

Quality parameters assessment of cakes produced from acorn-rice flour mixtures

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Abstract

Obtaining gluten-free products generally requires the optimization of certain technological processes aimed at achieving desirable properties to meet consumer expectations. This is necessary because raw materials often lead to unsatisfactory results in terms of taste, flavor, consistency and nutritional qualities. Therefore, various alternative ingredients have recently been explored with the intention of improving the result. In this context, the identification of new viable raw materials and their efficient use to produce flour-based products would be a suitable option for establishing a sustainable economy. Acorn flour are a good source of starch, protein, fat, minerals (such as P, K, Ca, and Mg), unsaturated fatty acids (i.e., oleic acid), and vitamins (mostly A and E) and numerous biologically active compounds (such as tannins, phenolic acids, and flavonoids). This flour possesses anti-inflammatory, mineralizing, anti-anemic, anti-rachitic, antitumor, antioxidant, diuretic, and energizing properties. Moreover, it promotes insulin secretion. The aim of this work consisted in the nutritional, physical and sensory evaluation of gluten-free cakes obtained from rice flour (RF), corn starch (CS) and acorn flour (AF), where RF was replaced by AF in proportion of 5, 15, 25%, using standard laboratory procedures. Four samples of cakes were obtained using different proportions of RF and AF and the same proportion of CS, as follows: 80:15:5%, 70:15:15%, 60:15:25% and 85:15:0% respectively. The results obtained regarding the proximate composition of the studied cake samples show the superior nutritional profile (higher ash, fiber and fat content but lower carbohydrate and protein) of all three cake samples with added AF compared to control sample. Moisture, fat, protein, carbohydrate, crude fiber, and ash content of the cakes ranged between 34.88 - 36.16%, 3.56 - 6.41%, 8.90 - 12.07%, 42.80 - 46.03%, 1.38 - 3.97, and 0.80 - 3.04%, respectively. The height, weight, and volume of the cake samples ranged from 3.5 to 4.5 cm, 45 to 56 g, and 620 cm³ to 1050 cm³, respectively. Following the sensory analysis of gluten-free cake samples, it was observed that the cake sample obtained from 70% RF, 15% CS and 15% AF had been given the highest scores on the Hedonic scale (appearance - 8.2; consistency - 8.7; taste - 8.5; flavor - 8.5; color - 8.2; overall acceptability - 8.4). Following the sensory evaluation of cake samples, we can recommend the use of the flour mixture 70%RF:15%CS:15%AF. Based on the results obtained in this study, it can be concluded that the addition of AF in gluten-free cake samples can significantly rise its nutritional value, alongside improving its sensorial qualities

Keywords: acorn flour, gluten-free cake, sensory evaluation, high nutritional value, health benefits

1. Introduction

In the last decade, consumer health has been a driving factor in the food industry, leading to a rapid development of healthy, nutrient-dense foods as alternatives to traditional products that have significantly lower nutritional values [1]. In the growing demand for innovative food products, organic and functional foods can also be mentioned,

being chosen by consumers for the health benefits they own. There had been observed a straight connection between the used raw materials and consumers' health, therefore a significant accent has been put on the quality and nutritional profile of these ingredients [2, 3]. Furthermore, demand for gluten-free food products also presents an ever-growing scale, as an increasing number of consumers with celiac disease seem to require them

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as part of a special diet. Therefore, it is necessary to find gluten-free alternatives to use in bakery in order to provide celiac patients with more options and different bakery products to meet their needs. It is important to mention that gluten-free diets are generally associated with

fundamental nutritional deficiencies (e.g., dietary fiber, proteins and specific micronutrients such as zinc, magnesium, iron, B vitamins, vitamin D, calcium, and folate) and also excesses (e.g., calorie intake, simple carbohydrates, saturated fats and lipids) [3]. As a result, obtaining bakery products that contain no gluten leads to a number of technological shortcomings and the quality of these foods is compromised [4, 5]. The main aim of this study is to analyse and study different unconventional flours as potential substitutes to traditional, gluten flours, in order to obtain gluten-free bakery products with high nutritional values. Due to its high starch percentage, AF is considered a beneficial unconventional flour and can be used in various baking technologies [6].

