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Effect of carob (*Ceratonia siliqua* L.) flour on the antioxidant potential, nutritional quality, and sensory characteristics of fortified durum wheat pasta

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ABSTRACT

This paper presents a study on the effect of carob flour addition from 1% to 5% (w/w) on phenolics content, antioxidant activity, nutritional quality, and sensory attributes of wheat pasta. An increase of about 2-folds, 18-folds and 3-folds in phenolics content, antiradical activity and reducing power for pasta fortified with 5% of carob flour was observed, respectively, compared to the control. Expected glycemic index (eGI) was increased proportionally to the substitution level and ranged between 72.2 and 83.9 for 1–5% of supplement, respectively. Furthermore, pasta fortification affected the *in vitro* bioaccessibility of nutrients. In case of 5% supplemented pasta, the digestibility of starch and protein decreased by about 9% compared to the control. The replacement of semolina with carob flour from 1% to 5% had no significant effect on pasta sensory attributes. In conclusion, carob flour seems to be a promising functional ingredient for pasta fortification.

1. Introduction

Carob (*Ceratonia siliqua* L.) is an evergreen tree belonging to the Leguminosae family, widely cultivated in the Mediterranean region, mainly Spain, Italy, Portugal, and Morocco (Dakia, Wathelet, & Paquot, 2007; Durazzo et al., 2014). Seeds and pods of carob fruit are used as a raw material in industries such as food, pharmaceutical, and cosmetics ones (Durazzo et al., 2014). The carob gum, also called locust bean gum (LBG) is obtained from seeds containing high amounts of galactomannans (Dakia et al., 2007). It is a valuable natural food thickener, stabilizer, and flavorant, which is commonly added to a variety of products, for example, ice creams, sweets, and soups (Biner, Gubbuk, Karhan, Aksu, & Pekmezci, 2007; Durazzo et al., 2014).

Pods of the carob fruit have long been used as a raw material for food additives production (Biner et al., 2007). Due to its sweetness and flavor similar to chocolate, as well as its low price—the seedless pods milled into flour are widely used in the Mediterranean region as cocoa substitute for sweets, biscuits, and processed drinks production (Ayaz et al., 2009; Bengoechea et al., 2008; Biner et al., 2007; Durazzo et al., 2014; Kumazawa et al., 2002). Additionally, the advantage of using carob powder as a cocoa substitute is that it does not contain caffeine and theobromine (Bengoechea et al., 2008).

Carob pods are characterized by high soluble sugars (about 40–50%, mainly sucrose), low protein (3–4%) and lipids (0.4–0.8%) contents (Kumazawa et al., 2002). Moreover, raw carob pods and carob pod flour contain substantial amounts of polyphenols (Avallone, Plessi, Baraldi, & Monzani, 1997; Kumazawa et al., 2002; Youssef, El-manfaloty, & Ali, 2013), especially condensed tannins (Ayaz et al., 2009; Kumazawa et al., 2002).

Polyphenols exhibit a wide range of biological properties, and among these, the antioxidant activity is the best known. Phenolic antioxidants prevent against oxidative damage of some important biomolecules like DNA, protein, and lipids, which is considered to be one of the main factors favoring the occurrence of degenerative diseases such as cancer, inflammatory, cardiovascular, and neurodegenerative diseases (Scalbert, Manach, Morand, Rémésy, & Jiménez, 2005).

Besides the high content of phenolic compounds, carob flour is regarded as a product that contains a high level of dietary fiber (Ortega, Macià, Romero, Reguant, & Motilva, 2011), minerals (Fe, Ca, Na, K, P and S), and vitamins (E, D, C, Niacin, B6 and folic acid) (Youssef et al., 2013). In view of its high nutritional value, the growing interest in using carob flour as a functional ingredient in producing pro-health foods is increasing. Carob flour is used to enhance the nutritional value of cereal-based products such as

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breads, biscuits, and cakes (Ortega et al., 2011). Nevertheless, not much information is available on the usefulness of carob flour in pasta fortification.

