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The effect of storage time on the physico-chemical, microbiological, and sensory properties of gluten-free snacks containing textured fruit juice

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Abstract

In the modern world, there is a high incidence of food allergies and intolerances. Half the world's population suffers from food intolerance, while in Romania, 8 out of 10 persons have food intolerance to some degree. Such examples are celiac disease, non-celiac gluten sensitivity, gluten ataxia, and dermatitis herpetiformis, which are pathologies correlated with the intake of gluten prolamins (e.g., proteins of wheat, rye, and barley). Thus, the aim of this study was to evaluate the effect of storage time on the physico-chemical, microbiological, and sensory properties of two gluten-free snacks containing textured fruit juice. There were obtained two gluten-free snacks with textured fruit juice that were analysed in order to determine their self-life. During the accelerated shelf-life test (ASLT) that was conducted at 35 °C in the climate chamber, the texture properties, the peroxide index, and the microbiological tests had the biggest variations. The shelf life in terms of sensory quality was 222 days for the gluten-free snack with textured mango juice and 186 days for the gluten-free snack with textured raspberry juice.

Keywords: gluten-free, snack, storage

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1. Introduction

Recent studies have focused on the importance of personalized nutritional needs for maintaining the health of different segments of the population, as well as in the prophylaxis of major diseases. Each group of people has different nutritional needs, which are based on factors such as age, a certain period of life (e.g., motherhood), certain diseases, allergies, and food intolerances [1].

In the modern world, there is a high incidence of food allergies and intolerances. As the World Health Organization reports [2], half the world's population suffers from food intolerance, even though only 1 billion people have been diagnosed. According to tests conducted by food intolerance specialists, 8 out of 10 Romanians have food intolerance to some degree. A food intolerance occurs when the body rejects certain foods that cannot be digested and assimilated. Thus, a local inflammatory reaction occurs that, if repeated, can damage the mucosa of the digestive tract in addition to causing a systemic inflammatory reaction that may affect the whole body [3]. Examples of food intolerances are celiac disease, non-celiac gluten sensitivity, gluten ataxia, and dermatitis herpetiformis. These are pathologies correlated with the intake of gluten prolamins (e.g., proteins fractions from cereals such as wheat, rye, and barley) among those people with genetic predispositions leading to intestinal lesions [4,5].

Thus, celiac disease is an autoimmune pathology that can start at any age, beginning in childhood. It is triggered by gluten and affects the small intestines of genetically susceptible individuals [6]. The disease currently affects many people around the

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world (1% of the population) and occurs in genetically predisposed individuals who develop inflammation in the small intestine after ingesting gluten. Studies show that celiac disease has been on the rise in recent decades, probably due to incorrect diets or the use of medications that may influence the gut microbiome [7]. Complications, including intestine lymphoma and malignancy, which are linked to untreated celiac disease and result in high morbidity, can be avoided with early diagnosis and adequate treatment [8]. Thus, flour products, which relate to the main element of the diet, become restrictive in the case of this food intolerance. These products must be free of allergens (e.g., gliadin) and should contain the necessary nutritional principles to correct the metabolic disorders caused by this disease. Consequently, consumer awareness of the role of food allergies in the health and illness of the population has led to the adoption of the Regulation on the Labeling of Food Allergens and Consumer Protection requiring the declaration of 14 allergens, including gluten, when added as an ingredient [9].

Along these lines, it is important for a gluten-free product to be very similar to a regular food commodity in terms of sensory and physico-chemical characteristics. As the production and consumption of ready-to-eat food products has increased worldwide in recent years and the convenience of these products fits the current consumers' needs and demands for such quality food products, a gluten-free snack based on gluten-free cereals and fruit would provide a dietary food product suitable for consumers who suffer from celiac disease, as well as a healthy and tasty ready-to-eat food product suitable for the alert lifestyle of young people and not only. Moreover, snacks represent a daily consumption habit, mostly for younger consumers [10].

Hence, the aim of this study was to diversify and optimize the assortments of current gluten-free products through the emergence of new commodities such as snacks with textured fruit juice, to meet the current requirements and needs of consumers.

The total quality of two different gluten-free snacks with textured fruit juice was evaluated regarding the differences that occurred throughout the storage period for the selected food commodities.

