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New cookie formulations using germinated pumpkin seed flour to increase its nutritional and sensory value

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Abstract

Using by-products for flour production is an alternative to optimise agricultural production through fully using raw materials, avoiding waste, and contributing to production sustainability. From this perspective, controlled germination is a process used to promote changes in seed composition, aiming to obtain a determined nutritional profile, and meet the specific demands of the market. Therefore, the present work aimed to produce cookies with the partial replacement of wheat flour with germinated pumpkin seed flours (GPSF) of three varieties (Crown, Jacarezinho, and Italian), evaluate their microbiological and sensory characteristics, proximate composition, mineral profile, and texture. Four cookie formulations were prepared: the control formulation, with 100% wheat flour; F1, by replacing wheat flour with 1.5% GPSF of the pumpkin variety Crown; F2, by replacing wheat flour with 1.5% GPSF of the pumpkin variety Jacarezinho; and F3, by replacing wheat flour with 1.5% GPSF of the pumpkin variety Italian. GPSF incorporation resulted in cookies with good acceptability by consumers. Moreover, GPSF incorporation changed the composition of compound cookies in relation to the control sample. This modification depended on the pumpkin variety used. GPSF incorporation resulted in cookies with high acceptability rates, similar to the control sample. Among the cookies formulated with GPSF, the products with the Italian variety showed the highest acceptability. The partial replacement of wheat flour with germinated seed flour of pumpkin could have the potential for cookie preparation, and is a viable alternative for the bakery industry.

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Introduction

Pumpkin is a widely appreciated cucurbit of worldwide cultivation, especially for pulp consumption. The pumpkin species grown in Brazil include the varieties Crown (*Cucurbita maxima* Duchesne), Jacarezinho (*C. moschata* Duchesne), and Italian (*C. pepo* L.).

Pumpkin seeds are usually discarded during processing, and considered waste, even though they have high nutritional value and economic potential. Furthermore, these seeds can be consumed after roasting, or processed to obtain pumpkin oil or a protein-rich pie. Several studies have been conducted to explore the potential of agro-industrial by-products incorporated into food formulations aiming to improve their nutrient content, and reduce environmental impacts with appropriate disposal (Erkel *et al*., 2015; Santos *et al*., 2018; Cunha *et al*., 2020).

Seed composition can change after controlled germination, providing new nutrients and bioactive properties. Germination allows economically, efficiently, and reliably improving the nutritional value of seeds by increasing nutrient bioavailability (Erba *et al*., 2018). Currently, the production and consumption of germinated products have increased in variety and quantity due to new worldwide trends for functional foods.

From this perspective, the use of germinated pumpkin flours is a simple and economical procedure to make use of, and add value to seeds, allowing its

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use to enrich foods products while providing gains in nutritional quality. Its application to partially replace wheat flour in the production of cookies, cakes, breads, and pastas constitutes an immediate application following the current demand for healthy products.

A widespread consumer product in the general population is cookie. Among its qualities are the ease of transport and conservation, as well as the possibility of complementing meals, and providing energy in a highly palatable product. Cookies have, as advantages, a long shelf life, great consumption, and good acceptance (Dias *et al*., 2016), in addition to being ready-to-eat, and low-cost products available in different flavours and textures (Ganorkar and Jain, 2014), which make them a suitable vehicle for improving the population's diet by incorporating nutritious flours into their composition.

Therefore, the present work aimed to differentiate biscuits produced with partial replacement of wheat flour by flour from germinated seeds of different pumpkin varieties (Crown, Jacarezinho, and Italian) through their acceptance, purchase intention, proximate composition, mineral profile, and texture.

Materials and methods

Raw materials

The raw materials used in the experiment were seeds of the pumpkin varieties Crown (*C. maxima* Duchesne), Jacarezinho (*C. moschata* Duchesne), and Italian (*C. pepo* L.) obtained from ripe fruits produced in Campina Grande, State of Paraíba, Brazil (7° 13' 50" S 35° 52' 52" W). The fruits were transported in polypropylene boxes to a laboratory at the Federal University of Campina Grande (UFCG), where they were cleaned, cut, and their seeds were manually removed. The seeds were washed under running water before germination to remove any traces of mucilage and pulp.

Seed germination

Germination was performed using Germitest® (J. Prolab) paper sheets as substrate. First, the seeds were placed between two paper sheets moistened with 2.5 times their dry weight. Then, the sheets with the seeds were rolled, placed in low-density polyethylene bags, and germinated in a BOD incubator (CienlaB, CE 300/350) at 25°C for 96 h.