Pasta is a widely consumed cereal-based food, conventionally made from durum wheat semolina, as the primary ingredient (Padalino et al., 2014a, 2014b). It is a good source of carbohydrates and a moderate source of protein and vitamins in human diet (Boroski et al., 2011). Beside this, it is low in sodium and fat and has no cholesterol (Chillo, Laverse, Falcone, & Del Nobile, 2008; Rajeswari, Susanna, Prabhasankar, & Rao, 2013). Conventional pasta is considered to be a slowly digestible starchy food with low glycemic index (GI) (Biney & Beta, 2014; Khan, Yousif, Johnson, & Gamlath, 2013; Osorio-Díaz, Agama-Acevedo, Mendoza-Vinalay, Tovar, & Bello-Pérez, 2008; Padalino et al., 2014b).

A diet rich in low-GI foods and promoting a small increase in blood glucose level after a meal can reduce the long-term risk of type 2 diabetes mellitus and can be beneficial for prevention and control of obesity and metabolic risk factors, such as coronary heart diseases (Giuberti, Gallo, Cerioli, Fortunati, & Masoero, 2015).

Wheat pasta is considered an adequate carrier pro-healthy components (Borneo & Aguirre, 2008; Boroski et al., 2011; Chillo et al., 2008). In recent years, many studies have investigated the effect of pasta supplementation with a wide range of supplements. The quality of pasta was effectively enriched with spirulina (Rodríguez De Marco, Steffolani, Martínez, & León, 2014), buckwheat flour and bran (Biney & Beta, 2014), sorghum flour (Khan et al., 2013), oregano and carrot leaves (Boroski et al., 2011), dry amaranth leaves flour (Borneo & Aguirre, 2008), parsley leaves (Sęczyk, Świeca, & Gawlik-dziki, 2015) and pea flour (Marinangeli, Kassis, & Jones, 2009; Padalino et al., 2014a).

The objective of this study was a determination of phenolics content, antioxidant activity, eGI, and nutrients digestibility of durum wheat pasta supplemented with 1–5% of carob flour. Additionally, the sensory characteristics of fortified pasta were also evaluated.

2. Materials and methods

2.1. Chemicals

Folin–Ciocalteau reagent, ABTS (2,2′-azino-bis (3-ethylbenzo thiazoline-6-sulfonic acid), potassium ferricyanide, α-amylase (EC 3.2.1.1), pepsin (EC 3.4.23.1), amyloglucosidase (EC 3.2.1.3), dinitrosalicylic acid (DNSA), pancreatin, 2,4,6-trinitrobenzenesulfonic acid (TNBS), were purchased from Sigma–Aldrich (St. Louis, MO, USA) company. All others chemicals were of analytical grade.

2.2. Pasta preparation

Durum wheat semolina (protein 10.1%, carbohydrates 7.5%, fat 1.3%) (Radix-bis, Rotmanka, Poland) and carob pod flour (protein 4.6%, carbohydrates 49%, dietary fiber 8% fat 0.6%) (Bio Planet S.A., Leszno, Poland) were purchased from a local store. Pasta was prepared with wheat flour at different concentrations of carob flour (0%—CP, 1–5%, P1–P5, respectively; w/w). For each formulation, semolina pasta flour and distilled water (flour: water, 2.5:1, w/w) were mixed using a domestic blender (Kitchen Aid, Mod K5SSWH) for 5 min, to obtain homogeneous dough. This dough was formed and cut in a pasta machine (Pasta machine, Kitchen collection, Mod 20171, Chillicothe, OH, USA). Pasta samples (about 2.5 mm thickness, 60 mm length) were dried in a laboratory dryer (SML30, Poland) for 24 h at 40 °C. Residual moisture of 12 g/100 g was present in the final dried pasta samples. Dried pasta (100 g) was cooked in 1000 mL of boiling distilled water. Optimum cooking time was determined by using AACC 66–50 method (AACC, 2000) and it was achieved at 360 s for control and at 430 s for fortified pasta samples (however, white core in the pasta was still present but disappeared after squeezing between two glass plates). After cooking, pasta was drained and cooled at room temperature. The cooked pasta was frozen at −20 °C and lyophilized in laboratory freeze drier (Labconco FreeZone, Kansas City, MO, USA). Then, freeze dried samples were milled and sieved to pass through 250 μm sieve. The ground pasta were stored in darkness at −20 °C.

