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Development of a Bread Product for Coeliac People Made form Rice, Chía and Amaranth Flour, by Mean of a Mixture Design

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Abstract

It was developed a bread for celiac people, based on a mixture of rice flour, burry amaranth and chía seed, as well as, hydrocolloids (alginate, guar and xantana gums) and sodium caseinate. Using the methodology of Mix Design. It was evaluated the volume and texture of the loaf, as well as, the quality of the crumb and the crust of the loaves and the taste of the loaves like response variables. The flours used were tested by immunological assays to ensure the absence of gluten in them. By means of the numerical optimization process, (Design Expert 5 statistical program) the best formula was obtained. The best result was obtained with the use of 9% sodium caseinate (based on% baker) and 2% guar gum, and it was possible to develop bread with good physical and sensory characteristics, safe for the consumption of celiac people.

Keywords: Coeliac Disease; Bread; Amaranth; Rice; Chía Seed; Hydrocolloids

Introduction

Celiac disease [1,2] consists in the development of a genetic and permanent intolerance to gluten and more specifically to the prolamin of wheat, as well as, to the prolamines of rye and barley, which involves the mucosa of the small intestine [2,3]. It is estimated that the prevalence of the population of people with celiac disease in México is 4.3% of the population, which means that 4.8 million people could suffer from the disease [4,5]. For the preparation of gluten-free breads, corn flour, rice, soybeans, flaxseed, sorghum, amaranth [6] have been used, particularly rice flour since its prolamine is not toxic for coeliac people [7]. Baking doughs prepared without gluten do not have the characteristics of gluten-containing doughs: the properties of viscoelasticity, the retention of carbon dioxide produced during the fermentation of bread, which results in unappetizing, very dense breads

[8], with crumb little cohesive, brittle; the gumsiness during mastication is usuallyanother problem [9-11], in order to replace the function of gluten it can be used hydrocolloids. The amaranth (*A. hypochondriacus* L.) belongs to the family Amaranthaceae, the main component of this grain is the starch, which represents between 50% and 60% of its weight; its protein content ranges from 10 to 15%, which it is rich in lysine; its fat content is between 7% and 8%, the main fatty acids found in amaranth oil are palmitic, in an average percentage of 18%, oleic acid, which together with linoleic acid make a total of 75% of total fatty acids, and linoleic acid present in 3% of total fatty acids [12]. Chia seed (*Salvia hispanica* L.) is the largest vegetable source of fatty acids of the omega-3 series, its oil has α-linolenic content between 60 to 63% [13,14], and it has a high antioxidant capacity due mainly to the phenolic compounds present in it [15-17]. In the present work, the effect of the use of hydrocolloids on some physical and sensory characteristics of a loaf of bread made from a mixture of rice flour, popped amaranth and chia seed was tested.

Materials and Methods

Raw Material

The seeds of chia and amaranth busted were acquired in a local market. The hydrocolloids that were tested were: guar gum, xanthan gum, alginate and a commercial mixture of hydrocolloids. The burst amaranth seed flour was prepared by grinding the trapped amaranth in a Skymsen brand industrial blender and passing it through a 30 sieve. The chia flour was prepared from clean seeds, toasted for 2 min in an oven at 180°C, and ground in an industrial blender, sieved in a sieve of 1 mm in diameter.

Proximal Analysis

It was done by the methods described in the Official Methods of Analysis of AOAC [18].

Gluten Detection Tests

Rice, amaranth, chia flours and corn starch were analyzed with immunoreactive strips for gluten detection: BIOKITS RAPID 3-DTM GLUTEN Test Cat. No. 901031P, which have a detection level of 4 ppm of gluten, in order to ensure its safety for the consumption of people with celiac disease.

Design Of Mixtures to Obtain the Base Formula

With the Design Expert 5 [19] program, a "simplex centroid" mix design was developed, which allows for a central point, where the components of the mixture were rice flour, chia seed, amaranth and corn starch, which gave as a result, a matrix of 28 experiments performed with 6 repetitions. The experimental variables were the proportion of chia flour and amaranth and corn starch, Table 1 and the responses to be measured were: the volume and hardness of the loaf, the crumb quality, the quality of the crust and flavor.

