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Canary seed flour as a promising new ingredient for gluten-free cookies

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Gozzi, M.S.[1]; Rosito, P.J.[1]; Cisternas, M.E.[1]; Fiorito, R.[1]; Játiva Gutiérrez, M.P.[1]; Ormando, P.[1,2]

ABSTRACT

Food science and gastronomy collaborate to provide healthy, customized options, including gluten- free food for people with celiac disease. Hairless canaryseed (*Phalaris canariensis* L.) is considered a gluten-free cereal with a good nutritional profile. The aim of this work was to characterize hairless canaryseed flour (CSF) as a new ingredient and the effect of its incorporation (0% Control, 15% [R15] and 35% [R35] to weight flour) in gluten-free cookies. The chemical composition, carotenoids, minerals, and color were determined. Also, the physical, mechanical and sensory parameters of the cookies were evaluated. The results showed an increase in protein (5.98-9.27%), ash (1.42-2.5%) and mineral content (iron 1.62-2.55 mg%, magnesium 15.63-46.13 mg%, potassium 70.75-125.60 mg%), and a decrease in lightness (74.30-60.93). Fortification increased the thickness of the cookies, while it also decreased the spread ratio and hardness. Sensorially, the enriched cookies had good acceptability, with color being the only parameter in which significant differences were observed. The results showed that CSF can be used to enhance the nutritional, physical, and sensory characteristics of gluten-free cookies.

Keywords: hairless canaryseed, protein source, bakery.

RESUMEN

La ciencia de los alimentos y la gastronomía trabajan en conjunto para proporcionar opciones saludables y personalizadas, lo cual incluye alimentos sin gluten para personas con enfermedad celíaca. El alpiste sin fibras de sílice (Phalaris canariensis L.) es considerado un cereal sin gluten con un buen perfil nutricional. El objetivo de este trabajo fue caracterizar la harina de alpiste sin sílice (CSF, por sus siglas en inglés) como un nuevo ingrediente y evaluar el efecto de su incorporación (0% Control, 15% (R15) y 35% (R35) de reemplazo en relación con el peso de la harina en galletitas sin gluten. Se determinaron la composición química, los carotenoides, los minerales y el color. Además, se evaluaron parámetros físicos, mecánicos y sensoriales de las galletitas. Los resultados mostraron un aumento en el contenido de proteína (5,98-9,27%), cenizas (1,42-2,5%) y minerales (hierro 1,62-2,55 mg%, magnesio 15,63-46,13 mg%, potasio 70,75-125,60 mg%), y una disminución en la luminosidad (74,30-60,93). La fortificación incrementó el grosor de las galletitas, redujo la relación de expansión y aumentó la dureza. Sensorialmente, las galletitas enriquecidas presentaron buena aceptabilidad, siendo el color el único parámetro en el que se observaron diferencias significativas. Los resultados demostraron que la CSF puede ser utilizada para mejorar las características nutricionales, físicas y sensoriales de las galletitas sin gluten.

Palabras clave: *alpiste sin fibras de sílice, fuente de proteínas, productos de panificación.*

[1]Universidad Argentina de la Empresa (UADE), Instituto de Tecnología (INTEC), Lima 775, Ciudad Autónoma de Buenos Aires, Argentina. Correo electrónico: mgozzi@uade.edu.ar

[2]Instituto Nacional de Tecnología Agropecuaria (INTA), Centro de Investigación de Agroindustria, Instituto Tecnología de Alimentos (ITA) Nicolás Repetto y De Los Reseros s/n. (1686) Hurlingham, Buenos Aires, Argentina.

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INTRODUCTION

Consumer demand for healthier options has led to a search for alternative ingredients to enhance the nutritional value of products. Diet has been proven to be a key factor in preventing chronic non-communicable diseases, as demonstrated by numerous studies (Lobefaro *et al*., 2021). In addition, individuals are turning to plant-based diets due to ethical and moral considerations. This requires an adaptation and an effort from all the actors in the agri-food sector to develop along with changes in society.

Nowadays, there is an interaction between food science and gastronomy, with the latter being regarded as science-based-cooking or scientific gastronomy (Navarro *et al*., 2012). This approach to gastronomy uses new ingredients and technologies that can interact with nutrients to offer healthier food and dishes, adapted to people's tastes and lifestyles and in line with sustainability.