2.3. Phenolics content and antioxidant activity

2.3.1. Extraction procedure

Powdered samples of pasta (1 g) or carob flour (1 g) were extracted for 1 h with 25 mL of 20 mmol/L hydrochloric acid in methanol: acetone: water solution (30:30:40; v/v/v). The extracts were centrifuged (6800*g*, 20 min.) and extraction procedure was repeated. Extracts were combined and stored in darkness at −20 °C until analysis.

2.3.2. Total phenolics content

The amount of total phenolics was determined using Folin–Ciocalteau reagent (Singleton & Rossi, 1965). To 0.5 mL of the extract, 0.5 mL H_2O, 2 mL Folin–Ciocalteau reagent (1:5 H_2O) were added, and after 3 min, 10 mL of 10% Na_2CO_3. The contents were mixed and allowed to stand for 30 min. Absorbance at 725 nm was measured in a UV–Vis spectrophotometer. The amount of total phenolics was expressed as a gallic acid equivalent (GAE) in mg/g of dry weight (DW).

2.3.3. ABTS radical scavenging assay

The experiments were performed using an improved ABTS depolarization assay (Re et al., 1999). The ABTS radical cation ($ABTS^{+\cdot}$) was produced by reacting 7 mmol/L stock solution of ABTS with 2.45 mmol/L potassium persulfate (final concentration). The $ABTS^{+\cdot}$ solution was diluted (with distilled water) to an absorbance of 0.7 ± 0.05 at 734 nm. Then, 50 μL of samples were added to 1.45 mL of $ABTS^{+\cdot}$ solution and the absorbance was measured at the end time of 3 min. The ability of the extracts to quench the ABTS free radical was expressed as a trolox equivalent (TE) in mg/g of dry weight (DW).

2.3.4. Ferric reducing antioxidant power

Reducing power was determined using the method described by Pulido, Bravo, and Saura-Calixto (2000). Extracts (0.5 mL) were mixed with phosphate buffer (0.5 mL, 200 mmol/L pH 6.6) and 0.5 mL of 1% aqueous solution of potassium ferricyanide $K_3[Fe(CN_6)]$. The mixture was incubated at 50 °C for 20 min. A portion (0.1 mL) of 10% trichloroacetic acid was added to the mixture, which was then centrifuged at 6800*g* for 10 min. The upper layer of solution (0.5 mL) was mixed with distilled water (0.5 mL) and 0.1 mL of 1 g/L $FeCl_3$, and the absorbance was measured at 700 nm. The ability of the extracts to reduce iron (III) was calculated as a trolox equivalent (TE) in mg/g DW.

2.3.5. Theoretical approaches

Predicted (PV) for total phenolic contents (TPC) was calculated as follow (Świeca, Sęczyk, Gawlik-Dziki, & Dziki, 2014):

$$PV = \left(TPC_{CP} - \left(TPC_{CP} \times \frac{N}{100\%}\right)\right) + \left(\frac{TPC_{CF} \times N}{100\%}\right),$$

where TPC_{CP} – phenolic content of control pasta, TPC_{CF} – phenolic content of carob flour, N – percent of carob flour supplement.

Predicted (PV) antioxidant activity (AA) was calculated as follow (Świeca et al., 2014):

$$PV = \left(AA_{CP} - \left(AA_{CP} \times \frac{N}{100\%}\right)\right) + \left(\frac{AA_{CF} \times N}{100\%}\right),$$

where AA_{CP} – activity of control pasta, AA_{CF} – activity of carob flour, N – percent of carob flour supplement.

2.4. In vitro starch digestion rate and expected glycemic index (eGI)

The digestion kinetics and eGI of the fortified pasta were calculated in accordance with the procedure established by Goñi, Garcia-Alonso, and Saura-Calixto (1997) with some modification.

Total carbohydrates (TC) content was determined after dispersion of the starch in 2 M KOH (50 mg sample, 6 ml KOH) at room temperature (30 min, constant shaking). Samples were centrifuged and pH was adjusted to 4.75 with 2 M HCl. The solubilized sugars were hydrolyzed with 80 μL (1 mg mL^{-1}) amyloglucosidase (14 U mg^{-1}; EC 3.2.1.3) in 0.2 M sodium acetate buffer pH 4.75 at 60 °C for 45 min (Goñi et al., 1997). The reducing sugar content was determined by using the standard 3,5-dinitrosalicylic acid (DNSA) method (Miller, 1959).

