and recycles air steam by a centrifugal fan. For the drying of the kernels the air velocity used was 2m/s at a temperature of 50°C. Each time 50gr of treated kernels were weighted and transferred in a tray where they were left to dry. The convection oven was equipped with a type K thermocouple which documents the external temperature as well as the temperature inside the oven. Also, the oven is equipped with a scale linked to a software which documents the weight of the kernels each minute and thus, the evaporation of water can be followed. After 4 hours 25 gr of the kernels were taken for texture analyzing measurements and moisture content analysis. Finally, after 8 hours the rest 25 grams were taken, and the texture and moisture content were again measured.

4.5 Moisture content analysis

To measure the moisture content, the LJ16 moisture analyzer by Mettler Tolledo company was used (picture 5). To do that, 25 grams of kernels were weighted and grounded manually in a mortar and then transferred to a small aluminum tray appropriate for use in the moisture analyzer. The instrument heats the grounded kernels to 130°C and measures the water that is evaporated. Finally, it shows the percentage of the moisture content.

Picture 5 . *Moisture analyzer (used for all moisture analysis in this experiment).*



Picture 6 *:Texture analyser used for all texture analysis in this experiment..*

4.6 Statistical analysis

For the statistical analysis of the results it was chosen to compare treatments which were performed as duplicates. The independent variables are the drying time, the time of microwave treatment and the enzyme treatment which was done in only one set-up due to the lack of material. The chosen statistical analysis was the One way Anova at $P< 0.05$, the null hypothesis was that all the means are significantly different from the others. In order to highlight which pairs of the data are significantly different the Turkey's pair wise comparison test was chosen.

5. Results & Discussion

Table 2*: Table showing the moisture content in the barley kernels after different treatments.*

Treatment	Moisture content
Untreated	9,11%
1 minute in microwave and 4h drying	8,13%
1 minute in microwave and 8h drying	7,14%
Enzyme treatment and 8h drying	8,75%

The present table (2) summarizes the moisture content of the untreated and treated barley kernels. We can see that there are slight differences in the moisture content between the different treatments, but they are all way bellow 15 % which is the acceptable limit for cereal products.

5.1 Positive force results

The following section presents the different results obtained from the experiments in forms of tables and figures. The results illustrate the positive force required to reach 20% of strain for the different treatments and the untreated barley kernels. Finally, the statistical analysis of the results is presented after each figure when a statistical evaluation is needed. The results are very promising for future studies.