2. Materials and Method

2.1. Production of gluten-free fruit snacks

Two gluten-free snacks with textured fruit juice were obtained in the cereal pilot plant of IBA-Bucharest (Figure 1). The products were selected from four batches obtained initially within this study.

To obtain the gluten-free products, the floury layer was made and baked in a hot oven at 180-190 °C for 25 minutes. After that, a textured juice layer was made and poured hot over the floury layer. The obtained products were left to cool and then sliced into 10x3 cm pieces (portions).

A gluten-free snack with textured raspberry juice was made with the following ingredients: whole meal rice flour (20%), corn flour (30%), buckwheat flour (20%), and millet flour (30%), sugar, eggs, expanded rice flakes with milk and fruit forest, cornflakes with corn, citric acid, and textured raspberry juice (raspberry puree, glucose syrup, pectin, citric acid, raspberry flavor).

The ingredients of the gluten-free snack with textured mango juice were the following: whole meal rice flour (70%), buckwheat flour (30%), sugar, eggs, expanded rice flakes with milk and berries, buckwheat flakes with corn, citric acid, and textured mango juice (mango puree, glucose syrup, pectin, citric acid, mango flavor).

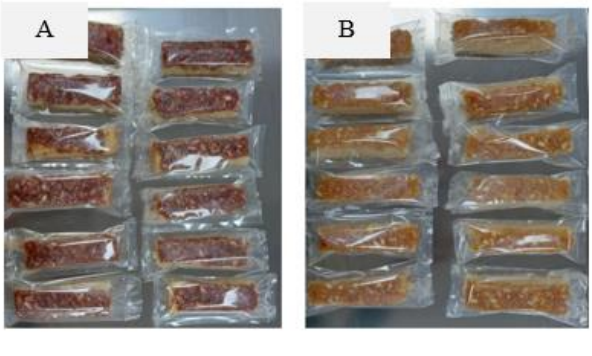


Figure 1. Gluten free snacks prepared for an accelerated shelf-life test: (A) gluten-free snack with textured raspberry juice; (B) gluten-free snack with textured mango juice

2.2. Packaging and storage conditions

A flexible multilayer film (PE/EVOX/PP) was used to pack the obtained gluten-free snacks. The characteristics of flexible multilayer film are: 2.39

g/m^2·24h water vapor permeability, and 5.38 cm^3 / m^2·24h·bar oxygen permeability.

The snacks were packaged using the Impulse Sealer Pfs 400 heat sealer and kept at real life (RL) at 25 °C and accelerated shelf-life test (ASLT) at 35 °C in the climate chamber for 30-37 days, the equivalent of 222 days of real life for the gluten-free snack with textured mango juice, and 180 days of real life for the gluten-free snack with textured raspberry juice, respectively.

2.3. Physico-chemical analysis

The following variables were analyzed: (1) Moisture content, (2) water activity, (3) textural properties, and (4) peroxide index. The materials and the conditions used were the following: (1) Moisture content measurement was performed using a Mettler LJ16 infrared dryer (Mettler-Toledo Ltd., UK). The working method consisted of drying 5 g of a well-ground and homogenized sample at 130 °C.; (2) Water activity was measured using the Aquaspector AQS-31 (NAGY Messsysteme GmbH, Germany) at a temperature of 24 °C. Grinded gluten-free snack samples were placed in the measurement cup and readings were noted; (3) Textural properties were analysed using the Instron texture analyzer (model 5944, Illinois Tool Works Inc., USA), equipped with a 500 N load cell. A puncture test was applied to the sample, using a cylindrical piston with a diameter of 9 mm. Using the Bluehill 3.13 program, hardness was measured and calculated; (4) The peroxide index was evaluated according to standard SR 13531:2008 for food products [11]. The peroxide index is a parameter that reflects the oxygen content in the form of peroxide (hydroperoxide) in a substance, and it is a parameter to measure the degree of oxidation of the sample.

