Addition of PromOat Beta-Glucan and Guar-gum to Gluten-Free Wheat Starch Pan Bread to increase Fiber while maintaining the Quality in Texture

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MASTER THESIS

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Addition of PromOat Beta-Glucan and Guar-gum to Gluten- Free Wheat Starch Pan Bread to increase Fiber while maintaining the Quality in Texture

Enhancing Gluten-Free Wheat Starch Pan Bread with PromOat Beta-glucan and Guar-gum for enhanced Texture and Nutrition

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Abstract

Gluten-free products have gained great attention in the last few years due to the growing incidence of celiac disease and the intolerance to gluten. The project is aimed to investigate the effects of adding PromOat, a good source of B-glucan to increase the fiber content and Guar-Gum to overcome the textural deformities in the gluten-free wheat starch Pan bread. Different levels of beta-glucan and guar-gum were divided into three treatments and the analysis of the moisture content, volume and water activity was done on the bread. The data was analyzed using the MATLAB in which ANOVA was implemented to check the statistical difference between the groups. There were differences in the moisture content values between the three treatments, especially in the third treatment which had both beta-glucan and guar-gum around 1% and 0.5% respectively. Differences were also seen in the water activity of the bread when guar gum was added. All the results that were obtained were summarized by the Principal Component Analysis (PCA), where it showed that moisture content was closely associated with guar-gum, beta-glucan and treatment. It was also concluded that the addition of the guar-gum doesn't have much influence on the volume of the bread.

Popular science Abstract

"Revolutionizing gluten-free baking; unleashing the secret to create a perfect gluten-free bread".

Gluten-free bread is a popular choice for those with gluten sensitivities. However, the problem with many gluten-free breads is that they are low in fiber as it is a vital nutrient that is important for a healthy digestive system. This is where beta-glucan comes into play as the addition of the beta-glucan can significantly increase the fiber content of the bread. But beta-glucan can also affect the textural properties of gluten-free bread. To mimic the behavior of gluten in a gluten free bread is quite a task because the presence of a structured protein gluten network is quite strong. Lantmännen, a food and agriculture company based in Sweden, has a very large facility for the production of PromOat and gluten-free starch. They have a number of projects to identify the appropriate combinations of these additives to their products...

Since there are not a lot of options available for the people who are gluten intolerant and hence working towards making more gluten-free options is one of the motives of the company. Therefore, the main aim of the project is to come up with a suitable gluten-free bread that mimics gluten bread by the addition of the suitable hydrocolloid. The analysis done on the bread will provide information about the physical characteristics of the gluten-free bread and how they can be improvised.

The results from the projects showed that guar-gum influences the moisture content of the bread, but it was surprising that it had no influence on the volume of the bread. The bread was similar to the Gluten bread and had a shelf life of about a week.

Acknowledgement

This thesis was only made possible by the efforts of those who worked in addition to myself, and I would like to express my gratitude to them through this brief note. Beginning with Stephen Burleigh, my supervisor at Lund University, whose constant guidance and feedback helped me work efficiently.

I would like to thank the team at Lantmannen, especially Mari Hyllstam for encouraging healthy discussions and all the efforts she has put into making me feel part of the team and helping me build this thesis.

Finally, I would like to thank my family and friends who were a constant source of motivation and support throughout this time. I am greatly indebted to everyone mentioned here.

Devika Singh

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1. Introduction

The project is focused on gluten-free baking of wheat starch pan bread with the addition of Lantmännen PromOat B-glucan and Guar-gum.

1.1 Why Gluten-Free Bread

Gluten is a protein naturally found in some grains like wheat, barley, and rye. It is present in the part of the grain called the endosperm. Gluten is made of two proteins that are known as gliadin and glutenin, and Fig. 1 shows the structure of glutenin and gliadin. The presence of these types of proteins in gluten helps to form a network and allows the dough to keep its shape during baking [1]. Gluten-free bread is for people suffering from celiac disease. Celiac disease is an immune mediated gastrointestinal disease that is triggered by the consumption of gluten. In a country like Sweden, where about three percent of the population is gluten-intolerant, gluten-free bread is a necessity for the market.

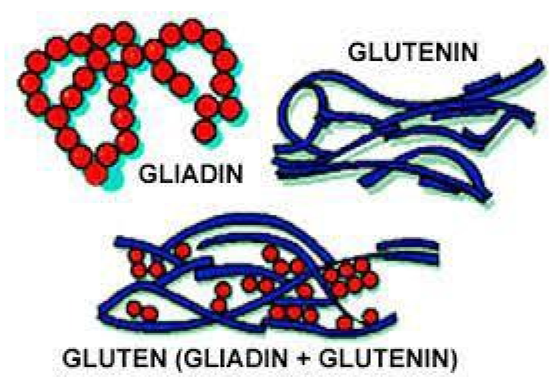


Fig.1 Structure of glutenin and gliadin. ***ChemMatters* February 2012**

1.2 Project background

Lantmännen is an agricultural cooperative and is primarily focused on agriculture, machinery, bioenergy, and food products. Producing fodder for animals is also one of the major byproducts that the company gets simultaneously during the production of starch and gluten. PromOat, a Lantmännen product, is a good source of beta-glucan [2]. PromOat beta-glucan is a highly concentrated, soluble oat bran fiber with the least amount of insoluble fibers and is a good replacement for gluten for people who are gluten intolerant. Hydrocolloids are needed to imitate gluten's viscoelastic behavior in gluten-free bread, and their properties differ from structure to structure [3]. Gluten is considered to have good gas retention, which gives decent volume to the bread. Lantmännen is working towards evaluating how the system functions when beta-glucan is added to the bread and creating a good gluten-free bread.

1.3 Aim

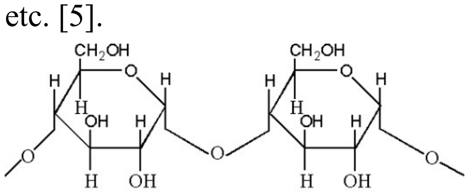
The project aims to create a gluten-free bread that is similar to gluten-bread and also investigate the water activity, moisture content, volume, and density. This includes the baking of the bread with three different treatments of beta-glucan and guar-gum that are discussed in more detail in the materials and method sections of this report.

2. Background

Since this project involves gluten-free baking, it is very difficult to recreate the gluten network. As compared to gluten bread, gluten-free bread requires a lot more ingredients to bake the same bread. A typical gluten-free bread can include psyllium husk, potato fiber, milk powder, yeast, butter, guar-gum, sugar, salt, and starch, whereas gluten bread needs wheat flour, granulated sugar, dry yeast, salt, and vegetable oil. As a result of this, it is essential to have a solid understanding of the ingredients that were utilized to simulate the behavior of gluten.