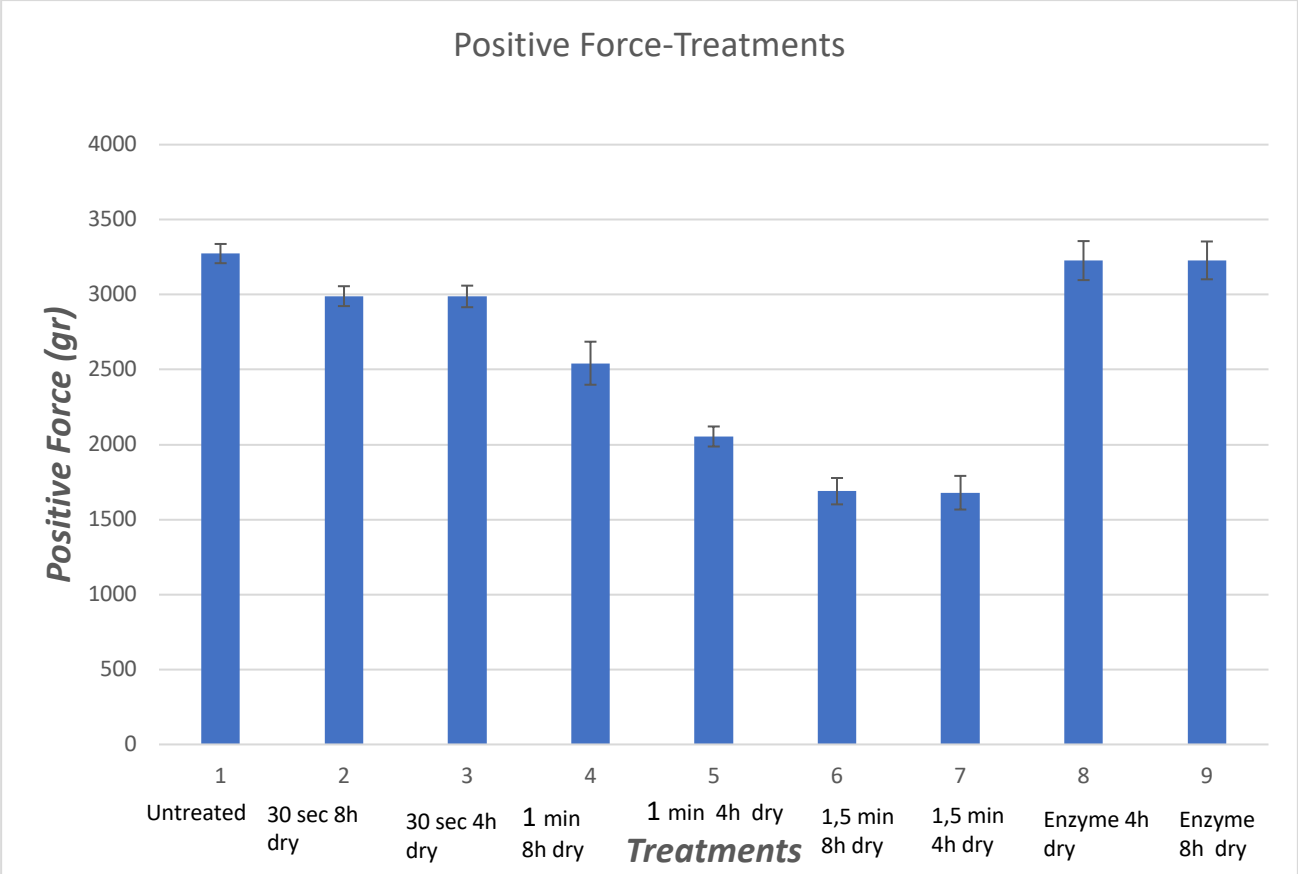


Figure 4: *Positive force required to press untreated and differently treated barley kernels.*

The measurements shown in figure 4 were performed to evaluate the hardness of the malted barley kernels. The best results are observed after 1 minute if microwave treatment as the results that we have concerning 1,5 and 2 minutes of microwave treatment are unreliable. That is because the samples after 1,5 minutes of treatment were partially burned and after 2 minutes they were totally destroyed.

Table 3*: ANOVA results based on data from untreated and treated in microwave kernels followed by 8h of drying regarding the positive force required to press the kernels.*

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	21608724,5	1	21608724,5	21,6338327	8,4401E-05	4,22520127
Within Groups	25969824,5	26	998839,404			
Total	47578549	27				

Based on the ANOVA as the F value is bigger than the F critical, we can reject the null hypothesis and conclude that there is significant difference between the two groups. The confidence interval is 95%.

Table 4*: ANOVA results based on data from 2 groups of treated in microwave kernels followed by 8h and 4h of drying respectively regarding the positive force required to press the kernels*

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	3742208,49	1	3742208,49	20,0792492	0,00013227	4,22520127
Within Groups	4845670,27	26	186371,934			
Total	8587878,76	27				

Based on the ANOVA as the F value is bigger than the F critical, we can reject the null hypothesis and conclude that there is significant difference between the two groups. The confidence interval is 95%.

Table 5*: ANOVA results based on data from 2 groups of treated with enzyme kernels followed by 4h of drying and untreated regarding the positive force required to press the kernels.*

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	1671148,81	1	1671148,81	8,84077831	0,00627829	4,22520127
Within Groups	4914710,84	26	189027,34			
Total	6585859,65	27				

Based on the ANOVA as the F value is bigger than the F critical, we can reject the null hypothesis and conclude that there is significant difference between the two groups. The confidence interval is 95%.

Table 6*: ANOVA results based on data from 2 groups of treated with enzyme kernels followed by 8h of drying and untreated regarding the positive force required to press the kernels.*

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	483184,116	1	483184,116	1,12686217	0,29820709	4,22520127
Within Groups	11148468,2	26	428787,237			
Total	11631652,3	27				

Based on the ANOVA as the F value is smaller than the F critical, we can accept the null hypothesis and conclude that there is no significant difference between the two groups. The confidence interval is 95%.

