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Physical and antioxidant properties of gluten-free bread enriched with carob fibre

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A b s t r a c t. There are no reports of addition of carob fibre to gluten-free bread, as only carob germ flour was used. The research task was to determine what level of carob fibre can be used and how it influences the physical and sensorial properties of gluten-free bread. Especially, the knowledge of the antioxidant properties of such bread is very valuable. The gluten-free bread from rice, corn, and buckwheat flour (35:35:30%) was prepared after mixing (5 min), proofing (40 min, 30ºC), and baking (45-50 min, 230ºC) of dough. Carob fibre was added in the amounts of 1, 2, 3, 4, and 5% of the total flour content. The results showed that increased content of carob fibre induced significant and favourable changes in the volume, colour, and texture (hardness and springiness) of the bread crumb. Carob fibre enriched the breads with lipophilic compounds able to chelate metal ions. The activity of hydrophilic compounds was significantly higher in the case of control bread and bread with the lowest percentage of the additive. In conclusion, the highest increase in antioxidant activity was found for breads with 1 and 2% of carob fibre. The most acceptable gluten-free bread can be obtained by adding up to 2% of carob.

K e y w o r d s: bread, carob, fibre, gluten-free, antioxidant activity

INTRODUCTION

Carob fibre is obtained from pods of the carob tree (*Ceratonia siliqua* L.), also called St. Josh bread, which has been grown throughout the Mediterranean region for thousands of years. The fruit of the carob tree is a brown 10-30 cm long and 1.5-3.5 cm wide pod containing two main components: 80-90% of pulp and 10-20% of seed (Ayaz *et al.,* 2007; Naghmouchi *et al.,* 2009).

Dietary fibre is produced by water extraction of carob pulp and mild physical processes, which retain their nutritional value and the most important components. Water soluble ingredients are isolated from the insoluble components and, as a result, insoluble fibre is obtained, which is 80% of the total (Haber, 2002), as well as insoluble tannins, small amounts of low molecular weight carbohydrates, minerals, and some water-soluble polyphenols (Klenow *et al.,* 2008). Papagiannopoulos *et al.* (2004) analysed the polyphenol composition of carob fibres using HPLC with UV. Carob fibre was found to contain 414.2 mg polyphenols/100 g and 19.18 mg tannins/100 g. They also showed a strong antioxidant activity of carob fibre, produced by cold-water extraction, measured by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method and Trolox equivalent antioxidant capacity (TEAC) assay.

Since carob fibre combines two positive nutritional ingredients, namely polyphenols and dietary fibre, it is a very special addition to healthy food. Studies on hypercholesterolemic patients have shown that daily consumption of food products enriched with carob fibre has beneficial effects on the human blood lipid profile, reduces LDL cholesterol, and lowers triglycerides in females (Zunft *et al.,* 2003).

Until recently, carob fibre has been used in wheat and rye bread in only few studies. The possible use of an additive carob fibre to rye bread, which had a positive impact on human health, was first examined by Haber *et al.* (2002). They have shown that a 6% addition of carob fibre to rye bread has a positive effect by lowering cholesterol levels, especially LDL. Other researchers have reported that

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addition of carob fibre does not change the procedure of wheat dough, increasing the dough water absorption and enhancing the rheological stability of the dough during mixing (Miś *et al.,* 2012). Moreover, textural properties and the final quality of wheat bread with carob fibre were studied (Siastała *et al.,* 2014; Wang *et al.,* 2002). In comparison with inulin and pea fibre, carob fibre exhibited the most promising potential in the development of fibre-rich bread in order to increase the daily fibre intake (Wang *et al.,* 2002).

Recent scientific studies have increasingly focused on GFB (Różyło *et al.,* 2015 a, b, c; Sakac *et al.,* 2011; Tsatsaragkou *et al.,* 2012, 2014 a, b), and a number of studies have addressed the impact of various additives on the quality of GFB.