2.1 Starch

Starch is a polymeric carbohydrate consisting of numerous glucose units joined together by α-(1-4)-D glycosidic bonds, Fig. 2 shows the structure of starch. Polysaccharide is produced by green plants for energy storage [4]. Pure dry starch is a white granular powder, and wheat flour contains 70%–73% starch and is found in the endosperm of the grain. Starch is called a "complex carbohydrate" because it is made up of various sugar molecules linked together. It has two main parts: amylose and amylopectin. Amylose is a straight line of sugar molecules joined together, whereas amylopectin is a branched chain of sugar. In the food industry, starch is used as a thickener, filler, and stabilizer in products such as custard powder, baby foods, bakery products, etc. [5].



Starch

Fig.2: chemical structure of starch ,2021 The Master Chemistry

Starch is a polymeric carbohydrate consisting of numerous glucose units joined together by α-(1-4)-D glycosidic bonds. Fig. 2 shows the structure of starch. Polysaccharide is produced by green plants for energy storage [4]. Pure dry starch is a white granular powder, and wheat flour contains 70%–73% starch and is found in the endosperm of the grain. Starch is called a "complex carbohydrate" because it is made up of various sugar molecules linked together. It has two main parts: amylose and amylopectin. Amylose is a straight line of sugar molecules joined together, whereas amylopectin is a branched chain of sugar. In the food industry, starch is used as a thickener, filler, and stabilizer in products such as custard powder, baby foods, bakery products, etc. [5].

2.2 Gluten-free baking and dough making

The formation of the dough is a crucial step during the baking process. The preparation of the dough is a complicated procedure to make the dough replicate the gluten network. Gluten-free bread lacks gluten proteins, which provide unique extensibility with viscoelastic behavior and excellent water and gas holding capacity; hence, replicating the same characteristics in a gluten-free bread is a challenging task. [6]

2.2.1 Importance of the starch flour in dough making

Starch plays a very important role in gluten-free dough and in replacing gluten. The gluten-free dough can contain guar gum and psyllium husk as water- and gas-holding agents. However, neither are cohesive nor elastic, and that is the reason they result in dry and non-cohesive crumbs that, in turn, make the bread stale quickly. The structure of the starch interacts with the gluten replacer (hydrocolloids) during the mixing, proofing, and baking processes and reduces the effects of hydrocolloids. The colloidal addition of the starchy flour and scratch granules will aid in better aeration during mixing and also help in better gas retention during proofing. Another factor that contributes to a better dough quality is the starch structure, which can withstand enlargement during the gelatinization as the starch granules swell and form a gel-like network that provides structure and stability to the dough. Also, the retrogradation of the starch will be responsible for the texture, as during retrogradation the starch molecules undergo a rearrangement, causing them to reassociate and form a more ordered structure. [7]

2.2.2 Gelatinization and the retrogradation of the starch

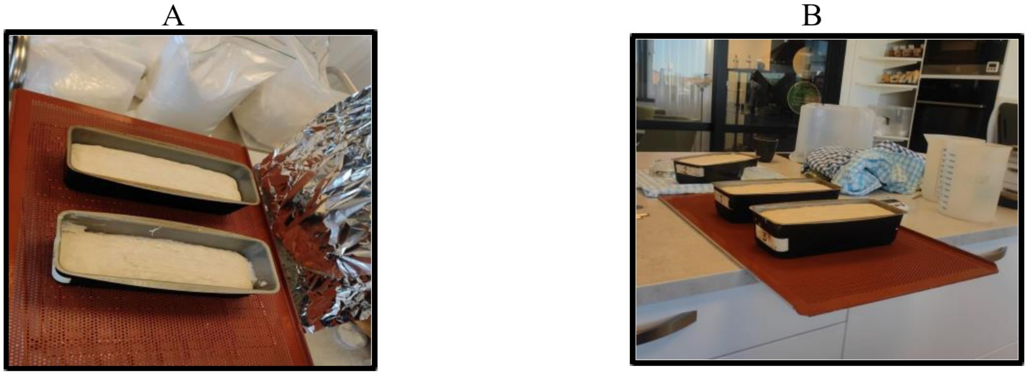
The gelatinization of the starch is an essential stage in the process of producing a regular bread crumb structure. When bread is baked, heated, or subjected to any form of heat, this is a common phenomenon that takes place over the course of the process. Gelatinization of the starch takes place in the presence of water and heat is an irreversible process [8]. During this process, the starch loses its crystalline structure and becomes water soluble. As a result of this process, the starch structure is disrupted. Gelatinization of the starch also tends to raise the viscosity of the continuous phase of the dough, which ultimately results in the bread structure becoming more stable when it is baked.

The process of re-crystallization of the starch molecules that are present in gelatinized starch is referred to as "starch retrogradation." The retrogradation of starch is a very common phenomenon in bakery products. This process is responsible for the staling of bread and can impact the crumb structure as well as the firmness of the bread. [9]

2.2.3 Fermentation and proofing of bread

The fermentation of the dough processed by the yeast is the most crucial phase in the making of bread. Major fermentation of the bread can be seen during the proofing and resting stages. To decide the proofing time, many factors have to be taken into account. In a study, it was found that the breads that were proofed for around 40 minutes at 27 °C had good hydration and looked the best out of the oven. Here is the picture. 2 shows the dough before and after proofing. Basically, the longer the proofing time, the coarser the crumb and the more intense the flavor. Flexibility is also increased with the proofing process. And hence, a minimum of 30 minutes were considered at a temperature of 30 °C to achieve the best results [29].

A B



picture.2 (A) before proofing (B) after proofing

2.3 Beta-Glucan

Beta-glucan is the dietary fiber readily found in oat bran and barley. These are non-starch polysaccharides that contain glucose monomers linked by β-glycosidic linkage. Among the cereals, the highest content of beta-glucan has been reported in barley. [10]. Dietary fibers are defined as the carbohydrate that cannot completely broken down by the human body. It helps lower blood cholesterol and glucose levels.

2.3.1 PromOat

PromOat Beta is a Lantmännen Product that is an oat bran fiber and a good source of beta-glucan as mentioned earlier. Fig.3 shows the schematic presentation of the production of PromOat beta-glucan in Lantmännen. When beta-glucan is added to the bread, it results in a reduced loaf volume and an increase in the firmness of the bread [11].