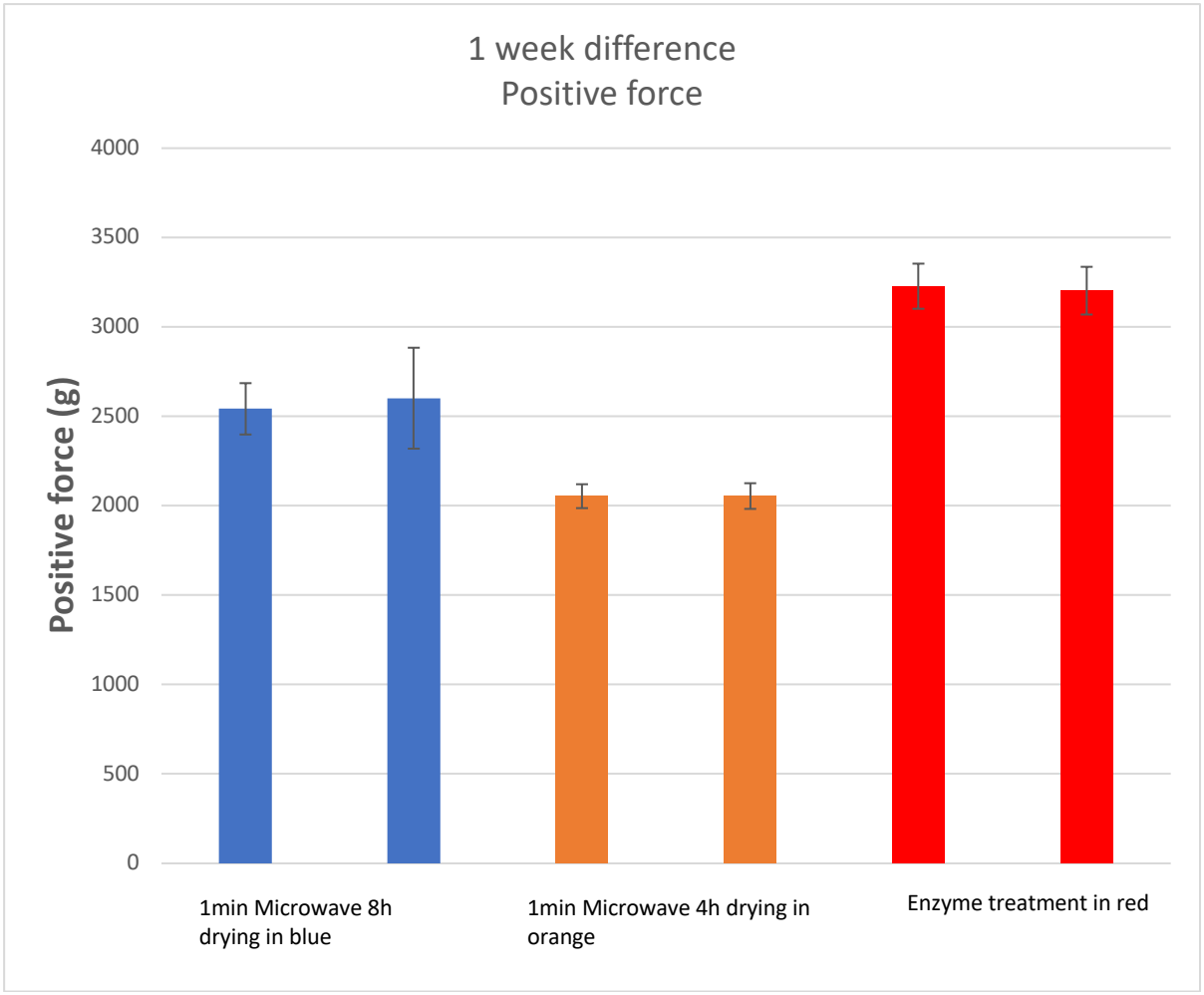


Figure 5: *Positive force after 1 week*

Figure 5 is comparing the force in grams required to press differently treated barley kernels directly after drying and after one week.

In this figure we can see the comparison between the measurement of the positive force performed immediately after drying the kernels and the same measurement after one week.