According to numerous studies, compared with other consumers, patients with celiac disease typically have a lower fibre intake below the recommended levels proposed by international organizations. This is related to the fact that the flour and starch used for the production of gluten-free breads have low fibre content in their composition, and dietary fibre is considered a hydrocolloid, included as a gluten substitute. To increase the dietary fibre content of gluten-free bread, different insoluble fibres (oat and bamboo, fine and coarse, potato and pea) and soluble fibres (Nutriose® and polydextrose) were added and their effect on gluten-free dough rheology and bread-making was studied (Martinez *et al.,* 2014). Recent studies have been focused on the production of gluten-free bread containing carob flour (from seed germ) at rates from 5 to 15% (Tsatsaragkou *et al.,* 2014a, b). In the literature, there are no reports relating to the production of gluten-free bread containing carob fibre. There is growing interest in the production of gluten-free bread; especially the results of antioxidant properties are very valuable.

The objective of this study was to determine changes in the physical properties and antioxidant activity of GFB caused by the addition of carob fibre.

MATERIALS AND METHODS

The raw material for making GFB included white rice flour, corn flour, buckwheat flour, and carob fibre. The white rice, corn, and buckwheat commercial flours were sourced from Glutenex (Poland). The carob fibre was sourced from Carob General Application (Valencia, Spain). The corn flour was characterised by: protein 5.9±0.26%, carbohydrate 78.1±4.1%, ash 0.45±0.27%, and fat 3.0±0.16%. The rice flour was characterised by: protein 7.2±0.33%, carbohydrate 79.2±3.8%, ash 0.22±0.01%, and fat 0.7±0.04%. The buckwheat flour was characterised by content of: protein 12.6±0.61%, carbohydrate 69.3±0.54%, ash 2.3±0.1%, and fat 3.1±0.16%. The carob fibre was characterised by: protein 6.1±0.2%, carbohydrate 82.3±1.1%, ash 0.72±0.03%, and fat 0.70±0.02%.

The protein, ash, and fat contents were evaluated according to ISO standards (ISO 20483:2006 (Kjeldahl method), ISO 2171:2007, ISO 11085:2008). Total carbohydrates were calculated as a difference between the protein, fat, ash and moisture contents in the flour.

Dried instant yeasts (Instaferm) were obtained from Lallemand Iberia, SA. Salt was purchased from a local market.

Gluten-free bread was baked in a laboratory oven equipped with a fermentation chamber **(**Sadkiewicz Instruments, Bydgoszcz, Poland). The control bread formulations used in this study were based on corn, rice, and buckwheat flour (35:35:30%). The corn and rice flour were used in formulations as the most popular and available gluten-free flour. The addition of buckwheat flour at the 30% level was chosen from a pool of previous studies (Sakac *et al*., 2011; Wronkowska and Soral-Śmietana, 2008; Różyło *et al*., 2015c). The carob fibre was added to the control bread in an amount of 1, 2, 3, 4, and 5%. In addition to flour, salt (2%), yeast (in an amount equivalent to 3% of compressed yeasts), guar gum (2%), apple pectin (2%), and water were used in the formulation (according to baking practice, the amount of flour is given as 100% and the ratio of the other components is converted to the weight of flour). The optimal water addition level was determined using empirical trial and-error testing (96.0, 96.5, 97.0, 97.5, 98.0, 98.5%) (Hager *et al*., 2012). The temperature of the added water was 30°C. The gluten-free dough was prepared after mixing all the ingredients in a 5-speed mixer (Kitchen Aid, St. Joseph, MI, USA) for 5 min. After mixing, the dough was immediately transferred into a pan (loaves of equal mass of 300 g) and then subjected to proofing (30°C and 75-88% RH) for 40 min.

The loaves were baked at 230°C for 45-50 min in a laboratory oven (live steam was injected immediately after the loaves were placed in the oven) (Sadkiewicz Instruments, Bydgoszcz, Poland). The GFB loaves were wrapped in polyethylene bags after baking and cooling. Baking tests were performed three times in three repetitions, *i.e.* on nine loaves of bread from which the average values were determined.

The gluten-free bread loaf volume was measured (one day after baking millet seed displacement method) and the bread loaf volume of 100 g of bread was calculated. The pH of the bread crumb was measured using a pH meter (TESTO 206-pH2, Pruszków, Poland) with a penetration probe for semi-solid substances (including baked products).

The colour measurements of bread samples were made in an area derived from the central part of the crumb using a Chromameter HP 2132 (Anticorr, Gdańsk, Poland). These analysis were based on the $L^*a^*b^*$ system for breads that were defined by the CIE (International Commission on Illumination), L* is the luminance or lightness component,

which ranges from 0 to 100, and parameters a* (from green to red) and b* (from blue to yellow) are the two chromatic components.

