New opportunities for gluten-free diet: teff (*Eragrostis tef*) as valuable fiber rich ingredient?

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Summary

This study assessed physicochemical parameters of high fiber and gluten-free breads made with teff and associated flours. Four breads samples were developed:wheat flour (T1), teff flour (T2), teff flour + cassava starch (T3 and T4). Hedonic evaluation of sensory attributes characterizing the samples was performed by celiac and non-celiac subjects. Breads made with different percentages of teff flour showed huge amount of total and insoluble fibers. The wheat bread presented the highest values for the texture parameters analyzed, except for crumb hardness and elasticity. The sensory analysis showed that all samples made with teff were well accepted by celiac and non-celiac subjects. Purchase intention and the acceptability index suggested a potential market success for the developed products. Teff flour showed promising use for its technological and nutritional values as well as sensory properties, supporting the hypothesis that it is possible to develop new gluten-free bakery products without decreasing consumers' satisfaction.

Keywords: gluten-free diet, acceptability, texture, purchase intention

Introduction

Gluten is the primary compound in bread production, since it is responsible for some relevant technological and sensory properties, such as: texture, crust and crumb appearance and acceptability (Arendt & Bello, 2011). Besides the mentioned relevant technological and sensory properties, the consumption of food products containing this protein complex is well known to cause gastrointestinal disorders related to celiac disease.

Celiac disease can be defined as a gluten-induced autoimmune enteropathy, occurring in genetically predisposed individuals (Leonard *et al*., 2017). Thus, this disease needs to be further studied to provide subsidies for health professionals and patients to recognize its signs, symptoms, and ideal diet.

When consumed by celiac individuals, gluten can cause several symptoms such as chronic diarrhea, weight loss, malaise and fatigue, among others. In addition, other diseases are related to gluten consumption, such as non-celiac gluten sensitivity, wheat allergy (Wgo, 2016), depression and diabetes (Haupt-Jorgensen *et al*., 2018).

According to the World Gastroenterology Organization (2016), the treatment for celiac disease is a gluten-free diet, besides a special care with the place and cookware for preparing food. Recently, an increase in gluten-free diet has been observed not only in subjects who reported gluten allergy or intolerance (Perrin *et al*., 2019), but also in people without diagnosis of any related disease (Kutlu, 2019), who follow gluten restrictions to contribute to a healthier diet. Indeed, the number of people adopting a gluten-free diet is increasing due to the belief of gluten avoidance may provide physical well-being, increase texture and flavour quality of gluten-free food (Calle *et al*., 2020).

Most of gluten-free bakery products available on both Italian and Brazilian market are mainly based on refined flour or starches from rice and maize (Di Cairano *et al*., 2020; Di Cairano *et al*., 2018; Santos *et al*., 2019).

It should be considered that a gluten-free diet has been related to both low fiber and higher amount of starch intake (Barone *et al*., 2016, Arslan *et al*., 2019). For this reason, the use of a broader range of gluten-free flours may aid in improving the nutritional quality of gluten-free products (Proserpio *et al*., 2020). In this context, teff (*Eragrostis tef*) is gaining relevance due to the absence of gluten, high fiber and its valuable nutrient content. Therefore, people are increasing its use in healthy food preparations, especially in the case of individuals who do not consume gluten (Gebremariam *et al*., 2014).

Some studies have already been carried out on the chemical and technological quality of teff and gluten-free breads (Tess et al., 2015; Coleman et al., 2013; Hager and Arendt, 2013), but hardly studies have investigated the sensory attributes, especially involving celiac people. The sensory characteristics of gluten-free breads need to be addressed and identified since they are determining factors for the food acceptance of celiac consumers (Pagliarini *et al*., 2010).

The dissatisfaction associated with gluten-free products, besides the lack of technological and sensory information about fibers-enriched breads made with teff cereal motivated this study.

In this context, the aim of the present study was to evaluate physicochemical parameters of high-fiber and gluten-free breads made with teff (*Eragrostis tef*) and associated flours. Hedonic evaluation of sensory specific attributes characterizing the samples was performed by celiac and non-celiac subjects. Moreover, purchase intention and acceptability index (AI) were also evaluated to deepen the study of consumers' responses towards these products.