2.4. Microbiological analysis

The (1) Total plate count, (2) Yeasts and molds, and (3) *Enterobacteriaceae* were monitored. An amount of 10 g of sample was aseptically removed from each package using a sterile spatula and was transferred to a sterile filter stomacher bag (Seward Limited, UK), which contained 90 mL of sterile homogenate solution (0.85% NaCl, and 0.1% neutralized bacteriological peptone). The samples were homogenized using a stomacher (Seward Limited, UK) for 30 s at room temperature. A tenfold dilution series was made in sterile peptone saline solution as needed for plating. The media and the conditions used were the following: (1) Plate count agar (PCA), (Oxoid, UK) was used for the total mesophilic bacteria and was incubated at 30 °C for 3 days, according to the ISO 4833-1/2014 standard; (2) A volume of 100 µL of inoculum was dispersed onto the entire surface areas of dichloran glycerol (DG-18) and the agar medium (Oxoid, UK) using an L-shaped spreader, and incubated at 25 °C for 7 days, according to the ISO 21527-2:2009 standard [12]; (3) Following the ISO 21528-2/2017 [13] standard, a volume of 1 mL of the appropriate sample dilution was plated on VRBG (Oxoid, UK) and was incubated at 37 °C for 24 ± 2h. All plates were examined visually for typical colony types and morphological characteristics associated with each growth medium. The analysis was conducted on duplicate plates. The microbial count was expressed as colony forming units per gram (cfu g^{-1}).

2.5. Sensory analysis

Sensory differences were determined by comparing the samples through a pair comparison method, as described in ISO 5495/2005 [14-16], in the sensory analysis laboratory by the Expergo Sensory Research team. The pair test has been chosen in order to determine whether there are considerable differences between the analyzed samples. Significant differences are determined by differences greater than one scalar unit between the intensity of the stability attributes of the analysed samples.

The samples had been stored in the climate chamber as follows: 30 days in the case of the gluten-free snack with textured raspberry juice and 37 days in the case of the gluten-free snack with textured mango juice, both at real life (RL) at 25 °C and at an accelerated shelf-life test (ASLT) at 35 °C. The experiment was aimed at determining whether there were significant differences in the main sensory descriptors during the entire period of the shelf life. Sensory tests were performed every week during the 30 and 37 days, respectively. Due to the fact that mango had good sensory features at the end of the 30-day period, the team decided to extend the period to 37 days in ASLT, which is equivalent to 222 days RL.

The evaluators completed monopolar scalar questionnaires with the following sensory descriptors: smell, firmness, crumbliness, sweet taste, sour taste, fruit taste, and after taste.

3. Results and Discussion

3.1. Moisture content

Moisture content and water activity are two physico-chemical attributes that have a great impact on the microbiological contamination of food products. These parameters were measured in order to assess the impact of the storage conditions on the gluten-free fruit snack type when samples were compared with each other.

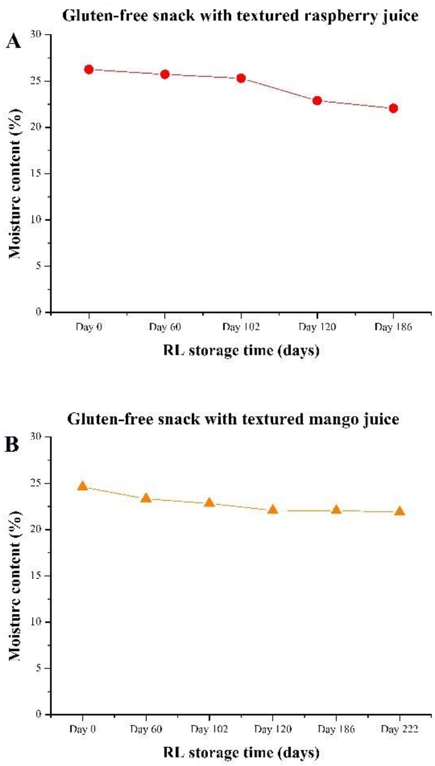


Figure 2. Moisture content during real life storage time: (A) gluten-free snack with textured raspberry juice; (B) gluten-free snack with textured mango juice

According to Figure 2A, the moisture content of the gluten-free snack with textured raspberry juice had the highest values both initially and during the storage time.

Throughout the analysis period, the moisture content of the gluten-free snack with textured raspberry juice had a decrease of 4.2% and only 2.72% for the gluten-free snack with textured mango juice (Figure 2B). The moisture content of the samples decreased during storage time. The products lost moisture, and after 30 days and 37 days, respectively, the moisture of both samples was around 22.00 %.