For textural measurements, the samples (30×30×20 mm) of the bread crumb (one day after baking) were compressed twice (ZWICK Z020/TN2S strength tester) using a capital equipped with a 30 mm plug until a 50% depth at a crosshead speed of 1 mm s^{-1} was achieved (Różyło, 2014; Różyło *et al.,* 2014a, b; 2015 a, b). Textural parameters were obtained from two-bite TPA curves (1 and 2 -curves): hardness (peak force 1), springiness (length of the base of area 2/length of the base of area 1), and cohesiveness (area 2/area l). The texture tests were performed in nine replicates (Gámbaro *et al.,* 2006).

For sensory evaluation (24 h after baking), the samples (1 cm thick) were sliced mechanically. Bread slices divided into four parts were presented on plastic dishes coded and served in randomized order (Matos and Rosell, 2012). The panel for sensory evaluation consisted of 73 untrained consumers (22 – 50 years old: 39 females and 34 males) who were habitual consumers of bread and who evaluated the overall acceptability of the bread. According to a nine-point hedonic scale (1: dislike extremely, 5: neither like nor dislike, 9: like extremely), the hedonic test was used to determine the taste, aroma, and texture of different types of bread based on the degree of liking or disliking (Lim *et al.,* 2011).

Buffer extract preparation: ordered samples of bread (1 g dry mass, dm) were extracted for 30 min with 20 ml of PBS buffer (phosphate buffered saline: pH 7.4) The extracts were separated by decantation and the residues were extracted again with 20 ml of PBS buffer. The extracts were combined and stored in darkness at -20°C. For estimation, the potential bioaccessibility *in vitro* digestion was performed according to the procedure described by Gawlik-Dziki *et al.* (2013).

Total phenolics (TPC) content was estimated according to Singleton and Rossi (1965) and calculated as gallic acid (GAE) equivalent (mg g^{-1} DM).

For antiradical activity (AA) analyses, an improved ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) decolourisation assay was performed (Re *et al*., 1999). Chelating (CHEL) and ferric reducing antioxidant power (FRAP) was determined according to the methods described by Guo *et al.* (2001) and Oyaizu (1986), respectively. FRAP was calculated as an EC_{50} value (mg ml^{-1}; the effective concentration at which the absorbance was 0.5 for reducing power obtained by interpolation from linear regression analysis). The OH• scavenging assay was performed according to the procedure described by Su *et al.* (2009). Antioxidant activities were determined as EC_{50} – an extract concentration providing 50% of activity based on a dose-dependent mode of action.

The following factors were determined for better understanding the relationships between biologically active compounds in the light of their potential bioaccessibility:

– the active compound bioaccessibility index (*ACB*):

$$ACB=C_{DE}/C_{BE}, \quad (1)$$

where: C_{DE} is the concentration of active compounds in the extract after digestion *in vitro*, C_{BE} is the concentration of active compounds in the buffer extract;

– the antioxidant bioaccessibility index (*BAC*), which is an indication of the bioaccessibility of antioxidant compounds:

$$BAC=A_{BE}/A_{DE}, \quad (2)$$

where: A_{BE} is the EC_{50} of buffer extracts, A_{DE} is the EC_{50} of extracts after digestion *in vitro*.

Statistical analysis was done at a significance level $\alpha = 0.05$ using Statistica by Statsoft. Measurement scores were subjected to analysis of variance (ANOVA). When significant differences in ANOVA were detected, the means were compared using the Tukey test.

RESULTS AND DISCUSSION

The pH of bread changed significantly as a result of carob fibre addition (Fig. 1a), this parameter decreased with the increasing content of carob fiber (CF). It is important to determine the pH of bread, because it determines the taste