Materials and Methods

Materials

The brown teff of the *Eragrostis tef (Zuccagni) Trotter* species came from the producer El Campo farm located in Pedro Juan Caballero, Paraguay (22 19′54.41″S, 55 52′ 22.35″W; 662m above the sea level), and the other ingredients to prepare the bread samples were purchased from local stores in Porto Alegre, RS, Brazil.

The teff grains were dried in an oven, at a temperature of 60 ºC for 12 h, and then crushed in a coffee grinder – Cadence, Di Grano (MDR302-127), with stainless steel blades and a power of 150 W – until reaching flour thickness, and then sieved in a 35 Tyler/Mesh (0.425 mm).

Samples preparation

Preliminary tests were carried out and adjustments were made, until obtaining the most promising formulations in term of technological performance. Consequently, four formulations were chosen to be evaluated in this study: T1- 100% wheat flour- standard; T2 - 100% teff flour; T3 - 75% teff flour, 12.5% rice flour and 12.5% cassava starch; T4 - 50% teff flour, 25% rice flour, and 25% cassava starch. The other ingredients were: refined salt, crystal sugar, soy oil, fresh white eggs, dry yeast, xanthan gum, and water (Table 1).

Teff flour (T2, T3, T4) soaking was carried out for 30 minutes with warm water at a temperature of 50 ºC, an adapted version of Sadik et al. (2012). The amounts of water were adjusted according to each formulation and determined after several previous tests to improve dough homogenization and to establish favorable sensory attributes such as texture and overall appearance.

Xanthan gum was added only to the treatments with teff flour and it has been used in many studies as an anti-staling agent, to produce higher specific volume, yield and softer crumbs (Hager and Arendt, 2013).

All ingredients were weighed on a digital analytical scale (0.01 g) Unibloc, Shimadzu, UX-6200 H. The dough production was carried out using the direct fermentation method. To bake the bread dough, a conventional oven was pre-heated for 20 min under 220 ºC. The loaves were baked for 25 min at 220 ºC, and this proceeding was taken four times. The loaf preparation process was adapted from César *et al*. (2006).

Total, insoluble and soluble fibres

Total fiber amount in breads was evaluated in duplicate using the enzymatic gravimetric method described by AOAC (2000), with the use of the Sigma-Aldrich Kit, TDF100A-1KT. Insoluble fiber amount was determined with the same method, although the samples were not subjected to 95% ethyl alcohol. The quantity of soluble fibers was calculated as difference between the total fibers and insoluble fibers.

Texture properties

Hardness was verified using a Stable Micro Systems® - London/UK texturometer TA.XT plus and a cylindrical probe with a 36 mm radius (code P/36R). This parameter was measured in 4 breads samples and three replicates, by the compression test performed on 12 slices, each slice had 2 cm thick. The maximum force was determined in the first compression cycle and defined from the following conditions: cylindrical texturometer, maximum test speed of 4.0 mm/s; minimum test speed of 0.01 mm/s; rupture distance of 0.001 mm. The other rheological parameters such as: elasticity, cohesiveness, resilience, chewiness and gumminess were calculated according to Calabuig (2012).

Hedonic and purchase intention evaluation

The acceptability and purchase intention analyses were carried out through a hedonic evaluation on different days involving 65 non-celiac and 11 celiac subjects from a Celiac Association **(blind review),** that provided an agreement to participate to the study. The inclusion criteria of both groups of subjects were: both genders, age between 18 and 50 years old, randomly and voluntarily recruited through invitations on social networks or advertising at a Medical School University **(blind review)**.

This study was submitted to the Ethics Committee of the University and it started only after its approval. According to Resolution 466/2012 of the National Health Council – **(blind review)**, this study is registered under protocol number 63481317.0.0000.5347 of the CAAE. Participants were assured of the confidentiality of their identifications as well as of the right to participate in the research through the Informed Consent Form (ICF), which explained the objectives of the study and emphasized that the data obtained are confidential and only used for research purposes. Before the analysis, all subjects signed an IFC, after receiving detailed information on the preparations and procedures.

Samples were offered on white disposable plates, coded with a random three-digit numbers. A slice of approximately 10 g of each bread formulation and a glass of water for rinsing the palate among samples were provided to the subjects.