Numerous *Quercus* species are known, including *Quercus dentata* and *Quercus ilex ssp. ballota*, which are used in the coffee industry, and *Quercus macrocarpa* and *Quercus alba*, which are used in pastry industry. Additionally, species such as *Quercus suber*, *Quercus acutissima*, and *Quercus arizonica* are highly regarded in the medical field [7]. Various Asian nations also process acorns into jelly (dotorimuk) and paste (dotori guksu), both of which are used in various national dishes. AF can be used to obtain various beverages, pastas, cookies, as a coffee substitute and confectionery [6, 7, 8]. AF is a gluten-free flour made by peeling acorns, drying and grinding their kernels. The process of obtaining AF often involves removing tannins, astringent biomolecules, as their presence in the final product can cause a dry reaction. Numerous studies have shown that AF has beneficial effects on the rheological properties of dough, improving firmness, cohesiveness and viscoelasticity [6, 7]. Food products based on AF showed higher dietary fiber and mineral content. The addition of AF also increased the values of phenolic compounds and antioxidant activity. AF has a high starch value (71-74%), is rich in proteins (5-7%) and fats (7-9%), containing significant quantities of fiber (10-12%) as well. The analysis revealed that AF contains high levels of micronutrients, specifically vitamins B2 (0.13-0.15 mg/100 g), B3 (1.5-2.3 mg/100 g), and B6 (0.03-0.7 mg/100 g), as well as significant

amounts of potassium (379-710 mg/100 g), magnesium (54-110 mg/100 g), calcium (42-160 mg/100 g), and phosphorus (90-102 mg/100 g) [7, 8]. AF contains a significant amount of amino acids, including increased quantities of aspartic acid (0.744 g/100 g), arginine (0.577 g/100 g), alanine (0.427 g/100 g), leucine (0.596 g/100 g), lysine (0.468 g/100 g), and valine (0.421 g/100 g) [8]. Products obtained from AF are characterized by a higher content of dietary fiber and minerals, as well as a higher content of total phenolic compounds and increased antioxidant activity [9]. Research into the use of AF suggests that it has a negative impact on the rheological characteristics of dough and the physical properties of products made with it. Based on the studies carried out, it is recommended that AF be mixed with wheat flour or rice flour in proportions of 15% to 20% [9, 10, 11]. Products obtained from such mixtures have superior nutritional characteristics, including high protein content and fiber, as well as improved sensory characteristics such as taste, odor, color, and general acceptability. However, they have lower technological characteristics, such as volume, porosity, elasticity, and texture [10, 11]. These values ensure that the obtained final product has a higher nutritional value, better sensory and technological properties, compliant with quality standards [12]. Due to its high content of fiber, vitamins and minerals, consumption of acorn flour has numerous benefits for the human body, such as: skin care, improves digestion, prevents diabetes, regulates overall cholesterol levels and prevents obesity, atherosclerosis and other dangerous conditions that threaten heart health, regulates metabolism, has a tonic effect, has a regenerative effect and prevents cancer [13, 14]. In addition, besides the objective of using AF to produce a gluten-free cake with better quality that is richer in bioactive compounds, there is also the advantage of adding value to the product, acorn, as an underexploited raw material. Therefore, due to its unique characteristics, AF can be considered a realistic alternative ingredient in the production of gluten-free cakes. This accomplishes the objective of improving the nutritional profile of the final product, as AF is high in dietary fibers, minerals, and essential amino acids [12]. Based on these observations, the purpose of this study was to obtain an assortment of cake with superior nutritional properties conferred by the addition of AF; establishing the optimal manufacturing recipe and

the optimal dose of AF that can be added to cakes without affecting their quality.

2. Material and methods

2.1. Materials

Acorn flour, rice flour, corn starch, date syrup, egg, milk, salt, butter, xanthan gum, baking powder, orange juice, figs and cranberries are used in preparing the cake dough. These materials were obtained from the local market from Timisoara, Romania.

Preparation of flour mixtures: To establish the optimal proportion of each type of flour to form a mixture, the physical, chemical, and technological

characteristics of each of these flours were taken into account. When forming the four mixtures, RF, CS and AF were used in variable proportions: Mixture (M1): 85%RF:15%CS:0%AF; Mixture (M2): 80%RF:15%CS:5%AF; Mixture (M3): 70%RF:15%CS:15%AF and Mixture (M4): 60%RF:15%CS:25%AF.

