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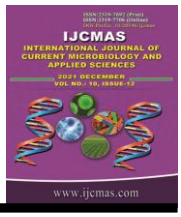

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Formulation and Characterization of Gluten Free Bread based on Quinoa and Rice Flour

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A B S T R A C T

The aim of the present study is to formulate gluten free bread enriched with quinoa and rice flour. Physicochemical properties of flour including water absorption capacity, bulk density, foaming capacity, swelling capacity and emulsion capacity were analysed. Then sensory score of the product such as colour, flavour, taste, and overall acceptability and nutritional compositions includes moisture, ash, protein, fat, carbohydrate, energy were analyzed by Hedonic scale and AOAC method. Data was analysed by Analysis of variance (Two-way ANOVA or Two-way classification) technique treatment of the developed food products. The result of sensory score reported that treatment (T_2) found to be best. The result of the present study revealed that rice flour and quinoa flour, enhance the nutritive value of gluten free bread.

Introduction

In last few years, the demand of gluten free diet has raised as the number of celiac patients is increasing. Celiac disease is auto-immune diseases that caused due to gluten insensitivity, can damage the stomach and small intestine (Crockett, 2009; *et al.*,). Celiac patients triggered to even only a small amount of gluten, a wheat protein that can lead to the small-intestine inflammation and thereby malabsorption of nutrients. Restriction of gluten diet for whole life is the only best treatment for celiac disease. Grains such as wheat, barley, rye are gluten rich and staple diet in most of the counties. Consuming alternative cereal grains and psedocereals such as sorghum, rice, teff, amaranth, buckwheat quinoa is one of the possible solutions to reverse the small intestine damage. In addition, theses grains have high nutritional quality and therapeutic properties. Gluten is the main structural protein in wheat which is required for desired volume and crumb

structurein dough system. Gluten is made up of glutenin and prolamin, which provides elasticity and extensibility respectively. They are the primary ingredient in cereal-based products especially bakery products and provides a continuous gluten network, resulting in a proper crumb structure. Gluten-free products, especially yeast leaved bakery products, have few technological problems such as poor appearance, texture, sensory properties and reduced shelf life. Lack of gluten, results in weak batters, decreased volume and crumbly structure, therefore its removal is the serious problems for bakers. Non yeast leavened bakery products such as cakes, muffins, biscuits are easier to obtain because the lack of visco-elasticity of their batters is not an issue, as gas is generated during baking and immediately retained. Bakery products are convenient, ready to eat and liked by all age group. Lack of gluten matrix is the major issue in formation of gluten free leavened bread.

The gluten-free market is becoming the most prosperous market in the field of food and beverage in the near future (Agriculture and Agri-Food Canada, 2011). Commercial gluten free breads are pale, have poor flavour, taste and lack fibre, vitamins and nutrients. There is urgent need to investigate new ingredients and technologies that improve the textural, sensory and nutritional quality of gluten free bread. Different cereals and pseudo cereal such as rice, amaranths, quinoa have been utilized for developing gluten-free food products including breads, pasta, cookies. Of these, rice flour as been used as basic ingredient in gluten-free bread, due to its lack of gluten, and easily digested carbohydrate. It is attractive, due to its unique properties, such as hypo allergenicity, colour lessness, bland taste and a high amount of easily digested carbohydrates, making it desirable in celiac patients. Quinoa (Chenopodium quinoa Wild) is pseudo cereal belongs to Chenopodiaceae family. It is richin high biological-value proteins, fibre, essential fatty acid, vitamins, and minerals. It has low glycaemic index and rich in various phytochemical compounds.

Therefore, it could be used as a functional compound in gluten-free bread with good rheological and sensory characteristic due to its desirable chemical composition and good nutritional value (Repo-Carrasco *et al.,* 2003). Thus, Quinoa and rice flour is one of the most emerging healthy alternatives to gluten containing grains in the gluten-free diet as it is a good source of proteins and a wide range of nutrients. They have shown positive effects on the rheological and sensory characteristics of bakery products, such bread and cookies with good nutritional quality. The aim of the present work is to formulate the gluten free bread by using rice flour, and quinoa flour. Then physicochemical, nutritional properties and consumer acceptability of formulated products were studied.

Materials and Methods

Development of products

The basic recipe of formulation for the breads was performed according to Aastha Deswal *et al.,* 2015. The treatment includes Rice flour (30-70%) and Quinoa flour (20-40%) that it is clearly present in table 1. Baking was done in an oven at baking temperature between 170 ^{0}C-190^{0}C and baking time 30-40 min. After baking the loaves were cooled at room temperature, sliced and packed in polyethylene bags.

