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RESEARCH ON OBTAINING ORGANIC GLUTEN-FREE COOKIES WITH AMARANTH FLOUR AND PUMPKIN PULP

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Abstract

Pumpkin pulp (Curcubita maxima), from organic farming, is an extremely versatile product that can be a basic ingredient in obtaining gluten-free cookies. In this paper, a cookie was obtained using amaranth flour and pumpkin pulp in two forms: fresh and baked. Following the analyses, the aim was to obtain a product with appropriate characteristics. Dough with amaranth flour and pumpkin pulp is characterized by a good ability to bind the dough and retains its shape when pouring. In the sensory analysis of the gluten-free cookies with organic baked pumpkin obtained in the product testing phase, the taste was better appreciated. From the analysis of the average values it is observed that the products obtained with the basic ingredient prepared in advance (baked pumpkin) showed a more intense perception on the attributes of smell, taste and aftertaste.

Key words*: amaranth, cookie, gluten-free, pumpkin pulp*

INTRODUCTION

The incidence of metabolic diseases among the active population has an upward trend, which requires the diversification of the assortment range of organic gluten-free products. The development of gluten-free products with organic pumpkin pulp meets the requirements of the consumer segment with gluten intolerance.

Proteins improve the nutritional value of gluten-free products. The choice of flour and possibly another source of protein affects the rheological properties of the dough and the water binding in the dough. Proteins interact with starch and lipids and together contribute to the stability of the dough and the structure of the product. They also give the impression of full product flavour. Proteins can be of plant origin (legumes, soya, gluten-free cereals, rapeseed, canola, sunflower, potato), animal origin (whey, egg, casein, caseinate) or microorganism-, algae-, seaweed- and insect-based (Skendi et al., 2021; Dragomir et al., 2019).

Pseudocereals, often used as substitutes for wheat, have a nutritional value that is closely related to their protein content and quality, and usually higher compared to cereal grains. This is especially the case with amaranth, which has the highest protein content, 13.1–21.0%, among pseudocereals (Alvarez-Jubete et al., 2010; Bhat et al., 2015; Venskutonis & Kraujalis, 2013).

Amaranth's name comes from the Greek for "immortal" or "everlasting." Grain amaranth species include *Amaranthus caudatus, A. cruentus* and *A. hypochondriacus*.

Considered a pseudocereal unrelated to wheat or other true grains, amaranth delivers high-quality protein -- roughly 13.1–21.0% by weight and contains plenty of other healthful nutrients. Amaranth flour is obtained from amaranth seeds (*Amaranthus*) with a protein content almost 2 times higher than wheat (up to 19%). High levels of the amino acid lysine help your body to properly absorb calcium from the digestive tract. It is a naturally gluten-free flour, versatile and with a moreish earthy flavor.

Generally, the protein content depends on variety and environmental conditions. In a study by Tömösközi et al. (2009) eight samples of *A. hypochondriacus* and *A. caudatus* lines, grown in Hungary and Austria, showed a range of protein content of between 14.23% and 17.40%. Amaranth proteins consist of about 40% albumins, 20% globulins, 25%–30% glutelins, and only 2%–3% prolamins (Venskutonis & Kraujalis, 2013). A lower proportion of 0.48%–0.79% prolamins was found by Muchova et al. (2000). For some amino acids the overview showed considerable variations, especially for lysine (3.3–9.2 mg/g protein) and leucine (3.6–7.9 mg/g protein).

Pumpkin (*Cucurbita maxima*) is an ingredient used in Romanian gastronomy, only in certain periods of the year, respectively in the winter season, and for this reason we propose the integration in products with added value and functional role. Organic pumpkin can be profitably used in a variety of value-added foods. Pumpkin is rich in β-carotene, carbohydrates, dietary fiber, vitamins (A, C, B1, B2 etc.), potassium, calcium, magnesium and iron, etc., with special sensory properties agreed by the Romanian consumer, with a low caloric content.

Pumpkin pulp has very good stability over time, in terms of color, influencing the color and favoring of the finished product.

MATERIALS AND METHODS

The objective of this study was the production of innovative functional cookies enriched with ecological pumpkin pulp and monitoring the impact of these enrichments on the nutritional, physicochemical and consumer acceptance.