2.2. The technological process to obtain gluten-free cakes

Table 1 presents the gluten-free cake formulas. The cakes were prepared using the method described by Gurbina *et al.* (2019) and Martinescu *et al.* (2020) [15, 16], with some modifications.

Table 1. Formulas for gluten-free cakes with RF, AF and CS

Ingredients (g)	Gluten-free cake samples			
	CCM1	CM2	CM3	CM4
RF	136	128	112	96
AF	0	8	24	40
CS	24	24	24	24
Date syrup	115	115	115	115
Butter	90	90	90	90
Xanthan gum	10	10	10	10
Milk	20	20	20	20
Salt	3	3	3	3
Baking powder	10	10	10	10
Egg	100	100	100	100
Figs	80	80	80	80
Cranberries	80	80	80	80
Orange juice	70	70	70	70

RF - rice flour; AF - acorn flour; CS - corn starch; CCM1 - control cake obtained from 85%RF:15%CS:0%AF; CM2 - cake obtained from 80%RF:15%CS:5%AF; CM3 - cake obtained from 70%RF:15%CS:15%AF; CM4 - cake obtained from 60%RF:15%CS:25%AF.

Four types of gluten-free cakes were obtained in this study: CCM1, CM2, CM3 and CM4. For the preparation of cakes with AF, RF was replaced by 5%, 15% and 25% AF. RF and AF were combined with CS, baking powder, xanthan gum, salt, orange juice, and chopped figs and cranberries. In a separate bowl, eggs were mixed with date syrup and orange juice. Once the mixture was homogenized, melted butter and milk were added and mixed first for 2 min at medium speed, then for 1 min at high speed and finally for 2 min at medium speed. The dry mixtures were then gradually added to the mixture, while continuously mixing, until the dough was formed. The four cake samples were baked in preheated electric oven (Kumatel, Turkey) at 40 minutes at 180°C.

After baking, the gluten-free cake samples were cooled at room temperature, then packed in in the

polyethylene packages and stored at temperatures of 12°C for 1 day [12].

2.3. Methods

2.2.1. Proximate composition of flours and gluten-free cake samples

The proximate composition of flours and cakes was determined using the approved AOAC 2000 standard methods [17]: moisture content, fat content, protein content, fiber content. Carbohydrate content was determined by difference using the equation: $100 - (\text{moisture content} + \text{fat content} + \text{ash content} + \text{protein content})$. All determinations were performed in triplicate, calculating their arithmetic mean of three separate determinations. The data were statistically analyzed using the program Microsoft Excel.

2.2.2. Physical characteristics of gluten-free cake samples

Each cake's weight (in grams) was determined individually two hours after baking and the mean value was noted. The volume of the cakes after cooling was estimated by rapeseed displacement method and the height was measured with the ruler (in cm) using AACC [18].

2.2.3. Sensory evaluation of gluten-free cake samples

The quality of freshly baked cakes was determined using the method described by Martinescu *et al.* (2020) [16]. A panel of 25 panelists, ages 21-70, have evaluated gluten-free cake using a 9-point hedonic scale, they rated the similarity of the

product's attributes as follows (1=dislike extremely, 5=neither like nor dislike, 9=like extremely). Gluten-free cakes were sliced into half, labeled, and offered to the panelists on a white plate at room temperature. All four cake samples were served, one at a time, to each taster. The general appearance, consistency, flavor, color, taste and overall acceptability, were evaluated [18].

3. Results and Discussion

3.1. Proximate composition of RF and AF

Table 2 shows the results obtained from proximate analysis of flours. These results highlight their functional potential.

Table 2. Proximate composition of RF and AF

Chemical composition (%)	Flour samples	
	RF	ALF
Moisture	9.22±0.08	8.83±0.12
Fat	0.68±0.12	8.99±0.04
Protein	7.24±0.06	5.11±0.14
Crude fiber	2.42±0.08	2.84±0.22
Ash	0.64±0.15	1.71±0.06
Carbohydrates	79.80±0.08	72.52±0.02

All determinations were done in triplicate and the results were reported as average value ± standard deviation (SD).