Germinated seed flour preparation

The germinated seeds containing radicles were ground in a processor (Philco®, PMX600, 600 W), after which the material was scattered in mesh baskets forming a 0.5 cm layer, and dried in a convective dryer at an airflow rate of 1.0 m/s and 70°C. The baskets were weighed at regular intervals during drying until reaching constant weight. The dry material was then removed from the baskets, and ground in a knife mill (Tecnal, Piracicaba, São Paulo, Brazil), thus obtaining the germinated seed flour of the three pumpkin varieties.

Cookie preparation

The ingredients used to prepare the cookies were: unleavened wheat flour, brown sugar, white sugar, baking powder, margarine, and the pumpkin seed flours. The formulations were defined after preliminary tests, with a control consisting of wheat flour, and three compositions consisting of the partial replacement of wheat flour with 1.5% germinated seed flour of the three pumpkin varieties.

The formulations with the proportions of the ingredients are as follows = control formulation: 120 g of unleavened flour, 35 g of brown sugar, 35 g of white sugar, 0.5 g of baking powder, and 80 g of margarin. The amount of brown sugar, white sugar, baking powder, and margarine were maintained for all formulations. The difference was the decrease in the amount of unleavened flour used, where in formulations 1, 2, and 3, around 118.2 g were used, and in the amount of pumpkin flour being added was 1.8 g of each studied pumpkin variety.

Formulation 1 replaced wheat flour with 1.5% germinated seed flour from the Crown pumpkin variety, Formulation 2 replaced wheat flour with 1.5% germinated seed flour from the Jacarezinho pumpkin variety, and Formulation 3 replacing wheat flour with 1.5% germinated seed flour from the Italian pumpkin variety. After preliminary tests with some concentrations of germinated pumpkin seed flour, the percentage was set at 1.5% since it favoured the texture and flavour aspects.

The processing stages of the formulations consisted of separating and weighing the ingredients, mixing and homogenising, modelling, roasting and cooling, packaging and storage. All stages of the process followed the Good Manufacturing Practices (GMP) established by RDC No. 216/04 (ANVISA, 2004). First, all ingredients were separated and weighed. Next, the margarine, brown sugar, and white sugar were mixed and homogenised in a bowl for 5 min until obtaining a homogeneous mixture. Then, the wheat flour, the respective pumpkin flour of each formulation, and baking powder were added, after which the dough was mixed for 10 min until the ingredients were perfectly incorporated.

The cookies were manually shaped by standardising the sizes with an approximate mass of 5 g. Then, the cookies were baked in an electric oven pre-heated at 200ºC for 15 min. After baking, the cookies were cooled at ambient temperature, put in laminated packages, and stored at ambient temperature until the microbiological, physicochemical, and sensorial analyses were done.

Microbiological analysis

Microbiological analyses were performed in triplicate to determine the most probable number (MPN/g) of total and thermotolerant coliforms, coagulase-positive staphylococci by direct plate counting, and *Salmonella* spp. (presence or absence) in 25 g of cookies following the methodologies proposed by the American Public Health Association (APHA, 2001). The results were compared with the standards established by RDC No. 331 of December 23, 2019, by the Brazilian Health Regulatory Agency (ANVISA, 2019).

Proximate analysis

The cookies were analysed following the analytical procedures of the Adolfo Lutz Institute (IAL, 2008) to determine the following parameters: moisture (%), determined by gravimetric analysis in a forced-air oven at 105°C until constant weight; and ash content, determined using a muffle furnace at 550°C, with results expressed as percentage (%). The protein content (%) was quantified by the micro-Kjeldahl method following the methodology recommended by AOAC (2016). Lipid quantification (%) followed the methodology described by Bligh and Dyer (1959). The carbohydrates were calculated as follows: % carbohydrates = 100 – (% water + % proteins + % lipids + % ash). The total energy value (kcal/100 g) was calculated using the Atwater factors (or heat of combustion) for lipids (9 kcal/g), proteins (4.02 kcal/g), and carbohydrates (3.87 kcal/g) (Atwater and Woods, 1896). The water activity (a_w) was measured by direct reading at 25°C using an Aqualab 3TE hygrometer (Decagon Devices, Washington, USA). The mineral profile was determined using an energy-dispersive X-ray spectrometer developed by Shimadzu®, model EDX-720.

Texture analysis

The cookies were analysed in triplicate for firmness and fracturability, 24 hours after processing. These parameters were determined using a texture analyser, model TA-XT2 (Stable Micro Systems, Surrey, United Kingdom), with an HDP/3PB probe (3-Point Bending Rig) and an HDP/90 platform. The conditions used in the tests were = pre-test speed: 1.0 mm/s; test speed: 3.0 mm/s; post-test speed: 10.0 mm/s; and a distance of 5.0 mm. The firmness and fracturability results were expressed as newtons (N) and millimetres (mm).