Methods of sample preparation and physicochemical analyses it was carried out at the laboratories of Research Center for Studies of Food Quality, University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania.

Ingredients

The experimental variants of gluten-free cookies were developed from the amaranth flour (AF) in mixture with ecological pumpkin pulp (*Curcubita maxima*): raw and baked.

The physicochemical characteristics and sensorial properties of the cookies were evaluated in a pursuit to identify an innovative pastry ingredient, easy to find, accessible; with high nutritional value and potential function that could be exploited by the food industry.

Pumpkin is a local raw material and a more economically advantageous ingredient.

The utilization it in production will allow ensuring the food safety of consumers suffering from celiac disease, the use of cheap and accessible local raw materials, the reduction of the cost of gluten-free snacks, and the widening of the assortment of gluten-free pastries.

Konjac flour - glucomannan is a beneficial, soluble and fermentable dietary fiber, gluten-free, derived from the rhizomes of the *Amorphophalli Konjac* plant.

Konjac Glucomannan bio is an essential prebiotic for health that increases the feeling of satiety.

Preparation of product

Organic gluten-free cookies were developed in the Bakery Pilot Station within the Faculty of Animal Productions Engineering and Management -USAMV Bucharest, recipes and technologies for the manufacture of gluten-free cookies to which was added in the recipe raw pumpkin pulp and baked pumpkin pulp (Table 1).

Table 1 Codes used for samples analysis

Code	Sample
A0	Organic cookie with amaranth flour
A1	Organic cookie with amaranth flour and raw pumpkin pulp
A2	Organic cookie with amaranth flour baked pumpkin pulp

Sensory Evaluation

A first sensory analysis was carried out using a semi-structured hedonic scale to evaluate the acceptability of the cakes made with the selected percentage of substitution. A total of 30 potential consumers evaluated biscuit color, texture, taste and overall acceptability according to a box-scale (1 - 5) anchored in the following steps: “dislike very much”, “indifferent” and “like very much”. Samples were presented to the evaluators randomly arranged and coded with three digit numbers.

Once the formula it was optimised, a sensory analysis was carried out where the panelists were asked to evaluate color, texture, taste, and overall acceptability of control and the selected amaranth flour and pumpkin pulp (fresh or baked) cookie formulations.

Elemental analysis C, H, N and determination of protein content

The analysis of the total N, H, C content was carried out using the Dumas method, using the Elemental Analyzer EA 3000. This method involves the total combustion of the sample in atmosphere of oxygen. The gases produced are reduced with copper to H_2O, N_2, CO_2 and SO_2 and quantified using a universal detector. From the ground sample, was weighed of 2–3 mg, enter in a tin crucible and subjected to combustion at 950°C. The amount of protein was calculated by multiplying the total nitrogen content by a factor of 6.25.

Total polyphenol content (TPC)

Depending on the type of sample, 1 g of material is mortared in the presence of quartz sand and 10 ml of 70% methanolic solution in water. The extracted sample is left overnight in the dark at room temperature. The next day, shake for 60 minutes to favor the extraction, centrifuge for 5 minutes at 5000 rpm and 4°C, after this transfer the supernatant to another bottle. Over the remaining sediment, add another 10 ml of 70% methanolic solution, shake and centrifuge. Repeat procedure. Finally, the 3 supernatants are combined, the final volume of the extract being 30 ml.

For the quantitative determination of the total content of polyphenols, the Folin-Ciocâlteu method following a protocol adapted from George et al. (2005).

Antioxidant activity

The antioxidant activity of the samples is determined based on the DPPH test, using the stable free radical 2,2-diphenyl-1-picrylhydrazyl – DPPH, after the method described by (Bujor et al., 2016).

To determine the antioxidant activity, a volume of 200 µl of the extract solution obtained for polyphenols is used, and 2 ml of DPPH solution (0.2 M) in methanol is added. Stir magnetically in the dark for 30 minutes.

After incubation, the absorbance is measured at a wavelength of 515 nm.

Determination of the dry matter (DM)

Determination of the dry matter (DM) was achieved by weighing 1 g of the sample, and then dries at 105°C, in an oven until a constant weight.

The determination of the dry matter content (DM) was carried out by the gravimetric method, through the removal of water by evaporation and weighing, according to the European Pharmacopoeia, edition 7, the results being expressed in percentages.

