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FUNCTIONAL PROPERTIES OF MUFFIN AS AFFECTED BY SUBSTITUING WHEAT FLOUR WITH CAROB POWDER

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ABSTRACT

Carob (*Cerationa soliqu*a L.) pod is the good source of dietary fiber, minerals and polyphenolic substances. The aim of this study was to prepare muffin where wheat flour was substituted with carob powder, and determine some physicochemical properties. Carob powder was prepared by milling dry carob pods to particles smaller than 600 µm. Then wheat flour in muffin dough was replaced by carob powder in 5, 10, 15 and 20% (w/w) and subsequently baked at 180 °C for 20 min. It was found that the height of the muffin fortified with carob powder decreased in comparison with that in control muffin sample. Although the height of muffins decreased with the increase in level of carob powder, the differences were not statistically significant. Weight loss was similar for all the muffin samples in this study. Moisture content of muffins with carob powder was significantly higher than that in control. Addition of carob powder had also effect on water activity of muffin. While 0.905 a_w was observed in control sample, significantly higher a_w values were determinated in fortified muffins (0.912 – 0.923 a_w). The antioxidant characteristics were determinated using spectrophotometric assays for total phenolics (TPC), total flavonoids (TFC), radical scavenging activities (DPPH, ABTS) and hydrogen peroxide scavenging (HPS). TPC values gradually increased with the increase in level of carob powder from 348.1 to 829.1 µg gallic acid.g^{-1} dry matter but TFC values significantly increased in muffin with 15 and 20% (w/w) of carob powder. All the antioxidant assays showed strong and positive association with the increase in level of carob powder. Addition of carob powder resulted in the increase of browning index and FAST index as a metrics of the formation of Maillard products.

Keywords: fortification; phenolics; antioxidant; browning

INTRODUCTION

Carob powder or flour is the product of the fruit of *Ceratonia siliqua L*. Carob powder is usually prepared from mature, dried carob pod (without seed) after milling to desired particle size. Carob powder is a good source of sucrose and other simple sugars (maltose, mannose), unsaturated fatty acids and minerals such as calcium, potassium and iron **(Ayaz et al., 2009)**. High content of dietary fibre, both soluble and non-soluble, is the most important parameter, which makes the carob powder applicable in various food products such as bread **(Durazzo et al., 2014)** and cookies **(Roman et al., 2017)**. Carob powder is also used as a replacer of cocoa in cocoa and chocolate-based products decreasing the content of caffeine and theobromine but keeps the cocoa-like aroma, particularly when roasting **(Loullis and Pinakoulaki, 2018)**. In addition, carob powder is the rich source of polyphenolic substances exhibited promising pharmacological actions such as antioxidant, antibacterial, anti-inflammatory and anti-diabetic activities **(Rtibi et al., 2017)**.

In a recent work of **Pawlowska et al. (2018)**, the effect of substitution of cocoa powder for carob powder at 5% (w/w) level in muffin dough was examined. Improved antiradical activity and higher content of phytosterols have been observed in their study. However, their research was focused on substitution of cocoa powder at a single level. Replacing wheat flour for carob flour/powder may lead to both cocoa-like aroma and gluten-free products **(Roman et al., 2017; Lauková, Kohajdová and Karovičová, 2016)**.

Scientific hypothesis

Increasing content of polyphenolic substances and increasing antioxidant status of muffins prepared by partial substitution of wheat flour for carob powder are expected.

MATERIAL AND METHODOLOGY

All the solvents (methanol, ethanol and acetone) and acids (sulphuric and acetic acids) were purchased from Lach-Ner s. r. o. (Neratovice, Czechia). Folin-Ciocalteau reagent solution, gallic acid, quercetin hydrate, (+)-catechin, vanillin, aluminium chloride, 2,2-diphenyl-1-

picrylhydrazyl (DPPH), 2,2´-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS), potassium persulphate, hydrogen peroxide, ferrous ammonium sulphate, 1,10-phenanthroline and (±)-6-hydroxy-2,5,7,8-ramethylchromane-2-carboxylic acid (Trolox) were obtained from Sigma-Aldrich Chemical Co., St. Louis, MO, USA). All the chemicals were of analytical grade.

