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Preparation of gluten free bread using the mixture of different cereals grain flour

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Abstract

This investigation aimed to produce gluten-free bread for coeliac disease patients. Due to coeliac disease, some individuals cannot tolerate the protein gliadin present in the gluten fraction of wheat flour. From a commercial perspective, there is a need for the development of gluten-free bread with texture and flavor properties similar to the conventional wheat flour loaf. The dough of Gluten-free bread made from rice flour, corn starch and barley starch at different levels with addition of 1.5 % xanthan gum. The use of rice flour, corn and barley starch was evaluated in several formulations aiming to find a flour mixture to replace wheat flour in the production of gluten free bread. Production parameters were evaluated through sensory analysis. Sensory evaluation, by serving the gluten-free bread to the panelists, was done and the secured scores were analyzed with ANOVA and DMRT at the significance level of $p<0.05$. At this level color, flavor, texture, taste and overall acceptability were found significant with LSD values of 1.3761, 1.5503, 1.4770, 1.3651 and 1.6359 respectively. The resulting breads treatment T2 and treatment T6 were best for color. Treatment T6 was best for flavor, texture, taste and overall acceptability. The overall study introduce that breads prepared with higher percentage of rice flour resulted in a softer product, presenting the better consistency with small alveoli homogeneously distributed. Production parameters were established based on these results and a mixture of flours, composed by 50% rice flour, 40% corn starch and 10% barley starch presented good results originating bread with crumb formed by uniform and well distributed cells, and pleasant flavor and appearance.

Keywords

Celiac Disease,
Gluten-Free Bread,
Corn Starch,
BarleyStarch,
Sensory
Characteristics.

1. Introduction

Cereals and cereal products are one of the major sources for human nutrition (carbohydrates, proteins, dietary fiber, many vitamins and non-nutrients) worldwide (Katina *et al.*, 2005 &Arendt *et al.*, 2010).

Bread has been regarded for centuries as one of the most popular and appealing food product both because of its relative high nutritional value and its unique sensory characteristics (texture, taste, and flavor). However, an increasing number of individuals are suffering from coeliac disease, the life-long intolerance to the gluten fraction of wheat, rye and barley. In particular, coeliac patients are intolerant to some cereal prolamins containing specific toxic oligopeptide sequences. It is

characterized by a strong immune response to certain amino acid sequences found in the prolamin or gliadin fractions of wheat, hordeins of barley, secalins of rye and possibly avenins of oats are involved in the coeliac disease mechanism (Hill *et al*., 2005).

When people with coeliac disease eat foods or use products containing gluten, their immune system gluten responds by damaging or destroying the intestinal villi leading to the malabsorption of nutrients, thus adversely affecting all systems of the body (Feighery, 1999).
Coeliac disease or gluten sensitive enteropathy is a chronic disorder of the small intestine caused by exposure to gluten in the genetically predisposed individuals (Hamer, 2005 &Laurin*et al.*, 2002). Intestinal symptoms can include diarrhea, abdominal cramping, pain and distention and untreated celiac disease may lead to vitamin and mineral deficiencies, osteoporosis and other extra intestinal problems.

Coeliac disease is common complex disease caused by a dietary intolerance to gluten proteins found in all wheat types and closely related cereals such as barley and rye (Katina *et al.*, 2005; Heap *et al.*, 2009 &Zandonadi*et al.*, 2009). The only effective treatment is a strict gluten-free diet throughout the life (Gobbett*et al.*, 2003). The term gluten-free does not refer to the total absence of gluten. In definition of gluten-free, some residual amount of gluten is allowed; this amount is strictly regulated (Arendt *et al.*, 2010). Coeliac disease is not only recognized as the most common food disease throughout Europe, but also in Middle East, Asia, Australia, America, and North Africa. Coeliac disease occurs in adults and children with rates approaching 1% of the population (Arendt *et al.*, 2010*).* According to Schober coeliac disease is one of the most frequent genetically based diseases occurring in 1 of 130-300 in European population and 1 of 111 of the US population (Schober*et al.*, 2003).