Determination of the content of carotenoid pigments by UV-VIS

The carotenoids pigments content was quantified after petroleum ether extraction method. In a mortar with pestle, 1 g of the sample was mortared in the presence of quartz sand, and washed quantitatively several times with petroleum ether. The ethereal extract was vacuum filtered and transferred quantitatively into a 50 ml volumetric flask.

It was dosed spectrophotometrically against a petroleum ether blank at wavelengths 452 and 472 nm, using the Specord 210 Plus UV/VIS spectrophotometer.

Results were calculated according to the formulas proposed by Rodriguez-Amaya et al., (2001) and Pelissarii et al. (2016).

The results obtained and presented are the average of three independent values and are expressed as mean ± standard deviation (SD).

Statistical analysis of obtained data was performed using Microsoft Excel for standard deviation; represent the average of three replicates with independent sample preparation.

RESULTS AND DISCUSSIONS

The objective of this study was the production of innovative functional cookies enriched with organic pumpkin pulp and monitoring the impact of these enrichments on the nutritional, physicochemical, texture characteristics and consumer acceptance. The gluten-free cookies were developed from the amaranth flour (AF) in mixture with organic pumpkin pulp.

The experimental variants (gluten-free cookies) were obtained by different methods of preparing pumpkin pulp: raw or baked.

The physicochemical characteristics and sensorial properties of the cookies were evaluated in a pursuit to identify an innovative pastry ingredient, easy to find, accessible; with high nutritional value and potential function that could be exploited by the food industry.

Organic cookies with amaranth flour and pulp pumpkin is was developed in the Bakery Pilot Station within the Faculty of Animal Productions Engineering and Management - USAMV Bucharest.

Obtaining gluten-free pastries is a major technological challenge, as gluten is essential in strengthening the structure of products, being essential in obtaining high quality pastries. As a result, in the first stage, tests were performed on the influence of amaranth flour on a fat-based cookie dough, in which konjac flour was used as a thickener and emulsifier to improve the texture of the dough and to maintain stability in time.

Preparation of product

It was obtained 3 samples of each product from amaranth flour, respectively a control sample that lacks pumpkin, a sample with the addition of freshly grated pumpkin and a sample with the addition of baked pumpkin in the form of paste (Tabel 2).

Table 2 Recipe used for samples analysis

Code	Sample
A0	62% amaranth flour, 16% butter, 10% unrefined brown sugar, 8% egg, 2% baking powder, 2% konjac flour.
A1	31% amaranth flour, 31% pumpkin pulp fresh, 16% butter, 10% unrefined brown sugar, 8% egg, 2% baking powder, 2% konjac flour.
A2	31% amaranth flour, 31% pumpkin pulp baked, 16% butter, 10% unrefined brown sugar, 8% egg, 2% baking powder, 2% konjac flour.

All products were purchased from retail specialty stores with organic products, except for organic pumpkin, which was purchased from the ecological farms. Organic pumpkin pulp was obtained in the local organic farming.

The product was obtained in the Bakery Pilot Station of the Faculty of Animal Productions Engineering and Management, from USAMV Bucharest, within the ECODONELA project support.

Amaranth flour is a great option in baking and works well with both sweet and salty flavors. It has an intense nutty taste, a complex and very dense aroma, difficult to work with. It is suitable for mixtures containing brown sugar or maple syrup. Due to their distinctive taste, crumbly texture and sandy feel, they are used sparingly.

Dough made from AF is characterized by a good ability to bind the dough and retains its shape when pouring. In the samples to be analyzed, where fresh or baked pumpkin is added, a higher humidity of the dough is observed due to the addition of pumpkin.

For the elaboration of the cookie recipes we started from the premise of valuing the pumpkin pulp in new assortments of cookies. The pumpkin was purchased in the harvest season (October) and the pulp was prepared as follows:

- Raw - grated pumpkin stern forming small particles;
- Heat treated - the pulp was cut into cubes and heat treated at 200°C/40 minutes in the convection oven and then mashed.

Repeated tests have shown that the high humidity of the raw or cooked pumpkin pulp requires the addition of substances that bind excess water and form a homogeneous mixture. Konjac flour –glucomannan - was used for this purpose.

Pumpkin pulp was not used in the preparation of the control sample.