The hedonic evaluation was performed using a 9-point hedonic scale, ranging from 1 ("Disliked extremely") to 9 ("Like extremely"), in order to assess the following attributes: appearance, colour, texture, flavour, odour, and global acceptance. Each subject also completed a purchase intention evaluation, with a 5-point scale ranging from 1 ("I certainly wouldn't buy it") to 5 ("I would certainly buy it").

The acceptability index (AI) was calculated according to the expression described by Viana (2009), for all treatments: AI (%) = A x 100/B

Statistical analysis

The results were evaluated through analysis of variance (ANOVA), followed by the comparison of the means performed by Tukey test, with a level of significance of 5 % of error probability, using the statistical software SPSS Statistics, version 21.0.

Results and Discussion

Total, insoluble and soluble fibres

As reported in Figure 1, T1 had significantly ($F=28.02$; $p<0.05$) the lowest total fibre content (13.9%) compared with the other samples with teff which showed high amount of fibres (T2:26 %, T3: 22.9%, and T4: 21.8%, respectively) and were comparable to each other.

For insoluble fibres, T1 showed the lowest content (8.9%) with statistically significant difference ($F= 37.37$; $p<0.05$) compared with T2, (25.5%) T3 (18%) and T4 (21.8%). As regards soluble fibres results, T1 was comparable to T3 (4.9%), and significantly higher than T2 (0.5%) and T4 (0.1%). T2 and T4 were not statistically different.

Many gluten-free products may not achieve the fibres recommended daily intake (Suliburska & Krejpcio, 2014). On the contrary, teff flour used in the present study depicted a higher amount of fibre. Gebremariam *et al*. (2014) suggested that food developed with teff flour showed higher amount of crude fibre than most of gluten and gluten-free cereals. For this reason, cereal-based formulations made using teff flour, which do not require to be fortified, could be used to reach the recommended daily fibre intake.

High fibre diet is important for health, because it can prevent many human diseases such as diabetes, coronary heart disease and colon cancer (Anderson et al., 2009).

It should be considered that the usual fibre enrichment in gluten-free breads can influence the resistance by entanglement of fibre (Demirkesen *et al*., 2010) and can modify loaf volume, viscosity, softness of the bread crumb and the firmness (Sangnark & Noomhorm, 2004). Moreover, insoluble dietary fibres in excess can affect gluten network formation (Ahmed *et al*., 2013) and can reduce bread quality (Kaack *et al*., 2006) due to gluten dilution or gluten-fibre interaction. While adding low amount of soluble dietary fibres, the breads quality can be improved (Sivam *et al*., 2011).

Texture properties

The crust hardness in T1 was significantly ($F=33.09$; $p<0.0001$) higher (188 g, force) than the other samples: T2 (48 g, f), T3 (40 g, f) and T4 (59 g, f), which were comparable to each other (Table 2).

Regarding crumb hardness it was observed that T1 showed significant ($F=6.20$; $p<0.05$) higher hardness compared to T3 (1681 g, f) and T4 (1716 g, f) and was comparable to T2 (2188 g, f). These results can be justified by the use of a hydrocolloid, xanthan gum, which was added in T2, T3 and T4 samples. Indeed, according to Hager & Arendt (2013), hydrocolloids foster a greater addition of water and consequently less hardness. Contrarily, Ronda *et al*. (2015) observed that with 30% and 40% of brown teff flour there was an increase in the crumb hardness in ciabatta breads. However, in the mentioned study a bread improver in 0.5% (containing mono- and di-glyceride of fatty acids, ascorbic acid, α-amylase and xylanase), beside xanthan gum, was used. In the present study, the increased hardness observed in T1 was also reported in muffins by Tess *et al*. (2015), in which they replaced rice flour by teff flour.

The crumb elasticity was significantly ($F=27.0$, $p<0.0001$) higher in T2 (1.10 mm), T3 (1.10 mm) and T4 (1.10 mm) compared to T1 (1.07 mm), on the other hand Phongthai *et al*. (2016) emphasized that ingredients with high concentration of proteins can provide high resistance and consistency of gluten free doughs, resulting in a limited elasticity.

Crumb cohesiveness for T1 (0.86 g.s) and T4 (0.78 g.s) was significantly higher ($F=11.89$, $p<0.05$) compared to the other samples. By contrast, T2 (0.61 g.s) showed the lower cohesiveness of the crumb. Cohesiveness was the unique rheological parameter that showed changing among the breads made with teff.