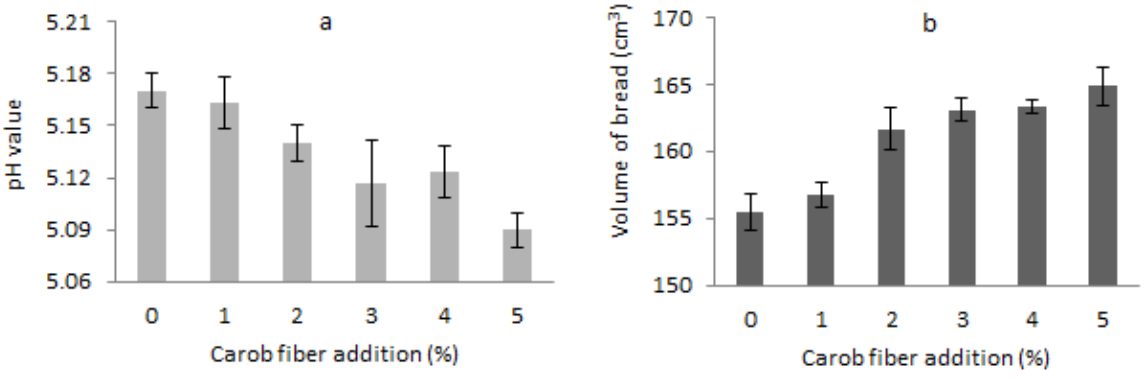

Fig. 1. Basic properties of gluten-free bread with different amount of carob fiber addition: a – pH value of dough and bread, b – volume of bread (n=54).

of the bread as well as suitability for long storage when it is lower. There were no significant differences in the pH of bread with 0 and 1% share of CF in the bread formulation. In other studies, the pH value of GFB with carob was not determined.

The results of the volume of the GFB loaves produced with different additions of CF in the range from 0 to 5% are shown in Fig. 1b. In comparison with the control bread (0% carob), a larger volume was obtained using 2% of CF. The mean value of the loaf volume increased with the increasing content (from 2 to 5%) of CF, but there were no significant differences in the volume of GFB with 2, 3, 4, and 5% share of carob in the bread formulation. Salinas *et al.* (2015) observed that breads with increasing levels of carob flour from seed germ and from pulp exhibited a lower loaf volume, whereas breads with flour from germ were smaller than breads with flour from pods. The hydration properties and rheological behaviour of wheat dough were affected according to the fraction type of the carob flour used. The authors explained that germ flour has higher protein content than pulp flour; these globular proteins may have greater affinity for water than components of pulp flour, the latter one containing high amounts of insoluble compounds belonging to the outermost layers of the pod (*i.e.*, fibre).

With the increased content of CF, significant changes were noted in the colour of the bread crumb (Fig. 2). Lightness component L^* (Fig. 2a) decreased with the addition of carob in the range from 0 to 5%. Parameters a* (Fig. 2b) increased with the addition of carob in the range from 0% to 5%. The highest redness (a*) (Fig. 2c) of the crumb colour was achieved with 5% content of carob. Colour b^* value of the crumb (yellowness) decreased with the increasing content of carob.

It is clear that carob fibre contains pigments that may have an impact on crumb colour changes. Colour changes were expected and obvious and did not relate to any essential phenomenon. In research presented by Salinas *et al*. (2015), breads with carob germ flour were yellowish-orange, while breads with carob pod flour were brown. With the increased content of CF, significant changes were noted in the textual properties of the bread crumb. The hardness (Fig. 3a) decreased significantly with the increasing CF addition (in the range from 0 to 2%), compared to the control bread. There were no significant differences in the hardness of gluten-free bread with 2, 3, 4, and 5% of CF. Springiness (Fig. 3b) increased with the carob addition. In comparison with the control bread (0% carob), significantly greater springiness was obtained even using 2, 3, 4, and 5% of CF. At these levels of CF addition, there were no significant differences in the springiness of GFB. Chewiness of the crumb (Fig. 3c) significantly increased after adding 4% of CF. There were no significant differences in the chewiness of gluten-free bread with the 0, 1, 2, and 3% share of CF in the bread formulation. Wang *et al*.