Besides this, the food science-gastronomy relationship could also help in the development of diets that meet special nutritional requirements, like in celiac disease. Providing a specific response is another factor to consider for those people who need gluten-free products, where a gluten-free diet is the only way to be healthy. Its prevalence in the general population ranges from 0.5% to 2%, with an average of about 1% (Catassi *et al*., 2022). In Argentina, 1 in 167 adults has celiac disease and the prevalence in childhood is higher (1 in 79) (ANMAT, 2023). Throughout 2025, the gluten-free market is projected to grow at a compound annual growth rate (CAGR) of 8.1% (Marketsandmarkets, 2020). Valitutti *et al*. (2017) reported that the consumption of gluten free crackers and biscuits is higher than that of gluten free bread. Rice flour is the most used gluten free flour for bakery goods, and it is often formulated with flour, starches, and proteins from cereals, pulses, pseudocereals, and other plant materials to achieve optimal dough properties and a high nutritional quality. Gastronomy, hand in hand with science, has the opportunity to provide alternatives to satisfy this demanding market.

Canaryseed (*Phalaris canariensis* L.), also known as annual canarygrass, is an emerging crop with unique nutritional, technological, and functional properties. The hull surface of the pubescent canaryseed grain is covered with trichomes (small, silicified hairs or spicules) that could cause skin irritations during harvesting and handling. To avoid this, new genetically modified varieties of canaryseed (called hairless or glabrous) were developed in Canada for human consumption and food applications (Patterson *et al*., 2018). According to the literature (Patterson *et al*., 2018; Abdel-Aal *et al*., 2011), on dry weight basis, the hairless canaryseed whole grain flour contains 55% of starch, 19-23% of protein, 5-7% of crude fat, 7-8% of total dietary fiber, and 2-3% of total ash. It also has bioactive properties and health-promoting effects, with superior antioxidant activity than those from oats and wheat (Mason *et al*., 2020).

Argentina has the necessary agroclimatic conditions for this crop. It is considered the third largest producer of canaryseed in the world, but it only has pubescent varieties, which are used mainly as bird feed (Cogliatti, 2012). Developing bakery products like cookies enriched with canaryseed flour could be a way to visualize the utility of this cereal flour and promote the possibility of growing canaryseed for human consumption in the country.

The aim of this research was to characterize hairless canaryseed flour and its incorporation into gluten-free cookies, including the evaluation of physico-chemical parameters and consumer acceptance.

MATERIALS AND METHODS

Canaryseed flour sample

The silica-free canaryseed flour (CSF) was kindly provided by InfraReady Products Ltd. (Saskatchewan, Canada). It was made from select hairless canaryseed (CDC Cibo variety, year 2020) that had been dehulled and milled through a process of impaction that creates a uniform, free-flowing flour. The granulation distribution was as follows: 425 µm, 5.0% max, 250 µm, 20.0% max, and less than 250 µm, 75.0% min. This was the first variety of yellow seed developed for human consumption, which is more palatable and aesthetically-pleasing.

Water- and oil-holding capacity of canaryseed flour

The water-holding capacity (WHC) and oil-holding capacity (OHC) of the flour were determined according to Rodríguez-Ambris et al. (2008), with slight modifications. Twenty-five milliliters of distilled water or commercial sunflower oil (density 0,910 g/ml) were added to 1 g of sample, stirred, and incubated at room temperature for 1 hour. After centrifugation (20 min, 4500 x g), the supernatant was removed. The tubes were placed upside down and tilted at 45° for 10 min. Then the residue was weighted, and the WHC and OHC were calculated as the mass of water or oil by sample (g water or oil/g sample).

Preparation of the cookies

The formula of the control cookies (C) was as follows: 170g of gluten-free mix (40% rice flour, 30% corn starch, and 30% cassava starch), 80g of whole egg, 40g of xylitol (sugar substitute), 30g of coconut oil, 10g of vanillin, 6g of baking powder and 1g of stevia (sweetener). The gluten-free mix was replaced with canaryseed flour to a level of 15% (R15) and 35% (R35) in the cookie recipe. All the ingredients were well mixed, and the dough was kneaded for ten minutes, sheeted to a uniform thickness of 4 mm, and refrigerated at -18ºC for 10 minutes. Then the dough was cut into a 30 mm diameter circular shapes and baked at 160ºC for 15 minutes in an electric convection oven (Pauna Convector Beta 21l, Argentina). After baking, the cookies were cooled at an ambient temperature and packed in polythene bags at room temperature until further analysis. Two batches were made for each formula.