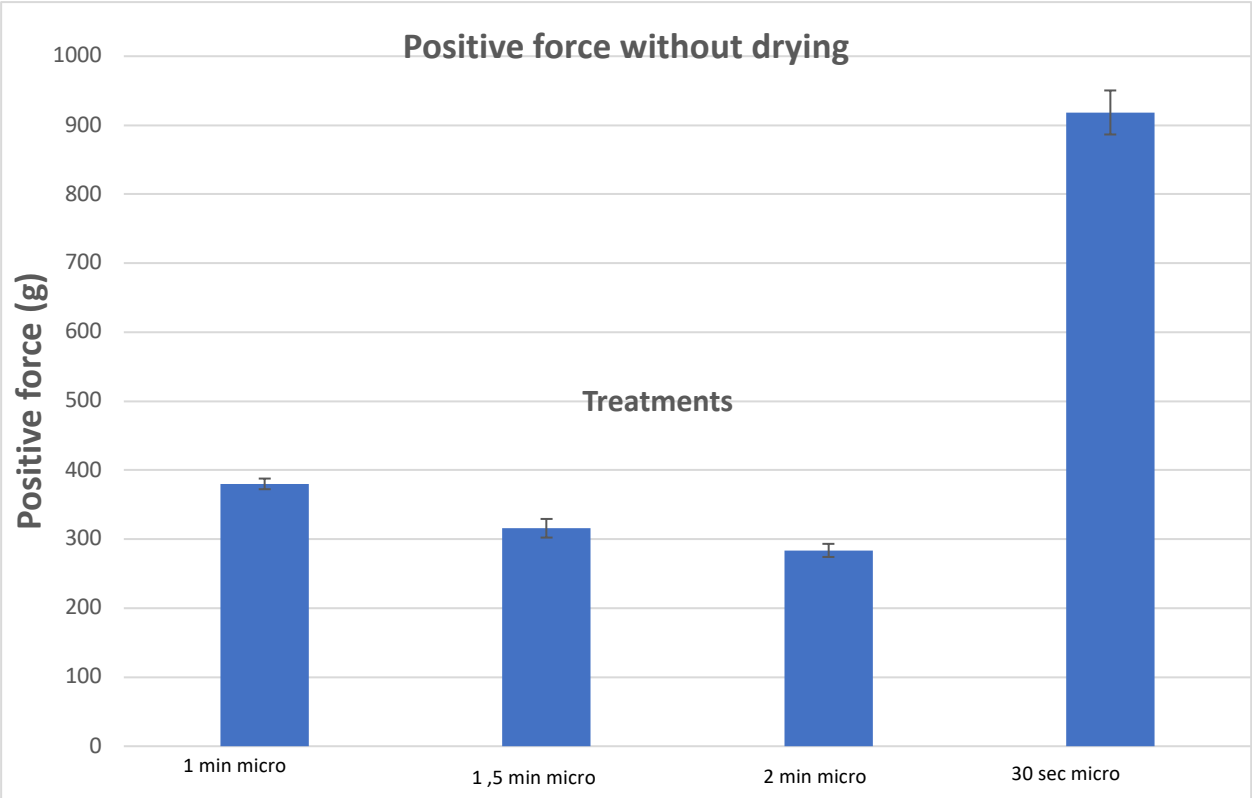


Figure 6*: Positive force without drying*

Figure 6 is showing the results of the positive force for different treatments times in the microwave before drying in the convection oven.

Table 7*: ANOVA results based on data from 2 groups of treated with microwave kernels for two different times of treatment (1 min and 1,5 min)*

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	28873,3121	1	28873,3121	15,8013877	0,00049886	4,22520127
Within Groups	47508,8727	26	1827,26433			
Total	76382,1848	27				

Based on the ANOVA as the F value is bigger than the F critical, we can reject the null hypothesis and conclude that there is significant difference between the two groups. The confidence interval is 95%.

Table 8*: ANOVA results based on data from 2 groups of treated with microwave kernels for two different times of treatment (1,5min and 2 min)*

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	7220,86511	1	7220,86511	3,50924809	0,07231084	4,22520127
Within Groups	53499,3504	26	2057,66732			
Total	60720,2155	27				

Based on the ANOVA as the F value is smaller than the F critical, we can accept the null hypothesis and conclude that there is no significant difference between the two groups. The confidence interval is 95%.

5.2 Gradient results.

The following section presents the different results obtained from the experiments in forms of tables and figures. The results illustrate the gradient of the force for the different treatments and the untreated barley kernels. Finally, the statistical analysis of the results is presented at after each figure when a statistical evaluation is needed. The results are very promising for future studies.