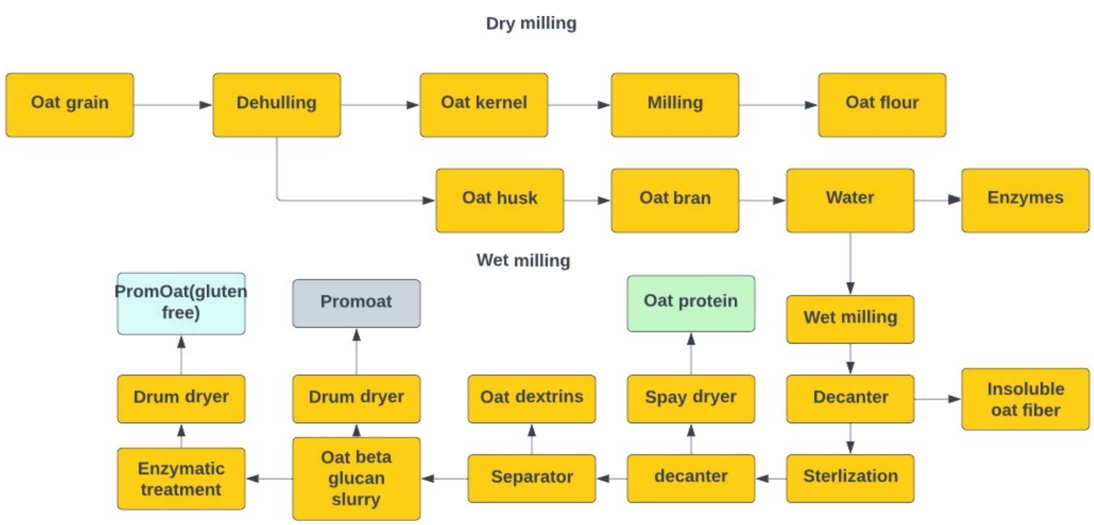


Fig:3 Schematic presentation of Production of the PromOat

2.4 Guar-gum

Guar-gum is extracted from the seeds of the drought tolerant plant Cyamopsis tetragonoloba, which is a member of the Leguminosae family. The addition of the Guar-gum to the bread may potentially have a good network as it is a hydrocolloid, and the bread has an absence of gluten. In studies, it has been found that 1% and 1.5% of the hydrocolloids, except the xanthan gum, result in a higher loaf volume and better color in the gluten free breads [11]. The studies also showed that the addition also resulted in an extended shelf life of the bread due to its ability to absorb moisture. The addition of guar-gum gave a thread like network to the dough, which was stiff but showed good results after baking. Binding agents such as guar-gum can also help in replicating the gluten characteristics in the dough.

2.5 Psyllium Husk

Psyllium comes from a shrub-like herb called Plantago psyllium, commonly found in India. The husk is the outer coating of the psyllium seed. Psyllium husk acts as a binder, and it gives gluten-free dough the elasticity and flexibility it needs as there is no gluten in it [32].

2.6 Analysis

2.6.1 Moisture Content

Moisture content can be defined as the total amount of water present in the sample. The primary method for determining the moisture content is loss on drying. It is an important parameter in the food industry as it determines the shelf life and the overall stability of the product [14]. Foods with low moisture content have lower enzymatic activity and reduced microbial growth. The wet basis is the standard basis for the moisture content. The moisture content of the wheat starch pan bread is around 41.23% [14].

2.6.2 Water activity (aw)

The amount of water that is in a free state or that is unbound in a sample is referred to as the "water activity." Since it serves as microorganisms' only source of free water, water activity can have a significant influence on the deterioration of food commodities. From a mathematical perspective, it is derived as the thermodynamic measure of water, which is expressed as the vapor pressure of water in a sample divided by the vapor pressure of pure water at a given temperature (aw = P/Po). The water activity of freshly baked pan bread is around 0.93 AW. The microorganisms that grow around the pan bread are salmonella, C. botulinum, and pediococcus [12]. The shelf life of a product is determined by its intrinsic food features such as water activity, moisture content, and pH, as these factors determine the amount of water that is in a bound and free state for microorganism growth, and the pH of the food determines the acidity, which is also crucial to the shelf life of a food commodity. [13]. The water activity needed for the bacteria to grow is around 0.91, whereas the fungi grow at least at 0.6 AW [31].

2.6.3 Volume and density

The volume of the bread can answer many questions about the quality aspects of the bread. For instance, a low volume in the bread suggests minimal enzymatic activity. It also shows good gas retention and fermentation when the bread is resting and being proofed. Having information about the volume can also aid in improving the dough mix, which is especially important when making gluten-free bread [15]. When testing the quality of bread, one of the most important quality parameters to look at is the bread's volume. If the bread has a decent volume, this also suggests that it has good aeration. A volume meter is a non-destructive method to measure the volume of bread [16].

2.7 Bread Form

The bread form describes the shape of the bread and the category it falls into. It is a beneficial way to categorize the bread into different forms, where the first form is a perfect curve with an appropriate height of the upper surface. Following the first form, the upper dome shape of the curve keeps on depleting as seen in Fig.4. Through this technique, we can examine the shape of the bread, which tells us about the textural properties of the bread. If the bread falls into the initial bread form, it is of better quality than the ones falling under the last categories.

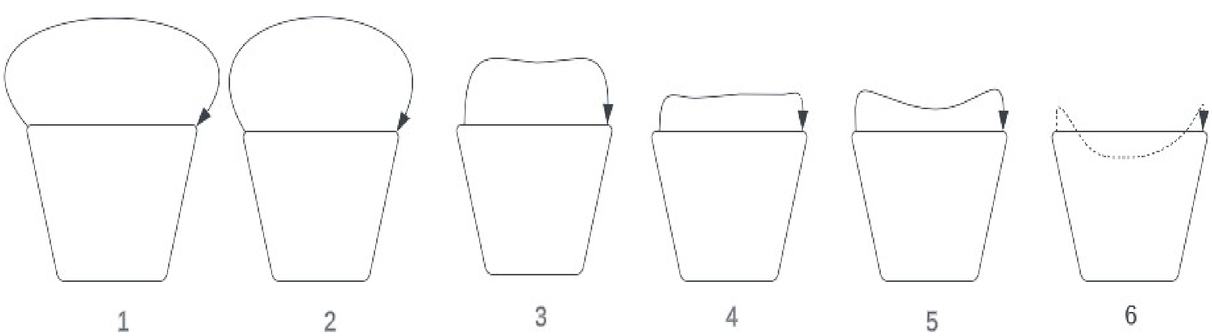


Fig.4 Different bread form

3. Materials and Methods

The ingredients utilized for the baking of the bread (starch, PromOat B-glucan, Guar-gum, Psyllium husk) were provided by Lantmännen, and the equipment for the analysis was provided by LTH Food Technology and the Nutrition Department. The recipe for baking the wheat starch pan bread was given by the company.

These are the ingredients that are typically included in the gluten-free wheat starch pan bread starch flour, potato fiber, milk powder, salt, sugar, Psyllium husk, butter, yeast, and water. The weight percentage of the ingredient is provided in Tabel No.1, which gives the weight in grams and the weight percentage of the individual ingredient. The addition of the promOat and the Guar-Gum was done according to the treatments that will be explained further in the methods sections of the report.

Table no.-1 Weight of the ingredients.

Ingredients	g (1 bread)	g	%
Water	336,00	1043,5	43.9
Starch	358,64	1040,5	43.7
Yeast	20,00	60,00	2,0
Butter	20,00	60,00	2,0
sugar	10,40	31,2	2,0
Salt	6,40	19,2	1,0
Milk Powder	24,00	72,0	3,0
Psyllium husk	6,00	18,0	1,5
Potato fiber	4,00	12,0	1,0

3.1 Three treatments with Guar-Gum and Beta-Glucan

The three treatments mainly focused on the different percentages of beta-glucan and guar gum. One batch had three breads with three replications of each treatment. The first treatment had nine breads with 0% beta-glucan and 0% guar gum. The second treatment also had nine breads with the addition of 1% beta-glucan. The last treatment had nine breads with the addition of beta-glucan and guar gum at 1% and 0.5% dry weight, respectively. The analysis was done such that the bread was baked and analyzed on the same day, for a total of nine days of baking. The analysis of the bread that involved moisture content, water activity, volume, and density was done on the very same day as baking and not on different timepoints or days; this was done to ensure that the results were achieved before the bread spoils from mold.