Physicochemical composition, mineral content, and color parameters

The determination of the moisture content (MC) was carried out using a moisture analyzer (model MB35, OHAUS, USA) at 105ºC until constant weight. Moisture percentage as a function of weight change was recorded. The water activity (aw) was measured using an AquaLab Lite® dew point water activity meter (Decagon Devices Inc., Pullman, WA, USA). The protein content was determined through the *Kjeldahl* method (N x 5,7) (Keltec K-454/355, Büchi, Germany), and the crude fat was analyzed using Soxhlet extraction with ether (SER-148; VELP Scientifica, Usmate Velate (MB), Italy). The ash content was gravimetrically determined after the incineration of the samples in a muffle furnace at 550ºC until constant weight. As for the CSF, the dietary fiber was determined using a combination of enzymatic and gravimetric methods (AOAC, 2000) (SIGMA Total Dietary Fiber Assay Kit TDF100A-1KT). The carbohydrates of the CSF were calculated by difference. All the results were expressed as g/100g.

The total carotenoid content (TCC) was determined according to Li and Beta (2012) with slight modifications. One gram of the flour was extracted with 25 mL of an acetone/ethanol solution (1:1, v/v) and sonicated for 15 min. Then the mixture was centrifuged for 10 min (4500 x g/4ºC). The supernatant was collected and placed in a 25 mL flask. The pellet was re-extracted. The supernatant was also placed in the flask, which was then filled to the mark with a solvent (solution. All manipulations were carried out in darkness to preserve the carotenoids. The absorbance was measured at 450 nm. Total carotenoids were calculated using the following equation (1):

Equation 1

$$TCC\ (mg/kg) = (Ab \times Vol \times 10^6) / (L \times A^{1\%} \times 100 \times W)$$

where *Ab* is the sample absorbance, *Vol* is the total final volume, *L* is the cell path length (1 cm), and *W* is the weight of the sample.

The mineral content (sodium, iron, calcium, magnesium, and potassium) was determined using a flame atomic absorption spectrophotometer (Model AA Analyst 100, Perkin Elmer), based on the adapted methodology of AOAC (2000). The results were expressed as mg/100g.

The chromatic parameters of the CSF and the cookies were measured using a colorimeter CR 400 Minolta (Minolta, Osaka, Japan). The equipment was set up for a D65 illuminant and 2° observer angle. The instrument was calibrated using a standard white reflector plate. Ten random samples were selected for each batch. The system selected was CIE L* a* b*, where L* represents the lightness (0-black to 100-white), a* represents the chromaticity from green (- values) to red (+ values) and b* represents the chromaticity from blue (- values) to yellow (+ values). The total color change (ΔE) was calculated according to equation (2):

Equation 2

$$\Delta E = ((L_0^*-L^*)^2 + (a_0^*-a^*)^2 + (b_0^*-b^*)^2)^{1/2}$$

where L_0^*, a_0^* and b_0^* represent the chromatic parameters of the control sample (C) and L^*, a^*, and b^* are the chromatic parameters of R15 and R35.

Physical and mechanical parameters of the cookies

The diameter (mm) and thickness (mm) of ten cookies were measured with a digital vernier calliper. The spread ratio was calculated as the diameter divided by the thickness of cookies. The bake loss of cookies was calculated by weighing ten cookies before and after baking. The difference in weight was average, and it was reported as a percentage bake loss (Chauhan *et al*., 2016). All tests were conducted in triplicate. Data are shown as average ± standard deviation.

The hardness (g.s) and fracturability (g.s) of the cookies were measured with a TA.XT Plus Texture Analyzer (Stable Micro Systems, Godalming, United Kingdom). Hardness (peak force) was calculated by measuring the maximum resistance of each cookie against a rounded-edge blade and occurred when the sample began to break. Fracturability was defined as the distance to peak force. A cylinder probe (SMSP/3 mm diameter) was used to penetrate an individual cookie. The test speed was 0.5 mm/s, and a 5-kg load cell was used.

Sensory evaluation

To assess the parameters of taste, sweetness, color, texture and flavor of the cookies, a sensory evaluation with 65 non-trained panelists that were drawn within the University community was performed, using a five-point hedonic scale ranging from 5 (like a lot) to 1 (dislike a lot). The global acceptability was evaluated by using a non-structured horizontal line scale of 10 cm, where the left side of the scale begins at zero (dislike a lot) and the right side of the scale ends at ten (like a lot). The tests took place in individual cabins, where the samples were presented codified with random numbers, and the panelists were given enough water to rinse their mouths between samples.