Sample preparation and baking procedure

Dry carob pods (*Ceratonia silique* L.) without seed were purchased in local supplier. Pods were milled using knife mill Grindomix GM 200 (Retsch®, Haan, Germany) to obtain powder. Particles smaller than 600 μm were separated by passing through analytical sieve and used for subsequent experiments. Carob powder was stored in evacuated plastic bag at room temperature until used.

Muffin formulation dough was adopted from the study of **Ambigaipalan and Shahidi (2015)** The control sample (without carob powder) contains 65 g of wheat flour and 37.5 g of sugar (Castello, Lidl Stiftung, Germany), 3.0 g of baking powder (Dr. Oetker, Bielefeld, Germany), 0.62 g of salt, 60.0 mL of 2.5% fat milk (Pilos, Louny, Czechia), 20.0 mL of canola oil (Promienna, Lidl Stiftung, Germany) and 1 egg (large). A portion of wheat flour was substituted for carob powder at 5, 10, 15 and 20% (w/w) levels to obtain fortified muffin. When carob powder content had been 25% and 30% (w/w), unacceptable taste was observed by three panelists. A dough was prepared by mixing all the ingredients, a batter (30 g) was transferred into muffin paper cups and baked in pre-heated oven at 180 °C for 20 min. Muffins were baked in quadruplicate, cool to room temperature and moisture content, water activity, weight and height loss were immediately determined. Thereafter, muffins were mixed and stored in evacuated plastic bag at -20 °C.

General chemical and physical analysis

Moisture content of carob powder samples and muffins was determined in moisture analyser (Kern MLB 50-3, Kern & Sohn GmbH, Balingen, Germany) at 103 °C to a constant weight. Water activity was measured at 25 °C in Aw Sprint TH 500 (Novasina AG, Lachen, Switzerland). Weight loss was calculated from the weight difference between dough and muffin using balance (sensitivity 0.001 g). Height loss was measured using digital caliper (sensitivity 0.01 mm) in three random positions. For measurement of those parameters, muffin samples were cooled down after baking for 1 h at ambient temperature.

Preparation of extracts

Aqueous-organic extracts of muffin or carob powder were prepared according to **Durazzo et al. (2014)** with slight modification. About 3 – 4 g of muffin or carob powder was placed in plastic tube with 20 mL of methanol/water (1:1, v/v). The tubes were placed in vertical shaker for 60 min followed by centrifugation at 5000 rpm for 10 min. The supernatant was removed and 20 mL of acetone/water (70:30, v/v) was added to pellet. After shaking for another 60 min, both methanolic and acetonic extracts were combined, centrifuged (5000 rpm, 5 min) and stored at -20 °C.

Determination of phenolics

Total phenolic content (TPC) was determined using Folin-Ciocalteau procedure as was provided in our previous study **(Brožková et al., 2018)**. The methanol/acetone extract (1.0 mL) was mixed with 0.5 mL of Folin-Ciocalteau reagent and 5.0 mL of distilled water followed by addition of 1.0 mL of 5% Na_2CO_3. After 60 min of incubation in a dark cabinet, the absorbance was measured at 765 nm. The same volume of distilled water was added instead of sample extract to obtain control measurement. The results were expressed as gallic acid equivalent (mg GA.g^{-1}) of dried matter (DM).

Total flavonoid content (TFC) was measured after formation of flavonoids-$AlCl_3$ complex in acetate solution followed by recording absorbance at 415 nm **(Brožková et al., 2018)**. The results were expressed as quercetin equivalent (mg QE.g^{-1} DM).

Determination of antioxidant capacity

Three various methods were used to assess antioxidant capacity of carob powder and muffin. Two assays are based on both electron and hydrogen atom transfer mechanisms involving the reaction of antioxidants with stable chromogen radical DPPH or ABTS. The procedures were adopted from our previous study **(Brožková et al., 2018)**. Briefly, 5.0 mL of DPPH methanolic solution (25 mg.L^{-1}) was mixed with 0.5 mL of methanol/acetone extract and the decrease of absorbance was observed after 35 min at 517 nm. ABTS radical cation (ABTS•+) was prepared from ABTS solution (5.0 mL, 50 mg.L^{-1}) and 100 μL of potassium persulphate (64 mmol.L^{-1}). The mixture was stored in a dark for 12 – 16 hours at laboratory temperature before use. Diluted ABTS•+ solution (3.0 mL) was mixed with 0.5 mL of methanol/acetone extract and the decrease of absorbance was recorded at 734 nm after 50 min of incubation. For both DPPH and ABTS assays, the results were expressed as Trolox equivalent antioxidant capacity (TEAC) in μg Trolox.g^{-1} DM.