The crumb resilience, chewiness and gumminess were significantly higher in T1 ($F=16.31$, $p<0.05$; $F= 25.69$, $p<0.0001$; $F=29.04$, $p<0.0001$, respectively) than bread samples with teff. This result is interesting since xanthan gum usually promote an increase in viscosity, according to Hager and Arendt (2013).

Hedonic and purchase intention evaluation

Hedonic scores provided by both non-celiac and celiac subjects are reported in Table 3.

As regards non-celiac subjects, no significant differences have been found in hedonic ratings for the attributes: appearance, colour, odour and global acceptance. However, significant lower ($F=10.86$; $p<0.0001$) hedonic scores were provided to T1 for bread texture compared to the other formulations. For this attribute, treatments with teff flour improved consumers' acceptability. These results are in agreement with other previous findings stating that dietary fibres can be added to gluten-free breads not only to be healthier, but also to improve sensory characteristics associated with flavour, dry mouth sensation and crumbling texture (Ziobro *et al*., 2012). In this context, thickener agents, such as xanthan gum and other hydrocolloids were found to improve the sensory characteristics of gluten-free bakery products (Preichardt, *et al*., 2009; Hager & Arendt., 2013). Teff grains soaking may have contributed to the acceptability of the breads, when compared to the sensory evaluations carried out in other studies, since the scores achieved were satisfactory in all assessments both for celiac and non-celiac groups.

Bread sample with 100% teff flour (T2) showed lower significant flavour hedonic scores than samples containing lower percentages. Tess *et al*. (2015) observed less acceptance for the flavour attribute in muffin samples with 75% and 100% of teff flour, maybe due to the different technological and sensory properties of the food matrix used.

As regards celiac subjects' significant difference ($F=2.581$, $p<0.05$) was observed only for the colour attribute between T2 and T4, with higher ratings provided to the sample with 100% of teff flour. On the contrary, Ezpeleta (2010) observed that breads with a higher teff content resulted in less acceptance when compared to breads with a lower teff content, justified by the darker colour. Based on the acceptability results an index for both group of subjects was calculated for each sample (Figure 2 a, b).

Acceptability indexes highlighted that the percentages were greater than 70%, regardless of the group of subjects, showing satisfactory indexes for the developed formulations according to all sensory attributes.

In the celiac group of subjects, the purchase intention scored 4.3 or above (on a scale ranging from 1 to 5) showing that they would likely buy the breads, while the scores among the non-celiac subjects were below 4.0.

Such difference between our results and the previous ones obtained by Ezpeleta (2010) may be a consequence of the increasing overtime consumers' awareness of the food quality and of "eating well". Indeed, according to FAO (2017), the search for a healthier diet grows gradually every year.

The purchase intention of non-celiac and celiac judges is represented as relative frequency of the assigned grades (Figure 3). It is observed that, for both groups, the highest grades were 4 and 5 (“Probably I would buy it” and “I would certainly buy it”, respectively).

Comparing hedonic scores provided by celiac and non-celiac groups of subjects, it was observed a significant difference ($p<0.05$) for texture attribute, with the latter showing greater acceptability (7.9) when compared to the celiac group (7.0), which seems to be more demanding about T3 texture attribute.

Regarding odour attribute, significant differences ($p<0.05$) were also found with the highest mean values came from the celiac group, indicating greater acceptability for T2 (7.7), T3 (8.0), and T4 (7.8). The non-celiac group was more demanding for these three samples, giving lower scores for T2 (6.7), T3 (7.0), and T4 (7.0).

Few studies compared non-celiac and celiac hedonic scores of gluten-free breads. Laureati *et al.* (2012) demonstrated no significant differences in hedonic responses to gluten-free bread comparing two groups, one of non-celiac (n = 85) and one of celiac judges (n = 21). The samples size of celiac subjects involved in both studies is small but proportional to the worldwide prevalence of 1% as reported in the global guideline of the World Gastroenterology Organization (2016). It is important to bear in mind that the participation of celiac judges in sensory tests is very relevant, since sensory evaluation is commonly used to improve the quality of gluten-free products. However, this can be not so helpful if the judges involved in sensory testing are not regular consumers of gluten-free products. In this context, it is reasonable to assume that following a diet, sometimes from birth or from an early age, can have a different impact on the dietary perception of celiac and non-celiac consumers (Laureati *et al.*, 2012). It was also noticed that the consumption of breads with gluten, prior to diagnosis by celiac people, can cause a different assessment when observing the evaluation of celiac people who never consumed gluten. The distinct period in which the judges obtained the diagnosis of celiac disease can cause inconsistent results (Pagliarini *et al*., 2010).