Pasta sample (50 mg) was incubated with 5 mL pepsin solution (0.2 g pepsin; 3500 U mg^{-1} per 100 mL 0.01 M KCl—HCl buffer, pH = 2) at 40 °C for protein hydrolysis. The sample solution was brought to 25 mL with 200 mM phosphate saline buffer (PBS), pH = 7.4. Carbohydrates hydrolysis was initiated by addition of 5 ml of PBS buffer containing α-amylase (50 U/mL). Then, the sample was incubated at 37 °C. At 15 min intervals, over 0–90 min, 1 ml of sample was transferred to another tube and α-amylase was inactivated immediately by incubating at 100 °C for 5 min. Then 3 mL of 0.2 M sodium acetate buffer pH = 4.75 and 60 μL of amyloglucosidase (1 mg/mL; 14 U mg^{-1}; EC 3.2.1.3) was added to each aliquot to hydrolyze the samples into glucose. Samples were incubated 45 min at 60 °C. Then the reducing sugar content was determined by using the DNSA method (Miller, 1959). The rate of digestion was expressed as the percentage of total sugars hydrolyzed at different times.

A non-linear model following the equation [$C = C1 \propto (1 - e^{-kt})$] was applied to describe the kinetics of starch hydrolysis, where C, $C1$, and k were the hydrolysis degree at each time, the maximum hydrolysis extent and the kinetic constant, respectively. The hydrolysis index (HI) was calculated as the relation between the areas under the hydrolysis curve (0–90 min) of the pasta sample and the area of standard material from white bread (reference sample). The eGI was calculated using the equation proposed by Granfeldt, Bjorck, Drews, and Tovar (1992), that is, eGI = 8.198 + 0.862HI.

2.5. Relative digestibility of nutrients

2.5.1. Relative digestibility of protein

Powdered pasta samples (0.3 g) were mixed with 5 mL of 200 mM PBS, pH = 7.4) and shaken (80 rpm) for 10 min at 4 °C. Samples were centrifuged (10 min 6800*g*; 4 °C), and supernatants were collected (time 0). Additionally, pasta samples (0.3 g) were mixed with 5 ml of pancreatin solution (150 mg of pancreatin; activity equivalent 4 × USP, per 100 mL of PBS) and samples were shaken (80 rpm) in darkness for 60 min at 37 °C. Thereafter, samples were centrifuged (10 min 6800*g*; 4 °C), and supernatants were collected. The content of free amino groups before (time 0) and after digestion was determined by using the trinitrobenzenesulfonic acid (TNBS) method (Adler-Nissen, 1979). Briefly, samples (0.025 mL) were mixed with 0.975 mL 0.2 M phosphate buffer, pH 8.0, and 0.5 mL of 0.1% TNBS. After 60 min of incubation at 50 °C in darkness, 1 mL 0.1 M HCl was added to stop the reaction. After cooling (30 min), absorbance was measured at 340 nm. The free amino group levels were expressed as a L-leucine equivalent (LE) in μg/g DW. The correction for free amino groups present in the study material was carried out before digestion (time 0). The difference between control and fortified pasta was expressed as the relative digestibility of the protein.

2.5.2. Relative digestibility of starch

Powdered pasta samples (0.3 g) were mixed with 5 mL of 200 mM PBS, pH = 7.4) and shaken (80 rpm) for 10 min. at 20 °C. Samples were centrifuged (10 min; 6800*g*; 4 °C), and supernatants were collected (time 0). Additionally, pasta samples (0.3 g) were mixed with 5 ml of pancreatin solution (150 mg of pancreatin per 100 mL of PBS) and samples were shaken (80 rpm) in darkness for 60 min at 37 °C. Thereafter, samples were centrifuged (10 min 6800*g*; 4 °C), and supernatants were collected. The reducing sugar content before (time 0) and after digestion was determined by using the DNSA method (Miller, 1959). Briefly, samples (0.05 mL) were mixed with 0.95 mL of distilled water and 1 mL of DNSA. Then, the mixture was incubated in 100 °C for 10 min. After cooling to room temperature, 10 mL of distilled water was added and the absorbance at 540 nm was measured. The free reducing sugar content was expressed as a maltose equivalent in μg/g DW. The correction for free reducing sugars present in the study materials was carried out before digestion (time 0). Relative digestibility of the starch was expressed as the difference between control and fortified pasta.