Statistical analysis

The proximate composition, mineral content, and physical parameters of the cookies were analyzed statistically using GraphPad Prism software (GraphPad Software, Boston, version 8.0.1). The differences between the mean values were determined using the analysis of variance (ANOVA) with a Tukey's test ($p<0.05$). The data are presented as means ± SD, and the values were obtained from triplicate determinations.

A statistical analysis of the color and mechanical parameters of the cookies was carried out with the open-source software R Core Team. For each cookie, the average of the L*, a*, b*, and ΔE color measurements and texture measurements (hardness and fracturability) were performed by the analysis of the data set. Generalized linear mixed models were built to evaluate the effect of percentage replacement on cookies. The replacement treatment and its three levels (Control – R15 – R35) were integrated as fixed factors, while the cookies were integrated as random effect factors. A deviance test with a threshold of 5% was performed to fixed and random effects. The data were analyzed using the packages lme4, lmerTest and flextable to build and compare the models.

For the sensory evaluation, the reliability analysis was carried out using Cronbach´s α (0,57). The normality of the sample distribution was assessed with the Shapiro-Wilk normality test. After that, the groups were compared using the Kruskal Wallis test and post-hoc comparisons were made using Dunn´s test.

RESULTS AND DISCUSSION

Canaryseed flour characterization

Hairless canaryseed flour has a unique nutritional profile, and it could be used as a novel food ingredient for bakery products, pastas, breakfast cereals and snacks. The chemical composition of the canaryseed flour is illustrated in table 1.

Canaryseed flour (CSF) stood out for its high protein content (16.2%), which was higher compared to other common cereals and legumes, such as wheat (13%), oat (10%), barley (13%), rye (11%) (Mason, 2019), rice (2.416%), corn (3.775%), and soy (4.454%) (Kohli *et al*., 2023). This emerging gluten-free cereal could be considered as an alternative source of plant protein,

Moisture (%)	6.1 ± 0.2
Nutritional values (g/100g)	
Ash	4.9 ± 0.1
Protein	16.2 ± 0.1
Crude fat	5.5 ± 0.1
Fiber	8.0 ± 0.1
Total carbohydrate**	59.3 ± 0.5
Total carotenoids (mg/kg)	9.4 ± 0.4
Mineral content (mg/100g)	
Sodium	37.5 ± 1.0
Iron	16.8 ± 0.3
Calcium	33.7 ± 0.6
Magnesium	147.9 ± 3.6
Potassium	149.2 ± 0.7
Color	
L	49.4 ± 1.8
a*	6.8 ± 0.2
b*	12.3 ± 0.1

Results are means ± SD (n=3)

**by difference

Table 1. Nutrient content and color of CDC Cibo canaryseed flour.

because of the overall quality of its proteins (rich in tryptophan aminoacid) and the health-promoting effects of some bioactive peptides (Urbizo-Reyes *et al*., 2021). It could also be a novel ingredient for improving the nutritional quality of non-gluten products.

The ash content reflected the high mineral content of this cereal, in agreement with Lima *et al*. (2021). Sodium and iron were higher, while potassium and magnesium were lower compared to other canaryseed varieties such as CDC Maria variety (Abdel-Aal *et al*., 2021). The iron and calcium levels were significantly higher than those in wheat (4.2 mg/100g Fe and 20 mg/100 g) (Abdel-Aal *et al*., 2021) and other gluten-free flours (1.11 mg/100 g Fe and 3.5 mg/100 g Ca for corn, 8.24 mg/100 g Fe and 3.2 mg/100g Ca for buckwheat, and 1.03 mg/100g Fe and 7.3 mg/100g Ca for rice) (Rybicka and Gliszczyńska-Swiglo, 2017). Since minerals have diverse functionalities and play a key role in metabolism and homeostasis, using canaryseed flour to prepare bakery products could be a good source of those minerals.

The fiber content, crude fat and total carbohydrate values were in accordance with the literature (Patterson *et al*., 2018). Carotenoids are a group of fat-soluble pigments and are well-known for their potential beneficial effects in disease prevention and health promotion (Ngamwonglumlert and Devahastin, 2019). The total carotenoids values were like those reported by Li and Beta (2012), where the canaryseed flour had the highest content (10.86-11.69 mg/kg) followed by the canaryseed wholemeal (7.53-10.03 mg/kg). The same authors reported that lutein, zeaxanthin, and β-carotene were the major carotenoids compounds identified in glabrous canaryseed.