3.2 Materials

3.2.1 Equipment used for baking.

The equipment used for baking in the kitchen is as follows: dough mixer, baking oven, three bread baking tray, proofer.

3.2.2 Equipment used for the analysis.

- Water activity meter
- Volume meter
- Analytical balance
- Drying oven.

3.3 Methods

The ingredients utilized for the baking of the bread (starch, PromOat B-glucan, Guar-gum, Psyllium husk) were provided by Lantmännen, and the equipment for the analysis was provided by LTH Food Technology and the Nutrition Department. The recipe for baking the wheat starch pan bread was given by the company.

3.3.1 Baking Methods

The recipe for baking the wheat starch pan bread was provided by "Lantmannen Reppe Gluten-free Wheat Starch Baking Recipe." Three breads were taken in total for one batch of baking that involved the following steps:

3.3.2 Preparing the Flour Mix

1040 g of starch flour (as given in Table No. 1), Psyllium husk (around 18 g), potato fiber (12 g), milk powder (72 g), guar gum (6 g), and PromOat (12 g) were used according to the different treatments. Then the dry ingredients were mixed and added to the mixer, where weighted sugar (31,2 g) and salt (19,2 g) were added afterwards to the dough mixer. The water added to the dough mix was maintained at around 28 °C to give the dough an appropriate temperature before mixing.

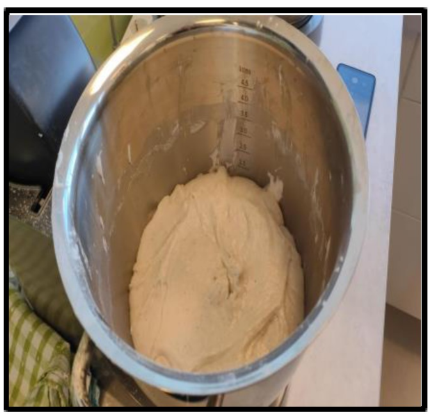
3.3.3 Mixing

After the addition of water, the butter 60 g and yeast 60 g were added to the mixer and the dough mix was kept at its minimum speed for about two minutes. After two minutes the temperature of the dough mix was checked to ensure that it should not exceed 30℃.

Following that the mixer speed was increased from the minimum speed level to one level above to the minimum speed so as to allow the dough to mix properly for about seven minutes. To achieve a proper mixing of the dough because it results in structure formation of the dough, homogenization, and air inclusion.

3.3.4 Resting

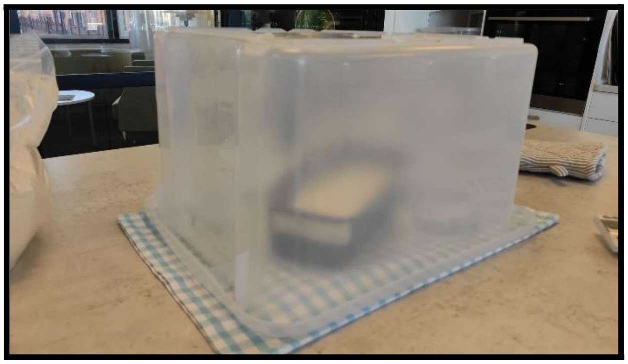
After the dough was left to rest for 30 minutes, During the resting phase, the yeast initiates the fermentation of the bread [19]. After the resting period, there were changes in the dough, as seen in picture 3. The temperature of the dough was measured, and subsequently, around 700 grams (the detailed amount of the weighted dough after the resting period, proofing, and baking are provided in Table No. 3 in the Appendix) of the dough were poured into each of the three different baking trays.



Picture 3: Before and after resting phase respectively

3.3.5 Proofing

Proofing is a process where the dough is kept resting for around 30 minutes at room temperature. As there was no proofer present, a set-up was carried out to complete the process. The three baking trays were placed on top of a normal baking oven on the stove and the oven was left at around 110°C so that the temperature at the top of reached around 30°C which is the proofing temperature. The trays were covered with a plastic box (as shown in the picture), with a cloth covering the box.



Picture 4: Setup for proofing.

3.3.6 Baking

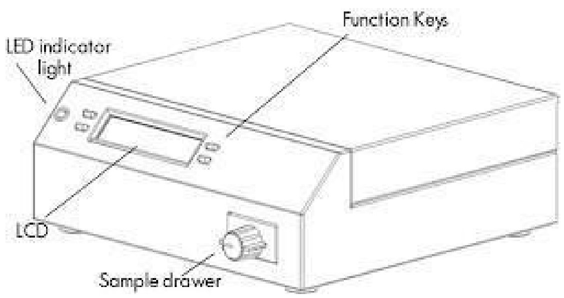
After proofing the dough, each tray was weighed again to check the difference in weight before and after baking as well as after proofing (see Table 3 in the Appendix). The bread was kept in the oven, which was set at 210 °C, for about 40 minutes. The bread was baked for 30-35 minutes. After the baking, the baked bread was weighed and kept to cool down for about an hour before the analysis was performed on it. During baking, a considerable amount of carbon dioxide is present in the dough produced by the yeast. As the temperature reaches above 60 °C, stable crumbs start to form as the starch granule swells, bursts, and forms a thick gel-like consistency. At 75 °C, the α -amylase gets destroyed, and the crumb is ready when it reaches 98 °C. The baking also results in a gradual decrease in yeast activity as it dies when the temperature reaches approximately 46 °C [20]. And after baking, the bread is left to rest for an hour because the baking and the analysis were done on the same day, so one hour was considered before starting the analysis.

3.4 Analysis

As discussed earlier the analysis of the bread was done in batches and there was a total of nine batches and the analysis was done one hour after the baking where each batch had three breads in total.

3.4.1 Water Activity

In the water activity meter *METER Aqualab PRE*, the sample is placed in a sample cup that seals inside the sample chamber. Inside, it has a fan, a dew point sensor, a temperature sensor, and an infrared thermometer. The dew point sensor measures the dew point temperature of the air inside the chamber, and the infrared thermometer measures the temperature of the sample. When the water activity of the sample and the relative humidity of the air are in equilibrium, the head space humidity measurement gives the water activity of the sample. In the case of bread, which is a baked food prepared in an oven, it is not initially at internal equilibrium. The surface of the bread has a lower water activity as compared to the internal surface, and hence it is suggested to keep the baked goods for at least an hour before the analysis [21].



(Fig.5 Water activity meter, 2018 Meter Group, Inc.)