2.6. Sensory evaluation

Control and fortified pasta were evaluated by a consumer panel consisting of 32 members (13 male, 19 female, aged 22–48 years). Before testing, all participants were enquired for possible food allergies to wheat or wheat components and/or carob flour. Pasta (500 g) were cooked freshly in water (5 L) for 360 s (control) and 430 s (fortified pasta samples), rinsed and cooled in water at 20 °C for 2 min. Cooked pasta were placed in plastic cups and presented to the panelists. Participants were instructed to rinse with water (20 °C) before they began testing and between samples. Pasta samples were evaluated for the color, taste, aroma, texture (mouth), and overall quality. Sensory attributes were evaluated using a nine-point hedonic scale, and the values ranged from 1 to 9, wherein: (1) *extremely unpleasant*, (2) *very unpleasant*, (3) *moderately unpleasant*, (4) *slightly unpleasant*, (5) *neither pleasant nor unpleasant*, (6) *slightly pleasant*, (7) *moderately pleasant*, (8) *very pleasant*, and (9) *extremely pleasant*.

2.7. Statistical analysis

All experimental results represented mean ± S.D. of three parallel measurements. One-way analysis of variance post hoc and Tukey's test were used to compare groups. α values = 0.05 were regarded as a significant.

3. Results

The results describing the effect of pasta fortification on its phenolics content and antioxidant activity are presented in Table 1. Phenolics content and antioxidant activity were positively correlated with the percentage of carob flour addition, and the highest values were obtained for pasta enriched with 5% supplement. In comparison to the control, levels of phenolics in fortified pasta were significantly higher from about 0.5 to 2-folds. Antioxidant activity was examined by two different mechanisms—free radical

Table 1
Phenolics content and antioxidant potential of pasta fortified with carob flour.

		TPC (mg GAE/g DW)	ABTS (mg TE/g DW)	FRAP (mg TE/g DW)
CP	EV	3.51 ± 0.26[a]	0.07 ± 0.01[a]	1.62 ± 0.19[a]
	PV	–	–	–
P1	EV	5.27 ± 0.24[b]	0.25 ± 0.08[b]	2.05 ± 0.15[b]
	PV	6.05	0.51	2.61
P2	EV	8.35 ± 0.37[c]	0.75 ± 0.07[c]	3.50 ± 0.38[c]
	PV	8.58	0.94	3.60
P3	EV	9.31 ± 0.75[c]	0.88 ± 0.03[c]	3.82 ± 0.19[c]
	PV	11.12	1.38	4.59
P4	EV	10.54 ± 0.26[d]	1.14 ± 0.02[d]	4.94 ± 0.13[d]
	PV	13.65	1.81	5.58
P5	EV	12.12 ± 0.51[e]	1.35 ± 0.07[e]	6.87 ± 0.06[e]
	PV	16.18	2.25	6.57

Means (±SD, $n = 9$) followed by different small letters in columns for selected features are significantly different at $\alpha = 0.05$.
EV – experimental value, PV – predicted value.
TPC – total phenolics contents, ABTS – ABTS radical scavenging capacity, FRAP – ferric reducing antioxidant power; GAE – gallic acid equivalent, TE – trolox equivalent, DW – dry weight.
CP – control pasta, P1–P5 – pasta fortified with 1–5% of carob flour, respectively.

scavenging ability (ABTS) and ferric reducing antioxidant power (FRAP). An elevation from about 2-folds to 18-folds in antiradical activity and from about 1-fold to 3-folds in the ability to reduce Fe^{3+} ions by supplemented pasta (1–5%) was observed, respectively. Additionally, predicted values (PV) for total phenolic content and antioxidant properties were also evaluated. Generally experimental values (EV) for measured parameters were lower than those predicted (Table 1).

Fig. 1 depicts profiles of the *in vitro* enzymatic starch digestibility of the pasta control and fortified pasta samples compared to the commercial white bread. Generally, the curves for all studied samples presented a first part where the hydrolysis rates increases (0–45 min) and a second one (45–90 min) where a plateau was reached. Pasta fortification increased total starch digestion rate from about 59% (CP) to a range of 62% (P1) to 70% (P5) measured after 90 min of *in vitro* digestion. Whilst, white bread used as reference showed a digestion rate of about 82% at the end of the hydrolysis process (90 min).