Hydrogen peroxide scavenging (HPS) assay represents the method describing the effect of antioxidant against reactive oxygen species **(Mukhopadhyay et al., 2016)**. Extract (0.5 mL) was mixed with 0.25 mL of 1.0 mM ferrous ammonium sulphate and 62.5 uL of 5.0 mM H_2O_2. After 5.0 min of incubation in a dark cabinet at laboratory temperature, 1.5 mL of 1.0 mM 1,10-phenanthroline was added and subsequently incubated for 10 min. The absorbance was red at 510 nm against blank. Distilled water was added to control sample instead of extract and H_2O_2 (total volume 1.562 mL). The increase in HPS activity was reflected by the decrease of absorbance. The % of H_2O_2 scavenging ability was calculated as $(A_{test}/A_{blank}).100$, where A_{test} is the absorbance of the solution containing sample extract, ferrous ammonium sulphate, H_2O_2, 1,10-phenanthroline and A_{blank} is the absorbance of solution containing only ferrous ammonium sulphate and 1,10-phenanthroline.

Determination of Maillard products

The reaction of reducing sugars and tryptophan during roasting of carob powder and baking of muffin may result in formation of advanced Maillard products. Those products were determined according to the FAST (Fluorescence of Advanced Maillard products and Soluble Tryptophan) method of **Birlouez-Aragon et al. (2001)**. Briefly, sample extract was prepared by sonication (30 min) of 1.0 g of sample in 40 mL 0.1 M borate buffer solution (pH 8.2). The extract was filtered through filter paper (Whatmann no. 2) and fluorescence of advanced Maillard products (F_{amp}) was recorded at excitation and emission wavelengths of 353 nm and 438 nm, respectively. The decrease of tryptophan amount (F_{trp}) was monitored at excitation and emission wavelengths of 353 nm and 438 nm, respectively. Acryl cuvettes and fluorimeter Fluorat® 02 Panorama (Lumex Instruments, Mission, Canada) were used. FAST index was calculated as (F_{amp}/F_{trp}).100 and the results were expressed in %.

The formation of brown pigment resulted in Maillard reaction was measured in 80% of ethanol at 420 nm against distilled water according to **Krishnan et al. (2010)**. The results were expressed as browning index (BI) in absorbance unit (A_{420}). UV/VIS Spectrophotometer DU 530 (Beckman Coulter Inc., Brea, CA, USA) was used for all colorimetric measurements.

Statistic analysis

The results were expressed as the average means with standard deviations of 4 replicates (n = 4). Non-parametric statistical tests were used throughout this study. *i.e.* Tukey´s multiply comparison method was to find differences between means. Association between carob powder levels and selected variables were determined using Spearman´s correlation coefficient (*r*). Statistical treatment was performed on the probability level of p = 0.05 (Statistica CZ, StatSoft CR s. r.o., Prague, Czechia).

RESULTS AND DISCUSSION

The main composition of carob powder was as followed (in g.100g^{-1} DM): crude protein (7.37 ±0.80), crude fat (0.43 ±0.05), reducing sugar (13.12 ±0.4), crude fiber (26.30 ±0.25), ash (3.29 ±0.22) and moisture (7.20 ±2.00), similarly to those found by **Mohamed, Hamed and Al-Okbi (2008)** for carob pods. Concerning fibre content, lower amount was found in our study (~26%) in comparison with carob powder dried in microwave oven (~50%) **(Tounsi et al., 2017)**. Those differences may be attributed to the different methods, which were used for fibre determination (enzymatic or chemical) or it may be caused by the variability in processing technology and particle size of powder **(Benković et al., 2017)**. Nevertheless, the crude fibre content found in this study fits to the range of 7.6 – 38% previously presented in a review article of **Loullis and Pinakoulaki (2018)** for carob pulp composition.