Firstly, the instrument was calibrated by choosing a calibration standard that is close to the water activity of the sample that was measured. The calibration standard is a specifically prepared salt solution having a specific molality and water activity constant that are accurately measurable. In this case, the wheat starch pan bread has a water activity of around 0.93 (as discussed in the introduction section), and hence distilled water was used as it's around 1.000± 0.003 (shown in Fig. 6). The vial is emptied of the chosen calibration standard into a simple cup and placed in the sample drawer. Now the drawer knob is turned to the read position, as illustrated in Fig. 5.

Calibration Standard @ 25 °C	Water Activity
17.18 mol/kg LiCl	0.150 ±0.003
13.40 mol/kg LiCl	0.250 ±0.003
8.57 mol/kg LiCl	0.500 ±0.003
6.00 mol/kg NaCl	0.760 ±0.003
2.33 mol/kg NaCl	0.920 ±0.003
0.50 mol/kg KCl	0.984 ±0.003
Distilled Water	1.000 ±0.003

(Fig.6 calibration standard table, 2018 Meter Group, Inc.)

After the calibration, the actual bread where four samples of each bread in a batch were kept inside the sample drawer individually and water activity was measured. Average values of each bread in a batch were taken and recorded in the data set in the *Appendix.*

3.4.2 Moisture content

The moisture content of the bread was measured using the method of loss on drying. It is the loss of weight expressed as a percentage w/w resulting from water and volatile matter.

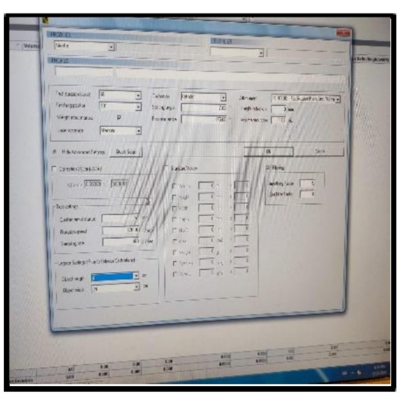
$$\text{Moisture content}_{\text{Wet basis}} = \frac{Wdry - Wwet}{Wwet} \times 100$$

W_{wet} = weight of the sample before drying
W_{dry}= weight of the sample after drying

For analyzing the moisture content of the bread, four samples of each of the three breeds of one batch were taken in an aluminum container and the weight of each sample was measured on the analytical balance and recorded. After measuring the weight, the samples were placed in the drying oven which was already set at a temperature of 105°C overnight [22]. The samples were weighed after the drying and then the values were recorded. The average values of each of the samples were presented in the data.

3.4.3 Volume and Density

The volume and the density were measured using the volume meter 0015 *preten* BVM. The instrument was calibrated by placing the tool approximately the size of the bread (a metal block) and the instrument was calibrated. After the calibration the specifications were provided in the volume meter about the height of the sample. The attachment for the bread was FSPC100: flat support circle 100mm (as shown in *picture:5*(b) Then the bread was placed in the volume meter and the readings of the volume of the sample bread with density was obtained. The average value of the sample was added in the data set in *Appendix*.



a) Picture.5 Volume meter settings

(b) attachment

3.5 ANOVA

ANOVA allows us to compare treatments to see if they are statistically the same or different. ANOVA one compares a single independent variable (such as ‘treatment’ vs a single dependent variable such as ‘moisture content’ [17]. The p-value of moisture content when analyzed against the treatment gave P-value less than 0.05 that means it gives a significant outcome. Hence, the ANOVA of moisture content and treatment shows that there is statistical difference between the groups.[17].

3.6 Principal Component Analysis (PCA)

One of the difficulties inherent in multivariate statistics is the problem of visualizing the data that has many variables. PCA generates a new set of variables called the “principal components” that can be compared to each other even if they have different magnitudes and units. Thus, measures can be compared side by side on the same figure to observe possible correlations [18].

4. Results

All the data collected during the baking can be seen in Table 2 in the Appendix section, which shows all the average values of the measured dependent variables (moisture content, volume, water activity, and density) that were analyzed against the dependent variables (beta glucan, reps, batch, treatment, and guar gum). Table 2 shows the standard deviation and the average values for the three treatments for moisture content, water activity, volume, and density.

Table.2 Shows the average values and standard deviation between the three treatments.

Treatment	Dependent factors	Average	Standard Deviation
1	Moisture content	41.055	5.765
1	Water activity	0.978	0.005
1	Density	0.630	0.087
1	Volume	1261.000	319.736
2	Moisture content	41.625	3.079
2	Water activity	0.975	0.003
2	Density	0.428	0.040
2	Volume	1487.333	121.275
3	Moisture content	43.630	5.205
3	Water activity	0.974	0.010
3	Density	0.436	0.035
3	Volume	1433.786	117.095

Note: - treatment no.1- had 0% beta-glucan and 0% Guar-gum, - treatment no.2- had 1% beta-glucan and 0% Guar-gum and treatment no.3- had1% beta-glucan and 0.5% Guar-gum,

After running an ANOVA in MATLAB, the following results were obtained that showcased the significant differences between some dependent variables with respect to the independent variables:

To determine if the difference between the groups is statistically significant, we can look at the p-value that corresponds to the F-statistics. If the p-value is greater than 0.05, then the null hypothesis is not rejected, and it can be said that we don't have enough data to say that there is a significant difference between the mean of the groups [23].

Independent variable	Volume	Moisture Content	Water Activity	Density
Treatment	0.0645	0.0026	0.0826	9.53E-09
Guar-gum	0.2177	0.0277	0.0277	0.036
B-glucan	0.0616	0.1412	0.1412	8.69E-10
Batch	0.061	0.2424	0.2424	0.9549
Reps	0.5691	0.4376	0.4376	0.3173

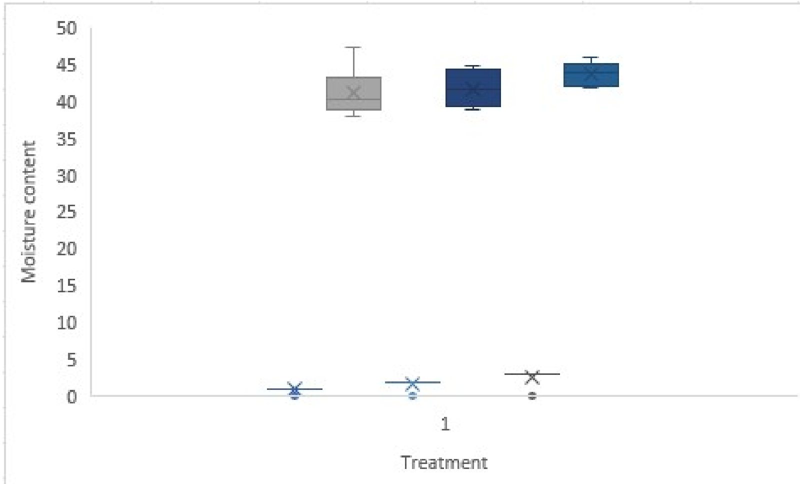
Table.3 P-values of the of volume, moisture content, water activity, density

The following terms involved in the experiment were as follows:

- **Treatment**: There were a total of three treatments in the experiment as explained in the materials and the method section.
- **Batch**: A total of three batches in each treatment 0, 1 and 2 where each batch had three batches.
- **Replications**: All the three treatments had three replications.