Gluten is composed of alcohol-soluble prolamins, which consist of gliadin fraction, and alcohol-insoluble glutelins, which consist of glutenin fraction, portions that trigger the disease. The gluten proteins in wheat have unique properties, such as good water absorption capacity, cohesiveness, viscosity and elastic properties. In a dough system, gliadin contributes to the viscous properties, while glutelin contribute to elastic properties. A proper mixture of both fractions is essential to impart the viscoelastic properties of dough. The adequate mixture of these fractions is only found in wheat, making this cereal the most valuable of all the food grains (Arendt *et al.*, 2010). Maize, rice, tapioca, sorghum, amaranth, buckwheat and potato flour, which are allowed in a gluten-free diet, are not able to supply the same technological characteristics as gluten (Pagliarini*et al.*, 2010). Replacement of gluten is one of the major challenges for gluten-free product development. The main task to food producers is production of high quality, tasty, inexpensive and easily available gluten-free product.

Gluten free- bread is rarely or not practiced in Bangladesh. Gluten free bread was very much liked by coeliac patient who do not like to have wheat bread. This may create a new image for processed of bakery product by using various gluten free cereal grains flour. This will have a direct impact on consumer's food selection and will increase their awareness of the health benefits of processed bakery product in the prevention of chronic diseases.

Gluten free- bread could satisfy consumer's need for convenience as they are readily available, and easy to use, and better nutritional and economical values. Consequently this may help consumer increase their intake of cereal grains to reduce the risk of chronic diseases. This work will also open up more opportunities for the food - processing industries.

Specific Objectives

The aim of this research was to study the influence of flour mixture on gluten-free bread quality and to develop a gluten-free bread loaf with similar quality characteristics to that of standard white bread on the existing processing lines at quality bakers. Addition of flour mixture affect gluten-free bread crumb color, structure of crumb, weight loss and dry off of bread.
Considering the above information as accumulated the present study was carried out to achieve the following objectives:

1. To prepare gluten-free bread.
2. To analyze chemical composition and the sensory characteristics.

2. Materials and Methods

The experiment was conducted in the laboratory of the Department of Food Engineering and Technologyunder the faculty of Engineering, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh.

2.1 Sample Collection

Gluten-free flours were purchased from a commercial market and transported to the laboratory. Sieving of

flours for the removal of physical impurities. All other ingredients were purchased from commercial sources or directly from the suppliers, keeping the same specification in all experiments. Distilled water was used for all analysis. Polypropylene bags, Aluminum foil paper and standard grade chemicals required for the work done were used form the laboratory stock.

2.2 Material

The ingredients used were: rice flour, corn starch, barley starch, granulated white sugar, dried milk, iodized refined salt, baking powder, egg, xanthan gum, yeast, distilled water & flavoring agents.

2.3 Preparation of Gluten-Free Bread

To prepare the dough's of gluten- free breads, 6% compressed yeast dissolved in warm water (40℃), 2% salt, 25% sugar and 6% baking powder were added separately to the prepared flours while xanthan gum was added at 1.5% to the gluten-free flour mixtures and mixed to form the dough's, which are left at room temperature for 40 minute to complete fermentation. The dough's are cut into loaves, which baked at 200℃ for 30 min in an electric oven. Measurements of the loaves were carried out after cooling to room temperature for 1 hr.

Finally the gluten- free breads were termed as:

Treatment T1: Gluten-free bread with 25% rice, 25% corn, 50% barley and 0.5% xanthan gum.
Treatment T2: Gluten-free bread with 35% rice, 35% corn and 30% barley, 0.5% xanthan gum and 8% whole egg.
Treatment T3: Gluten-free bread with 40% rice, 40% corn and 20% barley, 1% xanthan gum and 18% whole egg.
Treatment T4: Gluten-free bread with 45% rice, 40% corn and 15% barley, 1.5% xanthan gum and 18% whole egg.
Treatment T5: Gluten-free bread with 40% rice, 45% corn and 15% barley, 1.5% xanthan gum and 18% whole egg.
Treatment T6: Gluten-free bread with 50% rice, 40% corn and 10% barley, 1.5% xanthan gum and 18% whole egg.

2.4 Chemical Analyses

Gluten-free breads were analyzed to determine the proximate analysis such as moisture, crude protein, crude fat, ash and total carbohydrate.

2.4.1 Determination of Moisture Content

Moisture content was determined adopting AOAC (2005) method
At first, the weights of empty dry crucibles were taken and 5g samples were taken in each dried crucible. The crucible with samples were dried in an air oven at 100^0C for 24hrs or more until the weight become constant. The crucibles were cooled in desiccators. The crucibles were removed from desiccators and weighed soon after reaching room temperature.