Regarding the chromatic parameters, some authors found that the ash content had significant influences on the lightness value (L*) (Aprodu and Banu, 2017). The higher the ash content, the lower L* value. The canaryseed flour presented a high ash content (4.9%) and an L* value of 49.4. For wheat, rye and oat flour, the same authors reported lower ash contents of 0.43%, 0.54% and 0.85% with higher L* values of 92.62, 88.71, and 88.25, respectively. For gluten-free flours, Azza and Hanan (2015) reported ash content values of 0.66% for rice and 4,03% for sweet potato, with L* values of 74.33 and 68.90, respectively.

The water (WHC) and oil holding capacity (OHC) of CSF were 0.99 ± 0.02 and 0.91 ± 0.02 g water or oil/g sample, respectively. The WHC and OHC describe the ability of the different components of the flour (starch, protein, fiber) to retain water or oil, respectively, against gravity. It reflects the hydrophilic and hydrophobic balance of its components. Rikal *et al*. (2023) determined some of the functional properties of canaryseed CDC Togo dehulled flour (a hairless variety) and of the traditional commercial canaryseed white flour (with silicified hairs). The particle size distribution of both flours presented a higher peak through a 250 µm mesh. The WHC and OHC values were 0.97 and 1.17 for Togo´s dehulled flour and 0.91 and 1.06 for traditional commercial flour. Azza *et al*. (2023) used alternatives flours when preparing baladi bread and reported the values of WHC (1.03 g water/g sample) and OHC (0.68 g oil/g sample) of a fine canaryseed flour (particle size lower than 250 µm) obtained from a local grain market without mentioning the variety. As could be observed, the values of these functional properties vary, being slightly higher or lower than those of CSF. This behavior could be due to the differences in the proximate composition of all the canaryseed flours, which reflects the physical and chemical composition of the grain, the dehulling and milling process (equipment used and conditions) (Lima *et al*., 2021). According to Islam *et al*. (2024), the particle size distribution also seems to have an important role on the functional and nutritional properties of flour and on the properties of the foods produced from them. Reducing article size improved the color and texture of the baked wheat products (Islam *et al*., 2024) and increased the water-binding capacity of rice flour (Lapčíková *et al*., 2021).

Inglett *et al*. (2015) used amaranth and oat-bran flours to prepare gluten-free cookies, with using wheat flour for the control cookies. As for their water-holding capacity (WHC), they reported values of 1.37; 3.56; and 0.92 for amaranth, oat-bran concentrate, and wheat flour, respectively. These values seem to correlate with the type of ingredient (refined vs. wholemeal) and their fiber content (Inglett *et al*., 2015). The canaryseed flour (CSF) exhibited a WHC slightly higher than that of wheat flour but lower than amaranth and, particularly, oat bran concentrate flour. Oats have a high β-glucan content, a type of polysaccharide which is a component of dietary fiber and a common component of the cell walls in the endosperm. The WHC of β-glucans is related to their highly hydrophilic nature due to the abundance of hydroxyl groups that form hydrogen bonds with water, allowing the molecule to absorb water (Sztupecki *et al*., 2023). According to Demirbas (2005), who determined the β-glucan content of 14 selected cereal grains grown in Turkey, oat grains have the highest β-glucan content (ranging from 3.9% to 5.7%), while canaryseed showed a lower content (ranging from 1.1% to 2.3%).

Higher values of WHC are related to the water absorption of the flour in the dough, which modifies the rheological properties (hardness, fracturability) and the physical parameters of the product. It is desirable to improve yield in most food processing systems and provide good organoleptic properties that make foods unique and acceptable to consumers (Huang *et al*., 2019).

Physical and mechanical analysis of the cookies

The effects of the CSF substitution on the physical parameters of cookies, such as weight, diameter, thickness, spread ratio and bake loss, are presented in table 2. It was found to have a significant effect (p<0.05) on thickness (figure 1), spread ratio and bake loss. The cookies with CSF addition (R15 and R35) showed a higher thickness and a lower spread ratio than the control cookies. The bake loss decreased with increased levels of CSF.

The decrease in the spread ratio of the cookies was also reported by other authors after the addition of refined and wholemeal canaryseed flour (González Cobos, 2019), grape skin (Kuchtová *et al*., 2018), coffee extract residue (Han and Lee, 2021), and flour blends of minor millets in gluten-free cookies (Sharma *et al*., 2016). The spread ratio depends on the viscosity of the dough and fat content. Considering that the cookies were gluten-free with a similar fat content, the difference on the spread ratio could be due to the fiber content of CSF, which interacts with the water, making it less available, modifying the consistency of the dough (Mudgil *et al*., 2017). This also affects the bake loss. Besides this, the protein content also modifies the binding water. Chauhan *et al*. (2016) observed this effect on cookies made with different blends of wheat flour (10.56% of protein) and gluten-free amaranth flour (15.05% of protein), where the bake loss was decreased with a higher proportion of amaranth flour. This trend was also observed in the cookies with the highest amount of CSF, since, as mentioned before (3.1), CSF is a good source of proteins. Having less bake loss is also important because there is a higher yield in the baking process.