4.1 Moisture Content

In *Table-3* we can clearly say that there were differences between the three treatments in the moisture content values of the bread as the p-value is 0.0026. Hence, it can be said that there is a statistically significant difference between the means of the three groups. The major difference between the three treatments is the percentage of beta-glucan and guar-gum. As discussed earlier,the third treatment has 1% beta-glucan and 0.5% guar-gum. In the data set in *Appendix,* it can be observed that in treatment no.3 the moisture content was between 41% to 45%. This falls under the range of the moisture content of the wheat starch pan bread as discussed in the introduction. Hence all the three treatments had moisture content ranging from 38% to 45%.



(Fig.7 graph showing difference in the moisture content between the three treatments)

Fig. 7 shows the difference in the moisture content between the three treatments and also shows the range of the moisture content where gray indicates treatment-1, dark blue color indicates treatment-2 and light blue color indicates treatment-3.

It also gives an idea about the difference in the moisture content when guar-gum is added to the bread. As the p-value<0.05, that implies that the addition of the guar-gum resulted in differences in the breads. In *Fig.8* orange color indicates the range of moisture contentthat was around 38%to 45% without guar-gum and yellow color indicates the range of moisture content with guar-gum that was around 41% to 45%.

Fig.8 graph showing difference in the moisture content with and without guar-gum.

Histograms are a very common method of visualizing data which has range and frequency. Where range is the smallest and the largest values on the data set and frequency is measured by the area of the bar. The shape of the histogram gives a lot of information about the data set.

Fig.9 shows the histogram of the data set of moisture content ranging from treatment.1 to treatment.3 and the histogram is bell shaped, which implies the fact that the data is normally distributed. One of the conclusions that can be drawn from a bell-shaped curve is that most of the data are clustered around the center while the extreme values are on either side of the center [22]. The bell-shaped curve of the moisture content gives us an idea that there were differences in the values of moisture content between the different treatments.

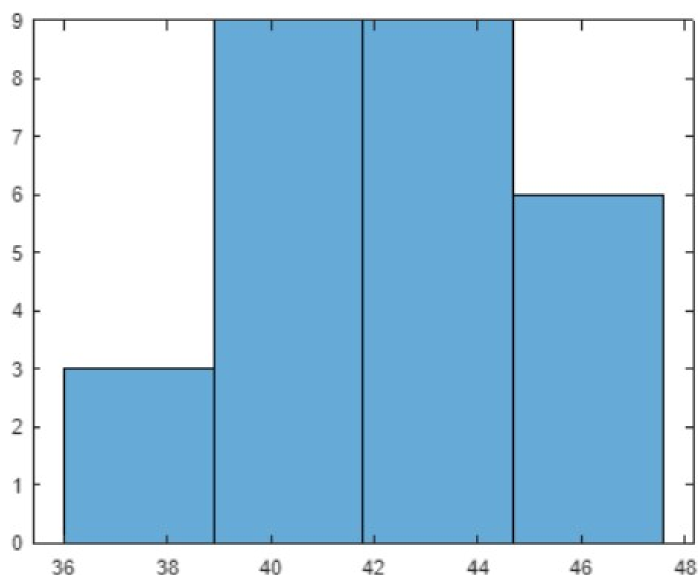


Fig.9 A bell shaped histogram showing normal distribution of moisture content values with respect to treatments.

If the P-value of an ANOVA is less than 0.05 then we reject the null hypothesis and, in this case, we can carry out a post-Hoc test to determine exactly what is the difference between the groups [27]. To understand how the post-Hoc test works Fig.*10* below shows the values of the moisture content with respect to guar-gum. The figure shows a clear difference between the moisture content values when there was absence and presence of guar-gum. It demonstrates different levels of guar-gum where the blue line indicates the guar-gum at 0% and the red line indicates guar-gum at 0.5%. And it is clearly visible that there is a difference between two levels as they don't overlap with each other at all, and this shows that guar-gum has influence on the moisture content of the bread.

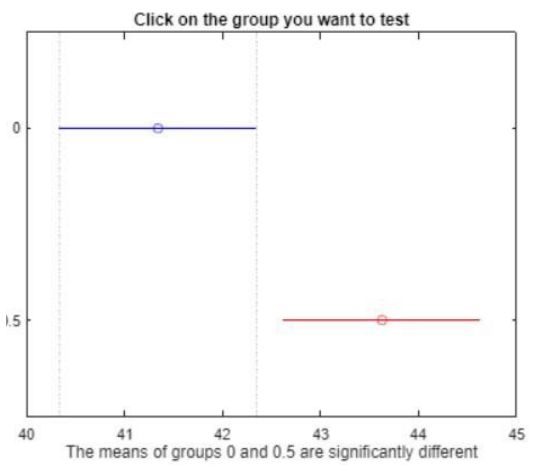


Fig.10 The graph shows the difference between the two levels of guar-gum 0% and 0.5%. The post-hoc test clearly shows the difference in the moisture content values at the two levels.

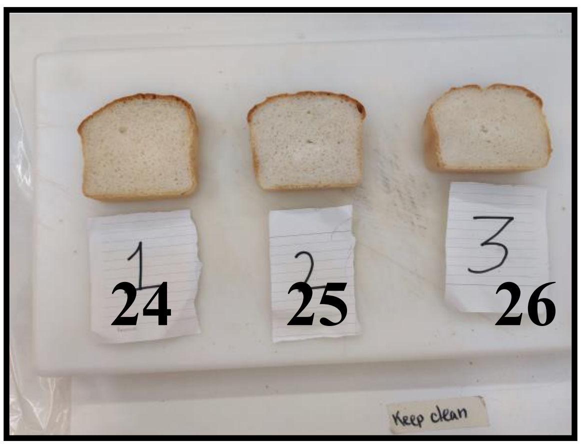
4.2 Volume and Density

Table.2 shows that there were significant differences in the density values. As we can clearly see in the table, the values for density with regards to treatment with guar-gum and beta-glucan were less than 0.05 which implies that we reject the null hypothesis showing that there is a significant difference between the means of the group. For the volume, the values didn’t show any difference between the treatment groups (guar gum and beta-glucan), as all the P-values were greater than 0.05, which means there was no significant difference between the groups.

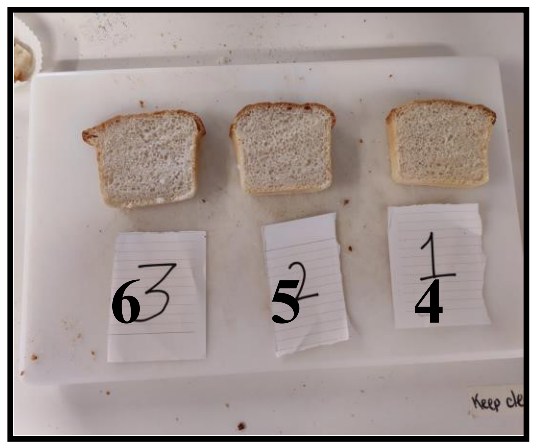
4.3 Water Activity (aw)

The significant difference can also be seen in water activity when guar-gum was added to the bread, as the p-value<0.05 indicates that there is a dissimilarity between the mean of the groups. The water activity went up to 0.98 (this can be seen in the data set in the appendix) when guar-gum was added. In Picture.6 the breads 24, 25 and 26 come under Treatment 3, which has guar gum and beta-glucan, whereas the breads 1, 2, 3 and 4, 5 ,6 belong to Treatment 1 in which there is no presence of beta glucan and guar gum and the breads 11, 12 and 13 had just beta-glucan. The picture clearly shows the difference in appearance of the bread with separate treatment, as the bread with guar-gum looks fluffier.