Component	Name	Tipo	Low value	High value
A	Amaranth flour	Mixture	0	1
B	Chía seed flour	Mixture	0	1
C	Corn starch	Mixture	0	1
			Total =	1

Table 1: Components and limits used to establish the Mixture Design [17].

For this experiment the formula showed in Table 2 was taken as is basis.

Components	Quality
Rice flour	100 g
Dry yeast	3.4 g
Sugar	13.6 g
Vegetal oil	6.8 g
Salt	3.4 g
Milk poder	5.1 g
Water	100 mL

Table 2: Formulation of the free gluten bread.

Where:

a) For each 100 g of rice flour, 70 g of a mixture of flours (70% baker) are added, formed by chia flour, amaranth flour and / or corn starch.

b) The sum of these 3 components (70 g) is equivalent in the design of mixtures to a value of 1, as shown in Table 1.

Table 3 shows the experimental runs that must be carried out to obtain the optimum base formula,

Run Number	Amaranth Flour	Chía Seed Flour	Corn Starch
1	1.000	0.000	0.000
2	0.500	0.500	0.000
3	0.000	0.000	1.000
4	0.167	0.667	0.167
5	1.000	0.000	0.000
6	0.500	0.500	0.000
7	0.667	0.167	0.167
8	0.000	0.500	0.500
9	0.167	0.167	0.667
10	0.000	1.000	0.000
11	0.500	0.000	0.500
12	0.333	0.333	0.333
13	0.000	0.000	1.000
14	0.000	1.000	0.000
15	1.000	0.000	0.000
16	0.500	0.500	0.000
17	0.000	0.000	1.000
18	0.167	0.667	0.167
19	1.000	0.000	0.000
20	0.500	0.500	0.000
21	0.667	0.167	0.167
22	0.000	0.500	0.500
23	0.167	0.167	0.667
24	0.000	1.000	0.000
25	0.500	0.000	0.500
26	0.333	0.333	0.333
27	0.000	0.000	1.000
28	0.000	1.000	0.000

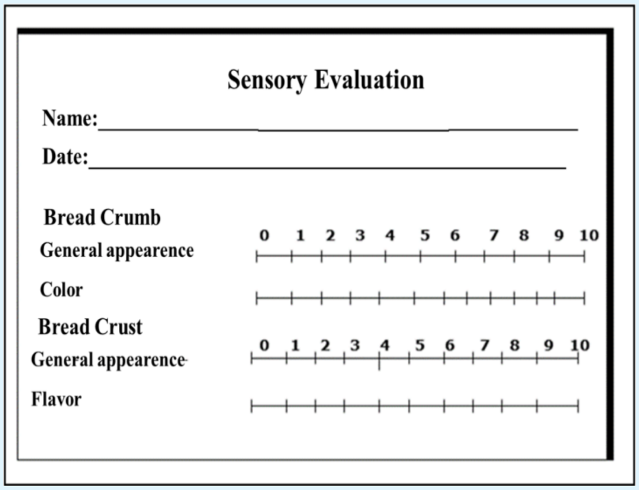
Table 3: Experimental runs according with the Mixture Design.

Methodology Used to Elaborate the Formulas of the Loaves Established by the Mixture Design

For the manufacture of the loaves the sponge mass method was used. To form the sponge, a part of the ingredients (rice flour, yeast, water and sugar) were mixed in a Kitchen Aid model KSM 90 mixer, the mixture was fermented for 3 h at 30°C in a Lab Line Instruments model 417 camera. Once the sponge is formed the rest of the ingredients were added to the formed sponge: salt, milk powder, vegetable fat, rice, amaranth and chia flours, as well as, corn starch, the mixture obtained was needed to form a dough that was fermented for 30 min at 30°C. The dough was cut into portions of 100 g each, molded and fermented at 30°C for another 30 min. Finally it was baked for 25 min in a Hamilton Beach convection oven, Model 31199, at 180°C.

Determination of the Physical and Physicochemical Characteristics of Bread

The loaves volume was made by the method of seed displacement [20]. The quality of the bread crumb, the bread crust, the color of the crumb and the crust, were evaluated by judges trained on a continuous scale from 0 to 10 (Figure 1).



Sensory Evaluation

Name:

Date:

Bread Crumb

General appearence 0 1 2 3 4 5 6 7 8 9 10

Color

Bread Crust

General appearence 0 1 2 3 4 5 6 7 8 9 10

Flavor

Figure 1: Format used to carry out sensory evaluation of the loaves.