Conclusion

Teff showed promising use for its technological and nutritional values as well as sensory properties. Thus, it could be a valuable ingredient for new food formulation in order to face the worldwide increasing celiac disease and the demanding of high-fibre and gluten-free diet. This study has addressed sensory comparison between celiac and non-celiac consumers trying to fill the gap in the knowledge about gluten-free bakery products involving specific targets of consumers.

In conclusion, the results of the present study suggest that it is possible to develop new gluten-free bakery products using an adequate concentration of teff flour without decreasing both celiac and non-celiac consumers' satisfaction.

Declaration of Interest

None

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Figure legends:

Figure 1. Total, insoluble and soluble fiber composition. Means followed by different letters show statistically significant difference ($p<0.05$).

Figure 2. Acceptability index of bread formulations evaluated by non-celiac (a) and celiac judges (b).

Figure 3. Relative frequency of grades reported for purchase intention by non-celiac(a) and celiac (b) judges.

Table 1 Ingredients for bread formulations made with wheat and teff flour (powder ingredients are reported in grams (g), liquids in milliliters (mL).

Ingredients	**Samples**			
	T1	**T2**	**T3**	**T4**
Wheat flour (g)	100	-	-	-
Teff flour (g)	-	100	75	50
Rice flour (g)	-	-	12.5	25
Cassava flour (g)	-	-	12.5	25
Xanthan gum (g)	-	2	2	2
Refined salt (g)	2.5	2.5	2.5	2.5
Crystal sugar (g)	5	5	5	5
Soybean oil (mL)	6	6	6	6
Biological yeast (g)	2.5	2.5	2.5	2.5
Total water (mL)	100	110	95	70
Egg (g)	48	48	48	48

T1: Standard - wheat flour;
T2: Teff flour (100%);
T3: Teff flour (75%) + cassava starch (12.5%) + rice flour (12.5%);
T4: Teff flour (50%) + cassava starch (25%) + rice flour (25%).

Table 2 Mean texture parameters (Mean ±SD) of the samples.

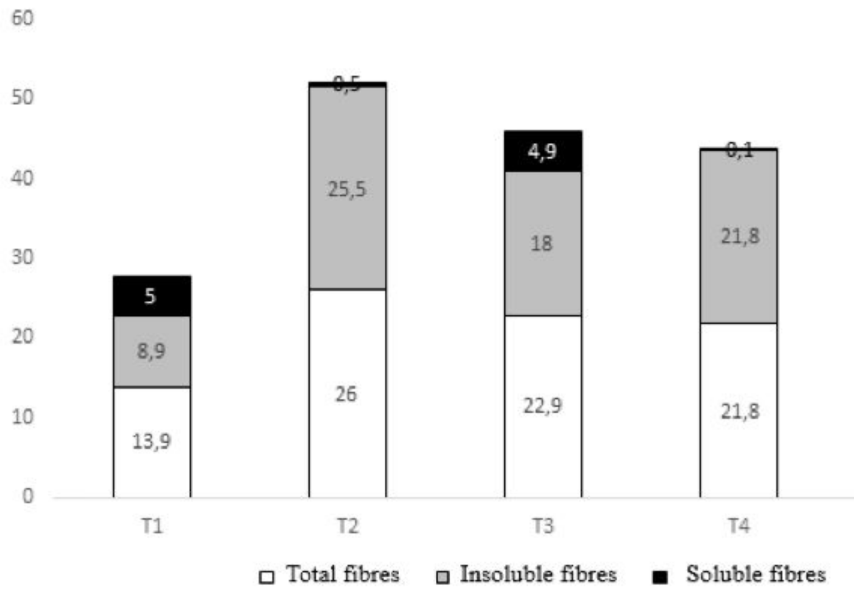
Parameters	Samples			
	T1	T2	T3	T4
Crust				
Hardness (g, force)	188± 39 [a]	48± 8 [b]	40± 5 [b]	59± 13 [b]
Crumb				
Hardness (g, force)	2430± 484 [a]	2188± 43[ab]	1681± 90 [b]	1716± 124 [b]
Elasticity (mm)	1.07± 1.1[b]	1.10± 1.1 [a]	1.10± 1.2 [a]	1.10± 1.1 [a]
Cohesiveness (g.sec)	0.86± 0.6[a]	0.61± 0.3[c]	0.69± 0.4[bc]	0.78± 0.7[ab]
Resilience (g.sec)	10415± 4371[a]	227± 4[b]	190± 6[b]	249± 15[b]
Chewiness (g)	2258± 316[a]	1462± 13[b]	1262± 43[b]	1421± 99[b]
Gumminess (N)	208.784± 2.9[a]	134.287± 1.13[b]	115.828± 3.6[b]	134.576± 9.0 [b]