3.2. Water activity

The water activity of food products represents an important parameter that influences undesirable chemical and enzymatic reactions as well as the shelf life of a food commodity [15]. In our study, all analyzed snack samples noted initial values for the water activity quality parameter of less than 0.95. After 10 days of storage, a slight increase in this parameter was observed for both samples compared to the initial values, which were recorded on the first day of analysis. Thus, an increase of 3.45% was noted for the gluten-free snack with textured mango juice, and 1.16% for the gluten-free snack with textured raspberry juice. On the seventeenth day of storage, a reduction in water activity was observed for the gluten-free snack with textured raspberry juice, while on the twentieth day of storage, a reduction of this quality indicator was observed for both analyzed samples (a reduction of 0.80% for the gluten-free snack with textured mango juice and 0.70% for the gluten-free snack with textured raspberry juice, respectively). On the thirty-first day of storage, there was noted a greater reduction of the water activity parameter for both samples, namely a reduction of 2.26% for the gluten-free snack with textured mango juice and 7.33% for the gluten-free snack with textured raspberry juice. This was the last storage day for the gluten-free snack with textured raspberry juice, the day on which the lowest value of this quality parameter was recorded for this sample. The gluten-free snack with textured mango juice could have been stored for up to 222 38 days, when this sample noted the highest reduction of the water activity parameter, which was recorded at 3.05% (Figure 3).

Most likely, the samples of gluten-free snacks and fruit content did not show visible signs of deterioration at the end of the thirty-first day of storage due to the maintenance of low values of the water activity quality indicator.

3.3. Variation of hardness

The hardness (resistance to pressure) parameter changed during the storage time of the gluten-free snacks with textured juice.

In Figure 4A, it can be observed that during the storage time (for self-life determination), the hardness of the floury layer of the gluten-free snack with textured raspberry juice was increased from

0.9265 on day zero to 1.7226 on the last day (186 day of RL). This means that during this period of 186 days of real life, the hardness was almost doubled, so the product has become denser and stronger on storage.

The hardness of the floury layer from the gluten-free snack with textured mango juice (Figure 4B) had a rapid increase in the first two weeks, from 1.717 on day 0 to 2.6591 on day 222 of real life then the increase was smaller and constant.

The increase in hardness during the storage period is due to the fact that the product dries, its consistency becoming stronger. Due to the fact that it has become crumbly, the product has lost its firmness slightly in the last week.

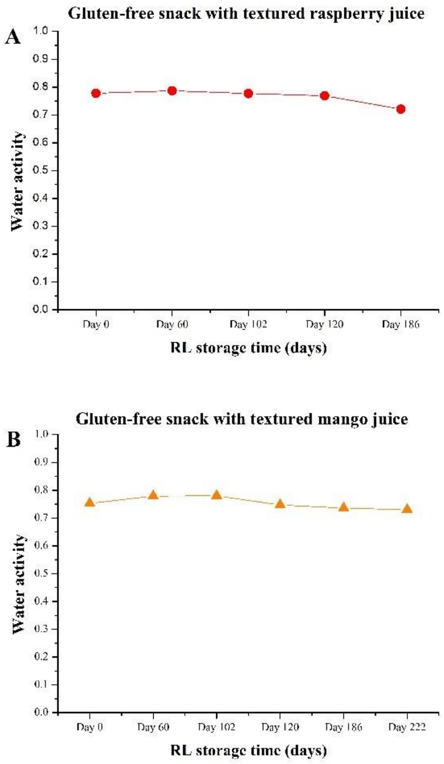


Figure 3. Water activity values during real life storage time: (A) gluten-free snack with textured raspberry juice; (B) gluten-free snack with textured mango juice

3.4. Peroxide index

The peroxide index changed during the storage time. Following the analyses performed, a linear correlation was calculated and obtained between the logarithm of the peroxide index and the storage time of the products analyzed.

