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CAROB SYRUP AND CAROB FLOUR (*CERATONIA SILIQUA* L.) AS FUNCTIONAL INGREDIENTS IN SPONGE CAKES

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ABSTRACT
The aim of this study was to evaluate the physicochemical and sensory characteristics of sponge cakes enriched with carob flour and carob syrup as functional ingredients and partial substitutes for wheat flour and sugar. Five formulations were prepared: a control cake, sponge cake with 25% carob flour, sponge cake with 50% carob flour, sponge cake with 25% carob syrup and sponge cake with 50% carob syrup. The replacement of wheat flour with carob flour resulted in a higher level of dietary fiber (2.45→ 18.28 g/100 g dry weight), protein (8.44 → 23.93 g/100 g dry weight) and carbohydrate content (65.40±5.20 → 86.10±2.70 g/100 g dry weight). The substitution of sugar with carob syrup increased the level of protein content (8.44 → 12.57g/100 g dry weight). Sensory evaluation of shape, color, cell size and uniformity, odor, sweetness, aftertaste, crumb tenderness was also performed.

1. Introduction

Consumer choices in the developed world are primarily dependent on socio-economic and cultural circumstances. Those consumers who have a consistently high intake of plant-derived foods have a much reduced incidence of cancer and possiblyother social and environmentally related diseases (Cencic and Chingwaru, 2010).

Functional foods are products that positively affect specific physiological functions in the body. The consumption of functional food results inimproved health, well-being, or performance beyond regular nutrition.

The beneficial effects of dietary fiber have been well-established by research data. Dietary fiber affects regulation of gastrointestinal motility, influences glucose and lipid metabolism, promotes fecal ouput, stimulates bacterial metabolic activity, detoxifies luminal contents of the colon and supports equilibrium of the colon ecosystem and integrity of the intestinal mucosa (Castillejo et al., 2006; Murakami et al., 2007; Santos et al., 2015). Dietary fiber thus qualifies as a functional food because it can affect one or more targeted functions in the body in a positive manner (Diplock et al., 1999).

Cakes are a popular dessert food for many consumers. However, most cakes have low fiber content and relatively high amounts of sucrose. Therefore, increasing the fiber content and reducing the sucrose in cakes would enhance this food’s nutritional quality.

Carob pods have generally been used as animal and human food. Nowadays, the seeds are used primarily for gum extraction. The pods are used after crushing and separation of the seeds from the pulp. The main products derived from carob pods are carob flour and syrup. Carob flour or powder could be added to cakes, bread, sweets, ice cream or beverages as a flavoring agent. Carob powder is already generally accepted as a substitute for cocoa, but it has advantages over cocoa because of lower calories and the absence of caffeine and theobromine (Hoda et al., 2006). The flavor of carob powder is not as intense as dark chocolate. In some countries such as Egypt, Turkey and Bulgaria, carob syrup is a popular drink obtained by extracting carob kibbles with water.

Carob bean seeds contain a protein called caroubin that exhibits rheological properties that are similar to those of wheat gluten (El Batal et al., 2012). This makes carob flour a promising alternative to gluten for people with celiac desease. Carob germ flour includes gallotannins, polyphenols, and proanthocyanidins which are known for their ability to inhibit reactive oxygen species and free radicals (Custódio et al., 2005).

The objective of this study was thus to evaluate the potential of carob syrup and carob flour for use in the production of sponge cakes and to determine the most appropriate level of these two foods as functional ingredients.

2. Materials and methods

2.1. Materials

2.1.1. Preparation of sponge cakes

The standard raw materials such as Type 500 wheat flour with 0.5% an ash content (GoodMills, Bulgaria EAD), granulated sugar (Zaharni zavodi AD) and eggs (local market) used in the current study, were authorized by the Ministry of Health as manufactured in Bulgaria. The functional ingredients, syrup and carob fruit flour (*Ceratonia siliqua* L.) from Turkey (Mersin province), were produced at the University of Food Technologies – Plovdiv, as previously described (Fidan et al., 2016). The sponge cake batter formulation used as the control was prepared according to traditional technology and formulation (Angelov et al., 1974). In particular, a double mixing procedure was applied by partitioning the whipping of egg whites and egg yolks. The carob syrup was added to the sponge cake as a sugar replacement at 25% and 50% levels. The sponge cakes with carob flour were prepared by replacing wheat flour with carob flour at 25% and 50 % levels.