The technological process of obtaining the recipe

The technological process of obtaining fat-based cookies gluten-free was obtained as follows: butter and sugar are mixed until a creamy consistency is obtained, the egg and other dry ingredients are added, and finally the pumpkin pulp is added by mixing at low speed, so as not to lose volume. The dough obtained is left to rest at 1-4°C for 30 minutes. The dough is modelling. Bake the cookies at 180°C/20-25 minutes.

Organic pumpkin pulp, used in the study, is: fresh grated pumpkin pulp and baked pumpkin pulp.

All samples had the same technological regime.

The analysis of the behavior of the dough during preparation revealed that the dough obtained from amaranth flour - is characterized by a good ability to bind the dough and retains its shape when pouring. In the samples to be analysed, where fresh or baked pumpkin is added, a higher humidity of the dough is observed due to the addition of pumpkin.

Sensory characteristics:

Cookies with AF stand out for their high palatability, thanks to the final earthy note, combined with the sweet taste of pumpkin pulp. The pieces are light brown, crispy on the outside and dense on the inside, with a slight tingling sensation at the end. The brown color of the cookies is due to the use of brown sugar and pumpkin pulp in the recipe. The taste is sweet, aromatic and towards the end with a touch of earth, a taste given by the presence of amaranth flour.

It is recommended to store in hermetically sealed packages and keep at room temperature, without large fluctuations in humidity.

The analysis of the behavior of the dough during preparation revealed that the dough obtained from amaranth flour - is characterized by a good ability to bind the dough and keeps its shape when pouring. In the samples to be analyzed, where fresh or baked pumpkin is added, a higher humidity of the dough is observed due to the addition of pumpkin (Figure 1).

Achieving sensory acceptability has been another big challenge when developing gluten-free bakery products. Gluten-free bakery products usually possess distinct appearance, color, texture, aroma, and taste in contrast to the products made of wheat flour. Color of gluten-free bakery products tends to be darker due to a complex formulation. Specific volume of gluten-free bakery products such as cakes are generally smaller with harder texture compared with the products made of wheat. Taste of glute-free bakery products is in high variability, depending on the gluten-free flours and formulations (Jingwen Xu et al., 2020).

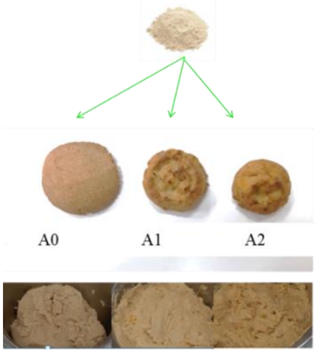

Figure 1. Cookies with amaranth flour and pumpkin pulp before baking (up) and after baking (down) (Original photo)

Table 3 Sensory analysis of gluten free organic cookies with amaranth flour and pumpkin pulp

Sample	Exterior appearance $\bar{X} \pm S_{\bar{X}}$	Section appearance $\bar{X} \pm S_{\bar{X}}$	Form $\bar{X} \pm S_{\bar{X}}$	Color $\bar{X} \pm S_{\bar{X}}$	Taste $\bar{X} \pm S_{\bar{X}}$	Flavor $\bar{X} \pm S_{\bar{X}}$	Texture $\bar{X} \pm S_{\bar{X}}$
A_0	3,979±0,153	3,766±0,137	3,702±0,182	3,681±0,167	3,299±0,206	3,191±0,221	3,128±0,174
A_1	3,594±0,205	3,656±0,188	3,688±0,217	3,594±0,228	3,062±0,233	3,375±0,245	3,250±0,229
A_2	3,800±0,166	3,633±0,159	3,550±0,183	3,283±0,154	2,700±0,178	2,876±0,192	3.267±0,166

Consumer acceptance

The sensorial evaluation of product was carried out in order to observe the impact of organic pumpkin pulp incorporation, on its sensory characteristics.

Consumer acceptance for cookies with amaranth flour and pumpkin pulp it was realized with a 5-point Hedonic evaluation scale, on a panel group by 30 persons. Objective it was to observe the impact of organic pumpkin pulp (raw and baked) incorporation, on its sensory characteristics. Sensory tests were performed taking into account: appearance, taste, color, flavor and smell, texture (Table 3).