Sensory analysis

Sensory analysis was performed with 100 consumers aged between 17 and 69 years, of which 59% were females and 41% were males. With regard to the frequency of cookie consumption, 0% of the assessors reported daily consumption; 15% reported consuming cookies at least once a week; 13% from two to three times a week; 53% from one to two times a month; and 19% from one to two times a year. The participants were placed in individual cabins at the sensory analysis laboratory, and informed about the contraindications (allergy or health problems) of the ingredients used. Subsequently, the assessors filled out the necessary free consent forms (TALE and TCLE), and only then they received the cookies.

The four cookie samples (control, F1, F2, and F3) were served in disposable trays covered with plastic film, and codified with random three-digit numbers, 2 h before or after meals, in the most appropriate period to perform the evaluation. The assessors were encouraged to use water at ambient temperature between samples to eliminate the sensory impressions of the previous samples.

Cookie acceptance was evaluated based on the sensory attributes of appearance, colour, aroma, flavour, texture, and overall impression using a 9-point hedonic scale ranging from “dislike extremely” (grade 1) to “like extremely” (grade 9) (Dutcosky, 2013), using a structured scale. The cookies were accepted when they achieved a mean grade of 5.0 or higher (hedonic term “neither like nor dislike”).

The purchase intention was evaluated using a structured 5-point hedonic scale with grades ranging from 1 (definitely would not buy) to 5 (definitely would buy) (Meilgaard *et al*., 1991).

The acceptability index (IA) was determined by considering as 100% the highest grade obtained in the overall acceptability of the samples (Eq. 1), and adopting as a criterion for satisfactory classification an acceptability index equal to or higher than 70% (Dutcosky, 2013):

$$IA = \frac{A}{B} \times 100 \quad \text{(Eq. 1)}$$

where, IA = acceptability index (%); A = mean grade obtained; and B = maximum grade given to the sample.

The present work was conducted with the previous approval of the Human Research Ethics Committee under protocol No. 4.107.734, meeting the ethical and scientific demands of Resolution No. 466/2012 of the National Health Council of Brazil (ANVISA, 2012; CNS, 2012).

Statistical analysis

The data on proximate composition, total energy value, water activity, mineral profile, and texture profile of the cookies were obtained using a completely randomised design with four treatments and three replications. The results were subjected to analysis of variance (ANOVA) by checking the presence of significance by the F-test, whereas the means were compared by the Tukey's test at 5% probability using the software Assistat, version 7.7 beta (Silva and Azevedo, 2016).

The sensory analysis data of appearance, colour, aroma, flavour, texture, and overall acceptability were also subjected to the completely randomised design (CRD) with comparison of means by the Tukey's test at 5% probability using the software Assistat, version 7.7 beta (Silva and Azevedo, 2016).

The frequency histogram was developed based on the sensory grades of purchase intention. Data distribution was analysed with a Box Plot graph. The sensory responses were evaluated by Principal Component Analysis (PCA). These analyses were performed using the software STATISTICA®, version 7.0.

Results and discussion

Microbiological analysis

Table 1 shows the results of the microbiological analyses of the cookies formulated with germinated pumpkin flours. The microbiological parameters of the cookies showed that the samples were within the standards established by RDC No. 331 of December 23, 2019, highlighting the hygienic quality of these products, and their fitness for consumption at the moment of sensory analysis.

Table 1. Microbiological analyses results of cookies formulated with germinated pumpkin flours.

Parameter	Formulation			
	Control	**F1**	**F2**	**F3**
Total coliforms (MPN/g)[a]	3.6	3.6	3.0	3.6
Thermotolerant coliforms (MPN/g)[a]	< 3	< 3	< 3	3.6
Coagulase-positive staphylococci (CFU/g)[b]	$< 1.0 \times 10^1$	$< 1.0 \times 10^1$	$< 1.0 \times 10^1$	$< 1.0 \times 10^1$
Salmonella spp.[c]	Absence	Absence	Absence	Absence

[a]most probable number per gram; [b]colony forming units per gram; [c]absence or presence in 25 g of cookies; Control: formulation with 100% of wheat flour; F1: replacement of wheat flour with 1.5% germinated seed flour of the pumpkin variety Crown; F2: replacement of wheat flour with 1.5% germinated seed flour of the pumpkin variety Jacarezinho; and F3: replacement of wheat flour with 1.5% germinated seed flour of the pumpkin variety Italian.

According to RDC No. 331, cookies must show an absence of *Salmonella* spp. in 25 g of sample, and a maximum of 10^3 CFU/g of coagulase-positive staphylococci. On the other hand, the same regulation establishes no limits for total and thermotolerant coliforms.