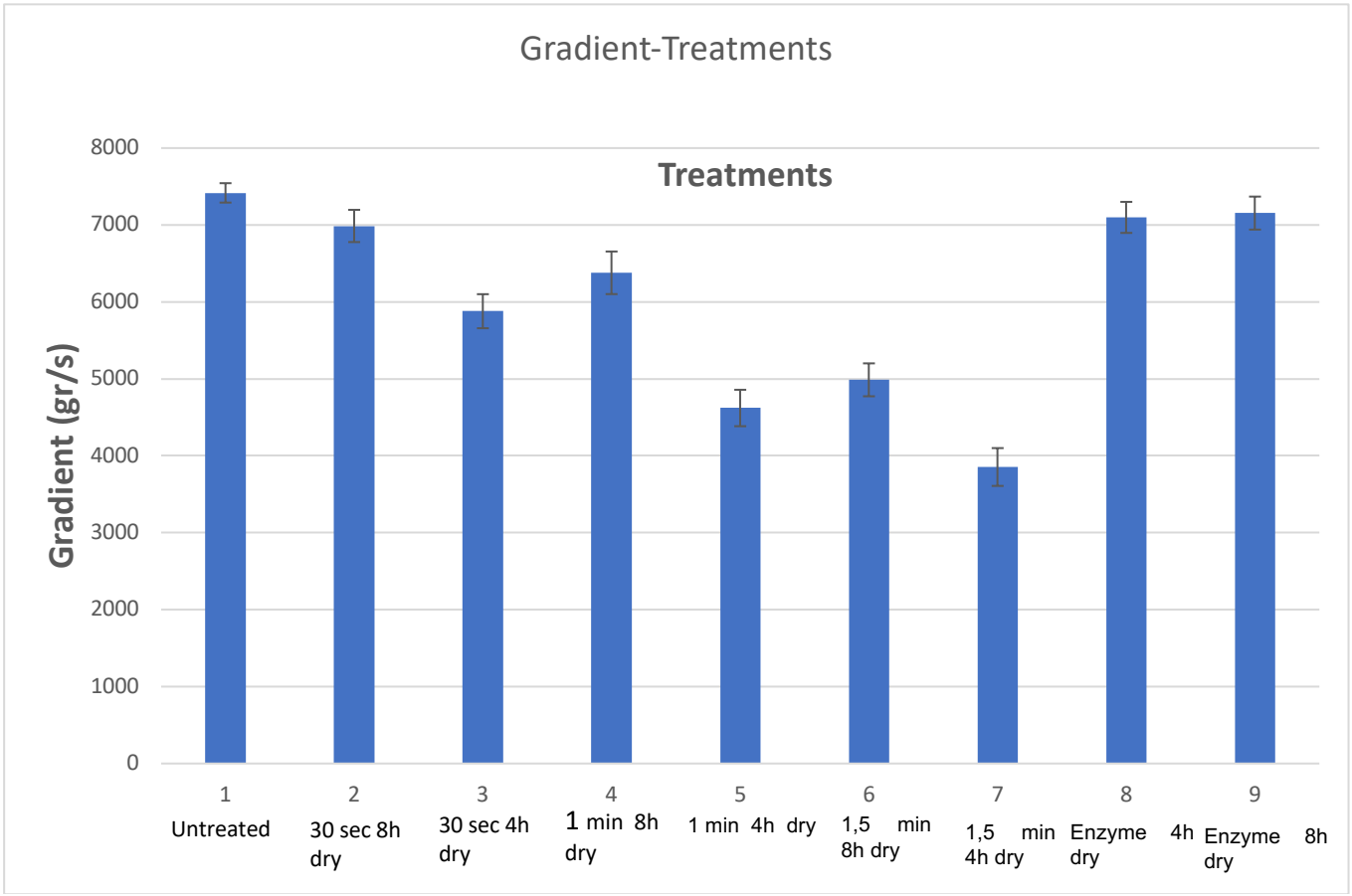


Figure 7*: Force Gradient during pressing of untreated and differently treated barley kernels.*

The measurements shown in figure 7 were performed to evaluate the gradient of force for the malted barley kernels. The best results are observed after 1 minute if microwave treatment as the results that we have concerning 1,5 and 2 minutes of microwave treatment are unreliable. That is because the samples after 1,5 minutes of treatment were partially burned and after 2 minutes they were totally destroyed.

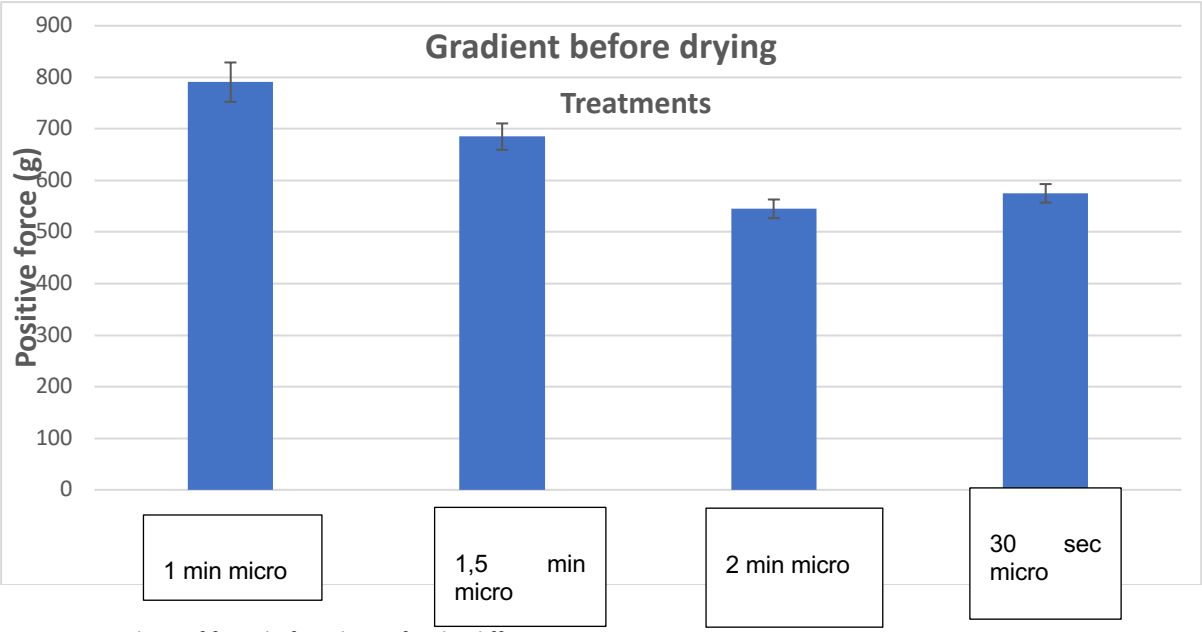


Figure 8: *Gradient of force before drying for the different treatments.*

Figure 8 is comparing the gradient of the force for the different treatments before drying with 33%moisture content.