The results obtained regarding the proximal composition of AF compared to RF highlight its nutritional potential, as a result of the higher content of fats, crude fibers and ash. Thus, AF presented high levels of fats - 8.99±0.04% compared to 0.68±0.12% in RF, of fibers - 2.84±0.22% compared to 2.42±0.08% in RF, of ash - 1.71±0.06% compared to 0.64±0.15% in RF and lower levels of proteins - 5.11±0.14% compared to 7.24±0.06% in RF and of carbohydrates - 72.52±0.02% compared to 79.80±0.08% in RF, results which are comparable to those obtained by Silva *et al.* (2016), Szabłowska *et al.* (2020) and Martinescu *et al.* (2020) [7, 12, 16]. The obtained results indicate that the partial replacement of RF with AF will significantly influence the chemical composition of the flour mixtures obtained, they will have a higher fat, ash and fiber content, and a lower protein and carbohydrate content. The humidity of AF was lower (8.83±0.12%) than that of RF (9.22±0.08%). Considering the lower moisture content of AF (8.83% <14% the acceptable limits that determine the extension of the shelf life of the products), it can be used to obtain bakery and pastry products [13, 19, 20].

3.2. Proximate composition of gluten-free cake samples

Results showed in Table 3 are the chemical composition of gluten-free cake samples substituted with different levels of AF. The results obtained regarding the chemical composition of the gluten-free cake samples analyzed in this study, show that the addition of AF in the manufacturing recipe, caused a significant increase in nutrient content, so the products obtained can be considered products with a high functional potential, being important sources of protein, fats, minerals, and fibers. The data presented in Table 3 shows moisture variation for the different samples of AF enriched gluten-free cake. The highest value belongs to the control cake (CCM1) being 36.16±0.12%. With the addition of AF in the composition, a progressive decrease in moisture can be observed, inversely proportional to the amount of AF that was added. Therefore, sample CM2 has a moisture value of 35.76±0.09%, sample CM3 has a moisture value of 35.11±0.06% and sample CM4 has a moisture value of 34.88±0.11%. The highest moisture value in the control cake is due to the higher water holding capacity of RF

compared to AF. The moisture values recorded for the studied gluten-free cake fall within the range of values indicated in the specialized literature for cake-type semi-finished products (25 - 35%), thus,

they can be kept for a longer period of time before microbiological degradation sets in, thus having a longer shelf life [10, 16].

Table 3. Chemical evaluation of gluten-free cake samples

Chemical composition (%)	Gluten-free cakes			
	CCM1	CM2	CM3	CM4
Moisture	36.16±0.12	35.76±0.09	35.11±0.06	34.88±0.11
Fat	3.56±0.04	4.61±0.22	5.56±0.14	6.41±0.21
Protein	12.07±0.12	11.03±0.26	9.93±0.01	8.90±0.03
Ash	0.80±0.22	1.47±0.16	2.28±0.33	3.04±0.05
Crude fiber	1.38±0.02	2.23±0.18	3.14±0.07	3.97±0.14
Carbohydrates	62.74±0.08	61.49±0.46	59.13±0.23	56.88±0.31

All determinations were done in triplicate and the results were reported as average value ± standard deviation (SD).

Table 3 shows the results of the analyzes regarding the protein content of the four samples of gluten-free cake. The decrease in protein content of the samples is caused by the addition of AF in recipe, the amount of protein decreasing with the increase in the percentage of AF used (5%, 15% and 25%). Thus, from the initial value of 12.07±0.12% in the control sample (CCM1), the protein content decreases to 11.03±0.26% in case of sample CM2, to 9.93±0.01% in case sample CM3, whereas CM4 reach the value of 8.90±0.03%. These results are similar to the studies made by Levent *et al.* (2023) and Sasani *et al.* (2023) [21, 22]. Along with the increase in the amount of AF and the decrease in the amount of RF in the cake recipe, an increase in the fat content was also observed, as can be seen in Table 3. Thus, the fat content in cake samples increased from 3.56±0.04% in the control sample, to 6.41±0.21% in the cake sample with 25% AF. These results coincide with studies carried out for example by Levent *et al.* (2023) [21], which shows AF has a higher fat content compared to RF. Thus, the addition of AF in food products will also increase their fat content [22]. As can be seen in Table 3, the addition of AF in the cake recipe had a great impact on the ash content of the product. While the cakes from the control sample made from 85% RF and 15% CS presented an ash content of 0.80±0.22%, the cake sample with 15% AF presented an ash content of 2.28±0.33% and the cake sample with 25% AF presented an ash content of 3.04±0.05%. Similar results regarding the ash content were also reported by by Levent *et al.* (2023) and Sasani *et al.* (2023) [21, 22] in the case of cakes with the addition of AF. Thus, a positive effect of the addition of AF on the ash content, and therefore on the mineral content, of food products