A

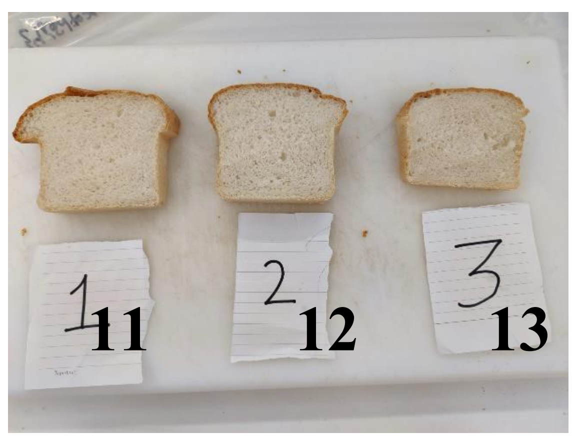


B



C

D



Picture 6: (A) Bread with guar gum and beta-glucan (B) without guar-gum and beta-glucan (C)without Guar-gum and beta-glucan (D) with beta-glucan and without guar-gum.

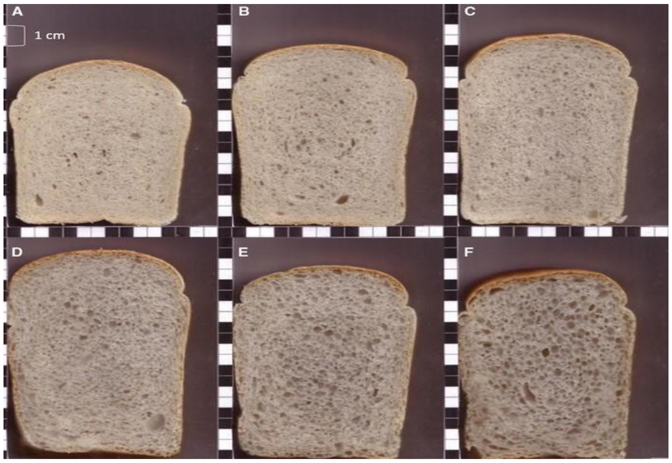
While comparing the sponginess of these breads it was observed that the breads with guar-gum belonging to treatment no.3 were spongier than the bread that were just with beta-glucan and the ones without beta-glucan.

4.4 Bread Form

According to the structure of the bread we can segregate them into different categories by comparing the upper section of the bread. The different forms of the bread show how well the bread is baked and the rising capacity, along with good gas retention and the bounciness of the bread. We can see the difference in the bread form of picture.6 (a) and picture.6 (b). The breads 24, 25, and 26 have a more prominent curve, which is elevated to some extent, and on the other hand, the breads 4, 5, and 6 have a comparatively flat upper surface.

The bread in the *picture.6 A* falls under the category 2 and 3 of the Fig.4 (section 2.7) whereas the bread in the*picture.6 B* falls under 3 and 4 which clearly states the bread structure is implying that theaddition of guar-gum to the bread gives it a considerable amount of bounce.

A closer look at the bread also implies that the pore or air bubble size between the two pictures is quite different. As we can see in picture.6, the cavity between the bread, including the size and the number, is way less in that picture. 6(a) compared to the picture.6 (b) [24] [25]. Also, we can observe that the bread in the picture.6(a) is more like the picture. 7A (taken as an example to show the difference) with little to no cavities present, whereas the bread in picture 6(b) is closer to that of the picture. 7F with a considerable number of cavities. These can be influenced by many factors, including leavening agents, mixing techniques, and dough hydration [21].



Picture.7 Different bread cavities, available from food research and technology, Feb 2017

4.5 Principal Component Analysis (PCA)

PCA gives information about the data set in one graph. The different quadrants of the graph suggest the positive and negative associations of a specific dependent variable with respect to that of an independent variable. In Fig. 11, we can observe that guar-gum, beta-glucan, and treatment have a positive influence on the moisture content. That again confirms that the addition of guar-gum and beta glucan influences the moisture content of the bread.

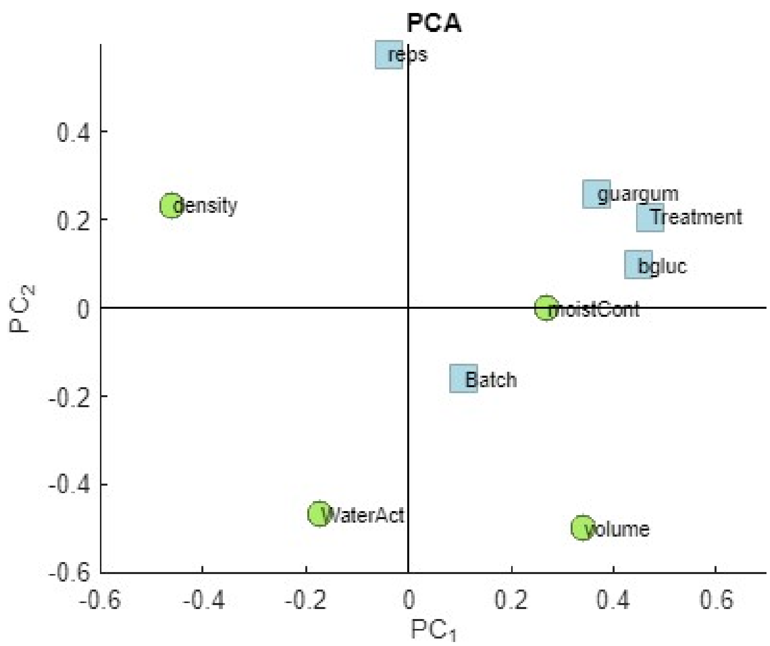


Fig.12 Principal component analysis showing the relationship between the dependent variables like moisture content and water activity and the influence of the independent variables on them.

If we talk about the water activity, it is negatively influenced by guar-gum (opposite and on the diagonal) and hence we can say that the more guar-gum we add to the bread the lesser the water activity. Volume and batch are slightly associated with each other as they are placed in the same quadrant but are negatively associated with beta-glucan, treatment, and guar-gum and hence we can say that the volume is affected by these factors but not very strongly. Here PC_1 has the most influence on the data set and PC_2 is the second most influential factor. The assumptions can be made about PC_1 that It might involve mass as you can see in PC_1 beta-glucan, treatment and guar-gum had the most influence on the moisture content that implies that if the mass of the factor is increased or a particular ingredient is added to the recipe like beta-glucan and guar-gum it influences the dependent factors like moisture content. On the other hand, it is hard to interpret what PC_2 is, as reps have the most influence on it. Hence PCA is the way to confirm the results that we got from ANOVA and is also an overview of the results.