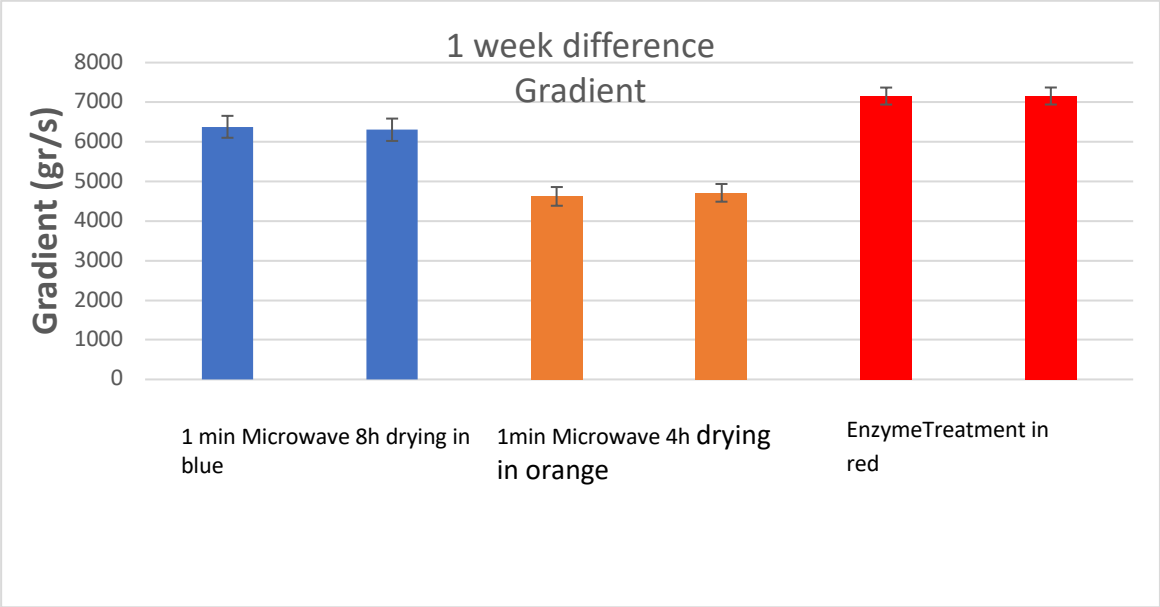


Figure 9: *Force gradient during pressing of differently treated barley kernels.*

Figure 9 is comparing the force gradient during pressing of differently treated barley kernels directly after drying and after one week. In this figure (9) we can see the comparison of the gradient of force during compressing the differently treated barley kernels immediately after drying and after one week.

