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A New Gluten-Free Product: Quality and Acceptability of Pumpkin Flour and Chips

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ABSTRACT

Pumpkin, a versatile ingredient known for its high nutritional content and abundance of vitamins, minerals, and antioxidants, was used in the development of flour and gluten-free chips. The physicochemical, microbiological, and sensory properties of both the pumpkin flour and gluten-free chips were thoroughly examined. The moisture, dry matter, and ash content of pumpkin flour ranged from 32.84% to 55.28%, 44.72% to 67.16%, and 4.57% to 6.88%, respectively, with a mean pH value of 6.77. Gluten-free chips made with pumpkin flour had a high dry matter content (89.97%) and low moisture content (10.03%). Additionally, due to the high mineral content, the product was slightly acidic (pH 5.79) with a high ash content (9.61%). The L*, a*, and b* values for pumpkin flour were 70.5, 11.3, and 58.0, respectively. In comparison, the values for gluten-free chips were 40.08, 11.02, and 21.59, respectively. The research results show that gluten-free chips are microbiologically safe, preferred by consumers, and resistant to spoilage during shelf life.

Introduction

Today, gluten sensitivity is common. This condition, called celiac disease, is caused by the consumption of gluten-containing foods, which damages the mucous membrane of the small intestine and causes inflammation. The disease results in chronic inflammation and the loss of villi in the intestinal mucosa, impairing the absorption of important nutrients such as iron, calcium, folic acid, and vitamins K, E, D, and A, leading to malnutrition. In the long term, it can affect many systems, from the liver to bone structure. It is the most common autoimmune disease worldwide, with a prevalence varying between 1 in 350 and 1 in 2000 in different countries. Celiac disease is more common in regions where wheat consumption is common. For example, while it is common in Northern Europe, North America, and Western Asia, it is almost completely absent in China, Japan, and some parts of Africa, as these societies do not consume wheat-based diets. In our country, the prevalence ranges between 1 percent and 3 percent. Celiac patients must consume gluten-free foods throughout their lives (Ammar and Abd El-Razik, 2013, Aly and Seleem, 2015). In addition to celiac patients, the number of people following a gluten-free diet due to gluten allergies (such as dermatitis or hypertyphima) or as a result of popularism is increasing. As a result, the market for various gluten-free foods within the sector is growing. However, the gluten-free foods produced are not sufficient in terms of protein, micronutrients, bioactive compounds, and dietary fiber (Voučko *et al.,* 2022). Consumers who demand gluten-free food demand that product variety be increased and that these products contain adequate nutrients. Driven by this consumer group's demand, developing formulations, processing techniques, and improving nutritional content in the production of gluten-free products has become a priority in the food industry (Tomić *et al.,* 2022). Pumpkin fruit and pumpkin products are of interest to various sectors, including the food industry, due to their richness in carotene, pectin, mineral salts, vitamins, and other health-beneficial substances. Pumpkin is a low-calorie food because of its low fat and high water content, making it a popular choice in diet menus. It is rich in bioactive substances such as carotenoids, sterols, and tocopherols, which are believed to help prevent diseases like diabetes, hypertension, cancer, high cholesterol, and coronary heart disease. Pumpkin has also been used to treat inflammation of the small intestine and indigestion. It is a common ingredient in a variety of foods including jams, beverages, pickles, desserts, and pastries. Recently, there has been a growing interest in healthy foods, leading to a focus on developing functional foods that contain pumpkin, especially in bakery products.

The fact that pumpkin is gluten-free not only contributes to its health benefits and nutritional content, but also positively impacts the flavor, aroma, and color of the product (Ammar and Abd El-Razik, 2013). Snacks are one of the food groups that require gluten-free nutrition. Consumer interest in ready-to-eat (RTE) snack foods is growing due to their convenience, value, attractive appearance, taste, and texture. Chips are among the most widely consumed and popular snacks. However, the use of maltodextrin, wheat starch, barley flour, malt vinegar, and malt extract in their production poses a risk of gluten exposure. Chips also contain refined sugar, salt, and oil. Gluten-free, healthy, natural, and functional snack foods have become a trend in the global market, and pumpkin is a product that can provide these qualities (Aydin, 2022).