can be observed [15, 16, 21, 22]. According to the results presented in Table 3, it can be appreciated that the cake samples obtained at a laboratory level have a high fiber content, up to 3.97±0.14% in the CM4 sample compared to 1.38±0.02% in the CCM1 sample. Among the three gluten-free cake samples with added AF, the highest value of fiber content was recorded in the CM4 sample (3.97±0.14%), followed by the CM3 sample (3.14±0.07%) and the CM2 sample (4.88%) respectively. Given the high fiber content of the studied gluten-free cake samples and due to the raw materials used to obtain them, it is possible to label the product with the statement "Rich in fiber" [11, 15, 16]. Fiber helps to lower the blood cholesterol levels and slows down the glucose absorption process, thus helping to keep blood glucose level under control [23]. Unlike the other characteristics analyzed regarding the proximate composition of the cake made from RF, CS and AF, no significant changes were found regarding the carbohydrate content present in the samples, as can be seen in Table 3. The carbohydrate content of the samples analyzed in this study decreased proportionally to the percentage of AF thus added, from 61.49±0.46% (CM2) to 56.88±0.31% (CM4), compared to the CCM1 sample which had a carbohydrate content of 62.74±0.08%. The results obtained regarding the carbohydrate content of the analyzed samples are higher than those obtained by Sasani *et al.* (31.22%), but they are still relatively small values, which will determine obtaining products with a relatively low glycemic index [22].

3.3. Physical characteristics of gluten-free cake samples

The height, weight, and volume results belonging to gluten-free cakes are shown in Table 4.

Table 4. Physical characteristics of gluten-free cake samples

Parameter	Gluten-free cake samples evaluated			
	CCM1	CM2	CM3	CM4
Height (cm)	4.5±0.09	4.3±0.24	3.9±0.02	3.5±0.14
Weight (g)	56.24±0.11	51.12±0.23	48.44±0.09	45.56±0.22
Volume (cm ³)	1050.35±0.08	824.86±0.44	7.62±0.26	620.21±0.15

All determinations were done in triplicate and the results were reported as average value ± standard deviation (SD).

Technological quality of cake samples, such as height, weight, and volume were affected by the level of AF in the products. The height of the cake samples decreased significantly as the concentration of AF increased. The height of the cake samples decreased significantly as the concentration of AF increased. The height of the control sample was 4.5±0.09 cm, while in the samples containing AF (5%, 15% and 25%) it decreased to 4.3±0.24 cm in CM2, to 3.9±0.02 cm in CM3 and respectively 3.5±0.14 cm at CM4. Similar results were reported by Molavi *et al.* (2015) [11]. The average weight of the cake samples (Table 4) with the addition of AF varied from 51.12±0.23g in the case of CM1 to 45.56±0.22 g in the case of CM4, compared to CCM1 which had an average weight of 56.24±0.11 g, thus finding that the weight of cake samples analyzed in this study, decreased in proportion to the ratio of AF incorporated in the dough. Similar results were reported by Molavi *et al.* (2015) [11]. Regarding the volume of the analyzed cake samples, it has decreased from 824.86±0.44 cm³ in sample

CM2 to 620.21±0.15 cm³ in CM4, compared to CCM1 which presented an average volume of 1050.35±0.08 cm³. The decrease in the height, weight, and volume is due to the relative decrease in the gluten content. AF dose not contain gluten therefore it causes the loss of air bubble from the cake batter and decreases the cake characteristics. The quick binding of free water molecules by the hydrophilic sites of non-wheat flours or other ingredients can increase the viscosity of the batter thus resulting in cakes which spread less [11, 15, 16, 22].

Sensory evaluation of gluten-free cake samples

Sensory characteristics of gluten-free cake obtained in this study, were influenced mainly by the raw materials and proportions used in the formulation, the results being presented in the Table 5. Experiments showed a direct correlation between the dough composition, working technological parameters and qualitative properties of these cake assortments.