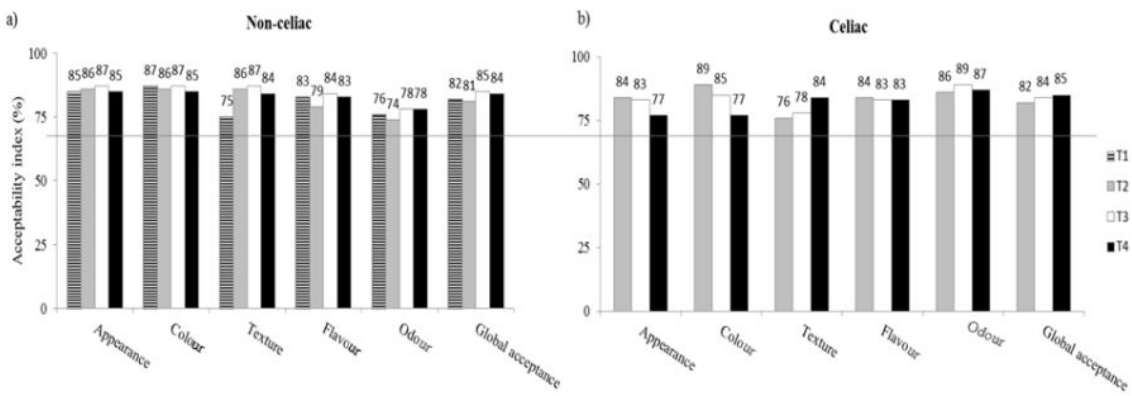
Means followed by different letters in raw show statistically significant difference ($p<0.05$).

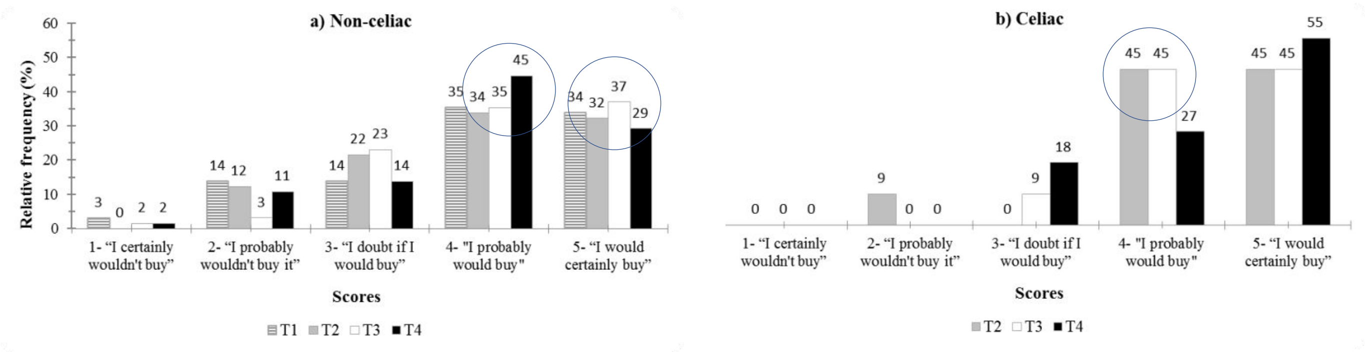
Table 3 Hedonic scores (Mean ± SD) provided by non-celiac and celiac subjects.