Proximate composition, total energy value, and water activity

Table 2 shows the mean values and standard deviations of the proximate composition, total energy value, and water activity of the cookies formulated with germinated pumpkin flours.

Table 2. Proximate composition, energy value, and water activity of cookies formulated with germinated pumpkin flours.

Parameter	Formulation			
	Control	F1	F2	F3
Moisture (%)	2.21 ± 0.11^{b}	1.96 ± 0.01^{c}	2.43 ± 0.11^{a}	2.16 ± 0.09^{b}
Proteins (%)	5.75 ± 0.20^{a}	5.71 ± 0.24^{a}	5.57 ± 0.24^{a}	4.67 ± 0.20^{b}
Lipids (%)	17.20 ± 0.47^{a}	17.24 ± 0.14^{a}	18.25 ± 0.74^{a}	18.29 ± 0.70^{a}
Ash (%)	3.74 ± 0.09^{a}	2.57 ± 0.09^{b}	2.04 ± 0.07^{c}	1.91 ± 0.05^{c}
Carbohydrates (%)	71.10 ± 0.24^{b}	72.51 ± 0.42^{a}	71.71 ± 1.00^{ab}	72.97 ± 0.56^{a}
Total energy value (kcal/100 g)	453.08 ± 2.76^{c}	458.76 ± 0.59^{bc}	464.17 ± 3.44^{ab}	465.77 ± 3.63^{a}
Water activity	0.272 ± 0.01^{a}	0.239 ± 0.01^{b}	0.260 ± 0.01^{ab}	0.251 ± 0.01^{ab}

Means followed by similar lowercase superscripts in similar row are not significantly different by Tukey's test at 5% probability ($p < 0.05$). Control: formulation with 100% wheat flour; F1: replacement of wheat flour with 1.5% germinated seed flour of the pumpkin variety Crown; F2: replacement of wheat flour with 1.5% germinated seed flour of the pumpkin variety Jacarezinho; and F3: replacement of wheat flour with 1.5% germinated seed flour of the pumpkin variety Italian.

The cookie samples showed moisture below 3%, with no statistical difference between the control and F3 samples, well below the 14% maximum established for cookies by RDC Resolution No. 263 of September 22, 2005, by ANVISA (2005). Low moisture in food products is essential to increase their shelf-life, inhibit microbial growth, and maintain texture (Madrona and Almeida, 2010). In another study, Barros *et al*. (2020) evaluated cookies produced with pine kernel flour, and also obtained moisture below regulation-established limits, ranging from 7.44 to 8.16%.

The protein contents remained at statistically similar values in the control, F1, and F2 samples, while decreasing in the Italian flour sample (F3), which differed statistically from the others. These values showed that the proportion of germinated pumpkin flour used in the cookies was insufficient in most samples to influence their protein content. In another study, Silva *et al*. (2019) observed that cookies made from avocado seed flour showed the highest protein content (9.46%) when adding 5% of flour than when adding 20% of this product, achieving 7.79%.

The lipid content remained statistically constant in all samples. Queiroz *et al*. (2017) added 5 and 10% coconut flour to cookies, and observed an increase in the lipid content of the formulations. In another study, Bick *et al*. (2014) demonstrated that the addition of 10, 20, and 30% quinoa flour to cooks did not interfere with the lipid content of the samples compared to the control. Close lipid contents were also observed in cookies enriched with *Spirulina platensis*, with values ranging from 14.21 to 14.92% (Donato *et al*., 2019).

The ash content, which represents the inorganic matter of the product, decreased with the addition of the three pumpkin flours compared to the control formulation, with no statistical difference between samples F2 and F3. Through CNNPA Resolution No.12 of 1978 (ANVISA, 1978), Brazilian regulations determine that cookies should have a maximum ash content of 3.0%. Based on this requirement, only the cookies prepared with germinated pumpkin flour met the established standards. Silva *et al*. (2015) found ash contents, ranging from 1.75 to 3.6% in cookies prepared with 0, 25, 50, 75, and 100% pumpkin seed flour, were close to the values of the present study. In another study, Kaur *et al*. (2017) observed that the ash content in cookies increased as 0, 5, 10, 15, 20, 25, and 30% flaxseed flour was added to the formulations, ranging from 1.24 to 1.71%.

The carbohydrate contents of the samples containing germinated pumpkin flour showed no statistical difference, whereas the control formulation showed the lowest percentage of this parameter. Gaspar *et al*. (2020) reported carbohydrate contents ranging from 61.6 to 68.6% in cookies with 10, 25, and 50% replacement of wheat flour with pumpkin peel flour.