Each sponge cake was comprised of 95 g of batter poured into metallic forms and baked in an electric oven (Rahovetz - 02, Bulgaria) at 180°C for 30 min. The sponge cakes were stored at standard conditions (at a temperature of 18°C and 75 % relative humidity) up to the sixth day following the production date according to standard requirements (BSS, 1982). The humidity and temperature were kept constant by means of a desiccator supplied with a psychrometer and placed in a thermostat with an accuracy of ± 0.5°C.

2.2. Methods

2.2.1. Physico-chemical characteristics of the sponge batters and cakes

The specific gravity of the sponge cake batter was calculated by dividing the weight of a standard batter cup to the weight of an equal volume of distilled water at batter temperature (20.0 ± 0.5ºC). The physical characteristics of the sponge cakes were determined 2h after baking. Volume was measured by the small uniform seed displacement method (AACC, 1999), and porosity was assessed according to the Bulgarian State Standard method (BSS, 1992). The porosity of the sponge cake was defined as the ratio of the volume of airpockets in the cake crumb to the volume of the crumb. Porosity determination was made using a cylinder driller, a device of Zhuravljov. The specific volume was expressed as the ratio of the sponge cake volume to its mass. The water-absorbing capacity of the sponge cake was measured by the extent of biscuit swelling according to the Bulgarian State Standard method (BSS, 1981). Shrinkage and Springiness were determined with an automatic penetrometer (model DSD VEB Feinmess, Dresden, Germany). A hemispherical body with a diameter 12.5 mm and total weight 300 g acted on the sectional surface of a 40 mm thick sponge cake sample, detecting shrinkage levels at 5 and 10 s. Relaxation was checked by means of a hemispherical body with a diameter 25 mm and total weight of 50 g acting upon a 40 mm thick cake crumb for 5 s. This procedure was used to determine crumb springiness (Vangelov and Karadjov, 1993). Total sample moisture was determined after drying the sample at 105^{o}C up to a constant weight according to the standard method (AACC, 2000).

Nitrogen level was determined by the Kjeldahl method (ISO 20483: 2014). A multiplication factor of 6.25 was used for the calculation of protein content.

The total insoluble and soluble dietary fiber levels were determined by the enzymatic gravimetric method (AOAC 985.28, 1990), using the total dietary fiber assay kit Bioquant 1.12979.0001 (Merck, Germany) with instructions provided by the manufacturer.

The total soluble carbohydrate content was estimated according to the spectrophotometric method of Dubois et al. (1956). In brief, 0.1 ml of each extract was mixed with 1 ml of 5% phenol and 5 ml of sulphuric acid. The samples were then placed in a water bath at 30 °C for 20 minutes. The absorbance was measured at 490 nm against a blank that was prepared using the same process as that used for distilled H_2O. The reducing groups were determinated by the PAHBAH method at 410 nm (Lever, 1972). The sample preparation was performed as previously described (Petkova et al., 2014). Chromatographic determination of sucrose, glucose and fructose was performed on a HPLC Elite Chrome Hitachi instrument coupled with a refractive index detector (RID) Chromaster 5450. The separation of sugars was performed on a Shodex® Sugar SP0810 (300 mm × 8.0 mm i.d.) with $Pb^{2+}$and a Shodex SP - G (5 µm, 6 × 50 mm) guard column at 85 °C in mobile phase distilled H_2O with a flow rate of 1.0 ml/minand aninjection volume of 20 µl.

2.2.2. Sensorial evaluation

The descriptive test for a quantitative sensory profiling was used to establish the sensory characteristics (shape, color, cell size and uniformity, odor, sweetness, aftertaste, crumb tenderness) of the sponge cakes 6 h after baking following the ISO 8586:2014 and ISO 13299:2011 methods. The sponge cakes samples were ready 1 h before the evaluation. Samples of different cakes were kept in coded plates covered with aluminium foil. Twelve trained panelists were selected in order to guarantee evaluation accuracy. The intensity of each sensory characteristic was recorded on a ten-point linear scale after 1 h orientation sessions for the panelists after whom terminology and anchor points on the scale were noted. The coded samples were shown simultaneously and evaluated in random order.