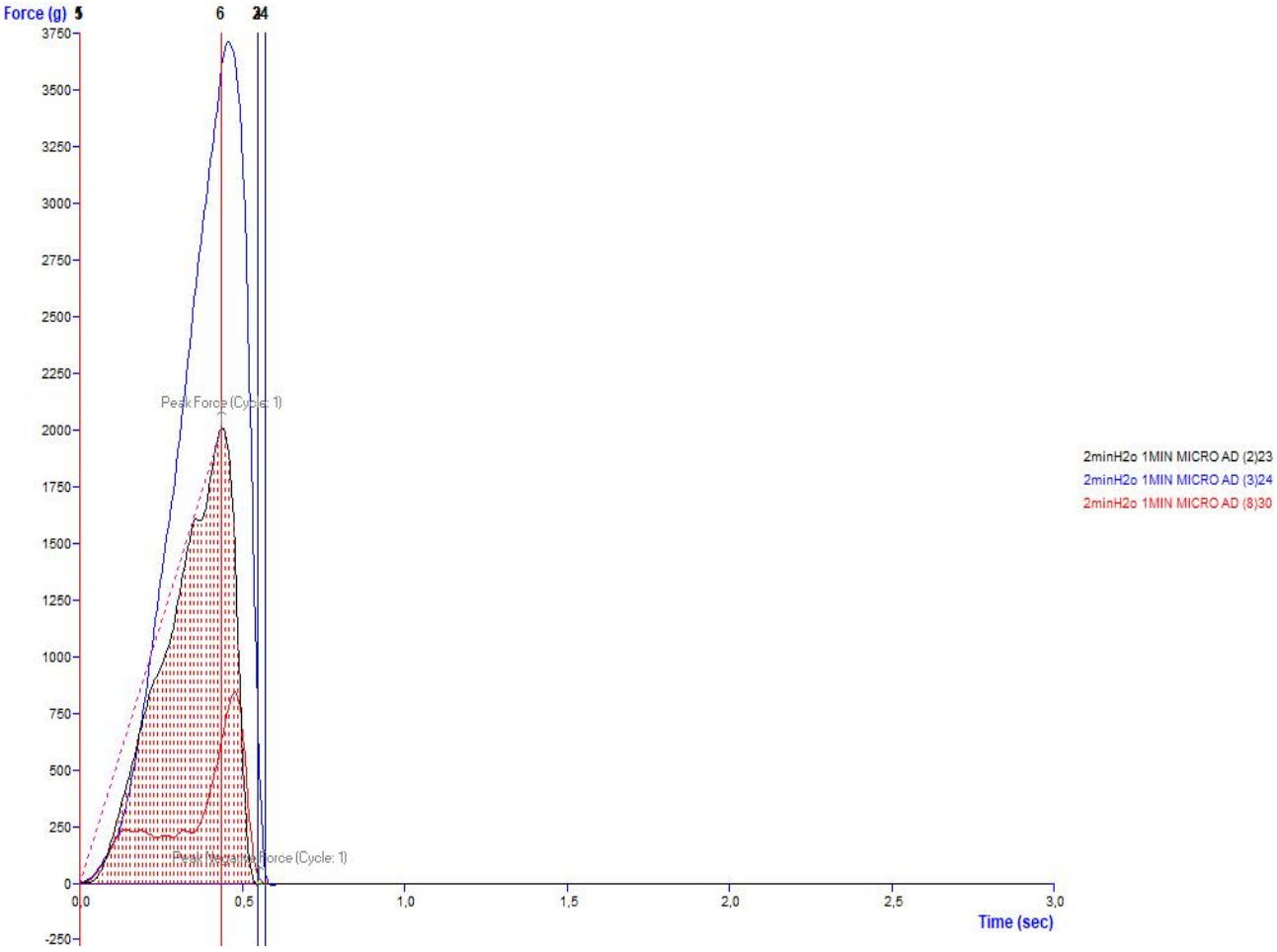


Figure 10*: Example of positive force peak and gradient graph.*

The above graph (figure 10) is showing an example of the positive force peak and the gradient in the case of microwave treated kernels dried for 8 hours. Exponent software was used to generate the graph from data given by the texture analyzer.

Table 9*: ANOVA results based on data from 2 groups of treated with enzyme kernels followed by 8h of drying and untreated regarding the positive force required to press the kernels.*

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	14446,2857	1	14446,2857	0,09569521	0,75952357	4,22520127
Within Groups	3924997,01	26	150961,423			
Total	3939443,29	27				

Table 10*: ANOVA results based on data from 2 groups of untreated and treated with microwave kernels followed by 8h of drying regarding the gradient of force required to press the kernels*

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	7556376,86	1	7556376,86	11,4538384	0,00227267	4,22520127
Within Groups	17152834,8	26	659724,416			
Total	24709211,7	27				

Based on the ANOVA as the F value is bigger than the F critical, we can reject the null hypothesis and conclude that there is significant difference between the two groups. The confidence interval is 95%.

Table 11*: ANOVA results based on data from 2 groups of treated in microwave kernels followed by 8h and 4h of drying respectively regarding the gradient of force required to press the kernels*.

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	21608724,5	1	21608724,5	21,6338327	8,4401E-05	4,22520127
Within Groups	25969824,5	26	998839,404			
Total	47578549	27				

Based on the ANOVA as the F value is bigger than the F critical, we can reject the null hypothesis and conclude that there is significant difference between the two groups. The confidence interval is 95%.

Table 12*: ANOVA results based on data from 2 groups of treated with enzyme kernels followed by 8h of drying and untreated regarding the gradient of force required to press the kernels.*

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	483184,116	1	483184,116	1,12686217	0,29820709	4,22520127
Within Groups	11148468,2	26	428787,237			
Total	11631652,3	27				

Based on the ANOVA as the F value is smaller than the F critical, we can accept the null hypothesis and conclude that there is no significant difference between the two groups. The confidence interval is 95%.

6. Discussion

6.1. Treatment techniques

The scope of this thesis was to evaluate the effect of different treatments on the texture of barley kernels. Specifically, since the present study was conducted inside a company's R&D department, the aim of this dissertation is to allow the improvement of the texture of the barley kernels in order to successfully to incorporate them in a new type of muesli. The techniques tried were microwave (600 W) and enzyme treatments (cellulase and xylanase) followed by 4 and 8 hours of drying. Better results were observed with microwave treatment.