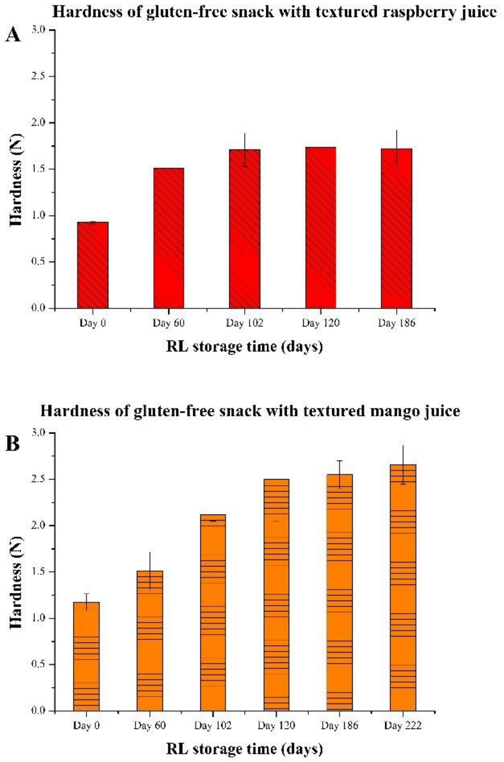


Figure 4. Variation of hardness during real life storage time: (A) gluten-free snack with textured raspberry juice; (B) gluten-free snack with textured mango juice

In Figure 5A, it can be seen that, in the case of the gluten-free snack with textured raspberry juice, after the first storage period, the peroxide index had values very close to the initial ones, of the fresh product (3.6, starting from the initial value of 3.4). During the whole period of storage, the value of the peroxide index increased constantly, reaching 4.8 on the 186 day of accelerated storage time.

Figure 5B shows that the peroxide index of the gluten-free snack with textured mango juice registered a faster increase in the first part of the storage interval than the increase after this period, which was lower and correlated with the loss of moisture of the products.

3.5. Microbiological analysis

Table 1 and Table 2 list the initial and final viable cell loads of different microbial groups, such as total plate counts, yeasts and molds, and *Enterobacteriaceae* during an accelerated storage time of 186 days of real life for the gluten-free snack with textured raspberry juice and for the gluten-free snack with textured mango juice, respectively. These microbiological results give an indication that no external contamination of the products occurred, as for all analyzed samples, the indicator *Enterobacteriaceae* noted values of less than 10 colony forming units per gram.

The gluten-free snack with textured raspberry juice noted microbial contamination since the tenth day of storage, when the total aerobic plate counts registered 1.6 x 10^2 cfu g^{-1}. Yeasts and molds were noted for the gluten-free snack with textured raspberry juice since the seventeenth day of storage, and the values of both these two parameters increased in direct relation to the storage time (Table 1).

On the contrary, the microbiological analysis revealed that the gluten-free snack with textured mango juice showed no microbial contamination during the entire storage period, as the microbiological results noted no contamination with total plate counts, yeasts and molds, or *Enterobacteriaceae*, respectively (Table 2). However, for this product, visible molds were noted only on the 40^{th} day of storage. These results are also supported by the physico-chemical results noted for this product. Thus, the gluten-free snack with textured mango juice noted the lowest moisture content values during the storage period, as well as lower water activity values when compared with the gluten-free snack with textured raspberry juice.

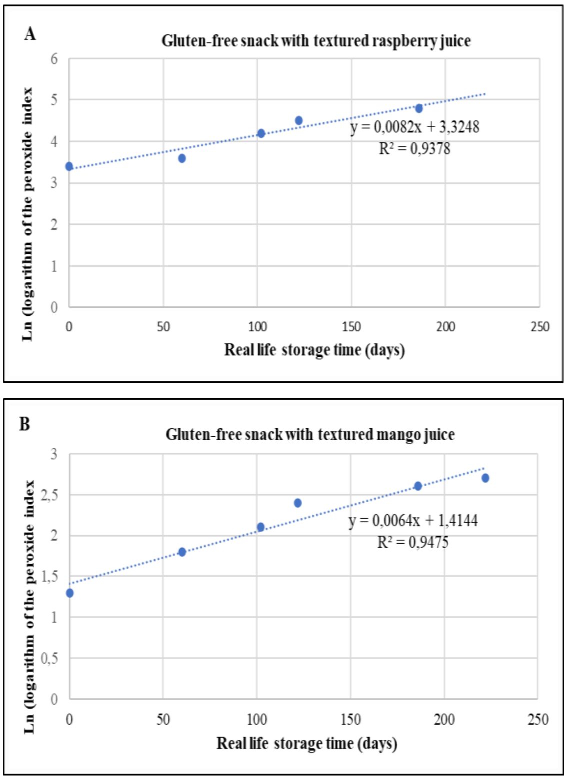


Figure 5. The variation of the peroxide during real life storage time: (A) gluten-free snack with textured raspberry juice; (B) gluten-free snack with textured mango juice