2.2.3.Microbiological analyses

The microbiological analyses were carried out according to the Bulgarian State Standard (BSS, 1986). Analyses for total plate count (TPC) (ISO 4833-1: 2013), molds and yeasts (ISO 21527-2: 2011), fecal coliforms (ISO 4831: 2006), *Salmonella* species (ISO 6579:2003) and coagulase-positive staphylococci (ISO 6888-1: 2000) were also conducted. Total plate count (TPC) in 1 g of sponge sample; total number of molds and yeasts in 1 g of sponge sample; coliforms in 1 g of sponge sample; *Salmonella* spp. in 25 g of sponge sample and coagulase-positive staphylococci in 1 g of product were determined.

2.2.4.Data analysis

Depending on the type of studied characteristic, 3 to 12 repetitions of each measurement were performed. The data were analyzed and presented as mean values ± standard deviation.

3. Results and discussions

3.1. Physical characteristics of sponge cakes

Changes in the physical characteristics of batters and sponge cakes containing carob syrup and carob flour in different amounts (25% and 50%) are summerized in Table 1.

The specific gravity of cake batter affects volume, porosity, water-absorbing capacity of the sponge cake and is important for the formation of crumb texture of the cake. The sponge batter, obtained by replacing wheat flour with carob flour, had a lower specific gravity than that of the control batter. Lower specific gravity is an indicator of more aeration, which is a desired property of cake batter. No significant differences were found in the specific gravity of the batter values between the control batter (0.71±0.01) and 25% carob syrup sponge cake batter (0.69±0.05), which could encourage the formation of larger bubbles during baking and therefore result in greater product height and volume. Another quality characteristics that is important for consumers is cake volume. The volume of cakes containing carob flour and syrup are shown in Table 1. Specific volume of cakes varied between 3.33±0.45 cm^3/g and 3.49±0.95 cm^3/g. For the control, which batter was characterized with higher value of the specific gravity, a smaller specific volume and porosity were measured. In this study, the specific volume of the control cake (3.29 ± 0.51 cm^3/g) was lower than the specific volume of sponge cake samples that contained carob flour. Berk et al. (2017) investigated the effects of partial replacement of rice flour by carob bean flour at different concentrations (10%, 20%, and 30%) on specific gravity and cake quality (specific volume, hardness). They reported that cakes prepared with 20% carob bean flour had the highest specific volume and lower hardness value and could thus be recommended for usage in gluten free cakes. According to Herranz et al. (2016) all cheackpea flour-based muffins had significantly lower specific volume than the control. The volume of the control cake (263.00±11.68 cm^3) was larger than that of sponge cakes with functional ingredients. The greatest porosity was observed in the cake with 50 % carob flour. The cake with 50 % carob syrup had the smallest volume (165.00±9.95 cm^3). In comparison with the control, a decrease in springiness was found when wheat flour was replaced by carob flour at 25% and 50% levels (25.80±4.92 PU and 21.80±2.39 PU) and by replacement of sugar with carob syrup at 25% and 50% levels (26.79±4.82 PU and 22.78±2.35 PU). Martinez-Cervera et al. (2011) reported that factors such as reological properties of the batter, the extent of air embodiment and continuance and speed of mixing and homogenisation affect the volume of bakery products. Finally, in comparison with the control, the sponge cake with 25% carob syrup had a significantly higher value of total moisture (33.65±0.98 %). It was observed that the total moisture content of the sponge cakes gradually increased with increasing levels of carob flour in the cake.

This might be due to the fact that carob flour enriches the sponge cakes with dietary fiber (see table 4). The fiber tends to hold water which could contribute to the higher total moisture content of the sponge cake.

In their study, Santos et al. (2015) noted that carob flour was a low glycemic index food and low glycemic load food. Carob flour was classified as a high fiber food, containing high levels of insoluble fiber. This high content of dietary fiber makes carob an ingredient with beneficial physiological effects. De La Hera et al. (2013) studied the batter characteristics and quality of cakes made with wheat-oat flour blends and reported that the inclusion of wheat-oat flour reduces cake volume and modifies their texture, reducing hardness, cohesiveness and springiness. Rosa et al. (2015) showed that increasing the substitution of cocoa powder by carob flour resulted in decreased cohesiveness (0.51 to 0.46), elasticity (0.95 to 0.79) and resilience in the cakes (0.26 to 0.24). Różyło et al. (2017) studied the physical and antioxidant properties of gluten-free bread enriched with carob fiber, and their results showed that increased carob fiber content induced significant favorable changes in the volume, color and texture (hardness and springiness) of the bread crumb.