HI and eGI are summarized in Table 2. At the highest carob flour substitution level, HI and eGI were increased. eGI of pasta fortified with 1–5% of carob flour ranged from 72.2 to 94.4. In comparison with control, an elevation from 3.6% to 18.2% in HI values and from 3.3% to 30.7% in eGI for fortified pasta samples was observed.

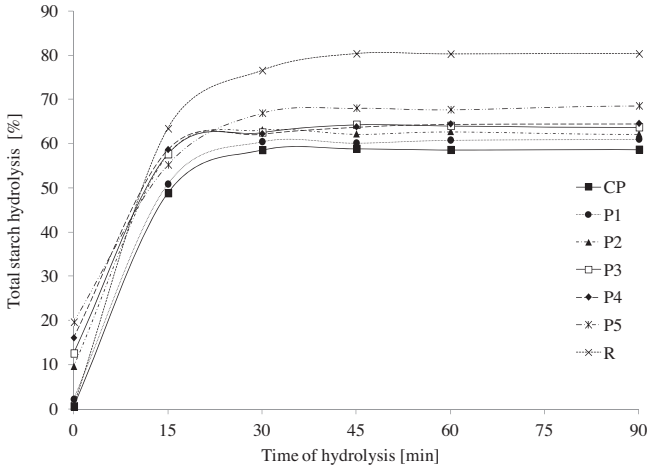

Fig. 1. *In vitro* starch hydrolysis rate. CP – control pasta, P1–P5 – pasta fortified with 1–5% of carob flour, respectively. R – reference (white bread).

The effect of pasta supplementation on relative digestibility of starch is presented in Fig. 2. In comparison with the control, only in the case of pasta fortified with 5% of carob flour a significant decrease (by about 9%) in starch digestibility was noted. In respect to the control, the protein digestibility was lower from 5% up to 9% for pasta enriched with 1–5% of supplement, respectively.

Evaluation of sensory characteristics (color, smell, taste, texture, and overall quality) of fortified pasta by the panelist is depicted in Table 3. Obtained results indicate that fortification of pasta did not negatively affect the tested sensory attributes. Pasta samples scored between 5 (*neither like nor dislike*) and 7 (*moderately pleasant*) for each evaluated characteristics. Generally, the higher rates were received for pasta supplemented with 5% of carob flour. However, mean rates did not differ significantly between all tested samples.

4. Discussion

As expected, the incorporation of carob flour substantially improved phenolics level and antioxidant activity of studied pasta (Table 1). Similarly to our current study, positive correlation between phenolics level, antioxidant potential, and the supplement level of fortified pasta was previously noted (Boroski et al., 2011; Fares, Platani, Baiano, & Menga, 2010; Khan et al., 2013; Prabhasankar et al., 2009; Rodríguez De Marco et al., 2014). Results obtained by Khan et al. (2013) show an increase in ability to reduce ABTS radical of cooked pasta enriched with 20–40% of red and white sorghum flour from 126% to 236% and from 26% to 58%, respectively. Similar trends were observed in case of total phenolics content (Khan et al., 2013). On the other hand, the study of Prabhasankar et al. (2009) reports an elevation of 89–200%, 81–169%, and 29–40% (in respect to control) in total phenolics content, total antioxidant activity, and reducing power of cooked pasta supplemented with wakame (5–30%), respectively. Beyond the type and level of functional supplements, other factors such as processing and cooking are involved in the creation of the antioxidant properties of pasta. A water soluble antioxidant can leach into the cooking water (Khan et al., 2013; Prabhasankar et al., 2009; Rodríguez De Marco et al., 2014). A decrease of phenolics level and antioxidant activity in cooked pasta, compared to the raw product (uncooked pasta) have been reported in pasta supplemented with sorghum flour (Khan et al., 2013), wakame (Prabhasankar et al., 2009), buckwheat flour, and bran (Biney & Beta, 2014). Additionally, it should be mentioned, that the action of oxygen, water, and heat treatment during pasta processing and cooking induce the oxidation of some sensitive phenolics antioxidants (Fares et al., 2010; Khan et al., 2013; Prabhasankar et al., 2009). Conversely, the study of Fares et al. (2010) show that cooking leads to release of some bound phenolics from the food matrix and enhances the antioxidant capacity of pasta enriched with debranning fractions of wheat. Other factors, which may affect the potential activity of fortified products are interactions between the food matrix components and bioactive compounds (phenolics) (Świeca, Gawlik-Dziki, Dziki, Baraniak, & Czyż, 2013; Świeca et al., 2014) and/or among bioactive compounds themselves, e.g. phenolic–phenolic interactions (Gawlik-Dziki, 2012). To estimate the effect of the food matrix on the potential biological activity of fortified pasta, PV for measured properties were estimated (Table 1). Similarly to studies of Sęczyk et al. (2015) and Świeca et al. (2013, 2014) the phenolics content antioxidant activity was lower than those the predicted one, which may indicate that the mentioned interactions are engaged in shaping the potential bioactivity of studied pasta products.