Table 1. The occurrence of microbial parameters for the gluten-free snack with textured raspberry juice during RL storage time

Real shelf life (days)	**Total plate count** (cfu g^{-1})	**Yeasts and molds** (cfu g^{-1})	***Enterobacteriaceae*** (cfu g^{-1})
0	< 10	< 10	< 10
60	1.6 x 10^2	< 10	< 10
102	2.6 x 10^2	7.0 x 10^1	< 10
120	3.2 x 10^2	2.6 x 10^2	< 10
186	3.6 x 10^2	2.7 x 10^2	< 10

Table 2. The occurrence of microbial parameters for the gluten-free snack with textured mango juice during RL storage time

Real shelf life (days)	**Total plate count** (cfu g^{-1})	**Yeasts and molds** (cfu g^{-1})	***Enterobacteriaceae*** (cfu g^{-1})
0	< 10	< 10	< 10
60	< 10	< 10	< 10
102	< 10	< 10	< 10
120	< 10	< 10	< 10
186	< 10	< 10	< 10
222	< 10	< 10	< 10

Table 3. Test results for the gluten-free snack with textured raspberry juice

Sensory attributes	Real shelf life (Days)				
	0	**60**	**102**	**120**	**186**
Smell	4.00	2.33	1.92	1.67	1.58
Firmness	2.90	2.42	2.33	2.00	1.92
Crumbliness	2.58	2.67	2.67	3.20	5.00
Sweet taste	5.00	5.25	4.30	4.00	3.75
Sour taste	2.08	2.50	2.75	3.00	3.17
Fruit taste	4.00	3.08	2.92	2.67	2.50
After taste	3.30	3.00	2.92	2.75	2.67

Table 4. Test results for the gluten-free snack with textured mango juice

Sensory attributes	Real shelf life (Days)					
	0	**60**	**102**	**120**	**186**	**222**
Smell	2.80	2.58	1.92	1.75	1.33	1.00
Firmness	3.40	2.75	2.50	2.50	2.33	2.33
Crumbliness	2.25	2.42	2.90	3.08	3.30	5.00
Sweet taste	4.40	4.50	4.25	3.75	3.75	4.00
Sour taste	2.25	2.25	1.67	1.50	1.33	1.25
Fruit taste	2.60	2.33	1.83	1.75	1.50	1.08
After taste	4.00	3.17	3.00	2.83	2.83	2.00

This difference between the two assessed gluten-free fruit-based snacks when comparing their microbial contamination might be caused by the different ingredients that were used, such as the fruit type, respectively. Thus, raspberry proved to be more sensitive than mango to resisting for a longer period of time when used as an ingredient in a gluten-free fruit-based snack.

3.6. Sensory analysis

The samples had been stored in the climate chamber as follows: 30 days in the case of the gluten-free snack with textured raspberry juice and 37 days in the case of the gluten-free snack with textured mango juice, both at real life (RL) at 25 °C and at an accelerated shelf-life test (ASLT) at 35 °C. The experiment was aimed at determining whether there were significant differences in the main sensory descriptors during the entire period of the shelf life. Sensory tests were performed every week during the 30 and 37 days, respectively. Due to the fact that mango had good sensory features at the end of the 30-day period, the team decided to extend the period to 37 days in ASLT, which is equivalent to 222 days of RL.

The evaluators completed monopolar scalar questionnaires with the following sensory descriptors: smell, firmness, crumbliness, sweet taste, sour taste, fruit taste, and after taste. The test results for the gluten-free snack with textured raspberry juice maintained at ASLT are specified in Table 3.

The test results for the gluten-free snack with textured mango juice maintained at ASLT are specified in Table 4.