Weight loss and height of muffins fortified with carob powder

The height and weight loss of the control sample was 3.57 cm and 5.69 g, respectively. The general decrease in height was presented in Table 1. As can be seen, the addition of carob powder caused the reduction of height in comparison with the control. The height of muffin fortified with carob powder significantly decreased from 3.38 to 3.03 cm (p <0.05) with the increase of carob powder level. The decrease in height may be attributed to the dilution of gluten and disruption of gluten network as was previously described by **Lauková, Kohajdová and Karovičová (2016)**, who observed the decrease in diameter and volume of biscuits enriched with apple powder. Similarly, addition of chicory syrup to muffin dough significantly reduced the loaf volume of the products **(Zacharová et al., 2018)**. Incorporating of dietary fibre into dough formulation also exhibited reduction in volume and height of muffin fortified with red capsicum pomace powder **(Nath et al., 2018)**. Although the content of crude fibre in fortified muffins was not evaluated in this study, the content of carob powder with 25% of crude fibre should not be omitted and it requires further investigation. Weight losses of fortified muffins were not statistically significant for all the carob powder samples in comparison with the control muffin sample in the present study.

Moisture content and water activity of muffins fortified with carob powder

Weight loss occurs usually due to the evaporation of water during baking and reflects the changes in moisture content. As can be seen from Table 1, moisture content of muffin samples with carob powder (except of muffin with 15% (w/w)) was significantly higher than this in control. Some authors also observed small but significant increase in moisture content of bread fortified with 10% and 20% of carob flour **(Salinas et al., 2015)**. In a study of **Karaca, Saydam and Guven (2012)**, addition of carob molasses in yogurt significantly increased water-holding capacity of samples. The addition of carob powder to muffin formulation significantly increased water activity from 0.905 (control) to 0.912 – 0.923 a_w for muffins with carob powder (Table 2). Despite those findings, a_w values still fell within the safe water activity range.

Table 1 Weight loss, height, moisture content and water activity of muffin with different levels of carob powder.

Level (%, w/w)	Height (mm ±SD)	Weight loss (g ±SD)	Moisture content (g.100 g^{-1} ±SD)	Water activity
control	3.57 ±0.1^{d}	5.69 ±0.17^{a}	30.19 ±0.84^{a}	0.905 ±0.001^{a}
5	3.38 ±0.11^{bd}	5.71 ±0.18^{a}	31.52 ±0.55^{b}	0.914 ±0.002^{b}
10	3.23 ±0.16^{c}	5.62 ±0.15^{a}	32.41 ±0.19^{b}	0.923 ±0.001^{c}
15	3.22 ±0.05^{c}	5.51 ±0.14^{a}	29.76 ±0.43^{a}	0.918 ±0.001^{b}
20	3.03 ±0.13^{c}	5.58 ±0.16^{a}	31.12 ±0.41^{b}	0.912 ±0.002^{b}

Note: Average mean ± standard deviation (n = 4); significant difference between means in column is indicated by different small letters (p <0.05).

Table 2 Antioxidant properties of muffin with different levels of carob powder.

Level (%, w/w)	TPC	TFC	$TEAC_{DPPH}$	$TEAC_{ABTS}$	HPS
control	193.2 ±18.1^{a}	35.7 ±7.1^{a}	ND	198.2 ±4.4^{a}	5.5 ±0.4^{a}
5	348.1 ±6.9^{b}	49.6 ±3.3^{a}	222.9 ±6.1^{a}	1048.8 ±58.5^{b}	19.8 ±0.3^{b}
10	435.7 ±4.9^{c}	49.1 ±2.0^{a}	393.5 ±8.6^{c}	1683.4 ±18.7^{c}	30.6 ±0.2^{c}
15	670.0 ±1.2^{d}	73.0 ±7.8^{b}	1099.5 ±23.9^{e}	3137.5 ±4.9^{d}	45.8 ±0.2^{d}
20	829.1 ±8.8^{e}	81.7 ±1.7^{b}	1228.3 ±65.6^{e}	3599.4 ±21.7^{e}	74.6 ±0.2^{e}

Note: TPC, Total phenolics content (μg gallic acid.g^{-1} DM); TFC, total flavonoids content (μg quercetin.g^{-1} DM); TEAC, Trolox equivalent antioxidant capacity (μg Trolox.g^{-1} DM); HPS hydrogen peroxide scavenging (%); significant difference between means in column is indicated by different small letters (p <0.05).