Results obtained from carbohydrates digestion show that all fortified pasta samples had higher rates of carbohydrate digestion

Table 2
Hydrolysis index and estimated glycemic index of wheat pasta fortified with carob flour.

Sample	CP	P1	P2	P3	P4	P5	R
HI	74.3 ± 1.3[a]	77.0 ± 1.1[b]	81.6 ± 1.2[c]	83.2 ± 1.1[c]	83.9 ± 1.4[c]	87.8 ± 0.9[d]	100.0 ± 1.8[e]
eGI	72.2 ± 1.3[a]	74.6 ± 1.0[a]	78.5 ± 0.9[b]	79.9 ± 1.3[b]	80.6 ± 1.1[b]	83.9 ± 1.1[c]	94.4 ± 1.6[d]

Means (±SD, $n = 9$) followed by different small letters in the same row are significantly different at $\alpha = 0.05$.
HI – hydrolysis index; eGI – expected glycemic index, CP – control pasta, P1–P5 – pasta fortified with 1–5% of carob flour, respectively, R – reference (white bread).

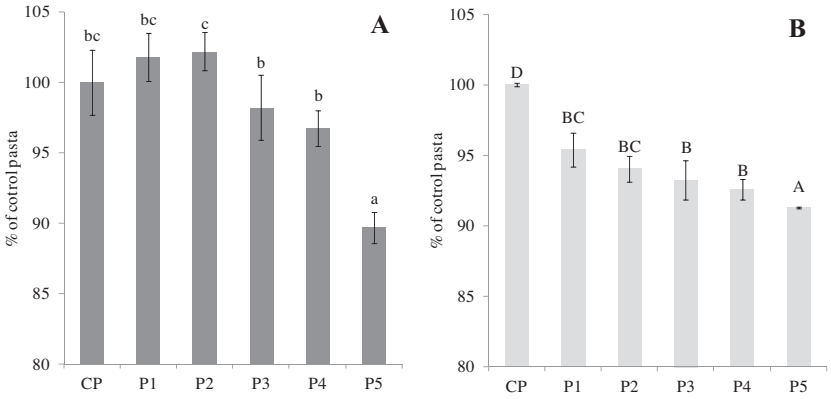

Fig. 2. Relative digestibility of starch (A) and protein (B). Bars represent means ± SD. Means ($n = 9$), followed by different small (starch digestibility) and capital letters (protein digestibility) in bars are significantly different at $\alpha = 0.05$. CP – control pasta, P1–P5 – pasta fortified with 1–5% of carob flour, respectively.

Table 3
Sensory characteristics of wheat pasta fortified with carob flour.

Sensory attribute	CP	P1	P2	P3	P4	P5
Color	6.00 ± 1.24	5.83 ± 1.40	5.22 ± 1.29	5.22 ± 1.48	5.78 ± 1.44	6.36 ± 1.09
Smell	6.14 ± 1.28	6.09 ± 1.44	5.95 ± 1.68	5.86 ± 1.49	5.86 ± 1.49	6.23 ± 1.63
Taste	5.76 ± 1.48	5.86 ± 1.46	5.67 ± 1.62	5.71 ± 1.35	5.90 ± 1.76	5.95 ± 1.56
Texture (mouth)	6.81 ± 0.93	6.29 ± 1.15	6.00 ± 1.38	6.05 ± 1.40	5.86 ± 1.49	6.33 ± 1.24
Overall quality	6.23 ± 1.27	5.82 ± 1.59	5.93 ± 1.66	5.91 ± 1.38	5.64 ± 1.59	6.45 ± 1.22