The total energy value (VET) was influenced by the incorporation of germinated pumpkin flour, with higher overall values in the F1, F2, and F3 formulations in relation to the control sample. TEV values close to those of the present work were reported by Pereira *et al*. (2016), ranging from 441.94 to 445.60 kcal/100 g in butter cookies prepared with jatobá flour.

Water activity was also low, with values under 0.3 in the four cookie samples, favouring their prolonged conservation. This parameter provides essential information about the shelf-life of a product and can be modified during production to increase stability (Gusmão *et al*., 2018). Moreover, changes in cookie crispness and durability significantly depend on this parameter (Jardim, 2010).

Mineral profile

Table 3 shows the mean values and standard deviations observed for the mineral profile of cookies formulated with germinated pumpkin flours.

Table 3. Mineral profiles of cookies formulated with germinated pumpkin flours.

Mineral (mg/100 g)	**Formulation**			
	Control	**F1**	**F2**	**F3**
Potassium (K)	137.68 ± 0.78[a]	111.43 ± 0.36[b]	104.89 ± 0.49[c]	94.38 ± 0.61[d]
Phosphorus (P)	670.93 ± 3.29[a]	484.62 ± 1.59[b]	477.48 ± 2.99[c]	355.21 ± 1.49[d]
Magnesium (Mg)	79.28 ± 1.67[a]	52.20 ± 1.40[b]	50.97 ± 1.02[b]	44.66 ± 0.59[c]
Calcium (Ca)	372.28 ± 1.85[a]	265.60 ± 1.25[c]	271.31 ± 1.71[b]	179.60 ± 1.15[d]
Zinc (Zn)	0.34 ± 0.01[a]	0.24 ± 0.00[b]	0.24 ± 0.01[b]	0.22 ± 0.00[c]
Iron (Fe)	1.97 ± 0.02[b]	2.14 ± 0.01[a]	2.18 ± 0.10[a]	1.97 ± 0.03[b]
Copper (Cu)	0.19 ± 0.00[c]	0.20 ± 0.01[b]	0.14 ± 0.00[d]	0.26 ± 0.00[a]
Manganese (Mn)	0.47 ± 0.01[a]	0.25 ± 0.01[b]	0.23 ± 0.01[b]	0.21 ± 0.01[c]
Sodium (Na)	2330.67 ± 3.80[a]	1893.33 ± 1.80[b]	1615.94 ± 3.14[c]	1553.48 ± 2.69[d]

Means followed by similar lowercase superscripts in similar row are not significantly different by Tukey's test at 5% probability ($p < 0.05$). Control: formulation with 100% wheat flour; F1: replacement of wheat flour with 1.5% germinated seed flour of the pumpkin variety Crown; F2: replacement of wheat flour with 1.5% germinated seed flour of the pumpkin variety Jacarezinho; and F3: replacement of wheat flour with 1.5% germinated seed flour of the pumpkin variety Italian.

The control formulation showed the highest content of potassium, phosphorus, magnesium, calcium, zinc, manganese, and sodium, statistically differing from the others. The highest iron content of the samples was observed in formulations F1 and F2, whereas the F3 sample showed the highest copper content. Among the formulations that received germinated pumpkin flour, the F1 sample, which contained the Crown flour, showed the highest content of potassium, phosphorus, magnesium, zinc, manganese, and sodium.

In the study conducted by Kaur and Sharma (2017), the iron content of the cookies supplemented with pumpkin flour was also higher than in the control samples. The cookies containing 30% pumpkin flour showed the highest iron content, with 2.36 mg/100 g; followed by the samples with 50% pumpkin flour, with 2.18 mg/100 g; and the control sample composed of refined wheat flour, with 1.31 mg/100 g. In another study, Kumari and Sindhu (2019) evaluated cookies produced with 0, 10, 20, and 30% germinated pumpkin flour, and observed increased concentration of minerals (calcium, magnesium, zinc, iron, potassium, and phosphorus) as the pumpkin flour levels increased in the formulation.

Texture profile

Table 4 shows the values obtained for the texture profile parameters of firmness and fracturability.

Texture analysis is an important parameter for the production process, the quality of the final product, and the development of new food products (Carneiro *et al*., 2011). Moreover, parameters such as firmness and fracturability determine consumer acceptability, with a preference for lower values.

The firmness of the control, F2, and F3 samples resulted in statistically similar values, with the Crown flour sample standing out with the highest value. Firmness values above those of the present work were reported by Almeida *et al*. (2020) when evaluating cookies prepared with different proportions of red rice four after 60 days of storage (0, 50, and 100%), ranging from 76.54 to 165.55 N. The authors observed that the cookies with 100% red rice flour showed the highest firmness and fracturability. However, significant differences were observed after 60 days due to the increase in water activity.