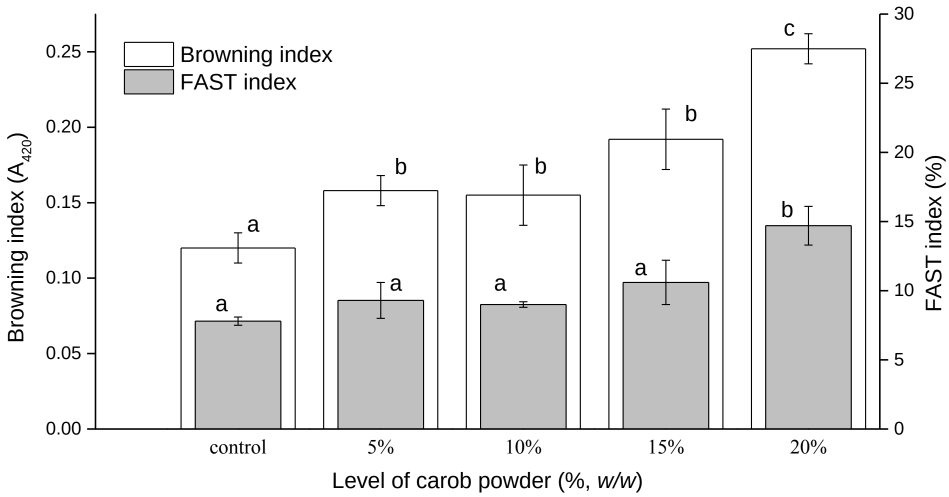

Figure 1 The effect of substitution of carob powder for wheat flour on browning index (white bars) and FAST index (gray bars) in muffin. Average mean ±standard deviation (n = 4).

Total phenolic, total flavonoid and condensed tannin contents in muffins fortified with carob powder

Total phenolic content of fortified muffins significantly increased in comparison with that in control (Table 2). While 193.2 μg GAE.g^{-1} DM was found in control sample, the substitution of wheat flour for carob powder resulted in gradual increase in TPC values from 348.1 to 829.1 μg GAE.g^{-1} DM with the increase of carob powder levels from 5% to 20% (w/w) (p <0.05). **Seczyk, Swieca and Gawlik-Dziki (2016)** also demonstrated that increase addition of carob flour to pasta resulted in the increase of total phenolic content. TFC values were significantly higher than in control (35.7 μg QE.g^{-1} DM; p <0.001) for all fortified muffins without regard of carob powder level. However, the increase in TFC values was not proportional to addition of carob powder. The addition of 5% and 10% (w/w) of carob powder led to the similar TFC values 49.6 – 49.1 μg QE.g^{-1} DM and to 73.1 – 81.7 μg QE.g^{-1} DM when 15% and 20% (w/w) of powders were added.

Antioxidant capacity of muffins fortified with carob powder

Antioxidant characteristics are presented in Table 3. While control muffin sample did not exhibit antioxidant capacity in terms of DPPH assay, the addition of carob powder at 5% (w/w) level to muffin recipe resulted in sharp increase to 222.9 μg Trolox.g^{-1} DM. Then, $TEAC_{DPPH}$ values increased with the increase in carob powder level. The highest $TEAC_{DPPH}$ values were obtained for muffin with 15% and 20% (w/w); *i.e*. 1099.5 and 1228.3 μg Trolox.g^{-1} DM (p <0.01), respectively. Antioxidant capacity measured by ABTS assay is based on similar principle as DPPH assay. Both radicals are able to accept hydrogen atoms or electrons provided by phenolic compounds in the extract. ABTS assay gave higher TEAC values than those in DPPH assay, probably due to the steric hindrance or different solubility of active substances in solutions, which were used for the preparation of stable radicals **(Apak et al, 2016)**. As can be seen from Table 3, ABTS assay always exhibited higher TEAC values at the same level of carob powder in comparison with those determined using DPPH assay (pairwise comparison test was not provided) in current study. It is known that each phenolic constituent contributes in different manner to total antioxidant capacity. We have found that rutin exhibited pro-oxidative effect during drying of buckwheat-based products **(Brožková et al., 2018)** and the same pattern was observed for quercetin, rutin or chlorogenic acid during processing of tomato paste **(Jacob et al., 2010)**. The addition of carob powder significantly increased the ability to scavenge H_2O_2 from initial 5.5% (control) to 19.8% followed by further increase with the increase of carob powder level (Table 3).