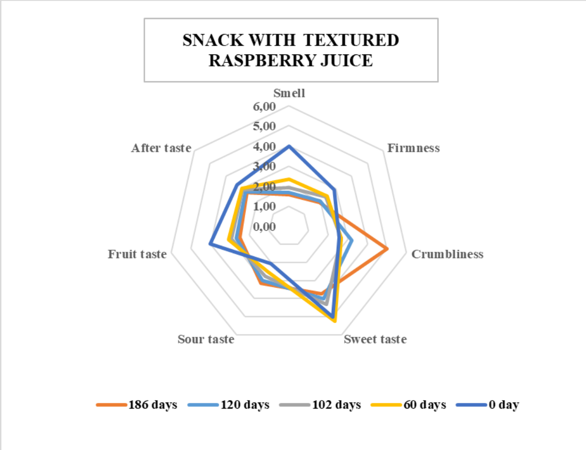
As shown in the Table 3 and Table 4, the intensity of the majority of the sensory descriptors decreased during the last lapse of accelerated aging test.

Based on the results specified above, the team determined the flavor profiles of the two gluten-free snack samples (with textured raspberry juice and with textured mango juice, respectively). These are specified in Figure 6.

Most of the sensory descriptors evaluated during the assessment did not have significant variations. They did not influence the shelf life studied by the accelerated aging method (ASLT). Note that these descriptors are characteristic of the textured fruit juice used.

The most important sensory descriptor that showed significant variations towards the end of the test period and that directly influenced the shelf life was the crumbliness. This descriptor represents a special feature in the case of sponge cake, for example.

In the case of the gluten-free snack with textured raspberry juice, the crumbling had a significant increase between 120 and 186 days, while in the case of the gluten-free snack with textured mango juice, the crumbling had a significant increase between 186 and 222 days.



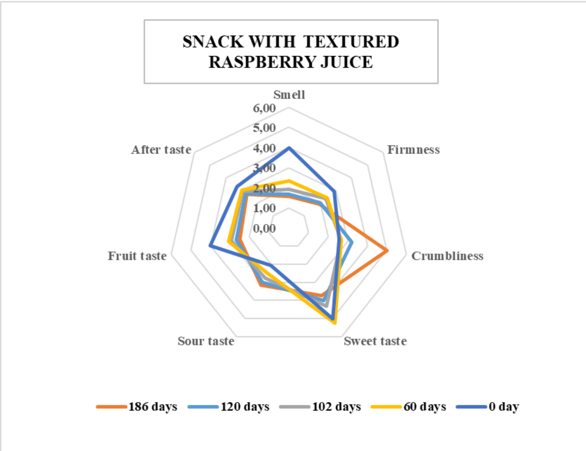


Figure 6. The flavour profile of the gluten-free snacks juices

4. Conclusion

In our study, the gluten-free snack samples noted initial values for the water activity parameter of less than 0.95, while on the thirty-first day of storage, there was a greater reduction of this parameter for both samples. The hardness (resistance to pressure) parameter changed in both sample types during the storage time due to the fact that the products dried and their consistency became stronger. Our results also noted that the peroxide index of the gluten-free snack with textured raspberry juice increased constantly during the storage period, while for the gluten-free snack with textured mango juice, it noted a faster increase in the first part of the storage interval than the increase after this period, which was lower and correlated with the loss of moisture of the products. The microbiological results gave an indication that no external contamination of the products occurred for any of the analyzed samples, as the *Enterobacteriaceae* indicator noted values of less than 10 colony forming units per gram during the entire storage period. Moreover, the gluten-free snack with textured mango juice showed no microbial contamination with total plate counts, yeasts and molds, or *Enterobacteriaceae* during the entire storage period. However, for this product, visible molds were noted only on the 40th day of storage (ASLT). On the contrary, the gluten-free snack with textured raspberry juice noted microbial contamination since the tenth day of storage, while yeasts and molds were noted since the seventeenth day of storage. Both of these two parameters increased in direct proportion to the storage time. The shelf life in terms of sensory quality was also longer for the snack with textured mango juice than for the snack with textured raspberry juice (222 days for the snack with mango, and 186 days for the snack with raspberry, respectively). Notably, the results obtained for the gluten-free snack with textured mango juice recommend this product as a safe product with a prolonged shelf life, that meets consumer demand for functional foods. At the same time, the consumption of such ready-to-eat gluten-free products would represent a safe and healthy way of snack consumption for those suffering from celiac disease and non-celiac gluten sensitivity.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human / or animal subjects (if exist) respect the specific regulation and standards. Authors declare that they present their own literature survey and results/discussion/conclusion in the article.

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