The loss in weights was taken as the moisture loss of the sample and the percent of moisture content in the sample were calculated by using the following formula:

$$\% \text{ Moisture} = \frac{w_1 - w_2}{w} \times 100$$

Here, w_1 = weight of sample with crucible
w_2 = weight of dried sample with crucible
w = weight of sample

2.4.2 Determination of Crude Protein

Protein content was determined using AOAC (2005) method. The accepted method was as follows:

Reagent:

1. Concentrated H_2SO_4
2. Digestion mixture.
- Potassium sulphate =100g
- Copper sulphate=10g and
- Selenium dioxide=2.5g
3. Boric acid solution=2% solution in water
4. Alkali solution=500g sodium hydroxide dissolved in water and diluted to 1 litre.
5. Mixed indicator solution= Bromocresol 0.1g and Methyle red 0.2 g dissolved in 100ml ethyl alcohol.
6. Standard HCl=0.1 N

2gm of each sample were taken in a 250ml of Kjeldahl flask. 2gm of digestion mixture was added with the sample. 25ml of concentrated H_2S0_4 was added for oxidation. The flask was placed in an inclined position on the stand in digestion chamber, heated continuously until frothing ceased and then simmered briskly. The solution became cleaned in 15-20mintues, continued heating for 45mintues.

After cooling, 100ml water was added and transferred quantitatively to a 1 litre round bottom flask; the final

volume was about 500ml. Added gently down the side enough NaOH solution to form a precipitate at cupric hydroxide and immediately concentrated the flask to stream-trap and condenser. To a 500ml conical receiving flask 50ml of boric acid solution, 50ml distilled water and 5 drops of indicator solution were added. Positioning the condenser distillation was carried out for 40-45 minutes or until about 250ml of distillate was obtained.

The content receiving was titrated against HCl acid solution, the end point was marked by a pink color and the readings for blank sample was also determined and deducted from the titration. A protein conversion factor was used to calculate the percent protein from nitrogen determination. Percentage of nitrogen and protein calculated by the following equation:

$$\%\ \text{of}\ N_2 = \frac{(A-B)\times \text{Normality of HCl} \times \text{Volume made up of the digest} \times 100 \times 14}{\text{Aliquot of the digest taken} \times \text{Weight of the sample} \times 1000}$$

% Crude Protein = % of N_2×Protein factor
Here; Protein factor = 5.5

2.4.3 Determination of Fat

AOAC method (2005) was used to determined crude fat content of the samples.

The dried sample remaining after moisture determination was transferred to a thimble and the top of the thimble was plugged with a wad of fat free cotton. The thimble was dropped into the fat extraction tube of a soxhlet apparatus. The bottom of the extraction tube was attached to a soxhlet flask. Approximately 75ml of or more of anhydrous ether was poured through the sample in the tube into the flask. The top of the fat extraction tube was attached to the condenser. The sample was extracted for 16hours or longer on a water bath at 70-80^{0}c. At the end of the extraction period, the thimble from the apparatus was removed and distilled off most of the petroleum ether by allowing it or collected in soxhlet tube. The petroleum ether was poured off when the tube was nearly full. When the petroleum ether had reached small volume, it was poured into a small, dry (previously weight) backer through a small funnel containing plug cotton. The flask was rinsed and filtered thoroughly using ether. The ether was evaporated on steam bath at low temperature and was then dried at 100^{0}c for 1 hour, cooled and weighed. The difference in the weights gave the ether soluble materials present in the sample. The percent of crude fat was expressed as follows:

% Crude Fat

$$= \frac{\text{Weight of petroleum ether soluble material}}{\text{Weight of the sample}} \times 100$$

2.4.4 Determination of Ash

AOAC method (2005) was used to determine the total ash content.