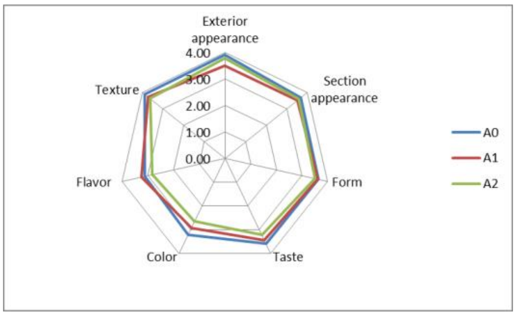

Figure 2. Consumer acceptability scores on a 5-point Hedonic scale for cookies with AF and pumpkin pulp

Following analysis, consumer acceptance was very good in all age segments, the new product being to the liking of consumers and consumer acceptability.

The score obtained for each attribute was processed, and the average values are presented in Figure 2.

The most appreciated attributes were those related to the appearance of the product (outer appearance, section appearance, shape) for all 3 samples analyzed.

In the case of sample A1, the value 3.594±0.205 lower than that of the other samples is due to the increased humidity of the biscuit and the oily sensation.

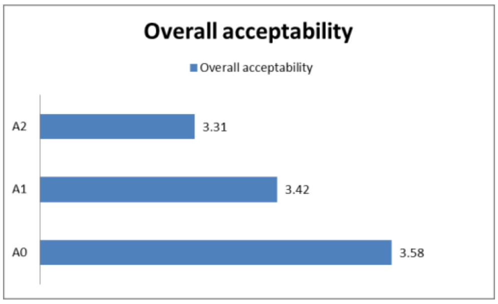

Figure 3. Overall acceptability for cookies with AF and pumpkin pulp

The taste was quite controversial, because many tasters found a pleasant, aromatic taste but did not appreciate the sandy sensation of the core when tasting. It is certain that samples A0 and A1 were the most appreciated products.

The flavor of sample A1 was appreciated the most with a value of 3.375±0.245, which shows that consumers appreciate the combination between the flavor of amaranth flour and fresh pumpkin flesh.

The acceptability of the samples by the consumer showed that the A0 sample has the highest appreciation from the tasters, followed by the A1 sample (Figure 3). The least appreciated is sample A2 with ripe pumpkin pulp, and this is due to a harder texture, a more intense color and a specific taste.

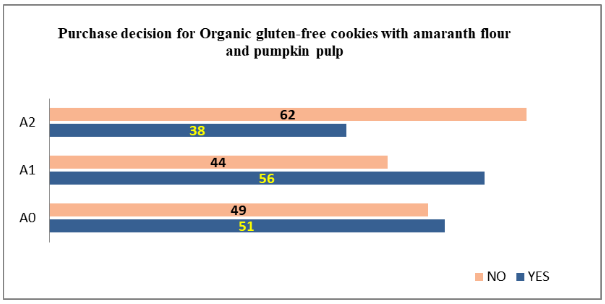

Figure 4. Purchase decision for organic gluten-free cookies with amaranth flour and pumpkin pulp

Following the analysis of consumers' purchase intention (Figure 4), regarding gluten-free cookies with amaranth flour and *Curcubita maxima* pumpkin pulp, a preference is observed in sample A1, respect for cookies with fresh pumpkin pulp, followed by sample A0, respectively gluten-free cookies bio with amaranth flour without the addition of pumpkin pulp.

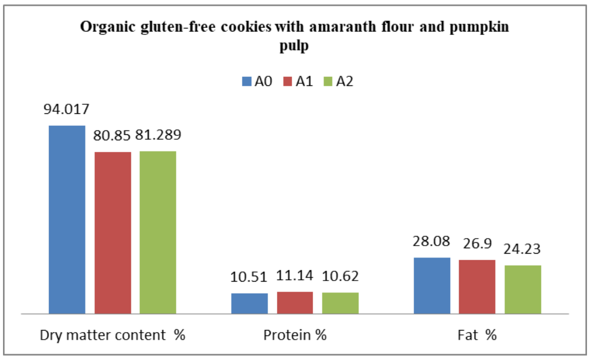

Figure 5. Chemical composition of organic gluten-free cookies with amaranth flour and pumpkin pulp

Following the analysis of the chemical composition, it was found that the dry matter, protein and fat content varied very little. The variation comes from the ingredients used in the recipe (Figure 5).