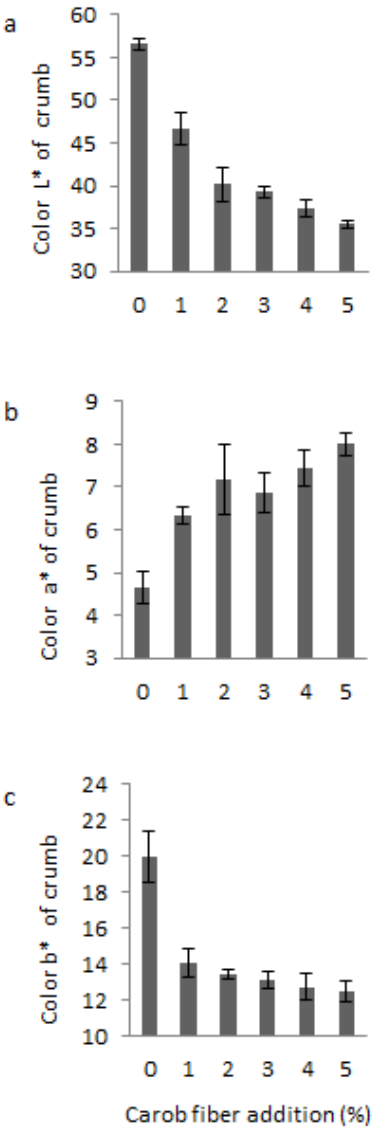

Fig. 2. Colour L*a*b* values of gluten-free bread crumb with carob fiber addition: a – colour L*value, b – colour a* value, c – colour b* value (n=36).

(2002) indicated that the addition of CF gives a softer wheat bread crumb, although fibre addition usually leads to crumb hardening. In some of the available studies, it was shown that wheat breads with carob flour from pods had a more compact crumb than control bread, and breads with carob flour from seed germ were characterised by a less structured and less compact crumb (Salinas *et al.,* 2015). According to these authors, harder, less elastic, and more chewable wheat crumbs were obtained with high levels (>10%) of carob flours. In contrast to the wheat bread used in the above-mentioned studies, in our research the control gluten-free bread with no additives was characterised by high hardness. Carob fibre had a positive impact on reducing the hardness GFB, which could be caused by the presence of natural hydrocolloids in carob. Smith *et al.*

a

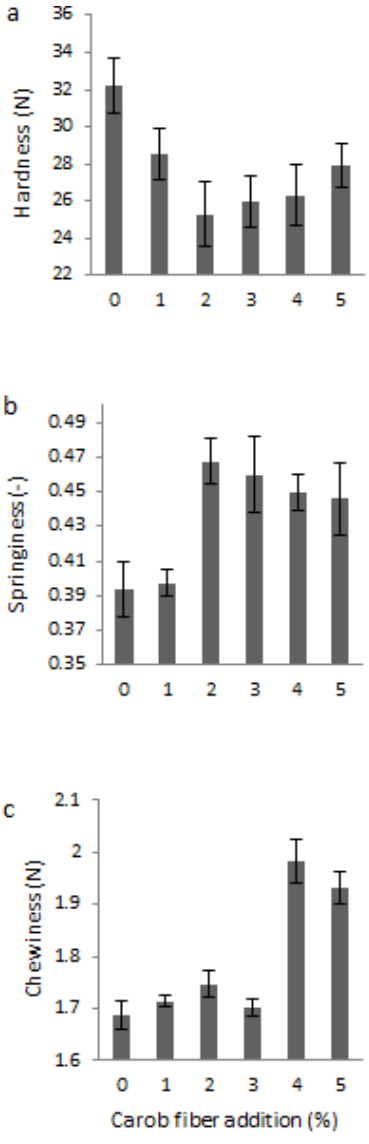

Fig. 3. Texture properties of gluten-free bread crumb baked with different amount of carob fiber addition: a – hardness of bread crumb, b – springiness of bread crumb, c – chewiness of bread crumb (n=54).

(2012) obtained high quality gluten-free bread similar to wheat bread using carob germ flour and HPMC hydrocolloids. The optimum formulation for obtaining gluten-free bread with low crumb firmness and improved porosity values was obtained in another study by combining carob flour and resistant starch (Tsatsaragkou *et al.,* 2014a). Gluten-free breads with carob flour from seed exhibited higher quality in terms of dough proofing, porosity, crumb firmness, and viscoelasticity (Tsatsaragkou *et al.,* 2012). The available investigations have attempted to use carob flour from seed for gluten-free bread, but there is no study with carob flour from pods (carob fibre).

The sensory evaluation showed that the control GFB achieved the highest scores for taste (Table 1). Taste evaluation decreased with the addition of carob. The most acceptable GFB can be obtained by adding from 1 to 2% of CF; greater levels of carob (3-5%) caused a slightly bitter aftertaste of the bread. It was also demonstrated that the sensory evaluation of the crumb texture of GFB made with the increasing additions of CF slightly decreased. Finally, it was concluded that the most acceptable GFB was obtained by adding from 1 to 2% of CF.