Table 3 The correlation analysis of increasing carob powder level *vs.* selected variables in muffin.

Parameters	*r*
Height	-0.756**
Weight loss	-0.006
Moisture content	-0.454
Water activity	-0.486
TPC	0.974***
TFC	0.824**
$TEAC_{DPPH}$	0.971***
$TEAC_{ABTS}$	0.971***
HPS	0.972***
Browning index	0.892***
FAST index	0.692**

Note: TPC, total phenolic content; TFC, total flavonoid content; TEAC, trolox equivalent antioxidant capacity; HPS hydrogen peroxide scavenging; * p <0.05, ** p <0.01, *** p <0.001.

Maillard reaction products in muffins fortified with carob powder

The higher content of reducing sugars and specific amino acids in food matrix may lead to the formation of various Maillard reaction products, which can be measured, in general, at specific wavelength or determination of compounds can be provided using HPLC techniques. Since carob powder is rich in reducing sugars (fructose, glucose) and reactive amino acids such as lysin or proline **(Ayaz et al., 2009; Benkovic et al., 2017)**, the formation of Maillard reaction products should be under control. With the increase in roasting temperatures and roasting times (130 – 150 °C for 5 – 30 min), the coloured Maillard reaction products, fluorescent compounds and hydroxymethylfurfural significantly increased in carob powder as was described in a study of **Cepo et al. (2014)**. The browning and FAST indices were determined in the present study. Addition of carob powder resulted in significantly higher BI when compared with the control muffin sample (p <0.05). BI values increased with the increase of the amount of carob powder up to 0.252 (A_{420}) for muffin with 20 % of carob powder (Figure 1). Surprisingly, FAST index did not differ in muffins fortified with carob powder from that found in control muffin sample (p >0.05) except in muffin with 20% level of carob powder, where significantly higher value was found (p <0.01).

The results of correlation analysis

The correlation analysis revealed that addition of carob powder instead of wheat flour to muffin recipe in the range from 5% to 20% (w/w) did not have effect on the weight loss, moisture content and water activity (Table 3). Strong and positive correlation coefficients ranged from 0.824 to 0.974 for antioxidant properties in terms of TPC, TFC, both TEAC values and hydrogen peroxide scavenging assay were obtained. While browning index showed strong positive correlation with the increase of carob powder level (r = 0.892, p <0.001), FAST index exhibited weak but significant association with the carob powder level (r = 0.692, p <0.01). However, this association is rather statistical than practical. It was evident from multiply comparison (see Figure 1) that FAST index values were not statistically different for control sample and muffins with 5 – 15% (w/w) of carob powder.

CONCLUSION

The additions of carob powder significantly affect the height of the muffins but not weight. Significant differences in moisture content of muffin samples were observed but it was not proportional to the level of carob powder. The addition of carob powder significantly increased water acitivity of muffins in comparison with the control sample. It was found that TPC gradually increased with the increase level of carob powder while TFC significantly increased in muffins fortified with higher levels of carob powder. Antioxidant capacity has increased with the increase level of carob powder in terms of DPPH, ABTS, and hydrogen peroxide scavenging assays. On the other hand, the addition of carob powder was associated with the formation of Maillard products. Browning index was significantly higher for muffin with the lowest carob powder level than that in control sample, but FAST index was significantly higher in muffin with the highest level of carob powder.

The results of this study showed that the replacement of wheat flour with carob powder increased the antioxidant status of muffins, however the higher the level of carob powder, the higher the potential to the formation of Maillard products.

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