Table 5. Sensory evaluation of gluten-free cake samples

Sensory evaluation	Gluten-free cake samples			
	CCM1	CM2	CM3	CM4
Appearance	7.14 ± 0.87	7.76 ± 1.22	8.22 ± 0.13	6.62 ± 0.02
Consistency	7.72 ± 0.14	7.98 ± 0.12	8.76 ± 0.09	6.34 ± 0.14
Taste	8.08 ± 0.17	8.13 ± 0.36	8.56 ± 0.14	7.23 ± 0.33
Flavor	6.56 ± 0.07	7.04 ± 0.13	8.50 ± 0.55	7.48 ± 0.09
Colour	7.04 ± 0.43	7.88 ± 0.66	8.20 ± 0.02	7.07 ± 0.04
Overall acceptance	7.36 ± 0.32	7.85 ± 0.18	8.40 ± 0.22	7.27 ± 0.24

All determinations were done in triplicate and the results were reported as average value ± standard deviation (SD).

Sensory analysis of gluten-free cake samples was carried out using the 9-point Hedonic scale, a method generally used to determine the acceptability of new or existing products on the food market. Sensory quality attributes were evaluated by a panel of 25 semi-trained members. Partial substitution of RF with AF causes significant changes in these attributes (appearance, consistency, taste, flavor, color, and overall acceptability) between CCM1 and gluten-free cake samples with the addition of 5%, 15% and 25% AF.

The gluten-free cake samples showed changes in flavor depending on the proportion of AF added, therefore the lowest score was obtained by the CM2 sample (flavor - 7.04 ± 0.13) and the highest score by CM3 sample (flavor - 8.50 ± 0.55), compared to CCM1 which presented a score of 6.56 ± 0.07. The results obtained regarding the appearance, consistency and taste of the gluten-free cake samples showed that the partial substitution of RF with variable proportions of AF directly influences these characteristics, thus, the scores assigned to the

appearance increase from 7.14 ± 0.87 in CCM1 to 8.22 ± 0.13 in CM3, those attributed to consistency increase from 7.72 ± 0.14 in CCM1 to 8.76 ± 0.09 in CM3, and those attributed to taste increase from 8.08 ± 0.17 in CCM1 to 8.56 ± 0.14 in CM3. Regarding the color, no big differences were found between the samples, however, a greater appreciation was observed with the increase in the amount of AF in the recipe, the best result being obtained by the gluten-free cake samples with 15% AF (8.20 ± 0.02), and the lowest by the control sample, made from 85% RF and 15%CS (7.04 ± 0.43). Color of the cake containing AF was brownish, due to reducing sugars that exist in AF and other ingredients, but it did not have an adverse effect on panelists' evaluation and they scored the sample similar to the control. Previous product attributes influenced the overall acceptability of the gluten free cake, the rate increasing in CM2 - 7.85 ± 0.18 and CM3 - 8.40 ± 0.22 samples compared with CCM1 (7.36 ± 0.32) but to CM4 sample, the hedonic rate decreased to 7.27 ± 0.24 . Summarizing all these data, it can be seen that the CM3 sample was the most appreciated by the panelists (appearance – 8.22; consistency – 8.76; taste - 8.56; flavor - 8.50; color - 8.20; overall acceptability - 8.40) , so it can be considered that the addition of 70% RF, 15%CS and 15% AF is the optimal proportion that must be used in order to obtain the most appreciated variety of gluten-free cake from the sensory point of view [11, 15, 16, 23 - 25].

Conclusion

The data obtained from this study demonstrate that AF can be used to design and develop food products with improved functionality. With the substitution of an amount of RF in the recipe with AF, an improvement in their nutritional properties was observed, an increase in fiber, fat and ash content and decrease in content of carbohydrates, with a positive impact on health and various diseases, having the potential to be incorporated into a wide range of functional food products. The scores for sensory attributes such as appearance, consistency, taste, flavor and color were generally higher. Therefore, the cakes with AF had better overall acceptability scores than the CCM1.

Correlating the results obtained in terms of sensory, chemical, and physical analysis of cake samples, we can appreciate that the recipe established for obtaining the cake with the addition of 85% RF: 15%Cs:15%AF can be successfully applied on an

industrial scale, thus obtaining both nutritionally and qualitative rich products.

Compliance with Ethics Requirements. The authors declare that they comply with the Ethics requirements of the journal. The authors declare that they have no conflicts of interest and that all procedures involving human or animal subjects (if any) comply with specific regulations and standards.

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