Table 1. Effect of different levels of replacement of sugar and wheat flour with carob syrup and flour on the physical characteristics of the batters and sponge cakes

Physical characteristics[1]	**Sponge cake type**				
	control sample	**with 25 % carob syrup**	**with 50 % carob syrup**	**with 25 % carob flour**	**with 50 % carob flour**
Specific gravity (for batter)[2]	0.71±0.01	0.69±0.05	0.58±0.02	0.64±0.00	0.59±0.01
Specific volume, [cm^3/g]	3.29±0.51	3.33±0.45	3.39±0.34	3.38±0.28	3.49±0.95
Volume, [cm^3]	263.00±11.68	173.33±11.55	165.00±9.95	236.67±15.28	226.67±13.95
Porosity, [%]	66.68±1.54	68.37±3.74	69.25±2.51	67.90±2.14	74.08±3.71
Water-absorbing capacity, [%], [PU][3]	329.00±18.05	303.19±9.02	336.02±14.05	344.00±11.01	329.00±14.91
Spinginess, [PU]	45.00±1.40	26.79±4.82	22.78±2.35	25.80±4.92	21.80±2.39
Shrinkage, PU	63.80±2.87	68.37±8.29	62.02±4.74	67.60±8.29	62.00±4.74
Total moisture, [%]	23.42±0.44	33.65±0.98	27.62±1.14	27.81±1.13	30.86±0.97

[1] The values are mean ± SD ($p \leq 0.05$).

[2] The temperature of the batter is on the average 20.0 ± 0.5 ºC.

[3] PU - Penetrometer Units.

3.2. Carbohydrate content of sponge cakes, supplemented with different concentration of carob syrup and carob flour

Detailed information about the carbohydrate profiles of sponge cakes - carob syrup and flour was presented. The carbohydrate content of cakes containing different levels of carob syrup, flour and the control cake are summarized in Table 2. The total carbohydrate content of different cakes varied from 65.4±5.2g/100 g dry weight to 86.1±2.7 g/100 g dry weight, being lowest for the control cake (65.4±5.2 g/100 g dry weight) and highest for the sponge cake sample with 50% carob flour (86.1±2.7 g/100 g dry weight). It was observed that total carbohydrate content increased in sponge cakes with increased levels of carob flour and carob syrup in the batter.

In all analyzed samples with carob ingredients the presence of monosaccharide glucose and disaccharide sucrose was detected. The content of reducing sugars varied between 1.3 and 5.3 g/100 g dry weight. The sponge cake, obtained by replacing 50% wheat flour with carob powder, was estimated to be a rich source of carbohydrates (86.1 g/100 g dry weight).

Rosa et al. (2015) studied the effect substituting of cocoa powder for carob flour in cakes made with soy and banana flour and found that the obtained cakes had a higher level of dietary fiber (16.42 the 25.89 %), a lower carbohydrate content (20.65 the 8.42 %) and lower calorie content (208.89 the 148.07 kcal).

For more detailed characterization of the carbohydrate profile, sponge cake samples were analyzed by the HPLC-RID method (Figure 1).

The protein and fiber content of four different cake samples processed with different levels of carob syrup and flour are shown in Table 3.

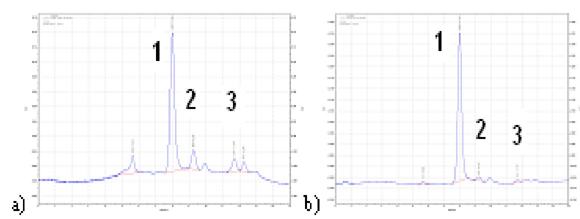

Figure 1. HPL-RID chromatograms of carbohydrates composition in sponge cakes, supplemented with different concentration of carob (*Ceratonia siliqua* L.) syrup and carob flour, showing peaks for sucrose (1), glucose (2), and fructose (3).

a) sponge cake with 50% carob syrup

b) sponge cake with 50% carob flour

Table 2. Means ± standard deviations of carbohydrate content in sponge cakes, supplemented with different concentration of carob syrup and flour (g/100 g dry weight)