Therefore, this study investigated the development of gluten-free chips produced using pumpkin flour. Firstly, flours of four different pumpkin varieties were produced and chips were obtained from the selected pumpkin flours. Pysico-chemical, microbiology and sensory analyses of the pumpkin flour and chips were carried out.

Materials and Methods

Materials

The pumpkins were purchased from a local market in Adana, Turkey. Two summer pumpkins (*Cucurbita pepo*) and two winter pumpkins (*Cucurbita moschata*) were bought (see Picture 1). The raw pumpkins are used in the production of pumpkin flour. The ingredients used in gluten-free chip production included pumpkin flour, potato starch, yogurt powder, and milk powder, along with additional pumpkin flour (see Figure 1). Spices and flavorings were added based on consumer preferences.

Methods

Raw pumpkin was used in the production of pumpkin flour. The production process of gluten-free snack chips involved using pumpkin flour, potato starch, yogurt powder, milk powder, salt, sugar, tomato powder, onion powder, and water (see Figure 1). Analyses were carried out in two parallels on two samples each of summer and winter squash. Moisture, dry matter, ash, pH, color, microbiological, and sensory analyses were carried out on both fresh and dried squash samples. Gluten-free chips were produced from pumpkin flour in three replicates, two parallels, and the same analyses were carried out on the chips.

Pumpkin flour preparation

The fresh pumpkins were washed, peeled, and cut into halves after removing the stem. The seeds and stringy bits were then removed, and the pumpkins were sliced into pieces measuring 1x1cm and 1x2cm thick. Slices were washed and dried (15 h, 60°C) in a hot-air dryer (APın Samheung). After the dried pumpkins were ground in a laboratory-type grinder (Isolab), they were passed through a 50 mesh sieve to obtain pumpkin flour. The resulting flour was packed in polyethylene bags and stored at -18°C until use (Ammar and Abd El-Razik, 2013).

Preparation of Gluten-Free Chips

The recipe for gluten-free chips was developed through preliminary trials using a variety of ingredients. Samples of gluten-free chips were made with the following ingredients: pumpkin flour, potato starch, yogurt powder, milk powder, salt, sugar, tomato powder, onion powder, and water. Initially, the necessary ingredients were gathered and weighed in appropriate proportions. Pumpkin flour, potato starch, yogurt powder, and milk powder were mixed in equal parts. Gluten-free chip samples were created by substituting wheat flour with pumpkin flour, potato starch, yogurt powder, and milk powder, all mixed in a 1:1:1:1 ratio. Salt, sugar, tomato powder, and onion powder were then added to the chip mixture, followed by water, and mixed for 5 minutes. The dough was placed in molds and baked in a hot-air dryer (APın Samheung) at 180°C for 15 minutes. After baking, the prepared chip samples were allowed to cool for 30 minutes, then packed in polyethylene bags at room temperature for analysis. In the preparation and cooking process, the studies of Brigagão *et al.,* (2021) and Ammar and Abd El-Razik (2013) were referenced.

Physicochemical Properties of Pumpkin Flour and Gluten-Free Chips

The chemical composition of the pumpkin flour and gluten-free chips was determined using the recommended methods of the Association of Official Analytical Chemists (AOAC, 2005). This included analyzing the moisture, dry matter, ash, and pH content. The dry matter content was determined by placing the pumpkin flour and gluten-free chips in an oven (APın Samheung) at 105 ± 1°C until a constant weight was achieved. The moisture content was expressed as g/100 g of sample and calculated from the dry matter ratio. Ash content was determined by incinerating the sample at 520 °C in a muffle furnace. pH values were measured using a pH meter (Hanna 98107). All analyses were performed in triplicate (Ardabili *et al.,* 2011; Brigagão *et al.,* 2021; Aktaş and Gerçekaslan, 2024).