6.1.1. Microwave treatment

As shown before in the results section we can see that microwave treatment has given significantly improved texture when applying 1 minute of microwave treatment on malted barley kernels after being soaked for 2 minutes in water. The results were evaluated by measuring the hardness of the kernels after 4 and 8 hours of drying and were expressed in grams necessary to press the kernels. The required force was, on average, 3273,5 gr for the untreated barley while the values were 2542,33 gr and 2053,73 gr for 8 hours and 4 hours of drying after the microwave treatment respectively. The same analysis was done before drying, with similar results. These results are insignificant for the industry because the moisture content is 33% which means that they cannot be utilized in the food industry before drying. On the other hand, they can serve as a starting point for further studies.

Similar results are observed when analyzing the gradient of the force. The average gradient for untreated barley was 7417,54 gr/s while the values where 6378,56 gr/s and 4621,59 for 8 hours and 4 hours of drying after the microwave treatment respectively. All the results were analyzed using one-way ANOVA and was shown that these differences were statistically significant and thus, it was proved that microwave treatment can soften malted barley kernels.

Additionally, it was observed that the softening effect is preserved for at least one week after the drying process, which is crucial for the shelf life of the product in which the kernels are intended to be incorporated. In order to furthermore establish the above hypothesis, the same measurements were evaluated after one week with the same results, which are presented in figures 1 and 2 in the results chapter of this study. All the above results regarding the microwave treatment on the hardness of

the barley kernels can also be confirmed by previous studies. According to the experiments performed by Grundas et al., (2008) on wheat grains it was observed that direct exposure to microwave radiation decreases the hardness of the third-generation wheat grains.

The authors on this study observed the best results when it came to the kernel hardness at exposure times of 120 and 180 seconds. During our experiment the best softening effect was showed for an exposure time of 1 minute. At this point it is important to highlight the fact that even though experiments with higher exposure times were conducted, the quality of the product was not acceptable for food applications due to pyrolysis reaction.

6.1.2. Enzymatic Treatment

During the enzymatic treatment, a homogenized mixture of cellulase and xylanase was added to the barley kernels in order to improve their texture by partially degrading the cell wall components. The kernels were left in the water bath at 50˚C for 12 hours and then dried in the convection oven for another 8 hours. The average positive force required to press the untreated kernels was 3273,5 gr and the gradient of the force was 7417,54 gr/s. Similarly, for the kernels that were treated with these specific enzymes the values were 3228,1gr and 7154,82 for the positive force and the gradient respectively. The gradient represents the slope of the positive force curve and practically it represents the crispiness of the barley kernels.

Statistical analysis was conducted on these results using a one-way ANOVA which confirmed that there is no statistically significant difference between the two groups proving that enzymatic treatment using cellulase and xylanase for that particular time of 12 hours does not give an improved texture of the barley kernels. Finally, as it was observed for the microwave treatment, the results, both for the positive force and for the gradient did not present any change after one week's period. According to A.K. Holtekjølen et al. (2006) cellulose content in barley is higher that arabinoxylan content with the values varying from 4,4% to 13,7% for arabinoxylans and from 8% to 17,7% for cellulose depending on the different varieties.

To furthermore support these results, we can refer to the Yoo et al., (2009) study in which also the enzymatic treatment using a cocktail of enzymes containing cellulase, xylanase and pectinase did not affect the hardness of tempered wheat kernels.

7. Conclusion

In this study microwave and enzyme treatments for improving the texture of malted barley kernels in order to use them in food applications were evaluated. The better results were obtained by soaking the barley kernels for 2 minutes in distilled water and then treating them with microwave radiation for 1 minute followed by 4 hours of drying in the convection oven (2m/s velocity of air and 50˚C air temperature). Acceptable results were also obtained after 8 hours of drying. The hardness of the kernels was evaluated using the texture analyzer in Lund University. Additionally, microwave treatment resulted in a very pleasant roasted taste, after tasting them, which is desirable in food products.

7.1 Future Work

The enzymatic treatment with cellulase and xylanase for 12 hours did not improve the hardness of the barley kernels and also gave them an unpleasant taste which made them totally useless for food applications.

Future studies could emphasize on testing different concentrations of the enzymes and also testing both optimal temperatures for each enzyme respectively, instead of using the middle temperature between the two optimum.

Other treatments used in previous studies in order to soften different types of cereals could also be used, such as ultra-sound treatment, pressure with mechanical means and use of different times and intensity of microwave treatment. Different varieties of barley with slightly different botanical characteristics such as fiber content could also be tested.

8. Acknowledgements

I gratefully acknowledge my supervisor professor Yvonne Granfeldt at Lund University for her support and advice throughout the project together with my examiner Ingegerd Sjöholm senior lecturer. also thank Shohreh Askaripour, researcher at Aventure AB for her enormous help in the experiments.

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