Type of sponge cake	Total carbohydrates	Reducing sugars	Glucose	Fructose	Sucrose
control	65.4±5.2	0.7±0.2	0.4±0.1	ND[1]	26.8±0.6
with 25 % carob syrup	67.1±3.5	2.8±0.2	1.4±0.1	0.4±0.1	27.7±0.2
with 50 % carob syrup	74.3±4.1	5.3±0.4	1.3±0.2	2.7±0.2	28.5±0.2
with 25 % carob flour	83.5±2.4	1.3±0.2	0.7±0.1	0.5±0.1	35.4±0.2
with 50 % carob flour	86.1±2.7	4.9±0.7	2.4±0.2	2.1±0.1	43.90±0.2

[1] ND = Not detected

Table 3. Mean values of the dietary fibers and of the protein of the sponge cake samples without and with functional ingredient (per g/100g dry weight)

Type of sponge cake	Dietary fibers [g/100 g dry weight]			Total protein*, [g/100 g dry weight]
	insoluble	soluble	total	
control	2.00±0.10	0.63±0.09	2.63±0.11	8.44
with 25 % carob syrup	1.89±0.15	0.56±0.09	2.45±0.15	10.63
with 50 % carob syrup	1.95±0.14	0.63±0.10	2.58±0.17	12.57
with 25 % carob flour	10.68±0.15	1.74±0.10	12.42±0.21	15.81
with 50 % carob flour	16.22±0.19	2.06±0.10	18.28±0.22	23.93

*Protein = % Nitrogen × 6.25

In the present study it was observed that the protein content of all samples (10.63-23.93 g/100g dry weight) was higher than that of the control sample (8.44 g/100g dry weight). It was also noticed that the protein content in sponge cake samples increased with increased substitution levels of carob syrup and flour. The results for total dietary fiber content revealed that increasing the replacement levels of wheat flour with carob flour from 25% to 50%, respectively, led to a concomitant increase in values for the fiber content in sponge cake samples from 2.63 g/100g dry weight (control) to 12.42 g/100g dry weight (sponge cake with 25% carob flour) and 18.28 g/100g dry weight (sponge cake with 50% carob flour). This could be due to the fact that carob flour contains a higher percentage of fiber (28.17 g/100g dry weight) than wheat flour (0.45 g/100g dry weight) (Fidan, 2017). A similar observation of dietary fiber content increase in sponge cakes enriched with powder from the outer leaves of white cabbage was made by Prokopov et al. (2015).

Table 4. Sensory characteristics of the sponge cake samples without and with functional ingredient

Sensory characteristics[1]	Sponge cake type				
	control sample	with 25 % carob syrup	with 50 % carob syrup	with 25 % carob flour	with 50 % carob flour
Shape	7.93±1.03	7.13±1.41	7.47±1.11	7.47±1.19	7.73±1.33
Colour	8.07±1.10	7.53±1.46	7.60±1.19	7.53±1.51	7.73±1.79
Cells size and uniformity[2]	6.93±1.03	7.40±1.18	7.07±1.24	7.47±1.25	6.87±1.30
Odour	7.20±1.37	7.53±1.13	7.33±1.32	6.67±1.68	6.47±1.64
Sweetness	6.67±2.06	7.00±2.04	6.67±1.12	6.87±1.55	7.33±1.76
Aftertaste	4.07±1.14	2.87±0.70	3.67±1.02	3.93±1.08	4.13±1.07
Crumb tenderness	6.67±1.63	7.53±1.19	6.93±1.16	7.40±1.12	7.80±1.37

1 The values are mean ± SD ($p \leq 0.05$).

2 A scale from 0 to 9 was used to evaluate sensory characteristics. Nine is ideal for the third sensory characteristic when the cells are small and equal in size

3.3. Sensorial evaluation of sponge cakes, supplemented with different concentrations of carob flour and carob syrup

Sensory analysis evaluations were performed in order to determine the optimum sensory characteristics of sponge cakes with regard to the panelists' preferences. The results of the sensory evaluation are given in Table 4.