Color Measurement

The color values of pumpkin flour and chips were measured using a colorimeter (Hunter Lab ColorFlex, USA). The dimension L∗ represents lightness, a∗ indicates redness, and b∗ indicates yellowness. Each experiment was carried out in duplicate (Kiharason and Isutsa, 2019; Poliszko *et al.,* 2019; Aydin, 2022; Mahmoud and Mehder, 2022).

Microbiological Analyses

Total aerobic mesophilic bacteria, yeast, and mold counts were carried out on the samples by inoculating them on Plate Count Agar (PCA) and Potato Dextrose Agar (PDA) using the spread method. The inoculated samples were then incubated on PCA at 30±2°C for 48 hours and on PDA at 25±2°C for 72 hours. Following the incubation period, counts were performed (Ike *et al.,* 2020).

Sensory Evaluation

The sensory evaluation of pumpkin chips was carried out with 10 panelists. These panelists, who were students in the Food Science program at the University, were invited to participate in the sensory analysis. Before each test sample, water at room temperature was provided to the panelists to cleanse their palate. Panelists used a hedonic scale of 1-5 to judge parameters such as color, taste, flavor, crispness, overall acceptance, and whether they would purchase the product (1=too bad, 2=bad, 3=moderate, 4=good, 5=very good). The final judgment was calculated by averaging the scores given by all the panelists (Reis & Minim, 2006; Kiharason and Isutsa, 2019; Brigagão *et al.,* 2021; Aydin, 2022; Mahmoud and Mehder, 2022).

Results and Discussion

The analysis results of fresh and dried (flour) pumpkin

The chemical composition of fresh and dried pumpkin, including pH, moisture percentage, dry matter percentage, and ash percentage, was studied and is presented in Table 1. The moisture, dry matter, and ash content of fresh pumpkin ranged from 93.35% to 97.81%, 3.17% to 6.65%, and 0.07% to 0.73%, respectively, with a mean pH value of 7.27. The moisture, dry matter, and ash content of pumpkin flour ranged from 32.84% to 55.28%, 44.72% to 67.16%, and 4.57% to 6.88%, respectively, with a mean pH value of 6.77. Drying decreases the moisture content of pumpkins while increasing the dry matter and ash content. The pH value in dried pumpkin samples was lower than in fresh samples. Additionally, summer pumpkin had a higher average moisture content and lower dry matter and ash content compared to winter pumpkin.

Usha *et al.,* (2010) reported that an evaluation of the nutritional content of fresh pumpkin and pumpkin flour revealed that fresh pumpkin had higher moisture and lower ash and β-carotene contents than pumpkin flour.

This is consistent with the results of our research, indicating that pumpkin powder would significantly alter the nutritional value of the mixture.

Dry matter is a crucial quality indicator that provides insight into a food's composition. The quantity of dry matter can vary based on the variety, region, and climate. Typically, pumpkins have a dry matter content ranging from 4.15% to 23.1%. The ash content in pumpkins is significant because it reflects the mineral levels present. Minerals offer a variety of health benefits. The mineral content in pumpkins is influenced by factors such as genotype, soil quality, environmental conditions, and nutrient availability. Studies in literature suggest that the ash content in pumpkins typically falls between 6.61% and 8.89% (Kulaitienė *et al.,* 2014). Similar ash content (6.05±0.26%) in pumpkin flour was reported by Ammar *et al.,* (2013). Our studies indicated that pumpkin flour contained 5.95% ash, which is higher than the 2.3% reported by Minarovičová *et al.,* (2017) in pumpkin flour.