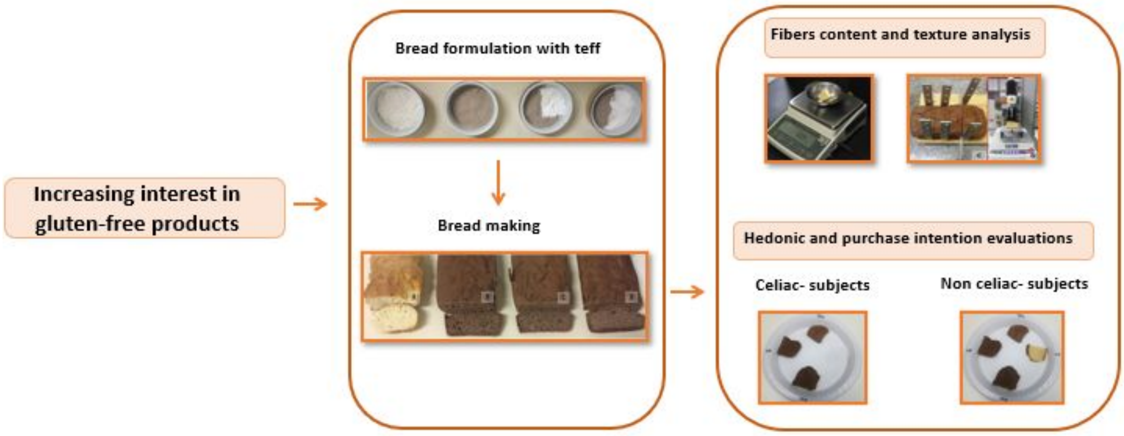
Attributes	Samples			
	T1	T2	T3	T4
	Non-celiac group			
Appearance	7.7±1.4 [a]	7.7±1.1 [a]	7.8± 0.97 [a]	7.7±1.2 [a]
Colour	7.8± 1.3 [a]	7.8± 1.2 [a]	7.8± 1.12 [a]	7.7± 1.1 [a]
Texture	6.7± 1.8[b]	7.7± 1.2 [a]	7.9± 1.06 [a]	7.5± 1.2 [a]
Flavour	7.5± 1.5 [ab]	7.1± 1.4[b]	7.6± 1.16 [a]	7.5± 1.2 [a]
Odour	6.8± 1.7[a]	6.7± 1.6 [a]	7.0± 1.54 [a]	7.0± 1.6 [a]
Global acceptance	7.3± 1.4 [a]	7.3± 1.3 [a]	7.6± 1.10 [a]	7.6± 1.1 [a]
	Celiac group			
Appearance	-	7.5±1.2 [a]	7.5±1.2 [a]	6.9±1.4 [a]
Colour	-	8.0±1.0[a]	7.6±0.9[ab]	6.9±1.4[b]
Texture	-	6.8±1.7 [a]	7.0±1.2 [a]	7.5±1.0 [a]
Flavour	-	7.5±1.4 [a]	7.4±1.7 [a]	7.4±1.7 [a]
Odour	-	7.7±1.3 [a]	8.0±1.0 [a]	7.8±1.0 [a]
Global acceptance	-	7.4±1.4 [a]	7.5±1.0 [a]	7.6±1.1 [a]

Means followed by different letters in raw show statistically significant difference (p<0.05).


60
50
40
30
20
10
0
0,5
25,5
26
4,9
18
22,9
0,1
21,8
21,8
5
8,9
13,9
T1
T2
T3
T4
Total fibres
Insoluble fibres
Soluble fibres


a)
Non-celiac
Acceptability index (%)
100
75
50
25
0
85 86 87 85
87 86 87 85
75 86 87 84
83 79 84 83
76 74 78 78
82 81 85 84
Appearance
Colour
Texture
Flavour
Odour
Global acceptance
b)
Celiac
84 83 77
89 85 77
76 78 84
84 83 83
86 89 87
82 84 85
T1
T2
T3
T4


a) Non-celiac
Relative frequency (%)
1- "I certainly wouldn't buy"
2- "I probably wouldn't buy it"
3- "I doubt if I would buy"
4- "I probably would buy"
5- "I would certainly buy"
Scores
T1 T2 T3 T4
b) Celiac
Scores
T2 T3 T4


Increasing interest in gluten-free products
Bread formulation with teff
Bread making
Fibers content and texture analysis
Hedonic and purchase intention evaluations
Celiac- subjects
Non celiac- subjects