In a study presented by Wang *et al.* (2002), carob fibre-rich wheat breads were acceptable by the sensory panel. Carob fibre was a product with promising potential in the development of fibre-rich bread in order to increase the daily fibre intake.

As presented in the Table 2, all bread samples contained phenolic compounds. In the case of chemically extractable phenolics, carob fibre addition slightly increased their content; however, their level was not correlated with the percentage of CF addition. The same tendency was found in the case of buffer-extractable phenolics. It is worth noting that their level was higher than that observed after chemical extraction. Interesting results were obtained after simulated digestion. Potentially bioaccessible compounds were released from all samples; however, higher results were obtained for samples C and C1. In the other breads, the CF addition did not increase the level of phenolics, which

T a b l e 1. Sensory attributes of gluten free bread made with the addition of carob fiber

Amount of carob fiber addition	Sensory evaluation attributes*			
	Taste	Flavour	Texture	Acceptability
0	6.6±0.5 a	6.5±0.42 a	6.0±0.35 c	6.1±0.45 a
1	6.4±0.43 a	6.3±0.41 ab	6.3±0.61 bc	6.6±0.52 a
2	6.0±0.64 ab	6.0±0.57 b	6.5±0.58 ab	6.2±0.49 a
3	5.2±0.63 b	5.7±0.73 c	6.6±0.64 a	5.7±0.52 a
4	4.4±0.31 c	5.5±0.32 c	5.8±0.72 c	4.6±0.35 b
5	3.5±0.33 d	5.2±0.29 d	5.7±0.43 c	4.3±0.36 b

*Nine-point hedonic scale with 1, 5 and 9 representing extremely dislike, neither like nor dislike, and extremely like, respectively. Means with different letter in the same column are significantly different ($\alpha < 0.05$) (n=438).

T a b l e 2. Total phenolics content and antioxidant properties of bread

Parameter	Sample	Kind of extract		
		CE	BE	DE
TPC (mg GAE g^{-1} DM)	C	6.0±0.21 Aa	7.6±0.16 Ab	34.1±0.43 Dc
	C1	8.1±0.32 Ba	10.3±0.35 Bb	38.4±0.36 Ec
	C2	8.3±0.28 Ba	11.9±0.21 Cb	33.7±0.59 Dc
	C3	7.5±0.18 Ca	21.2±0.56 Db	31.0±0.28 Cc
	C4	8.7±0.26 BDa	20.2±0.68 Cb	26.1±0.23 Bc
	C5	9.1±0.36 Da	24.3±0.19 Ec	24.1±0.09 Ab
ABTS (EC_{50} mg ml^{-1} DM)	C	46.8±2.22 Bb	53.0±2.33 Bc	23.3±1.65 Aa
	C1	66.8±0.2.6 Eb	83.8±3.28 Cc	22.3±0.98 Aa
	C2	49.5±2.66 Cb	56.3±2.56 Bc	27.7±1.26 Ba
	C3	53.2±1.67 Db	54.5±2.68 Bb	27.0±0.92 Ba
	C4	48.4±1.97 BCa	49.7±1.18 Aa	52.9±2.19 Db
	C5	42.5±2.12 Aa	48.8±1.96 Ac	44.5±2.13 Cb
CHEL (EC_{50} mg ml^{-1} DM)	C	51.7±2.12 Ec	29.5±1.33 Bb	15.4±0.37 Ca
	C1	33.0±1.23 Dc	21.1±0.99 Ab	11.8±0.50 Aa
	C2	22.9±0.96 Ab	22.4±1.66 Ab	12.4±0.36 ABc
	C3	26.1±1.12 Bb	42.4±1.93 Cc	13.3±0.87 Ba
	C4	28.6±0.87 Cb	45.2±2.06 Dc	13.2±0.64 Ba
	C5	27.8±0.49 Cb	43.6±2.13 CDc	13.1±1.13 Ba
RED (EC_{50} mg ml^{-1} DM)	C	10.9±0.32 Aa	27.1±1.26 Db	30.5±1.23 Dc
	C1	13.8±0.45 Ba	27.5±1.18 Db	26.9±1.01 Cb
	C2	13.7±0.21 Ba	25.5±1.34 Cb	24.4±0.95 Bb
	C3	16.1±0.38 Ca	23.1±1.56 Bb	24.0±1.12 Bb
	C4	16.0±0.58 Ca	21.6±0.98 Bb	23.4±0.58 Bb
	C5	17.5±0.61 Da	18.9±0.66 Ab	17.4±0.47 Aa
LPO (EC_{50} mg ml^{-1} DM)	C	88.4±3.22 Db	98.5±4.02 Dc	55.5±2.52 Da
	C1	62.3±2.98 ABb	95.6±4.23 Dc	29.3±1.58 Ba
	C2	61.0±1.97 Ac	43.2±1.78 Cb	22.0±1.05 Aa
	C3	64.9±2.54 Bc	38.4±1.94 Bb	32.8±1.67 Ca
	C4	80.6±3.24 Cc	37.9±2.11 Bb	32.5±1.73 Ca
	C5	86.7±2.94 CDb	29.0±1.54 Aa	31.9±1.43 BCa