The hardness of the bread was measured as the force required compressing 50% of the bread, by means of a Universal Texturometer brand Instron model 5565 using a cylindrical plunger of 25.5 mm of dynamometer as compression device, which moved into the loaf to a speed of 100 mm / min with penetration of 50%.

Aging of the Bread

The aging of the bread was measured as the increase in hardness of the loaf, after baking, the loaves were allowed to cool for 1 h at room temperature, stored in polyethylene bags and placed on a shelf at room temperature: For the determination of hardness samples were taken at different times after baking: 1, 24 and 96 h.

Obtaining the Base Formula through the Design Expert 5 Program

Bread formulas proposed by the Mixture Design Table 3 were prepared as described and each one was evaluated in accordance with its physical and physicochemical characteristics. The results were analyzed statistically using the Design Expert 5 [19] program: with the results of the statistical analysis, the mathematical optimization was carried out to obtain the Optimum Base Formula, using the same software.

Incorporation of Gums to the Base Formula

Once the base formula was established, the hydrocolloid incorporation was evaluated: xanthan gum, guar, alginate, a mixture of hydrocolloids in a proportion of 1 and 2% [8,9,21-23] in a system with cabinet from sodium to 9% baker [8].

Statistical Analysis

The effect of the addition of hydrocolloids on the quality of the loaf of the base formula was determined with analysis of variance, with a confidence level of 95% [24].

Results and Discussion

Proximal Analysis

The proximal composition of the rice, amaranth and chia flours is shown in Table 4. Amaranth and chia flours have a high protein content, which can help to strengthen the networks that trap the gas generated during the fermentation of the loaves. The high fiber content of the chia contributes to obtain a product rich in this component. In addition the high content in fats of chía seed are excellent sources of omega 3fatty acids, and in second place of omega 6 fatty acids [14].

Proximal Chemical Analysis * (%)	Rice Flour	Chía Seed	Amaranth Seed
Ethereal Extract	0.64	32.89	9.10
Proteíns	8.91	21.31	18.29
Ashes	0.54	4.62	2.49
Fibre	2.00	26.27	4.42
Carbohydrates	87.90	14.91	65.69

*Dry base.

Table 4: Proximal composition of the flours used in this research.

Detection of the Presence of Gluten in Raw Materials

Table 5 shows the result of the detection of the presence of gluten in the raw materials, using wheat flour as a positive control. In all cases a negative result was obtained, which means that they have less than 4 ppm of gluten, so the raw materials are considered safe for use in this study, according to the Codex Alimentarius, which states that a food naturally Gluten Free must contain less than 20 ppm of it [25].

Raw Material	Result
Rice flour	Negative
Amaranth flour	Negative
Chía seed	Negative
Corn starch	Negative
Optimal formula	Negative
Wheat flour	Positive

Table 5: Detection of gluten content in raw materials, through the use of immune-chromatographic strips.

Development of the Base Formula, Through the Analysis of Statistical Optimization DX5 Softwear [19]

The loaves were prepared according to the mixture design shown in Table 3 and the effect of the experimental variables on the response variables was evaluated using the DX5 Softwear.It was found that in all cases the proportion of the ingredients significantly affected the characteristics of the loaves evaluated ($p \leq 0.0001$). Table 6 shows the results of the analysis of variance. The special cubic model was selected, since it allows the analysis of each of the study variables separately, as well as, their interactions.

	Response variables	Units	Low value	High value	Mixture Model	P	R^2
Y1	Loaf volume	cm3	100	180	Special Qubic	≤ 0.0001	0.8817
Y2	Bread crust quality	-	0	9	Special Qubic	≤0.0001	0.9708
Y3	Bread crust color	-	0	8.5	Special Qubic	≤0.0001	0.9282
Y4	Bread crumb quality	-	0	8.4	Special Qubic	≤ 0.0001	0.9403
Y5	Bread crumb color	-	1	8.0	Special Qubic	≤ 0.0001	0.9461
Y6	Flavor	-	1	7.4	Special Qubic	≤ 0.0001	0.9217
Y7	Hardness	Kgf	1.833	19.934	Special Qubic	≤ 0.0001	0.8933

168 experimental runs were made, Mixture Design: Model Simplex Centroide
Table 6: Analysis of variance of each of the evaluated responses *(DX5).