Umuhozariho *et al.,* (2020) analyzed fresh and dried forms of pumpkins, finding moisture content was 85.33%, and and ash content was 3.21% in fresh products, and 7.62% and 6.25% in dried ones, respectively. Ravi *et al.,* (2010) also reported that fresh and dried pumpkin had moisture content of 87.3%, 14.95%, and ash content of 2.48%, 5.78%, respectively. Pereora *et al.,* (2020) found in raw pumpkin samples a moisture content between 84.6 ± 0.3% and 95.5 ± 0.3%. With drying, moisture content decreased (6.8±0.3% - 10.7±0.5%), making the product more stable and maintaining the quality and conservation of the fruit. They also found a range of ash content (5.8 ± 0.4% - 9.4 ± 0.3%) in pumpkin flour. Drying foods has been practiced for centuries to preserve them for longer periods. Drying pumpkins and using them in flour form also helps with preservation. Additionally, the reduced water content results in a product that is more resistant to chemical degradation (Park *et al.,* 2001).

Results of the color of fresh and dried pumpkin are shown in Table 5. The mean L* values for fresh summer and winter squash were 49.6 and 47.1, respectively. The fresh pumpkin a* values range from 2.3 to 9.4, and the b* values range from 44.7 to 46.9 for summer and winter pumpkins, respectively. L*, a*, and b* values increased in dried samples. Thus, dried samples had a brighter and darker orange color than fresh samples. In all samples, the a* value was higher in winter squash. Winter squash has a* darker orange color than summer squash. The higher a* value in winter pumpkins is proportional to the carotenoid content. Therefore, the carotenoid content of winter pumpkins can be interpreted as higher than that of summer pumpkins. The presence of more carotenoids in winter pumpkins is likely the main reason for the higher a* value. Additionally, the higher a* value in dried pumpkins compared to fresh ones can be attributed to the concentration of carotenoids increasing as a result of drying, leading to a more intense color.

Similar to the research results, Pereora *et al.,* (2020) reported that the L* value of pumpkin flours was found to be between 73.7 and 84.1. In another study, Noor Aziah and Komathi (2009) reported similar results as 63.4 and 64.9 L* values in pumpkin flours. The a* values were found to be between 0.8 and 4.7, and the b* values were found to be between 28.1 and 46.7. They explained that higher a* values indicate that pumpkin flour contains more carotenoids.

The pigments condense after drying, and the product becomes more intense in color. Flour color is an important factor in terms of its marketing and use as an ingredient in various product formulas. The yellow-orange color of pumpkins can be associated with the presence of natural carotenoids.

Roongruangsri and Bronlund (2016) reported that the L* value of dried pumpkin powder was between 92.90 and 96.55. They stated that increasing the drying temperature decreased the L value of pumpkin flour, resulting in a darker color. Roongruangsri and Bronlund (2016) also reported that the L* value of flour obtained from dried pumpkin at 65°C hot air for about 12 hours overnight was determined as 64.94, a* value as 13.53 and b* value as 49.45.

The microbial qualities of pumpkin are detailed in Table 4. Yeast-mold counts (cfu/g) of fresh and dried pumpkin (flours) ranged from <10 cfu/g to $3.3x10^4$ cfu/g and $1.4x10^4$ cfu/g to $2.5x10^4$ cfu/g respectively. Ike *et al.,* (2020) investigated the microbial properties of pumpkin flours and reported that yeast and mold counts (cfu/g) in the flours ranged from $3.59x10^2$ ±1.81 cfu/g to $3.92x10^2$±1.12 cfu/g, respectively. Yeast and molds can be transmitted from pumpkin surfaces and various environmental conditions during processing. Therefore, implementing hygienic conditions during processing is crucial. Microbiological quality is an influential factor in food shelf life.

Analysis Results of Gluten-Free Chips

Gluten-free chip samples were prepared with pumpkin flour, potato starch, yogurt powder, milk powder, salt, sugar, tomato powder, and onion powder, and then analyzed for their physicochemical, microbiological, and sensory properties. The results for moisture, dry matter, ash content, and pH are presented in Table 4.