Physicochemical properties of flour

Water absorption capacity

Water absorption capacity was determined by following the procedure of Saetung (2010) with little modification. One gram of each

flour sample was mixed to10ml of distilled water. Then, slurry was vortexes for one min and allowed to stand for 30 min and then centrifuged at 3000rpm for 10 minutes. Excess water was decanted and samples were allowed to drain.

Bulk Density

It was measured by following the procedure of Saetung (2010).Take 10gms of tested flour in a 25ml graduated cylinder and pack it by giving a gentle tapping of the cylinder. The final volume of the test flour was measured and expressed as g/ml.

Foaming capacity

The foam capacity was determined using the method of Saetung (2010). Take 2 g of the flour and blend into100ml of distilled water for 6 min at room temperature and then mixtures were immediately placed in a 250 ml measuring cylinder. The volume of the foam was recorded. It was expressed as the % increases in the volume.

Swelling Capacity

Procedure

10 ml of water taken into measuring cylinder and add 1g of sample then mark the bed volume. Incubate for 18 hours, again take bed volume.

Calculation

$$\text{Swelling Capacity} = \frac{\text{final volume} - \text{initial volume} \times 100}{\text{Weight of sample}}$$

Emulsion capacity

The emulsion capacity was determined by the method of Martha Padial *et al.,* 2010 with little modification. Emulsion (1 g sample, 10 ml distilled water, and 10 ml soybean oil) was prepared in calibrated centrifuged tube and the mixture was centrifuged at 2000 g for 5 min. The ratio of the height of emulsion layer to the total height of the mixture was calculated as emulsion activity in %. The capacity of the emulsion of the sample was estimated after heating it contains in calibrated centrifuged tube at 80°C for 30 min in a water bath followed by cooling with tap water for 15 min and then centrifuged at 2000 g for 15 min. The emulsion capacity (%) was calculated as the ratio of the height of emulsified layer to the total height of the mixture.

Sensory characteristic of the gluten free bread

Sensory evaluation of the food products for their acceptability was done by panel members of 10 judges. Panel members are selected based on their performance in initial evaluation trails. Sensory descriptors o the samples were appearance, colour, aroma, taste, texture and overall acceptability. The nine-point hedonic scale score card, purposefully made for analysis was used for sensory evaluation (Sri Lakshmi 2014).

Nutritional analysis of gluten free flour and bread

The chemical analysis of the flour and developed bread ingredient was done by the standardized procedure of AOAC (2010).

Shelf life of bread

Shelf-life of optimized (T_2) gluten free bread includes TPC, Coliform, Yeast and Mould count was done at 1,3,6,9 days. The total plate count (CFU/g) was determine by the standard plate count method on MRS agar plates and incubating for 48 h at 37 °C. The yeast and mold count of gluten free bread was determine using potato dextrose agar medium after incubating for 48 h at 37°C. The coliform

bacteria on the MacConkey agar was done after incubated at 37°C for 48-72 hours.

Statistical analysis

The data was statistically analysed by using Analysis of variance (Two-way ANOVA or Two-way classification) technique. A significant difference between the treatments was determined by using CD (Critical Difference) test (Appendix). 't' Test was performed for comparing the difference in the nutritional content between control and best treatment of the developed food products (*Chandel, 2006*).

Results and Discussion

Physicochemical Properties of Gluten Free Flour

The physicochemical properties of gluten free flours were shown in figure 2.The result of our study reported that quinoa flour (15.50%), exhibit higher water absorption capacity than rice flour (12.7%).

Good water absorption capacity is a desirable consistency to formulate sauces, dough, bakery products. High water absorption capacity is due to the presence of more hydrophilic constitutes and it suggest that the flour could be used for bakery products especially breads and cakes.

The value of swelling capacity and emulsion stability of quinoa flouris 19.43 and 47.03 respectively as shown in the below figure 3. Swelling capacity indicate the ability of grain to absorb water and swell to give desirable volume.

It indicates the extent of associative forces in the starch granules and is considered a quality measure in bakery products. Lower value of swelling capacity (16.76) and emulsion stability (42.84) of rice flour might be attributed on size of particles and species varieties. Although, rice flour has high foaming capacity (18.63), and bulk density (0.813) as shown in table 3.

High foaming stability signify the stability of large air bubbles. This indicate that air bubbles of rice flour particles are not easier to collapse and surrounded by flexible protein film.