Fracturability, which represents the tendency of a material to fracture, break, or disintegrate as a force or impact is applied, increased with the incorporation of germinated pumpkin flour, although showing no statistical differences between samples. This behaviour evidenced a variation within the range expected for the product prepared with 100% wheat flour, maintaining the typically accepted standard. Fracturability values close to the present work were reported by Gusmão *et al*. (2018) in cookies produced with different concentrations of mesquite flour (5, 15, and 25%) stored for 120 days, ranging from 0.41 to 0.53 mm.

Table 4. Texture profile parameters of cookies formulated with germinated pumpkin flours.

Parameter	Formulation			
	Control	F1	F2	F3
Firmness (N)	32.80 ± 1.12^{b}	43.36 ± 2.81^{a}	31.74 ± 2.75^{b}	34.04 ± 2.93^{b}
Fracturability (mm)	0.46 ± 0.03^{a}	0.62 ± 0.12^{a}	0.51 ± 0.13^{a}	0.48 ± 0.13^{a}

Means followed by similar lowercase superscripts in similar row are not significantly different by Tukey's test at 5% probability ($p < 0.05$). Control: formulation with 100% wheat flour; F1: replacement of wheat flour with 1.5% germinated seed flour of the pumpkin variety Crown; F2: replacement of wheat flour with 1.5% germinated seed flour of the pumpkin variety Jacarezinho; and F3: replacement of wheat flour with 1.5% germinated seed flour of the pumpkin variety Italian.

Sensory analysis

Table 5 shows the mean values and standard deviations found in the sensory analysis of the cookies formulated with germinated pumpkin flour. In general, with regard to all parameters evaluated, the cookies scored from 7.15 and 8.16, ranking between "like moderately" and "like very much" on the scale, thus showing good sensory acceptance. These findings were further confirmed by the acceptability index (IA), with values above 80% in all samples.

As seen in Table 5, the attributes of appearance, colour, aroma, and texture showed no statistical difference between cookies, showing that the incorporation of germinated pumpkin flours did not interfere with their evaluation by the assessors in the proportions used.

For the flavour attribute, the F1 formulation differed statistically ($p > 0.05$) from the others, showing the lowest score among all parameters analysed. This lower acceptance with regard to the flavour of the F1 sample was probably due to the residual taste of the sample, with greater bitterness, as reported by various assessors. The F1 sample was also less appreciated than the others in the overall acceptability. In contrast, the control, F2, and F3 samples showed no statistical differences.

Table 5. Sensory evaluation mean scores of cookies formulated with germinated pumpkin flours.

Parameter	Formulation			
	Control	F1	F2	F3
Appearance	8.04 ± 1.09^{a}	7.97 ± 1.13^{a}	7.94 ± 1.10^{a}	7.86 ± 1.35^{a}
Colour	8.16 ± 0.99^{a}	8.06 ± 1.07^{a}	8.03 ± 1.18^{a}	8.16 ± 1.03^{a}
Aroma	7.83 ± 1.13^{a}	7.56 ± 1.23^{a}	7.78 ± 1.14^{a}	7.64 ± 1.33^{a}
Flavour	7.97 ± 1.31^{a}	7.15 ± 1.71^{b}	7.75 ± 1.47^{a}	7.76 ± 1.36^{a}
Texture	7.71 ± 1.51^{a}	7.59 ± 1.44^{a}	7.72 ± 1.38^{a}	7.97 ± 1.15^{a}
Overall acceptability	7.89 ± 1.11^{a}	7.47 ± 1.23^{b}	7.81 ± 1.07^{ab}	7.88 ± 1.17^{ab}
Acceptability index (%)	88.15	84.77	87.11	87.55

Means followed by similar lowercase superscripts in similar row are not significantly different by Tukey's test at 5% probability ($p < 0.05$). Control: formulation with 100% wheat flour; F1: replacement of wheat flour with 1.5% germinated seed flour of the pumpkin variety Crown; F2: replacement of wheat flour with 1.5% germinated seed flour of the pumpkin variety Jacarezinho; and F3: replacement of wheat flour with 1.5% germinated seed flour of the pumpkin variety Italian.

Among all samples, the control formulation showed the highest scores for most attributes, whereas the cookies prepared with the Italian flour showed the highest scores for colour, flavour, texture, and overall acceptability among the three compound samples. In another study, Maskey *et al*. (2020) incorporated jackfruit seed flour into cookies, and observed that the flavour attribute received the highest scores in the control formulation (100% wheat flour) and in the formulation that contained the lowest content of jackfruit seed flour (12.5%). The authors justified that the decrease in the scores of the flavour attribute in the other samples was due to the increase in the jackfruit flour content, and the consequent greater bitterness.