Ratings test revealed that sensory characteristics including shape, color, cell size and uniformity, odor, sweetness, aftertaste and crumb tenderness were perceived without significant differences between the control cake and those supplemented with different concentrations of carob flour and carob syrup. Our investigation showed that the control sample and the cake with 50% carob flour both had approximately similar shape and color. In terms of their shape, the cakes with the addition of carob flour were perceived very well. The data showed that there was not a great difference in the values of size and uniformity of cells and crumb tenderness for the investigated cakes with 25% carob flour and 25% carob syrup. The cells of the new sponge cakes with carob ingredients were small and equal, uniformly distributed in the crumb, and were thin-walled. The control sample had a lightly yellow color, while the other cakes had a medium to dark surface and a brown crumb due to the presence of carob flour and syrup. Despite its color, the sponge cake made with the addition of 50% carob flour ws perceived very well by the panelists. Rosa et al. (2015) reported that in terms of color, the control and those made with 25% and 50% carob flour had the highest average scores with no significant difference between them. The researchers concluded that with regard to color, the cake with 100% carob flour had the lowest acceptability, a factor that may have been associated with the dark color caused by the carob flour. With regard to aftertaste, there were differences in the control and sponge cake with 25% carob syrup which could be explained by the fact that carob is rich in condensed tannins, which, when used in excess, tend to give an astringent aftertaste to products (Silanikove et a., 2006). The sample with 25% carob syrup had the least aftertaste (2.87±0.70). These results were in agreement with Hafez (2012) who reported that there was no remarkable difference among samples with and without marjoram powder substitution for the scores of crumb color, texture and overall acceptability, while in taste there were differences in the control and samples with other concentrations. As a result of sweetness, samples supplemented with 50% carob syrup (6.67±1.12) had the same score as the control (6.67±2.06). The mean score of sponge cakes with functional ingredients evaluated in terms of crumb tenderness was higher than that of the control sample.

3.4. Microbiological analysis

In this study, the storage (within six days from production at a temperature of up to 18 °C and relative humidity $\varphi < 75\%$) of sponge cake samples supplemented with different concentrations of carob flour and carob syrup was considered for microbial analyses.

The microbiological characteristics of the sponge cakes during storage are presented in Table 5.

No pathogenic bacteria such as coagulase-positive staphylococci in 1 g of *Salmonella* spp. in 25 g and fecal coliforms in 1 g, respectively, were detected. Up to the 6th day of storage at room temperature, no evidence of mold was detected on the samples. At the end of the storage period, the microbial loads in the control and the variants were similar, and this was likely due to secondary air contamination on the surface of the samples.

Table 5. Microbiological characteristics of the sponge cake samples without and with functional ingredient during sixth-day storage

Storage time, [day]	**Type of sponge cake**	**Total plate count, [CfU/g][1]**	**Coliforms, [CfU/g][1]**	**Coagulase-positive staphylococci, [CfU/g][1]**	***Salmonella* spp. in 25 g**	**Molds and yeasts, [CfU/g][1]**
0	control sample	0	ND[2]	ND	ND	0
	with 25 % carob syrup	0	ND	ND	ND	0
	with 50 % carob syrup	0	ND	ND	ND	0
	with 25 % carob flour	0	ND	ND	ND	0
	with 50 % carob flour	0	ND	ND	ND	0
3	control sample	2.2×10^4	ND	ND	ND	0
	with 25 % carob syrup	0	ND	ND	ND	0
	with 50 % carob syrup	0	ND	ND	ND	0
	with 25 % carob flour	0	ND	ND	ND	0
	with 50 % carob flour	0	ND	ND	ND	0
6	control sample	2.1×10^4	ND	ND	ND	1.3×10^4
	with 25 % carob syrup	5.6×10^2	ND	ND	ND	0
	with 50 % carob syrup	0	ND	ND	ND	4×10^1
	with 25 % carob flour	0	ND	ND	ND	0
	with 50 % carob flour	1.9×10^3	ND	ND	ND	0

[1] CfU/g - Colony forming Units per gram

2 ND = Not detected

4.Conclusions

The results of this study showed that it is possible to design and prepare sponge cakes that contain high protein and dietary fiber. The successful replacement of wheat flour with carob flour and replacement of sugar with carob syrup, was also demonstrated. The physical analyses revealed that partial replacement of wheat flour by carob flour increased the total moisture content of the sponge cakes. The chemical composition of the cakes with carob flour showed higher dietary fiber and protein content compared to the control sponge cake. The cakes with carob flour and syrup as functional ingredients showed differences in cell size and uniformity as well as sweetness and crumb tenderness, confirming that the replacement ingredients positively influenced sensory characteristics.

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