2g of each sample were taken in dry, clean porcelain dishes and weighed accurately. Hot air oven method was applied to remove the moisture. Then the samples were burned on an electric heater. These were done to avoid the loss of sample in the muffle furnace under higher temperature. Then samples were transferred into the muffle furnace and burnt for 4-6hours at a temperature of 550^{o}c and ignited until light grey ash resulted (or to constant weight). The samples were cooled in desiccators and weighed. The ash content as expressed as:

$$\%\text{Ash} = \frac{\text{Weight of Recidue}}{\text{Weight of the sample}} \times 100$$

2.4.5 Total Carbohydrate

Total carbohydrate content of foods, for many years, been calculated difference, rather than analyzed directly. Under this approaches, the other constituents in the food (protein, fat, water, ash) are determined individually, summed and subtracted from the total weight of the food. This is referred to as total carbohydrate by difference and is calculated by the following formula:
100-(weight in grams [protein+fat+water+ash] in 100gm of food)

It should be cleared that carbohydrate estimated in this fashion includes fiber, as well as some components that are not strictly speaking carbohydrate, e.g. organic acids. Total carbohydrate can also be calculated from the some of the weights of individual carbohydrates and fibers after each has been directly analyzed.

2.5 Sensory Evaluation of Gluten-Free Bread

For statistical analysis of sensory data six different types of gluten free bread were evaluated for color, flavor, texture and overall acceptability by a panel of 10 testers. All the testers were the students and teachers of the faculty of Engineering, Hajee Mohammad Danesh Science and Technology University, Dinajpur. The panelists were briefed before evaluation. Six types of

gluten-free bread were presented as randomly coded sample to the 10 panelists. The test panelists were asked to rate the different gluten free bread presented to them on a 9 point hedonic scale with the ratings of: 9 = Like extremely; 8 = Like very much; 7 = Like moderately; 6 = Like slightly; 5 = Neither like nor dislike; 4 = Dislike slightly; 3 = Dislike moderately; 2 = Dislike very much and 1 = Dislike extremely. The results were evaluated with Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT) procedures of the Statistical Analysis System.

3. Results and Discussion

Demand of gluten-free breadis growing as more people for diagnosed with coeliac disease and other types of gluten sensitivity. The gluten-free bread is a ready to eat bakery product prepared from different gluten-free flours. Gluten-free breads were studied for its formulation, acceptability and determined its chemical composition. The acceptability and proximal chemical composition were evaluated through sensory evaluation and chemical testing procedure.

The quality of bread depends on quality of flour, so it is important to choose high-quality flour. One of the most important characteristics of the flour quality is moisture content. Moisture content of flour depends on the grain milling technology and flour storage conditions. Moisture content of flour must not exceed 15%; otherwise the flour contains free water, which contributes to the development of microorganisms and effect positively enzyme activity. The process of nutrient degradation begins as a result of enzyme activity and causes adverse changes in quality of flour. Initial chemical composition of flours used in the formulation of gluten-free bread shown in Table 1.

Table 1: Initial Chemical Composition of Gluten-Free Flours

Chemical composition	Rice flour	Corn flour	Barley flour
Moisture (%)	11.0	12.25	10.6
Ash (%)	0.7	0.55	0.6
Fat (%)	0.8	1.82	1.2
Protein (%)	7.5	6.68	9.9
Carbohydrate (%)	80.0	78.7	77.7

3.1 Chemical Analysis

The proximate chemical composition of gluten-free breads samples (moisture, crude protein, crude fat, crude fiber and ash contents) was determined using the method of AOAC (2005), while total carbohydrates were determined by difference subtracting 100g minus the sum of moisture, protein, ash and fat expressed in grams/100 grams (FAO, 2003)and the results were Presented in Table 2:

Table 2: Chemical Composition of Prepared Gluten-Free Bread per 100g

Chemical Composition	T1	T2	T3	T4	T5	T6
Moisture (%)	15	15.5	16	15	12	10
Ash (%)	1.80	1.50	1.90	2.10	2.30	2.50
Fat (%)	2.67	3.01	3.35	2.53	2.97	2.00
Protein (%)	5.731	6.014	6.341	6.622	6.314	7.084
Carbohydrate (%)	74.799	73.976	72.409	73.748	76.416	78.416

T= Treatment

Chemical composition of the prepared gluten-free bread was analyzed and the results were mentioned in Table 2 &Figure 1:

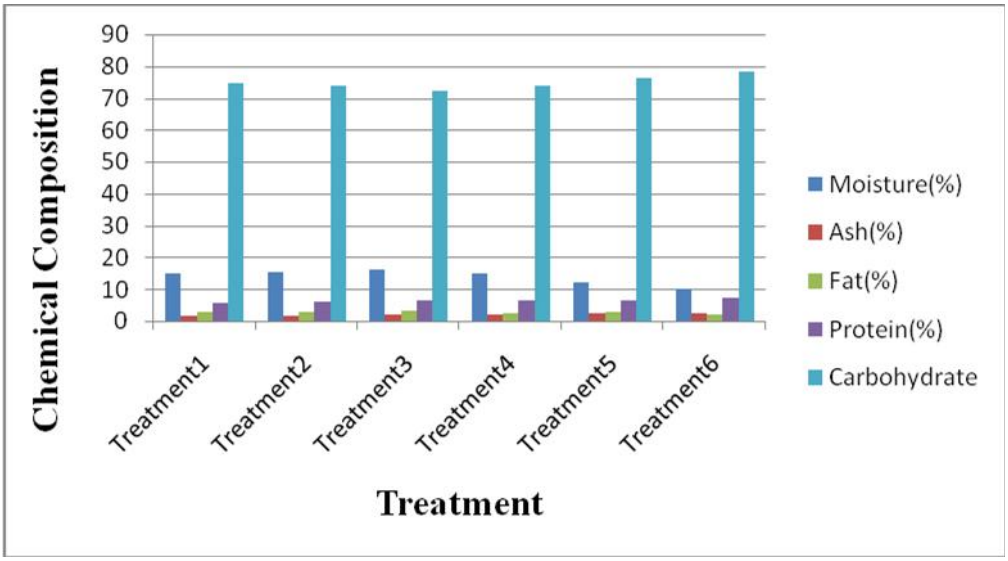

Figure 1: Chemical Analysis of Gluten-Free Bread

From (Fig:1) overall analysis treatment T6 secured the best score for carbohydrates, protein, moisture, ash and fat content. In this study, the gluten-free breads shows the great variation in the nutrient composition, being starchy based foods low in proteins and fat content and high in carbohydrate content. The possible reasons for securing this acceptability is explained below in respect of total carbohydrates, moisture, ash and fat content.

The different proximal composition of gluten-free bread commercial samples studies could be affected by many factors such as the wide range of complex ingredients added and their combinations, besides the additives used to improve the structure, mouth feel, acceptability and shelf-life of these products. Moller *et al.*, (2013) stated the chemical composition of gluten-free commercial sweet bread, which contained 2.18g of proteins, 7.80g of fats, 0.86g of ash and 76.26g of carbohydrates. According to Yazynima*et al.*, (2008) reported the nutritional composition of two kinds of gluten-free crispy breads, which contained 3.5-6.0g of proteins, 3.0-6.5g of fats,1.0-5.5g of ash and 71-80g of carbohydrates. As stated by Segura *et al.*, (2011) the protein, fat, mineral and carbohydrate content of the gluten-free bread showed great variation, ranging from 0.91g to 15.05g, 2.00g to 26.10g, 1.10g to 5.43g, and 75.6 g to 92.5g respectively.

The present study shows that the marketed gluten-free breads are carbohydrate based products. They have the great variation in their protein, fat and mineral content, in contrast to the carbohydrate content in the proximate composition observed in gluten-free bread products (Rosell, 2011).

3.2 Sensory Evaluation

The sensorial properties were determined by trained panelists and the results of the sensory analysis of the gluten-free bread samples are presented in Figure 2.The panelists scored showing their degree of preference in respect of color, flavor, texture, taste and overall acceptability of the gluten-free bread. The results were evaluated with Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT) procedures of the Statistical Analysis System at the significance level of $p<0.05$. Results highlighted that the addition of hydrocolloids (Xanthan gum) and whole egg improved the sensorial quality of gluten-free breads in most of the cases. In fact, the sensorial data showed that the treatment T6 with higher percentage of rice flour, hydrocolloids (Xanthan gum) and whole egg had an overall quality values higher than that of other treatment, whereas the samples added with lower percentage of rice flour, hydrocolloids (Xanthan gum) and whole egg. As stated by Padalino *et al.*, (2011) to improve the sensorial quality of gluten-free maize bread with 20% oat bran, hydrocolloids and white egg were used in the experimental step.