All values are mean ± SD, $n = 32$.
No significant differences at $\alpha = 0.05$ between control and fortified pasta for selected attributes were found.
CP – control pasta, P1–P5 – pasta fortified with 1–5% of carob flour, respectively.

with regard to control and eGI (Table 2). Furthermore, the expected postprandial glycemic response increased relatively quickly after the start of the digestion process (Fig. 1). Thus, this type of fortified products with high eGI (ranged between 74.6 and 83.9) (Table 2) generally should not be recommended for people predisposed to obesity, diabetes, and cardiovascular diseases (Giuberti et al., 2015). Nevertheless, this type of pasta, with a high glycemic index, seems appropriate, as a good source of rapidly digestible carbohydrates, for athletes and people with an active lifestyle, who have higher energy requirements than others.

Similarly to our studies an increase of eGI from 83.6 (control pasta) to 93.3 (pasta fortified with 30% of whole yellow pea flour) was observed in the *in vivo* study of Marinangeli et al. (2009). The study of Rodríguez De Marco et al. (2014) showed that the eGI of pasta enriched with 5% (eGI = 72), 10% (eGI = 75), and 20% (eGI = 72) of spirulina were similar to the control (eGI = 75). Osorio-Díaz et al. (2008) reported that the addition of chickpea flour to pasta resulted in a significant decrease of estimated GI. In the mentioned study eGI were 80.68, 70.7, and 61.45 for control pasta and those supplemented with 20% and 40% chickpea flour, respectively (Osorio-Díaz et al., 2008).

The glycemic index of pasta products is shaped by many factors related with methods of pasta processing and its chemical composition: among others pasta structure, the encapsulation of the starch granules by a protein network and dietary fiber, starch physico-chemical properties (degree of gelatinization and retrogradation; amylose/amylopectin ratio), as well as the presence of polyphenols should be emphasized. The modification of one or more of these elements by supplementation of pasta may lead to change in its glycemic response (Petitot, Barron, Morel, & Micard, 2010). Rodríguez De Marco et al. (2014) suggested that fortification of pasta with functional ingredients can affect the arrangement of components in the pasta structure and modify the integrity of the protein network. A disruption of the protein network and the entrapping of starch granules could increase the susceptibility of starch to amylosis, starch digestibility and glycemic index (Rodríguez De Marco et al., 2014).

Contrary to the results obtained for the total carbohydrates (TC) hydrolysis rate (Fig. 1), results summarized in Fig. 2 show that replacement of semolina with carob flour can adversely affect the *in vitro* digestibility of starch. Furthermore, protein digestibility was also lower than the control in enriched pasta (Fig. 2). Decreased digestibility of nutrients in fortified products may be associated with different content and susceptibility to hydrolysis of compounds derived from the functional supplement, compared to the conventional pasta components. Additionally, limited *in vitro* bioaccessibility of available starch and protein may be a consequence of interactions between phenolics compounds and

food matrix (Sęczyk et al., 2015; Świeca et al., 2013, 2014). Phenolics may interact with protein/starch and form indigestible complexes and/or inhibit the activity of digestive enzymes (Świeca et al., 2013, 2014).

Besides nutritional and nutraceutical properties of the fortified products, the sensory attributes are important factors for consumer's acceptability. Improving food functionality by fortification with such phenolics-rich ingredients as a carob pod flour, which is generally characterized by a high content of tannins (Ayaz, 2009; Kumazawa et al., 2002), can lead to lower consumer acceptance as a consequence, bitter, acrid, and astringent taste linked with the presence mentioned compounds (Drewnowski & Gomez-Carneros, 2000). However, obtained results presented in Table 3 show that the addition of carob flour at the level from 1% to 5% to pasta had no significant influence on its sensory attributes and consequently on consumer's acceptance.

5. Conclusion

Wheat pasta supplemented with carob flour exhibited significantly higher content of phenolic compounds and antioxidant activity, than the control. The fortification leads to increase of eGI, however it decreases digestibility of studied nutrients. Additionally, the incorporation of carob flour to pasta had no significant influence on its sensory attributes. Therefore, carob flour seems to be a promising functional ingredient for pasta fortification.

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