Optimization of the Results of the Experimental Variables Studied, to Obtain the Base Formula [19]

The optimization serves to find the answer that provides the best result; for this, it was necessary to enter the program the desired limits for each of the variables studied as it is shown in Table 7.

Response variables	Low limit	High límit
Loaf volume (cm3)	160	168
Hardness (kgf)	1.8	2.5
Crust quality	8	8.4
Crust color	7.5	8
Crumb quality	8	8.2
Crumb color	7.5	8.1
Flavor	7	7.3

Table 7: Limit values of the variables used to obtain the base formula through the numerical optimization of the model used (DX5).

The suggested proportions of amaranth (X1), chia (X2) and corn starch (X3), with a prediction of 0.966 are shown in Figure 2.

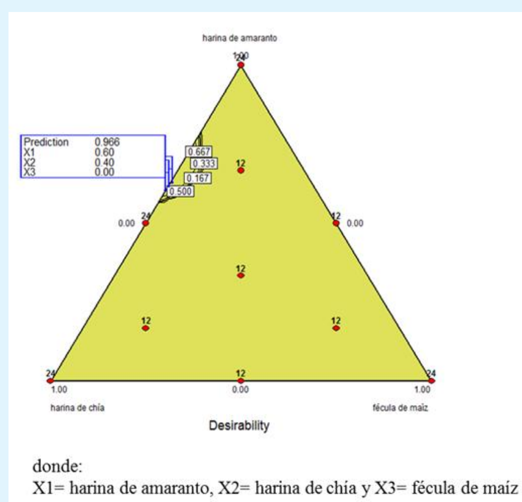


Figure 2: proportions of amaranth (X1), chia (X2) and corn starch (X3), with a prediction of 0.966.

Table 8 shows the predicted values of the evaluated responses: flavor, loaf volume (cm^3), quality and color of the crust, quality and color of the crumb, hardness of the loaves (kgf).

Responses	Predicted value	Standard deviation
Loaf volume (cm3)	167.35	±1.83
Crust quality	8.6	±0.096
Crust color	8.1	±0.136
Crumb quality	8.3	±0.130
Crumb color	8.3	±0.120
Flavor	8.3	±0.110
Hardness (kgf)	2.51	±0.385

Table 8: Predicted values of the evaluated responses.

The optimum base formula was made with amaranth and chia flour in a ratio of 72 and 43baker percentage respectively, it should be mentioned that the volume of breads made with the optimal formula was increased by 40.6% compared to those made with rice flour only, as it is shown in Table 9.

Formula:	Loaf volumen* cm3
Base formula	163.3±5.2
Bread made from rice flour (control)	141.7±4.1

*Average of 6 repetitions

Table 9: Volume of the loaf of the loaves made with the base formula (optimum) compared to those made with rice flour.

Incorporation of Gums and Sodium Caseinate in to the Base Formula

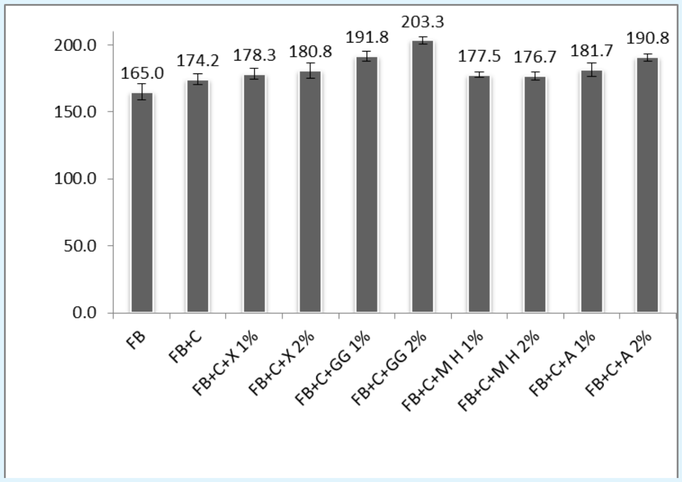
To improve the characteristics of the breads made with the base formula, the incorporation of gums and protein concentrates was tried since they have been used by other authors to mimic the viscoelastic properties of gluten, as well as to improve sensory characteristics and shelf life. of gluten-free breads [7,8,22]. It has been reported that sodium caseinate strengthens the ability to trap water when adding gums, Lazaridou A, et al. [22] incorporated sodium caseinate-carboxymethylcellulose and sodium caseinate-xanthan, achieving an increase in the volume of the loaf in comparison of the control elaborated without gums at 6%. McCarthy, et al. [26] reported an increase in volume in bread made from potato starch, rice flour and protein concentrates using hydroxypropyl methylcellulose.

