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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.