The bulk density is the function of mass and volume; it could determine the handling requirement and closeness of packaging as shown in figure 2. In consistent to present study, ogungbenle (2010) found that quinoa has a high-water absorption capacity and low foaming capacity and stability that lead to the good leavened baked product.

Sensory characteristic of gluten free bread

Colour and appearance

Figure 1 shows that the mean of sensory scores obtained for gluten free bread in relation to colour and appearance, express that T_2(8.1)has the highest score (8.1), followed by T_1 (7.8), T_0 (8.1) and T_3 (7.75). It indicates that the treatment T_2 (30 was liked very much by the responders, whereas T_o was liked moderately regarding the colour and appearance of gluten free bread by the responders.

Texture

Figure 1 shows that the mean of sensory scores obtain and analysed for gluten free bread in relation to texture, shows that T_2 has the highest score (8.1), followed by T_1 (7.8), T_o (8.1) and T_3 (7.75). It indicates that the treatment T_2 was liked very much by the responders, whereas T_o was liked moderately regarding the colour and appearance of gluten free bread by the responders.

Taste and flavour

Figure 1 shows that the mean of sensory scores obtain and analysed for gluten free bread in relation to taste and flavour, shows that T_2 has the highest score (8.1), followed by T_1 (7.8), T_o (8.1) and T_3 (7.75).

It indicates that there is a significant difference between the all three treatments of gluten free bread regarding their texture, so it can be easily concluded that gluten free bread incorporated with 50% quinoa flour, 40% rice flour, 5% xanthan gum, 5% yeast, 10% skim milk powder i.e., T_2 (8.2) has good consistency and is best from other 2 treatments.

Overall acceptability

Figure 1 shows that the mean of sensory scores obtained for gluten free bread in relation to overall acceptability, indicates that T_2 has the highest score (8.2), followed by T_1 (7.1), T_0(8.1) and T_3 (7.75). It indicates that there is a significant difference between the all three treatments of gluten free bread regarding their consistency, so it can be easily concluded that gluten free bread incorporated with 50% quinoa flour, 40% rice flour, 5% xanthan gum, 3% sugar, 5% yeast, 10% skim milk powder i.e., T_2 (8.2) has good in overall acceptability.

Nutritional composition of the developed products

The control and treatment samples were analysed and calculated for their various nutrient contents including moisture, ash, protein, fat, and energy calories. Figure 4 present the nutritional composition of gluten free bread and shows that highest moisture content was found in T_0(19.99±0.22g) followed by T_1(19.04±0.36g) and T_2(19.36±0.07g), T_3(19.81±0.31). Lowest moisture content was found in control T_1(19.04±0.36g). Although, no significant difference were found in moisture content of T_1 and T_2. It signifies that T_1 and T_2 have higher shelf life due to lower moisture content followed by T_3 and control has lowest shelf life in comparison to all formulated gluten free bread. The might attribute due to the presence of refined wheat flour in control.

The ash content of gluten free bread varies (0.08 to 0.88g). Ash content of all treatments were found significant different from each other. The ash content of treatment T_1 (0.88±0.07g) was found to be highest followed by T_0 (0.08±0.31g), T_2(0.88±0.07g), T_3 (0.66±0.31g) as shown in figure 4. The higher ash content signifies the higher mineral content in bread.

Highest ash content in T_1 signifies the highest mineral content in treatment T_1, attributed to higher mineral in quinoa than rice. Minimum ash content found in control, this might be low mineral content in refined wheat flour than quinoa and rice flour (Paulina sarbak *et al.*,).

The highest protein content found inT_3(13.98±0.68g) followed by T_0 (8.8±0.18g), T_1(9.66±0.88), and T_2(8.15±0.22g) as shown in figure 4.The protein content was found highest in T_3, this might be due to higher protein content of Quinoa and lowest protein content found in control attributed to lower protein content wheat. Breads are low in fat and in present study it ranges from 8.8g to 8.15.

The figure 4 illustrate control have lower fat content and T_1 ((3.33±0.28g) has highest fat content followed by T_0(0.72±0.31), T_2 (2.14±0.31g) and T_3 (1.56±0.22g). (Maria Estela *et al.*, 2013*)* reported the quinoa and rice flour high in PUFA content than refined wheat flour. It signifies that gluten free bread have high fat content, although they are good fat for health.