As for the mechanical parameters, the linear mixed model for hardness presented a $R^2 > 0.768$; and for fracturability, 0.301. Regarding the textural parameters (table 2), hardness refers to the ease with which the product will break, and it has a close association with the human perception of freshness (Azza and Hussien, 2015). The hardness tended to decrease with a higher replacement of CSF, but only the R35 showed a significant difference (p<0.05) from C cookies. These results agreed with several authors like Azza and Hussein (2015) for gluten-free cookies from broken rice flour and sweet potato and Kutchová *et al*. (2018) for cookies with grape skin and seed flour. This could be due to the high protein content of CSF and its water retention properties, which are likely to contribute to decreasing hardness. Mancebo *et al*. (2016) found that the addition of pea protein to sugar-snap cookies lowers the hardness, in contrast to 100% rice flour cookies. Gerzhova *et al*. (2016) also observed an increase in the thickness and a decrease in the spread ratio and hardness in gluten-free biscuits with canola protein concentrate. It is well recognized that the hardness of cookies is much affected by the composition and particle size of flour, the interaction among ingredients, and the different process parameters.

Physicochemical, mineral and color analysis of the cookies

The proximate composition, mineral content and color of the cookies are presented in table 3.

The cookies had low moisture content, being considered as low-moisture-food (Giuberti *et al*., 2018). Besides, the samples had low water activity (0.377 ± 0.061 for C, 0.289 ± 0.006 for R15, and 0.220 ± 0.04 for R35). Water activity (aw < 0.6) is considered one of the most important parameters in food preservation, since it contributes to a greater microbiological and chemical stability, more than moisture content (Syamaladevi *et al*., 2016). The ash content of the cookies was significantly different, increasing with higher flour replacement. In other studies, using different cereals and flour blends (such as wheat, niger, millet and birdseed) in breads (Lima *et al*., 2021) and lentil extract in crackers (Polat *et al*., 2020), the ash content increased when compared to the control samples. The same results were found for the protein content of the cookies. R15 had 22% more protein content than C, while R35 had 55% more. There is a growing global demand in the world for new plant-based protein sources, so the use of hairless canaryseed flour could be a good alternative for the development of novel gluten-free functional foods with potential health promotion properties. The analysis revealed 3.7 mg% of total carotenoids in cookies, with no significant differences between formulations. Carotenoids are lipid-soluble antioxidants, important for human health, and they must be obtained through the diet because animals cannot synthesize them in their bodies (Li and Beta, 2012).

Parameter	Control	R15	R35
Weight (g)	2.46 ± 0.23^a	2.56 ± 0.34^a	2.58 ± 0.21^a
Diameter (mm)	30.42 ± 0.47^a	30.76 ± 0.54^a	30.85 ± 0.86^a
Thickness (mm)	6.88 ± 0.78^b	9.52 ± 1.25^a	9.76 ± 1.22^a
Spread ratio	4.42 ± 0.40^a	3.53 ± 0.50^b	3.50 ± 0.50^b
Bake loss (g/100g)	27.11 ± 0.10^a	25.20 ± 0.50^b	23.80 ± 0.19^c
Hardness (g.s)	2810 ± 360^a	2053 ± 353^a	1422 ± 322^b
Fracturability (g.s)	3214 ± 682^a	3194 ± 860^a	2986 ± 667^a

Results are means ± SD (n=3). Means (two repetitions) followed by different letters, in the same column, are significantly different to each other (p<0.05).

Table 2. Physical and mechanical parameters of the cookies.