The same finding was reported by Bello *et al*. (2020) when producing cookies composed of banana flour and germinated pumpkin flour. The control sample (100% wheat flour) showed the highest acceptability, with the highest scores in all evaluated attributes, whereas the formulation with the lowest concentration of banana flour and germinated pumpkin flour (5% of each flour) ranked second. According to the authors, the low acceptability of the cookies with higher flour contents could be due to the increase in polyphenols, which impart a bitter flavour to the samples.

The results of the purchase intention analysis are shown in Figure 1A, meaning the intention of the assessors to purchase the tested product if available on the market. The Figure 1A shows the frequency distribution of the scores based on the scale used for evaluation.

The control sample showed the highest concentration of score 5, corresponding to "definitely would buy," followed by samples F3, F2, and F1. For score 4, corresponding to "probably would buy," the highest concentration was observed in formulations F2 and F2. Sample F1 showed the highest concentration of scores 3 and 2, corresponding to "may or may not buy" and "probably would not buy", respectively. Sample F2 obtained the highest number of score 1 (definitely would not buy). These results agreed with the observations for the sensory attributes, in which the control and F3 cookies stood out among the formulations, and if marketed, would be better accepted by consumers, with a more favourable purchase intention.

High purchase intention grades were also obtained by Monteiro *et al*. (2020) for cookies produced with West Indian Cherry flour, with purchase intention means ranging from 4 (probably would buy) to 5 (definitely would buy). Sousa *et al*. (2020) also evaluated the purchase intention of cookies developed with pineapple peel flour, and observed that the sample with 10% peel flour showed 57% of purchase intention.

The distribution of sensory data was analysed by a Box Plot graph, as seen in Figure 1B. The figure shows information regarding the centre of data in relation to the mean or median, and the range of the data for minimum and maximum values. The mean and standard deviation indicate data variability around the mean, whereas the mean and standard error indicate the confidence interval.

The flavour attribute showed the lowest mean, and the colour attribute showed the highest mean among the analysed samples. These results can be compared with the overall mean (Table 5), whose values were 7.66 and 8.10, respectively.

With regard to the variability of sensory grades, flavour and overall acceptability showed the highest variabilities, and consequently, the highest standard deviation values. Conversely, the colour attribute obtained the lowest variability and the lowest standard deviation.

Data distribution was symmetric, which was justified by the absence of a significant statistical difference between most attributes. When data distribution is symmetric, the line representing the median will be located more or less in the centre of the rectangle, and the two lines that emerge from the ends of the rectangle will have approximately the same length.

Figure 1C shows the graphs of the Principal Component Analysis (PCA). As seen in Figure 1C, each cookie sample is represented by a dot, with each dot corresponding to the mean value attributed by the sensory team. In Figure 1C, the samples that show similarity occupy nearby regions in the graph, and are characterised by closer vectors (attributes). The samples show different sensory characteristics since they are located in different quadrants (Figure 1C), except for the control and F2 samples (replacement of wheat flour with 1.5% germinated seed flour of the pumpkin variety Jacarezinho).

Most of the variation between samples was explained by Principal Component 1. However, when