Table.1 Different flours and ingredients taken to make gluten free bread

Ingredient	Amount
Rice flour	30-70%
Quinoa flour	20-40%
Skim milk powder	10g
Oil/butter	5g
Yeast	6g
Sugar	5g
Xanthan gum	0.5g

Table.2 Control and treatment combinations of gluten free bread

Treatments / Ingredients	T_0	T_1	T_2	T_3
Refined wheat flour(g)	80	-	-	-
Rice flour(g)	-	35	50	60
Quinoa flour(g)	-	45	30	20
Xanthan gum(g)	0	0.5	0.5	0.5
Skim milk powder(g)	10	10	10	10
Sugar(g)	5	5	5	5
Oil/ butter(g)	2	1.5	1.5	1.5
Yeast(g)	3	3	3	3
Total	100	100	100	100

Table.3 Shelf life of optimized gluten free bread (T_2)

	(Day 1)	(Day3)	(Day6)	(Day9)
Total plate count (TPC)- log CFU/g	2.55	3.25	5.38	7.13
Yeast / Mould (CFU/g)	$1.1x10^1$	$1.9x10^1$	$2.6x10^2$	$3.1x10^4$
Coliform	0	0	0	0

Fig.1 Average sensory scores of controls and treated samples of bread

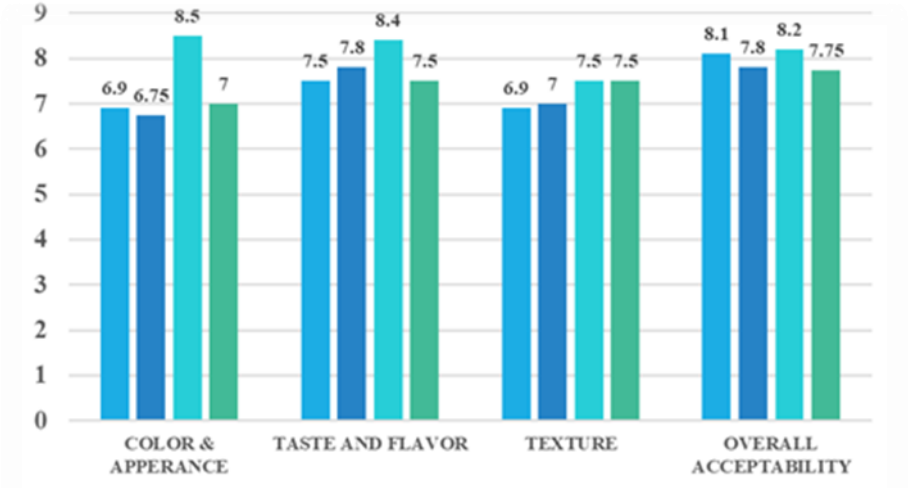
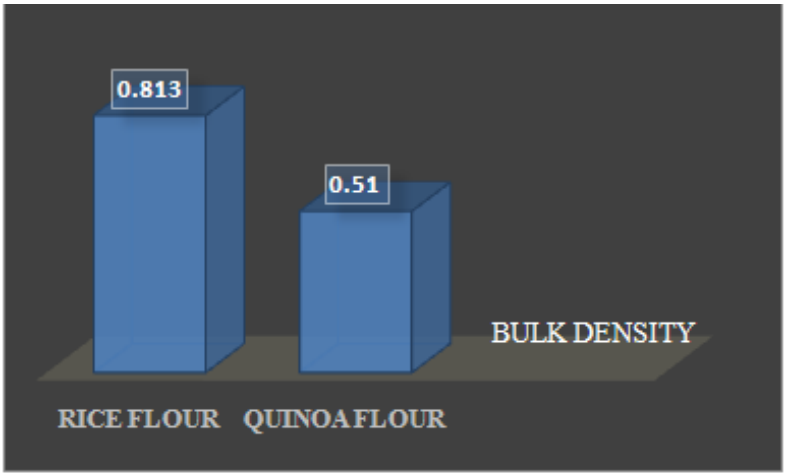