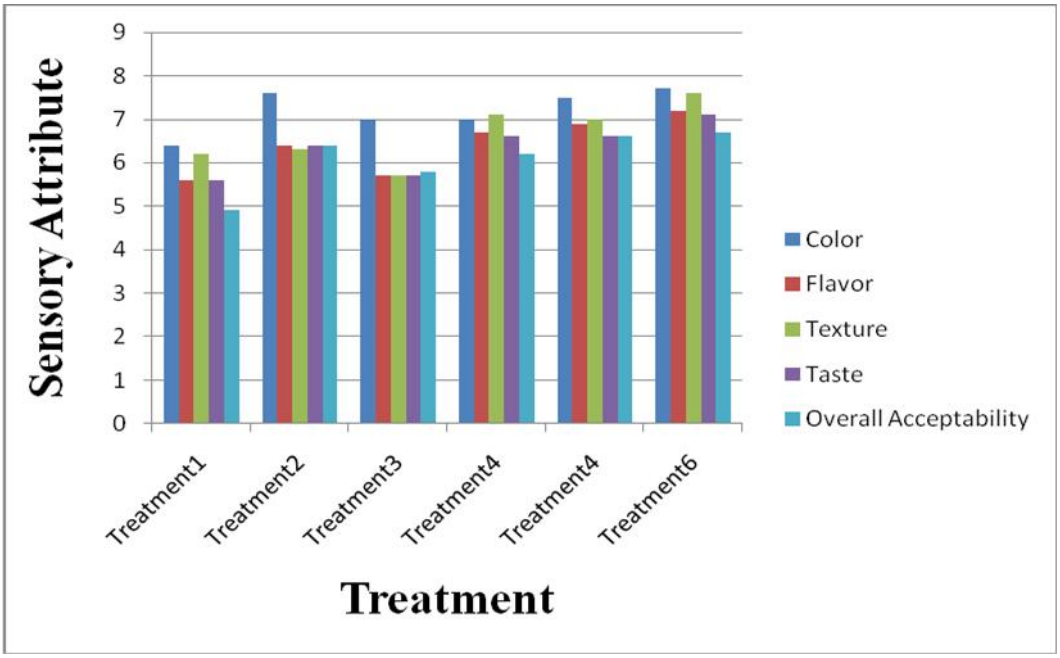

Figure 2: Sensory Evaluation of Gluten-Free Bread

From(Fig:2) the overall analysis treatment T6 secured the best score for color, flavor, texture, taste and overall acceptability closely followed by treatment T5 and T4. Treatment T6 was best for color and T2 was very near to T6. Treatment T1 was less liked among all the treatments and T5 was least liked. So, it can be concluded that T6 is the best product in this researched.

The mean scores for color, flavor, texture, taste and overall acceptability of six types of gluten-free breads are shown in Table 3:

Table 3: Mean Scores of Gluten-free Bread for Different Sensory Attributes

Treatment Code	Sensory attributes				
	Color	Flavor	Texture	Taste	Overall Acceptability
T1	6.4^{b}	5.6^{b}	6.2^{bc}	5.6^{b}	4.9^{b}
T2	7.6^{a}	6.4^{ab}	6.3^{bc}	6.4^{ab}	6.4^{a}
T3	7.0^{ab}	5.7^{b}	5.7^{c}	5.7^{b}	5.8^{ab}
T4	7.0^{ab}	6.7^{a}	7.1^{ab}	6.6^{a}	6.2^{a}
T5	7.5^{a}	6.9^{a}	7.0^{ab}	6.6^{a}	6.6^{a}
T6	7.7^{a}	7.2^{a}	7.6^{a}	7.1^{a}	6.7^{a}
LSD P<0.05	1.3761	1.5503	1.4770	1.3651	1.6359

The results obtained in the experimental step highlighted that the overall quality of gluten-free bread enriched with the highest rice flour concentration had a score below or close to acceptability threshold for most of the examined sensorial attributes. Therefore, in order to improve the sensorial quality of gluten-free bread with 50% rice flour, hydrocolloids (Xanthan gum) and whole egg were used in the experimental step.

4. Summary and Conclusion

Addition of hydrocolloids such as guar gum, xanthan and cellulose can improve mechanical structure of gluten-free breads. In this study, xanthan gum addition at 1.5% w/w rice flour gave the best improvement of dough rheological properties.

Sensory evaluation showed that treatment T2 and treatment T6 were best for color. Treatment T6 was best for flavor, texture, taste and overall acceptability. The study found treatment T6 as the best product.

The nutritional evaluation of different commercial gluten-free breads revealed that they are mainly starchy foods with great divergences in fat and protein composition, due to the occasional protein enrichment.

In consequence, these products have very low contribution to the recommended daily protein intake, but higher contribution to the carbohydrate dietary reference intake than their gluten containing counterpart.

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