	Control	R15	R35
Moisture (%)	4.67 ± 0.54[a]	5.08 ± 0.51[a]	3.46 ± 0.35[b]
Nutritional values (g/100g)			
Ash	1.42 ± 0.15[c]	1.93 ± 0.11[b]	2.50 ± 0.15[a]
Protein	5.98 ± 0.08[c]	7.32 ± 0.04[b]	9.27 ± 0.12[a]
Crude fat	14.30 ± 0.12[c]	14.54 ± 0.08[b]	15.39 ± 0.16[a]
Total carotenoids (mg/kg)	4.1 ± 0.5[a]	3.7 ± 0.5[a]	4.5 ± 0.4[a]
Mineral content (mg/100g)			
Sodium	32.85 ± 0.25[a]	32.77 ± 0.35[a]	34.53 ± 2.39[a]
Iron	1.62 ± 0.26[c]	2.13 ± 0.12[b]	2.55 ± 0.24[a]
Calcium	143.60 ± 2.60[a]	143.10 ± 1.17[a]	140.00 ± 2.92[a]
Magnesium	15.63 ± 0.39[c]	29.38 ± 0.92[b]	46.13 ± 1.86[a]
Potassium	70.75 ± 3.05[c]	93.90 ± 2.12[b]	125.60 ± 7.28[a]
Color			
L	74.30 ± 1.37[a]	67.23 ± 1.45[b]	60.93 ± 1.36[c]
a*	6.07 ± 0.57[a]	5.26 ± 0.43[b]	5.54 ± 0.69[a,b]
b*	33.63 ± 1.07[a]	27.40 ± 0.77[b]	26.00 ± 0.98[c]
ΔE	-	9.48 ± 1.45[b]	15.43 ± 1.22[a]

Results are means ± SD (n=3). Means (two repetitions) followed by different letters, in the same row, are significantly different to each other (p<0.05).

Table 3. Proximate composition, minerals, and color of the cookies.

Several studies have demonstrated that a gluten-free diet could be poor in minerals causing certain deficiencies in people with coeliac disease. One of the reasons could be the scarcity of fortified products and the use of refined flours (Rybicka and Gliszczyńska-Swiglo, 2017; Theethira and Dennis, 2015). These flours lack bran, which is where the minerals in the grain are found. In Argentina, only wheat flour must be fortified with iron (Ley 25.630, Argentina), so other gluten-free flours lack this addition. So, it is important to find new gluten-free ingredients that provide minerals. The sodium, iron, calcium, magnesium, and potassium contents of the gluten-free cookies are shown in table 3. Iron, magnesium, and potassium were significantly different (p<0,05), since their levels increased in correlation with the amount of flour replacement used. It was found that, in a gluten-free diet, the most common mineral deficiencies were those of Iron and magnesium, among other minerals, such as zinc and selenium (Vici *et al*., 2016). Iron is involved in various bodily functions and is mainly used to make hemoglobin; magnesium also plays an important role in the metabolism of proteins, nucleic acids, glucose, fats, and transmembrane transportation (Vici *et al*., 2016). Iron deficiency can cause anemia and related diseases, while magnesium deficiency can cause neurological or neuromuscular diseases The FAO/WHO (2001) recommended an intake (DRI) of iron (people 19-65 years old) of 14 and 29 mg/day for men and women, respectively. As for magnesium, the DRI is 260 and 220 mg for men and women, correspondingly. A 30 g portion of the R35 cookies can provide about 5.5 and 2.6% of the recommended intake of iron for men and women, respectively, and can cover 5,3 and 6,3% of the magnesium IDR for men and women, respectively. It is worth mentioning that canaryseed, like other cereals, legumes, and oilseeds, contains phytate, which could chelate minerals, such as calcium and iron, reducing their availability for digestion and absorption in the small intestine (Mason *et al*., 2018). Abdel-al *et al*. (2011b) evaluated the phytate content in bran, wholegrain flour, and white flour of hairy canaryseeds, hairless canaryseeds, and wheat. Phytate was mainly concentrated in the bran fractions of the three cereals, being higher in canary seed bran (32.5 mg/g) than in wheat (21.7 mg/g). Food processing can reduce phytate content by the phytase-induced degradation of phytic acid (Brouns, 2022). On the other hand, diets high in plant-based foods are commonly recommended for people with chronic kidney disease because they may decrease hydroxyapatite formation, vascular calcification, and calciphylaxis (Ekramzadek *et al*., 2024). The effects of phytate on vascular calcifications, urolithiasis, osteoporosis, cognitive function, metabolic health, cancer and some potential applications were also shown (Pujol *et al*., 2023). For this reason, nowadays phytate is more considered as a nutraceutical because it could prevent disease or disorders through a variety of bioactive functions (Pujol *et al*., 2023). Phytate is present in quantities low enough that do not outweigh their positive health benefits.