The study revealed that gluten-free chips made with pumpkin flour had a high dry matter content and low moisture content. Additionally, the product was found to have a high ash content and a slightly acidic structure.

Moisture content in food significantly impacts its quality. Microbial activity, enzymatic reactions, and oxidation in food are all linked to moisture content. When moisture content is low, these reactions slow down or cease entirely, preserving the food's quality for a longer period and extending its shelf life. Moisture content and dry matter content are inversely proportional.

Therefore, we can predict that the gluten-free chips we produce will have a long shelf life, with low moisture content, and their quality can be preserved without spoilage (Pereora *et al.,* 2020).

Our research also found high levels of ash content in pumpkin flour. The ash content of a food sample gives an idea of the mineral elements present in the food. Pumpkin flour is rich in calcium, iron, zinc, copper, potassium, and magnesium.

Pumpkin, a mineral-rich product, and its dried form, pumpkin flour chips, contribute significantly to the high mineral content. Mineral substances have significant effects on our nutrition. Calcium is one of the main minerals for bone health, and it also plays an important role in regulating blood pressure. Magnesium and potassium are the most important minerals, especially essential for heart health.

In addition, these minerals help regulate blood pressure, similar to calcium. Voučko *et al.,* (2022) reported that in their study on gluten-free bread production using buckwheat, rice, millet, and pumpkin flours, the highest mineral content (approximately five times higher) was found in pumpkin flours. Ammar and Abd El-Razik (2013) examined the moisture and ash content of gluten-free cake samples prepared with cassava, pumpkin, and potato flours and found them to be approximately 19.64% and 2.02%, respectively, in cakes made with pumpkin flour.

Picture.1 Pumpkins

Picture.2 Preparation of pumpkin flour

Figure.1 Preparation of Gluten-Free Chips

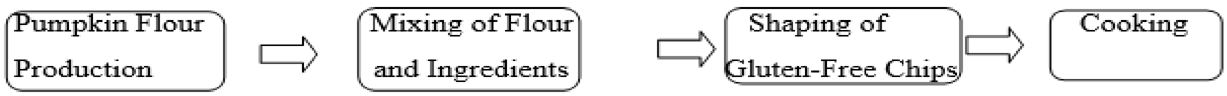

Figure.1 Sensory evaluation of the gluten-free chips

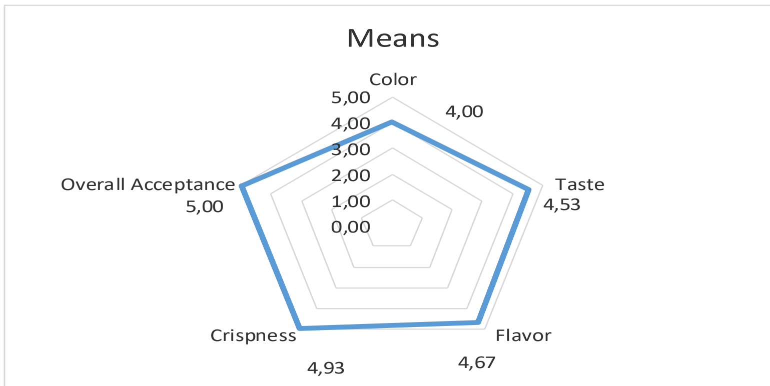

Table.1 Chemical composition of the fresh and dried pumpkin

Pumpkin	**pH**		**Moisture (%)**		**Drymatter (%)**		**Ash (%)**	
	Fresh	Dried	Fresh	Dried	Fresh	Dried	Fresh	Dried
Summer 1	7,01	7,13	93,35	43,80	6,65	56,20	0,46	6,16
Summer 2	7,53	6,66	97,81	55,28	2,19	44,72	0,07	4,57
Mean (Summer)	7,32	6,62	95,58	49,54	4,42	50,46	0,27	5,37
Winter 1	7,11	6,57	96,83	32,84	3,17	67,16	0,73	6,88
Winter 2	7,42	6,70	93,54	40,04	6,46	59,96	0,56	6,19
Mean (Winter)	7,22	6,91	95,18	36,44	4,82	63,56	0,65	6,53
General Mean	7,27	6,77	95,38	42,99	4,62	57,01	0,46	5,95