Fig.2 Physicochemical properties of gluten free flour



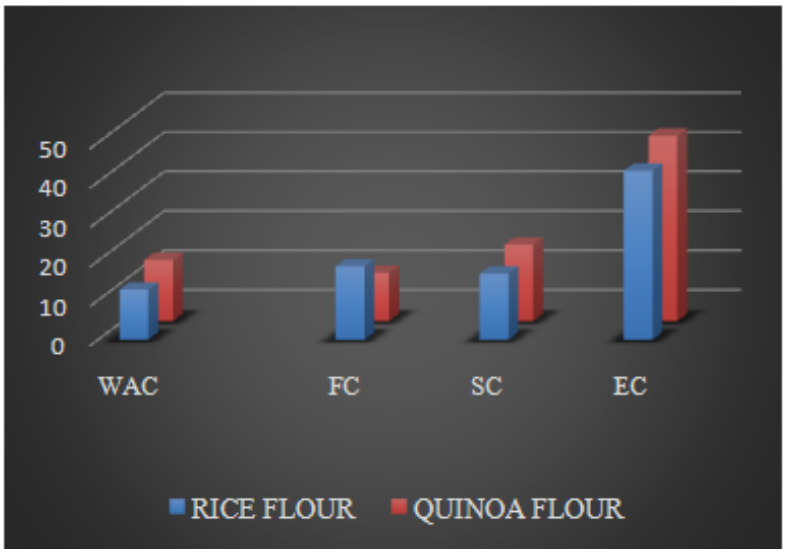


Fig.3 Physicochemical properties of flour

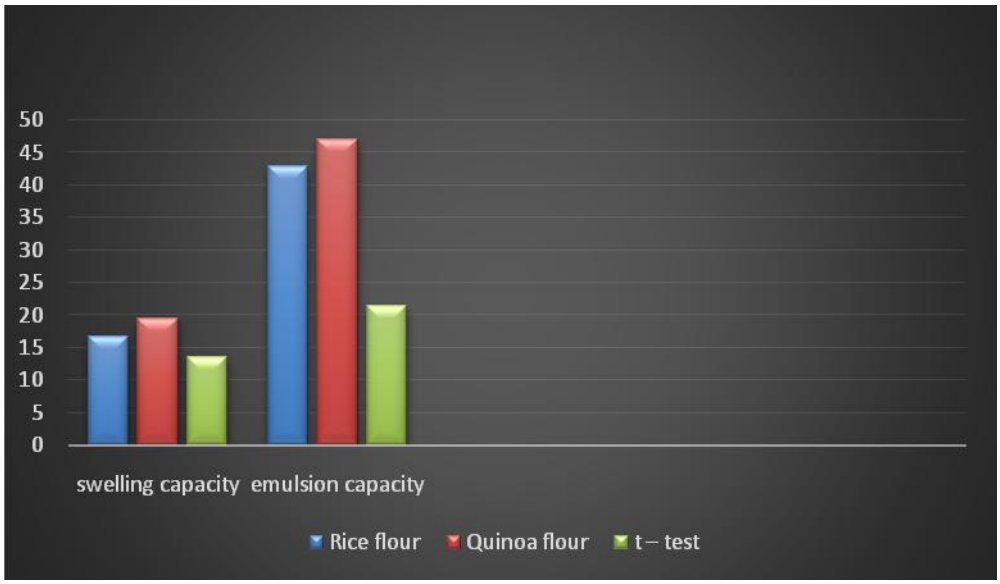


Fig.4 Nutritional composition of gluten free bread

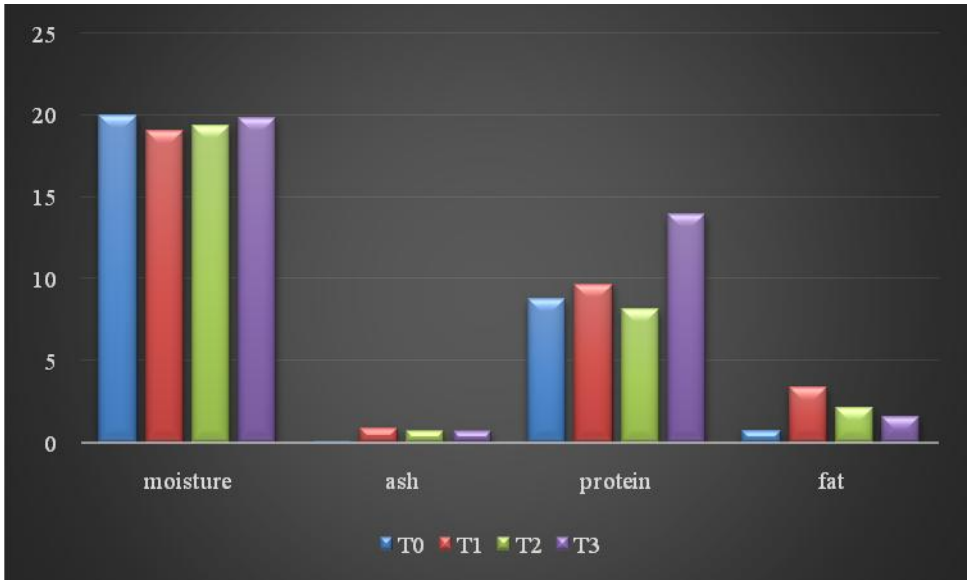
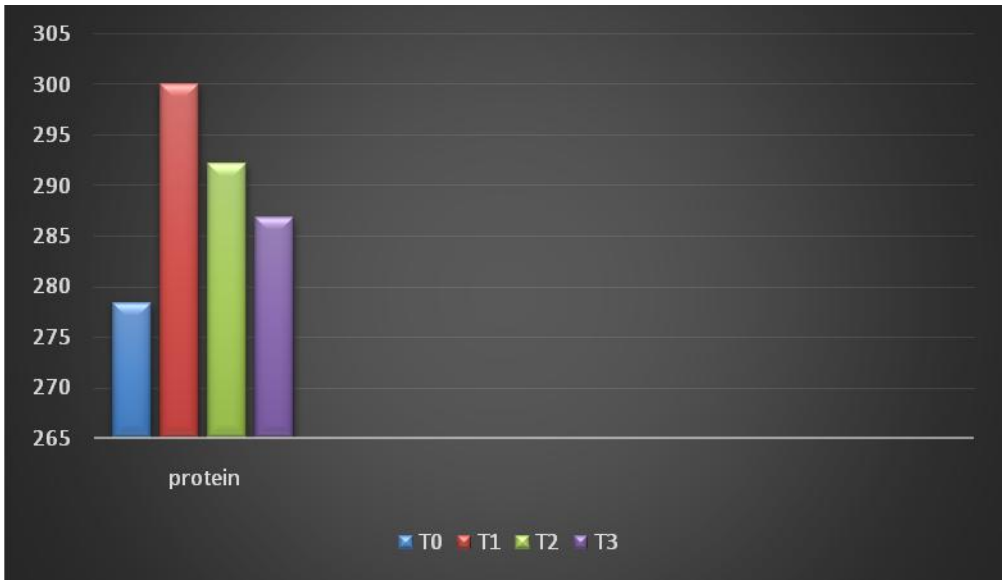


Fig.5 Nutritional composition of gluten free bread



Energy content is high in T_1 (300.1±0.25g) followed by T_0 (278±0.28), T_2(297.2±0.28g), and T_3 (286±0.19g), respectively as shown in figure 5. Although no significant difference is found in energy content of T_1 and T_2. The low energy content in control is attributed to low content of macronutrients. It signifies that gluten free bread has the good energy content because the quinoa flour and rice flour are rich in protein and fat. The nutrient contents shows that the incorporation of quinoa flour, rice flour in the product of gluten free bread, was good to enhance the nutritional values of it and also good for preventing celiac diseases.

Shelf life of gluten free bread

The shelf-life of optimized (T_2) gluten free bread includes TPC, Coliform, Yeast and Mould count is presented in table 3**.** The total plate count on day 1, 3, 6 and 9 of optimized gluten free bread (T_2) is 2.55 log CFU/g, 3.25 log CFU/g, 5.38 log CFU/g and 7.13 log CFU/g respectively. According to AOAC, the TPC for bread should be>0.88-6.13log CFU/g. In the present study, we found that on 6^{th} day the TPC is lower than recommended safety level and on 9^{th} day, the plate count is higher than recommended. Therefore, the present study found that the optimized bread is safe up to the 6^{th} day. The mould spoilage accounts for between 1-5% depending on the season. The total yeast and mould count of the bread ranged from $1.1x10^{1}$– $8.0x10^{4.}$ The yeast and mould count of optimized bread is $1.1X10^{1}$ in day one, $1.9X10^{1}$ in day 3, $2.6X10^{2}$ in day 6, $3.1x10^{4}$ in day 9. Therefore, the present study found that the optimized bread is safe up to 6^{th} day. The yeast and mould count found within recommended range up to 9^{th} day and coliform count was nil from day 1-9.These results consistent with other reported results of shelf-life of gluten free bread.

The finding of present study reveals that Quinoa and Rice flour enhance the nutritive value of gluten free bread. The treatment T_2 (50:30) was highly acceptability on the basis of overall acceptability for the gluten free bread. The nutritional composition of all treatments in the developed products was higher in comparison with control. The present study found that the optimized bread is safe up to 6^{th} day. The results of the study arouse the need of further improvement and optimization of gluten free bread.

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