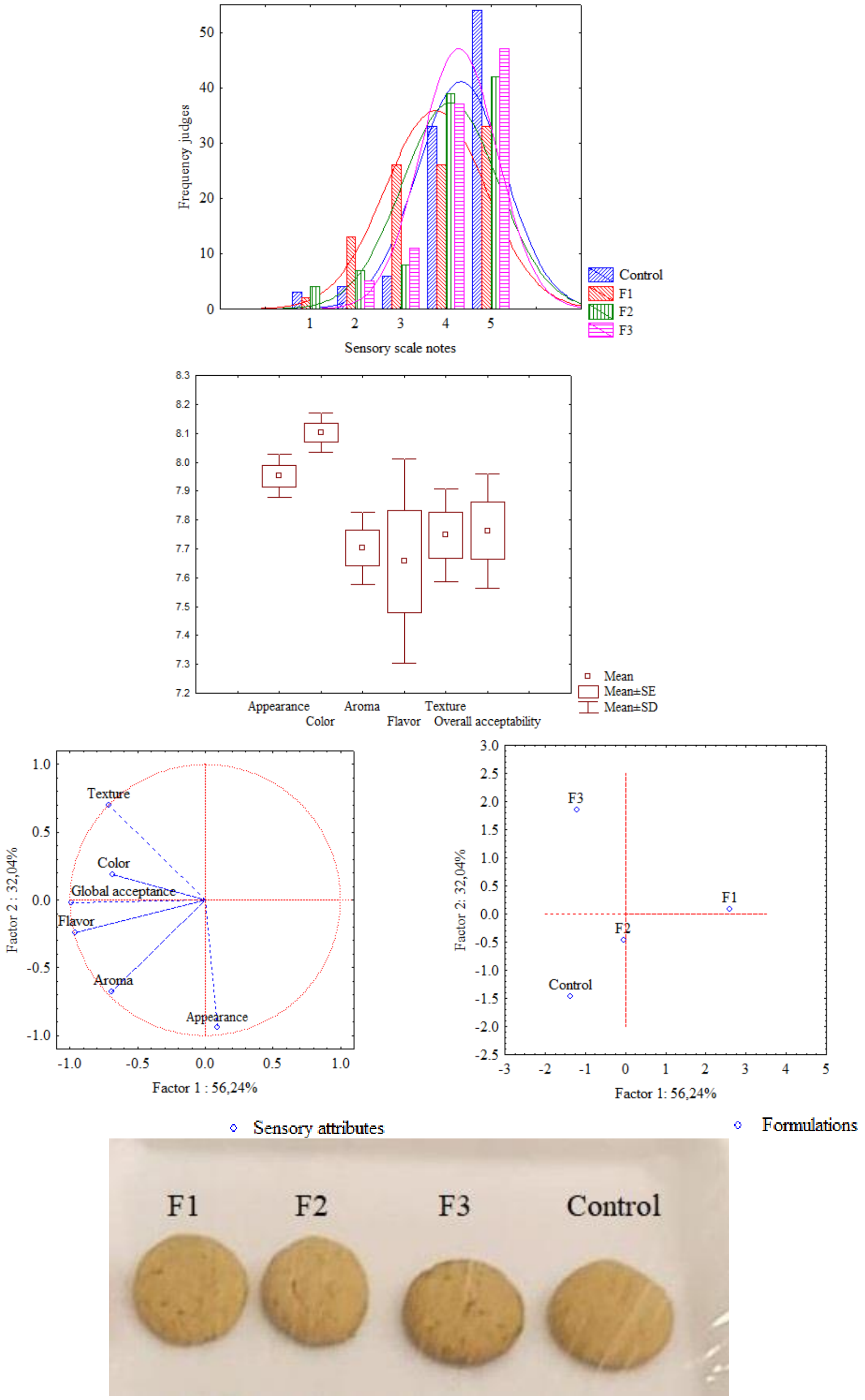

Figure 1. Frequency histogram of purchase intention **(A)**; box plot graph for attributes analysed in sensory analysis. Mean = mean; Mean ± SE = mean ± standard error; Mean ± SD = mean ± standard deviation **(B)**; two-dimensional principal component analysis (ACP) of the sensory attributes of cookies formulated with germinated pumpkin flours **(C)**; and picture of the cookies **(D)**.

associated with Principal Component 2, the two components explained 88.28% of the information contained in the mean values of the sensory attributes, meaning that the variability between samples can be explained using these two axes.

Figures 1C and 1D show that the F3 formulation (replacement of wheat flour with 1.5% germinated seed flour of the pumpkin variety Italian) differed from the other samples with regard to texture and colour. In contrast, the control and F2 samples (wheat flour replacement with 1.5% germinated seed flour of the pumpkin variety Jacarezinho) stood out by their flavour, aroma, and overall acceptability. The F1 sample (replacement of wheat flour with 1.5% germinated seed flour of the pumpkin variety Crown) was not highlighted by any of the sensory attributes since it was located distant from the attributes in the vectorial space and an opposite quadrant, indicating a negative correlation with regard to sensory attributes. Although not discriminated by any sensory attribute, the F1 formulation obtained good sensory acceptability. However, this sample showed the lowest scores for most attributes (aroma, flavour, texture, and overall acceptability) and was less preferred by the assessors.

Conclusion

The incorporation of germinated pumpkin seed flours (GPSF) from the Crown, Jacarezinho, and Italiana varieties in a proportion of 1.5% resulted in cookies with good sensory acceptability, with maintenance of firmness, fracturability, protein, and lipid content, and an increase in carbohydrates and energy value in relation to the control sample. Among the cookies with the incorporation of GPSF, the one with the highest acceptability was with the incorporation of GPSF from the Italian variety, and the one with the lowest was with the GPSF from the Crown variety. In view of the analyses carried out, it was demonstrated that the partial replacement of wheat flour by the GPSF of the Crown, Jacarezinho, and Italian varieties presented potential for the preparation of cookies as a viable alternative for the bakery industry.

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