The color characteristics of bakery products are important because they are the initial parameter of acceptance of the products by consumers. The linear mixed model for L*, b*, and ΔE presented a R^2 > 0.985, and for the parameter a*, the value was 0.822. It was observed in this study that the parameters of the cookies decreased with a higher replacement of CSF (p<0.05), presenting a lower luminosity (L*-value) and a higher tendency to blueness (b*-value) and greenness (a*-value) (figure 1).

Sharma *et al*. (2016) observed this tendency for the L* and b* values in cookies with raw and germinated millet flour

Figure 1. Appearance of the cookies at different CSF substitution levels (C: control; R15: 15%; R35: 35%).

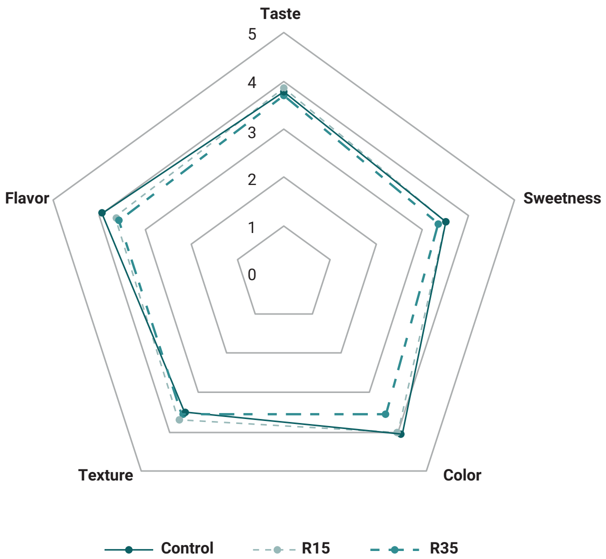

Figure 2. Sensory evaluation of the cookies.

blends, compared to control samples. The germinated millet flour blend had the lowest values, perhaps due to its higher protein content. The proteins are important in the Maillard reaction, which takes place during the baking process, and their content has been negatively correlated with luminosity (Sharma *et al*., 2016). Additionally, these changes in color could be attributed to the presence of natural pigments (Zouari *et al*., 2016). The total color difference (ΔE) was calculated to establish whether the color differences between the control and the supplemented CSF gluten-free cookies could be appreciated by the human eye. The total color change of the cookies had a greater difference with a greater CSF replacement. The same tendency was observed by Sotiles et al. (2015) for cookies made with mixed flours of green banana and birdseed. As was mentioned before, this could be due to the high ash and protein content of CSF.

Sensory evaluation

An effective testing method was used to establish consumer acceptability or preference for the cookies enriched with CSF. The sensory scores of the samples were shown in a spider web diagram (figure 2). The sensory scores for sweetness, taste, flavor, and texture were similar for the control and enriched samples, with a slight increase in R15 for taste and texture. The addition of CSF affected the color appearance of the cookies, highlighting that R35 presented a lower score compared to the control ones ($p<0.05$). This can be explained by the influence of the color of the CSF used as a replacement. Overall, the non-trained panelists gave, in this study, an average acceptability of 6.1 (liked slightly). Despite this, global acceptability was highest for R15 (6.6 score), followed by R35 (6.1 score), and then C (5.8 score). There were no noticeable differences in acceptability between the various treatments ($p>0.05$). In another study on gluten-free cookies, Simon and Hall (2017) evaluated the consumer acceptability of cookies containing raw cooked and germinated pinto bean flours. The taste panelists, consisting of 31 individuals, graded the cookies with an overall value of 6 on a 9-point hedonic scale. This rating was also not significantly different from that of the formulated cookies. The sensory scores and consumer acceptability were assessed by non-trained panelists, who rated the gluten-free cookies as slightly liked.

CONCLUSIONS

In the present study, hairless canaryseed flour was used as a replacement (up to 35%) to make gluten-free cookies. The canaryseed flour presented a good nutritional profile, highlighting the protein and mineral content, improving the nutritional profile of the added cookies. Sensorially, the enriched cookies had good acceptability, with color being the only parameter in which significant differences were observed. The results obtained show that it is possible to use hairless canaryseed flour as an unconventional source to develop gluten-free cookies with good nutritional, physical, and sensory characteristics. This could expand the quantity and the quality of products for people who must follow a gluten-free diet.

This work could also help to disseminate the characteristics and properties of hairless canaryseed flour, demonstrating its easy handling and ability to make baked products. It is necessary to carry out more studies to continue investigating its nutritional and technological properties and its interaction with other gluten-free flours to optimize formulations and its nutritional profile.

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