5. Discussion

The major differences were observed in the moisture content values between the treatment which implies guar-gum and beta-glucan influences the moisture content of the Wheat Starch Pan bread and specially guar-gum because considerable number of changes were observed in the last treatment compared to that of first and second treatment in which there was absence of the gur-gum. The percentage of moisture content varies from treatment one to treatment three in which treatment three had a moisture content that ranged from 41%-45% whereas both treatment one and two had a moisture content ranging from 38%-45%. ANOVA clearly shows the P-value<0.05 for moisture content with respect to treatment and guar-gum and thus it can be said that there was a statistical difference between the means of the group. The water activity values also varied when the guar-gum was added, and this implies that guar-gum influences water activity as it went up to 0.98 when it was added. The p-value for density, for beta-glucan, guar-gum and treatment, was less than 0.05, which shows that there were differences in the density of the bread when beta-glucan and guar-gum were added.

The histogram showed a bell curve for the moisture content values for the treatment which shows that the data is normally distributed, which in turn allows to do ANOVA and compare treatment means. And if the data is not distributed normally, we perform the Kruskal-Walli's test. The histogram for volume with respect to beta-glucan was quite uniform showing that it doesn't have much influence on the volume of the bread.

Post-hoc tests also showed differences in the third treatment moisture content values with respect to different levels of guar-gum as the lines were not overlapping with each other at all.

The results from PCA showed that moisture content was influenced by the addition of beta-glucan, guar-gum and treatment factor as they were placed in the same quadrant of the PCA graph. The density was negatively influenced by guar-gum, beta-glucan and the treatment which implies that more we add beta-glucan and guar-gum less is the density of the bread. Same can be said about the volume as it was closely associated with the batch, but beta-glucan and guar-gum didn't have that much influence on the volume of the bread. It was assumed that PC_1 that has the most influence on the variance can be mass as we cannot say only assumptions can be made.

In the screen plot the two unknown factors PC_1 and PC_2 explain about 60% of all the variation in the experiment. PC_1 projects more influence than PC_2. Then it can be said that mass is the main driving force of the results here. PC_2 has little less contribution

Therefore, we can draw the conclusion that the Guar-gum influences the moisture content as well as the water activity of the Wheat Starch Pan Bread; nevertheless, there is not a significant variation in the volume of the bread when guar-gum is added.

6. Conclusion

To summarize the project, the results showed that the addition of the guar gum to the bread didn't have much influence on the volume of the bread, which was a bit surprising as I hypothesized during the start of the project that guar gum would considerably increase the volume of the bread. But the texture of the bread after the addition of the guar gum was quite similar to that of gluten bread. The color of the bread was somewhat white, which is one of the factors that made it look very similar to Gluten-bread. The shelf life of the bread was less than a week. The bread was overall very similar to gluten bread, and the addition of guar gum to the bread had a positive effect on the texture of the bread, except there was no such change in the volume of the bread. Moreover, guar gum gave the dough a more elastic and thread like texture. The presence of guar gum contributed to a chewiness and resilience that are mostly associated with gluten-bread. Additionally, it has a neutral taste, which allows it to overpower the taste of other ingredients, giving the bread a good taste. As gluten-free breads are becoming really common these days, the utilization of ingredients like guar gum results in a bread that is very similar to gluten bread.

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Appendix

ID	B-glucan	Guar-Gum	replications	Batch	Moisture content (W)%	Water Activity (aw)	volume (V) m^3	Density (ρ) Kg/m^3
1	0	0	1	0	38.27378299	0.975	851	0.7775
2	0	0	2	0	40.5267859	0.975	819.5	0.76
3	0	0	3	0	39.32288384	0.976	796	0.79
4	0	0	1	1	41.29467449	0.987	1399	0.62
5	0	0	2	1	40.26999381	0.981	1400	0.62
6	0	0	3	1	39.47815983	0.977	1542.25	0.61
7	0	0	1	2	37.98393723	0.979	1574.5	0.55
8	0	0	2	2	47.34854916	0.976	1499	0.58
9	0	0	3	2	44.99659774	0.975	1467.75	0.62
10	1	0	1	0	38.95884495	0.976	1659.5	0.37
11	1	0	2	0	40.37075721	0.973	1517.5	0.4175
12	1	0	3	0	42.04705085	0.977	1302	0.4925
13	1	0	1	1	38.86330202	0.975	1516	0.4025
14	1	0	2	1	39.51659379	0.971	1477.25	0.4175
15	1	0	3	1	41.54949233	0.975	1326.5	0.47
16	1	0	1	2	44.15803132	0.978	1581.5	0.4
17	1	0	2	2	44.7755505	0.974	1567.75	0.41
18	1	0	3	2	44.38376268	0.972	1376	0.47
19	1	0.5	1	0	45.84925952	0.98	1721.5	0.37
20	1	0.5	2	0	45.36843143	0.979	1578.75	0.41
21	1	0.5	3	0	44.14790978	0.971	1389.5	0.47
22	1	0.5	1	1	43.79755279	0.98	1518.25	0.4125
23	1	0.5	2	1	44.86434581	0.978	1469.75	0.43
24	1	0.5	3	1	42.633996	0.977	1350.25	0.46
25	1	0.5	1	2	41.86972987	0.96	1539.75	0.42
26	1	0.5	2	2	42.26947738	0.9712	1425.25	0.46
27	1	0.5	3	2	41.86502677	0.967	1343.75	0.49

Table.3 Data set (The average values of four samples of each batch with three replications measured during the experiment including moisture content, water activity, volume and density)

Days	Bread	Before Proofing(g)	After Proofing(g)	After Baking(g)
1	1	754	751.34	673.35
1	2	699.98	698.63	623.63
1	3	700.1	700.21	629.65
2	4	700	921.78	865.25
2	5	700.01	931.29	873.06
2	6	779.03	1007.66	944.69
3	7	730.06	721.46	865.63
3	8	730.52	701.06	875.9
3	9	730.5	704.47	916.81
4	10	700.49	696.74	610.72
4	11	700.91	698.64	634.72
4	12	703.64	702.49	640
5	13	700.08	694.88	612.87
5	14	700.49	698.69	621.7
5	15	701.54	698.29	622.63
6	16	700.06	697.03	637.15
6	17	700.49	698.69	646.87
6	18	700.61	700.02	649.25
7	19	700.4	693.71	638.95
7	20	700.32	698.91	648.45
7	21	704.43	701.94	654.9
8	22	701.22	697.14	627.11
8	23	701.15	699.76	632.76
8	24	704.7	702.71	627.01
9	25	700.23	697.16	646.66
9	26	700.27	698.94	650.45
9	27	709.01	707.92	659.44

Table.4 (Weight before and after proofing and after baking)