The effect of the use of sodium caseinate and the gums on the loaf volume of the loaves is shown in Figure 4.The addition of 9% calcium caseinate to the base formula (FB + C) produced an increase in the volume of the loaf of the same, from a value of 165 cm^3 to 174.2 cm^3, which is equivalent to a 5.4% increase of the volume. The addition of 2% guar gum to the base formula added with caseinate (FB + C + GG2%), caused an increase in the volume of the loaf from 174.2 to 203.3 cm^3, which is equivalent to an increase in volume of the same. 23% with respect to the base formula.

Effect of The Use of Gums on the Texture of the Loaves at Different Times

The average hardness of the loaves made with the base formula: 3.621 ± 0.36 kgf after 1 h after leaving the oven, the addition of calcium caseinate to the base formula allowed obtaining softer loaves with a hardness of 2.43 ± 0.2 kgf ($p \leq 0.05$) (24) are shown in Figure 4. The addition of the gums in general improved the texture of the loaves making them softer; the best result was obtained with the addition of 2% guar gum ($p \leq 0.05$), with a hardness of 1.71 ± 0.15 kgf. These results coincide with some studies that propose that gums improve the texture of gluten-free breads [8,11,16,17,20,22,23]. Gambus, et al. [27] Incorporated guar gum into a bread made with potato starch, corn starch improving the texture of the same. Lazaridou, et al. [24] evaluated the effect of pectin, carboxymethylcellulose, agarose, xanthan and β-glucan in a bread made from rice flour, finding that the loaves to which agarose was incorporated showed a lower hardness compared to added with carboxymethylcellulose. Pedrosa, et al. [11] developed gluten-free breads from extruded rice flour incorporating guar gum, which had a lower hardness than those made only with extruded rice flour. Korus, et al. & Wang et al. [28&29],[30] found that guar gum Increased volume, sensory acceptance, and slow the staling of bread.

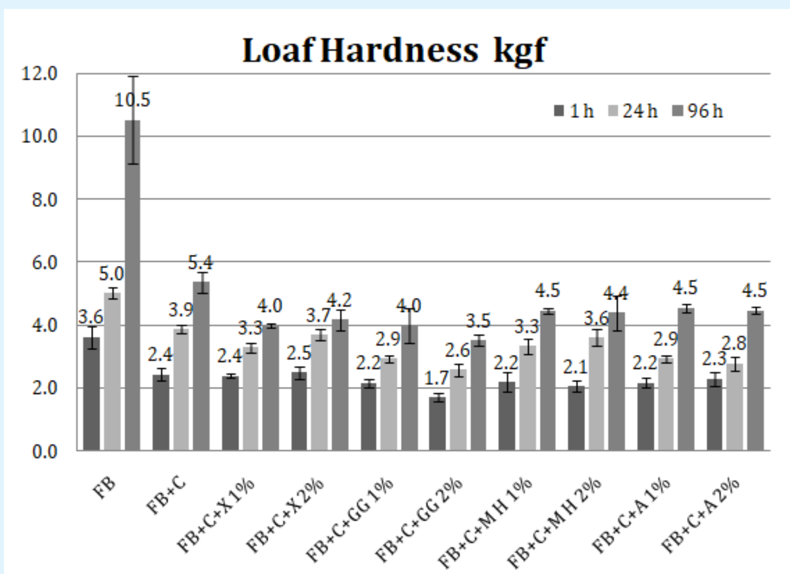
Another advantage of the use of gums is that they increase the shelf life of gluten-free breads [Korus y Wang]. In Figure 5 it can be seen that the addition of sodium caseinate to the base formula significantly decreases the hardness of the loaves at all times tested [23].The addition of the gums to the formula added with caseinate, increases the softness of the loaves at all times tested and improves the shelf life of the same, the best result was obtained with the addition of 2% guar gum ($p \leq 0.05$) [24].