The dry matter of the samples was consistent with the type of pulp added in the sample. Thus, control sample A0, without the addition of pumpkin, has a dry substance of ≈94%, the addition of pumpkin pulp decreases the percentage of dry substance in accordance with the percentage of pumpkin pulp added in the recipe.

The dry matter (DM) of samples A1 and A2 decreases compared to the control A0 (DM= 93.22 ± 0.68%) for all samples with addition of pumpkin pulp, being between 69.27 ± 2.79% (addition of fresh pumpkin pulp) and 73.46 ± 0.50% (added baked pumpkin pulp).

The increase moisture in products with fresh pumpkin pulp was high because its moisture was also on average 95.45% and for baked pumpkin 91.69%. In conclusion, the state of the pumpkin greatly influences the technological, sensory and stability characteristics of the obtained products.

Carotenoids are susceptible to various degradation and isomerization reactions, mainly due to carbon-carbon double bonds, causing discoloration and reduction of the biological activities of the relevant food products (Fratianni et al., 2017). In particular, thermal treatments such as cooking pumpkin puree (Provesi et al., 2011), steaming or boiling pumpkin flesh (Ribeiro et al., 2015) and hot air drying of pumpkin slices (Lago-Vanzela, 2013) could induce degradation and structural changes (eg, cis isomerization) of carotenoids. In most studies relevant to pumpkin carotenoids, only provitamin A carotenoids (mainly β-carotene) have been investigated (Ribeiro et al., 2015). The thermal stability of carotenoids without vitamin A activity in pumpkin pulp has generally remained unclear, although some of these carotenoids have been linked to reduced risk of degenerative diseases such as macular degeneration, which may be prevented by macular pigments, including lutein and zeaxanthin (Ziegler et al., 2015).

Pumpkin is a good source of carotenoids (Bergantin et al., 2018) because the color of their pulp varies from yellow to orange, which is associated with different carotenoid compositions (Marek et al., 2008). As precursors of vitamin A, carotenoids are essential components of the human diet and play a significant role in reducing the risk of cancer and stimulating the immune system (Beutner et al., 2001). Carotenoids are effective antioxidants to protect cells from damage caused by free radicals. The intake of carotenoids from pumpkin is an important supplement to the diet of a segment of consumers where vitamin A deficiency is a serious concern.

The recommended daily dose is currently either 2 mg β-carotene (recommended by the DGE, Germany, in addition to 1.0 (0.8) mg retinol equivalents for vitamin A requirements) or 5–6 mg β -carotene (recommended by NCI, USA). The recommended daily intake can only be reached by consuming (100-200 g/day) vegetables and fruits with particularly high carotene content (Müller et al., 1996).

The bioactive substances in pumpkin pulp are from the category of antioxidants, such as alpha-carotene, beta-carotene and beta-cryptoxanthin that support the effect of the body's immune response and improve the activity at the cell level (Ami Ben-Amotzsi et al., 1998). Pumpkin pulp has a high antioxidant activity (containing α-carotene, β-carotene and lutein) beneficial to the consumer with an approximate content of 202 µg/g beta-carotene (Hagos et al., 2022; Javeria et al., 2013). The presence of β-carotene provides the body with the necessary vitamin A.

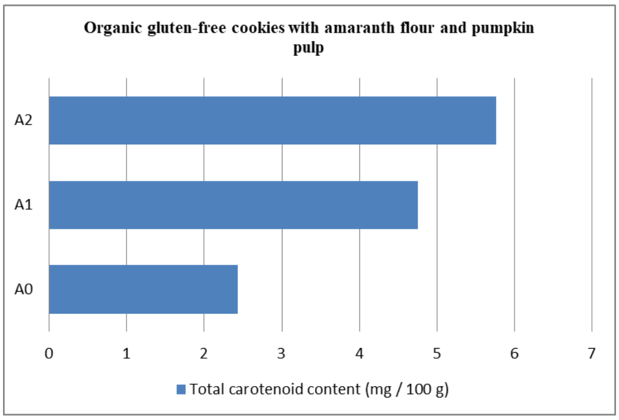

Figure 6. Total carotenoid content for Organic gluten-free cookies with amaranth flour and pumpkin pulp