Table.2 Color of fresh and dried pumpkin measured using the Hunter Lab system

	Hunter Lab											
Pumpkin	**C**						**D65**					
	Fresh			Dried			Fresh			Dried		
	*L**	*a**	*b**	*L**	*a**	*b**	*L**	*a**	*b**	*L**	*a**	*b**
Summer 1	47,8	1,2	42,6	72,8	6,7	56,9	44,3	8,2	34,6	68,9	11,5	50,4
Summer 2	51,4	3,4	46,7	72,6	7,2	56,4	47,9	9,9	41,3	68,7	11,8	50,0
Mean (Summer)	49,6	2,3	44,7	72,7	7,0	56,6	46,1	9,0	38,0	68,8	11,7	50,2
Winter 1	46,4	9,5	46,5	74,0	9,1	53,9	44,0	14,7	41,6	70,5	13,2	48,0
Winter 2	47,8	9,4	47,4	62,8	22,3	64,6	44,7	15,4	43,9	58,6	26,4	58,0
Mean (Winter)	47,1	9,4	46,9	68,4	15,7	59,3	44,4	15,0	42,8	64,6	19,8	53,0
General Mean	48,4	5,9	45,8	70,5	11,3	58,0	45,2	12,0	40,4	66,7	15,7	51,6

Table.2 Microbiological quality of fresh and dried pumpkin system

Pumpkin	Yeast Mould Count (cfu/g)	
	Fresh	Dried
Summer 1	<10	1,8 x10^4
Summer 2	1,3x10^4	2,5 x10^4
Mean (Summer)	<6,8x10^3	1,4 x10^4
Winter 1	<10	2,3 x10^4
Winter 2	3,3 x10^4	2,2 x10^4
Mean (Winter)	1,6 x10^4	2,2 x10^4
General Mean	<1,1x10^4	2,2 x10^4

Table.4 Chemical composition of the gluten-free chips

Pumpkin	pH	Moisture (%)	Drymatter (%)	Ash (%)
Means	5,79±0,06	10,03±1,56	89,97±1,56	9,61±0,42

Table.5 Color of gluten-free chips measured as color of the Hunter lab system.

Pumpkin	Hunter Lab					
	C			D65		
	*L**	*a**	*b**	*L**	*a**	*b**
Means	40,08±4,73	11,02±0,47	21,59±4,51	38,20±4,41	12,08±0,82	18,36±4,08

Table.6 Microbiological quality of gluten-free chips

Pumpkin	Total aerob mesofilic bacteria	Yeast Mould Count (cfu/g)
Means	<6,6x10^2	<10

Table.6 Significance Value of Correlation between Sensory Quality and Overall Acceptability.

Color and Overall acceptance	**0,23**
Taste and Overall acceptance	0,50
Flavor and Overall acceptance	0,74
Crispness and Overall acceptance	0,43

Color is one of the most important quality attributes of food. Table 5 displays the color analysis of gluten-free chips. As shown in Table 5, the gluten-free chips sample had an L* value of 40.08±4.73, which is lower than the L* value obtained from pumpkin flour. Similarly, the b value was determined to be 21.59±4.51, also lower than that of pumpkin flour. The a* value was determined as 11.02±0.47, which was higher than dried summer squash but lower than dried winter squash. Overall, the color values of gluten-free chips are less intense than those of pumpkin flour. Therefore, they are considered to be darker in color and lighter in orange than pumpkin flour. This is attributed to changes in carotenoids during heat treatment and other ingredients used in chip production (potato starch, yogurt powder, milk powder).