where:

FB = base formula, FB + C = base formula added sodium caseinate, FB + C + X 1% = base formula added of caseinate and 1% xanthan gum, FB + C + X 2% = base formula added of caseinate and 2% xanthan gum, FB + C + GG 1% = base formula added of caseinate and 1% guar gum, FB + C + GG 2% = base formula added of caseinate and 2% guar gum, FB + C + MH 1% = base formula added of caseinate and 1% hydrocolloid mixture, FB + C + MH 2% = base formula added of caseinate and 2% hydrocolloid mixture, FB + C + A 1% = base formula added of caseinate and 1% alginate, FB + C + A 2% = base formula added of caseinate and 2% alginate.

Figure 3: Volume of the loaf (cm3) of the breads made with the addition of caseinate and gums.



where: FB = base formula, FB + C = added base formula of sodium caseinate, FB + C + X 1% = added caseinate and 1% xanthan gum, FB + C + X 2% = added caseinate and 2% xanthan gum, FB + C + GG 1% = added caseinate and 1% guar gum, FB + C + GG 2% = added caseinate and 2% guar gum, FB + C + MH 1% = added caseinate and mixture of 1% hydrocolloids, FB + C + MH 2% = added caseinate and 2% hydrocolloid mixture, FB + C + A 1% = added caseinate and alginate 1%, FB + C + A 2% = added caseinate and 2% alginate.

Figure 4: Texture of the loaves, after 1 h to remove them from the oven.

The use of gums and sodium caseinate also improved the volume of the loaf and the structure of the crumb as it is shown in Figure 5, which it can see the cut the loaves of the bread made with rice flour only, the base formula without caseinate and without gums, and the selected formula added with 9% sodium caseinate and 2% baker guar gum, where it is evident, as already mentioned, a greater volume of the loaf prepared with the selected formula, as well as, a better structure of the crumb.

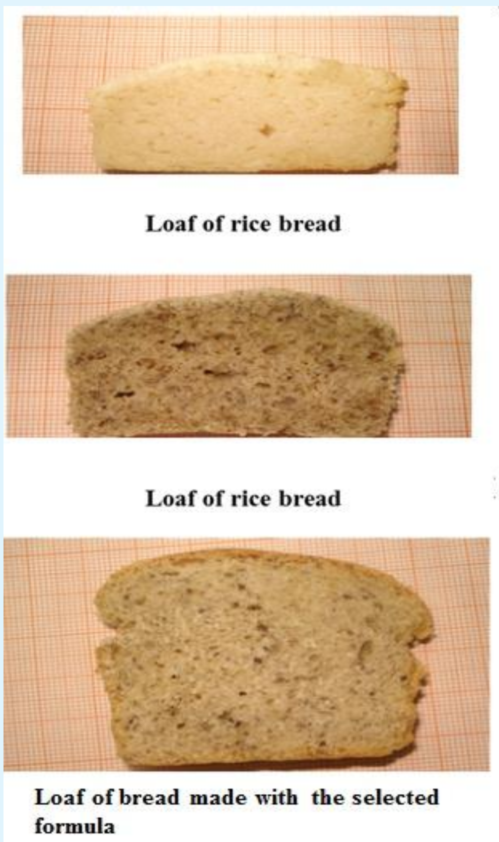


Figure 5: Appearance of the crumb of the bread selected in comparison with that made with the base formula and with rice flour.

Conclusions

The incorporation of amaranth and chia seed improves the nutritional quality of the bread due to the properties of each of these. Regularly gluten-free breads usually have poor amounts of fiber. Gallagher, et al. [5] incorporated 8 g / 100 g of inulin into a bread based on wheat starch, increasing the fiber content 0.4 to 3.5 g / 100 g. Gambus, et al. [6] replaced corn starch with amaranth flour to increase fiber and protein content, replacement of 10%, increased protein and fiber levels by 31% and 152%, respectively, while sensory quality was not It was affected. The present work fiber increased by 3.6g/100g compared with rice bread with 0.7 g/100 and 0.9 g/100 of a wheat bread.

The addition of caseinate and gums especially guar gum allowed to obtain gluten-free breads with good physical and sensory characteristics, with a considerable contribution of fiber and safe for the consumption of people with celiac disease.

Thanks

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