Determination of total carotenoid content in baked pumpkin (Figure 6) show a total carotene content of 2.388 ± 0.120 mg total carotenes/100 g and 0.776 ± 0.019 mg total carotenes/100 g for fresh pumpkin pulp, determined by the UV-VIS method, which confirms that the pumpkins used, from local organic farms, fall within the limits found in specialized literature. Hagos et al., 2022 found the β-carotene content higher in the pumpkin skin (340-445 µg/g), followed by the pumpkin pulp (317-341 µg/g) by the UV-VIS method, values comparable to those obtained in the determinations ours, respectively 238.8 mg/100 g total carotenes.

Following the results obtained, an increase in the total carotene content was observed in the samples containing baked pumpkin pulp.

The carotenoids contents of samples A1 and A2 increase compared to the control A0 (5.024 ± 0.034 mg / 100 g total carotens) for all samples with addition of pumpkin pulp, being between 6.435 ± 0.161 mg / 100 g total carotens (A1) and 6.629 ± 0.570 mg / 100 g total carotens (A2).

Following the results obtained, an increase in the total carotene content was observed in the samples A2, respectively 6.629 ± 0.570 mg /100 g.

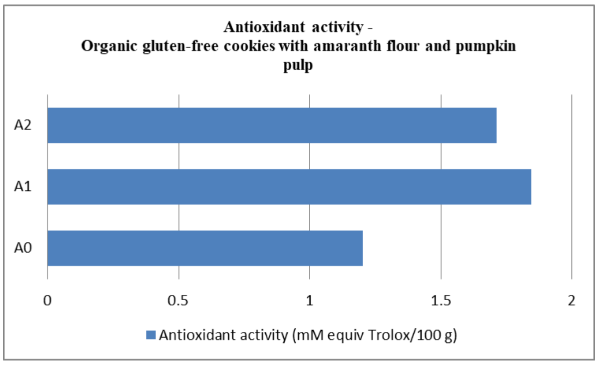

Figure 7. Antioxidant activity for Organic gluten-free cookies with amaranth flour and pumpkin pulp

As expected, the antioxidant activity is higher in organic gluten-free cookies with amaranth flour and fresh pumpkin pulp (Figure 7). The sample without pumpkin pulp has the lowest antioxidant activity.

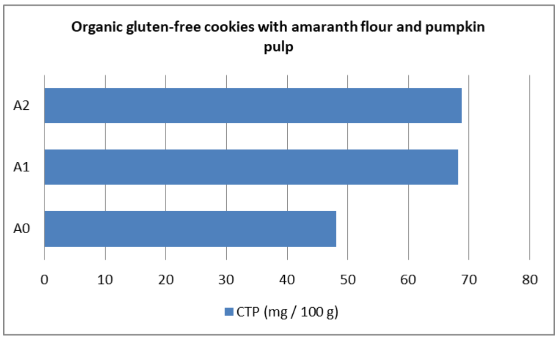

Figure 8. Total content of polyphenolic compounds for organic gluten-free cookies with amaranth flour and pumpkin pulp

The total content of polyphenolic compounds in gluten-free cookies with amaranth flour is observed to be higher in samples with pumpkin pulp (Figure 8), which highlights that *Curcubita maxima* pumpkin pulp, as an ingredient, comes with a high intake of valuable compounds high bioactive.

We can conclude that the addition of pumpkin pulp has a major positive impact on the nutritional value of the products obtained in our study.

CONCLUSIONS

The cookies are rich in nutrients and can be consumed by all individuals to help meet daily needs, especially those of people who have increased needs for these essential nutrients. The obtained results indicated that the developed cookies were not only improved in terms of nutritional value and health benefits, but also had high potential of being accepted by consumers.

In conclusion, each type of product was distinguished by an attribute, and following the sensory evaluation of the samples of gluten-free cookies with organic pumpkin pulp, the formulas used to finally obtain the sensory characteristics desired by the consumer will be reviewed and optimized, obtaining technology. The use of konjac flour has substantially improved the characteristics of the dough, but will especially optimize recipes for gluten-free cookies with the addition of fresh pumpkin pulp.

This work suggested that the amaranth flour and pumpkin pulp should be good potential candidates as gluten-free materials to replace wheat flour in some food products

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