*Values designated by the different small letters in the rows of table are statistically significantly different ($p<0.05$) (n=18); the values designated by the different capital letters in the columns of table are statistically significantly different ($p<0.05$); CE – chemical extrcts, BE – Buffet extracts, DE – extract after digestion in vitro, TPC – total phenolics content, ABTS – antiradical activity, CHEL – chelating power, RED – reducing power, LPO – inhibition of lipid peroxidation.

could be explained by interactions between food matrix components. In summary, all samples contained relatively high content of phenolics. Most importantly, these compounds demonstrated a hydrophilic character and seem to be potentially bioaccessible. Antiradical activity in the case of chemically extractable (lipophilic) compounds decreased with the percentage of CF addition. Only in the case of sample C5, a slight increase in the activity (relative to the controls) was found. A similar relationship was determined in the case of hydrophilic compounds. A slight increase in the antiradical activity was found for samples with the highest CF addition. Compounds released after simulated digestion demonstrated the highest antiradical activity; however, CF addition strongly decreased their level.

As presented in the study, the CF addition significantly enriched the breads with lipophilic compounds able to chelate metal ions; however, no simple relationship between the percentage of addition and chelating activity was found. The activity of hydrophilic compounds (extracted with PBS buffer) was significantly higher in the case of the control bread and products with the lowest percentage of the additive (1 and 2%). The activity decreased with the increase in the supplementation.

Most importantly, compounds able to chelate metal ions were highly bioaccessible *in vitro*. Additionally, the CF addition increased this activity; however, no relationship between the CF percentage and the activity was found.

The reducing power of lipophilic compounds decreased with the percentage of CF addition. The opposite was observed in the case of compounds extracted with PBS buffer and released after digestion *in vitro*; however, their activity was significantly lower than that found for methanolic extracts. The analysed breads were not good sources of lipophilic and hydrophilic compounds able to protect lipids against oxidation. The CF addition increased the activity in the case of buffer extracts and the activity was positively correlated with the CF addition. The highest increase in the activity was found for breads with low CF content (1 and 2%).

CONCLUSIONS

1. In comparison with the control bread, a larger volume was obtained using the 2, 3, 4, and 5% of carob fibre. There were no significant differences in the volume of gluten-free bread with 2, 3, 4, and 5% share of carob.

2. With an increased content of carob fibre, significant changes were noted in the colour of the bread crumb – the lightness and yellowness of the bread crumb decreased and the redness increased.

3. Carob fibre significantly decreased the hardness and increased the springiness of bread, compared to the control bread. There were no differences with the 2, 3, 4, and 5% share of carob fibre.

4. Carob fibre enriched the breads with lipophilic compounds able to chelate metal ions. The activity of hydrophilic compounds was significantly higher in the case of the control bread and products with the lowest percentage of the additive. The highest increase in the antioxidant activity was observed for bread with 1 and 2% of carob fibre.

5. With respect to the sensory evaluation, acceptable gluten-free bread can be obtained by adding up to 2% of carob fibre. Greater levels of carob from 3 to 5% caused a slightly bitter aftertaste of the bread.

Conflict of interest: The Authors do not declare conflict of interest.

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