Amar *et al.,* (2013) reported the L* value as 27.4, a* value as 11.11, and b* value as 12.96 for cakes prepared with sucrose (85 g), whole fresh egg (85 g), milk powder, and pumpkin flour. The decrease in L* value was explained by caramelization. Aly and Seleem (2015) reported that L values (64.87) decreased, while a* (3.75) and b* (27.45) values increased in breads made with rice flour, cassava flour, and extruded soy protein compared to the control when pumpkin flour was added. Furthermore, in the color analysis of biscuits produced in the same study, L* values were determined as 71.66, a* value as 3.71, and b* value as 27.46.

The microbial safety quality of the gluten-free chips is shown in Table 6. In chip samples, yeast-mold count was determined as <10 cfu/g, and total aerobe mesophilic bacteria count was determined as <6.6x10^2 cfu/g. Low moisture content and the heat treatment applied during production are the most important factors in limiting low microbial growth. Ike *et al.,* (2020) isolated *Bacillus* sp., *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli, Penicillium* sp., *Mucor* sp., and *Aspergillus* sp. in wheat flour and pumpkin seed flour mixtures. It has been explained that the isolated microorganisms may be associated with soil/environmental contaminants and inadequate hygienic practices during production. Fürnkranz *et al.,* (2012) reported that *Bacillus* sp. and *Pseudomonas* sp. were commonly isolated in a study investigating the microflora of squash plant development.

Sensory evaluation of color, taste, flavor, and crispness, as well as overall acceptance of gluten-free chips, is depicted in Figure 1. All sensory evaluations received high ratings (4 points). The highest score was for crispness (4.93), while the lowest was for color (4.0). The gluten-free chips produced in the study were highly preferred by the panelists.

Research has shown that the use of pumpkin flour in various studies is effective in achieving higher sensory evaluation scores for products. The correlation study shown in Table 6 reveals that the taste and aroma of the product have a greater impact on overall acceptance. Dabash *et al.,* (2017) noted that sensory evaluation of bread made with pumpkin flour did not decrease compared to bread made with rice flour. Purwandari *et al.,* (2014) found that in gluten-free noodles made with a combination of tapioca and pumpkin flour, the sensory scores for noodles made with pumpkin flour were higher than those made with different vegetable flours (carrot, eggplant, and green pepper). Aly and Selem (2015) studied gluten-free bread and biscuits made with pumpkin flour, and found that those made with 2.5% and/or 5% pumpkin flour received the highest overall acceptance scores. Mustika and Kartik (2020) stated that cookies prepared with pumpkin were appreciated by the panelists. A similar result was reported by Chhabra *et al.,* (2025), Fathonah *et al.,* (2018) and Minarovičová *et al.,* (2017).

In conclusion, by removing water through the drying process, the moisture content of pumpkin flour is reduced, resulting in a more microbiologically and chemically stable product. Therefore, pumpkin flour can be used as a functional ingredient additive in many food products. In the study, gluten-free chips were produced using pumpkin flour, potato starch, yogurt powder, milk powder, salt, sugar, tomato powder, and onion powder. The gluten-free chips produced were high in nutritional value with high dry matter and ash content. Additionally, due to their resistance to microbiological and chemical spoilage, they can be stored for long periods at room temperature without the need for a cold chain. Moreover, pumpkin flour's positive contributions to the sensory enhancement, light orange color, taste, and flavor of the gluten-free chips were found to have significant effects on consumer acceptance. The gluten-free chips developed in this study are a good alternative not only for celiac patients but also for people with various allergies, dieters, and athletes. Furthermore, their lightweight texture makes them suitable for mountain climbers and military personnel.

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Author Contributions

Emine Aksan Aldanmaz. The research was prepared by the author

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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