LWT - Food Science and Technology 58 (2014) 578–586

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Optimization of roasting conditions as an useful approach for increasing antioxidant activity of carob powder

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ARTICLE INFO

Article history:
Received 19 April 2013
Received in revised form
27 March 2014
Accepted 3 April 2014
Available online 24 April 2014

Keywords:
Carob
Roasting
Antioxidant activity
Gastrointestinal solubility
Maillard reaction

ABSTRACT

Antioxidant activity, gastrointestinal solubility of polyphenols and formation of different classes of Maillard reaction products (MRPs) that occurs during thermal processing of carob powder was investigated in relation to different roasting time-roasting temperature combinations applied. Principal component analysis (PCA) was used to group carob powders with the aim of proposing optimal roasting conditions for obtaining the one with the highest biological activity that can be expected after undergoing the simulation of gastrointestinal digestion. Results showed that antioxidant activity of carob powder can be significantly increased by roasting due to improved polyphenol solubility and formation of the early-stage MRPs. Fluorescent MRPs, HMF and furfural, known for their toxic properties, were the most abundant in samples roasted under more severe time/temperature conditions. Carob powder roasted at 130 °C for 30 min yielded the highest antioxidant activity and the lowest toxic MRP formation.

1. Introduction

Carob (*Ceratonia siliqua* L.) is one of the most useful native Mediterranean trees and its pods have traditionally been used as animal and human food. Currently, the main use is the production of carob bean gum from the seed endosperm which is used as the food additive (stabilizer and thickener) in food- and pharmaceutical industry. Carob pulp is used for the production of carob powder which is used in the food industry as the extender for cocoa due to certain advantages such as fewer calories and neither caffeine nor theobromine (Craig & Nguyen, 1984).

Chemically, obtained powder consists of about 46% of total sugar, (14% of reducing sugar), 7% protein and small amounts of numerous minerals and vitamins and is thus quite nutritious (Whiteside, 1981; Yousif & Alghzawi, 2000).

During the production of carob powder, kibbling of carob pods is followed by roasting using various temperature ranges (120–180 °C) and time intervals (10–60 min), depending mainly on the targeted sensory characteristics of the final product such as taste, colour and aroma, that change significantly during the roasting process. However, recent investigations showed that antioxidant properties, responsible for the majority of observed biological effects of carob flour, can too be significantly influenced during roasting (Sahin, Topuz, Pischetsrieder, & Ozdemir, 2009). Namely, certain phenolic compounds can degrade during roasting or they can become bound to the polymer structures; on the other hand, high temperatures can degrade certain polymer compounds releasing polyphenolic components to the matrix and making them available for absorption. Additionally, formation of certain Maillard reaction products (MRPs) that occurs during roasting can also significantly contribute to the increase of antioxidant activity – radical scavenging and reductive properties of fluorescent MRPs derived in different model systems has been proved by several authors (Morales & Jimenez-Perez, 2001; Vhangani & Van Wyk, 2013). However, in spite of their proven antioxidant activity, simulation of MRP formation during processing is not always acceptable since certain MRP classes also possess mutagenic, prooxidative and proinflamatoric effects (Goldin, Beckman, Schmidt, & Creager, 2006; Surh, & Tannennbaum, 1994; Wang, Qian, & Yao, 2011). Having in mind the fact that diet derived MRPs can be the major contributors to the body's MRP pool, as has been proven for AGEs (Uribarri et al., 2005), monitoring their formation and solubility during thermal processing of food is important, not only from the aspect of controlling food stability/quality but also from the aspect of their biological health-related properties. Therefore the balance between positive and negative effects should be taken into account.

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http://dx.doi.org/10.1016/j.lwt.2014.04.004
0023-6438/

The knowledge about the impact of carob processing (roasting) on the content of different bioactive compounds in the final product is very important considering some novel aspects of carob powder utilization. Namely, water extraction of the carob powder yields a product that contains mostly dietary fibre, as well as a phenolic fraction consisting of water-soluble and insoluble tannins, flavonol glycosides, and high contents of different forms of gallic acid (GA): free GA, gallotannins, and methyl gallate (Papagiannopoulos, Wollseifen, Mellenthin, Haber, & Galensa, 2004). Recent scientific investigations showed that carob pod extracts possess strong antioxidant activity (Kumazawa et al., 2002), modulates blood lipid profile in humans (Gruendel et al., 2007) reduces growth of different cell lines (Klenow, Glei, Haber, Owen, & Pool-Zobel, 2008) and modulates gene expression and protects colon adenoma cells from genotoxic impacts (Klenow, Jahns, Pool-Zobel, & Glei, 2009) making it an interesting raw material to be used in development of food additives, functional foods and dietary supplements. Commercially, such extracts are already being used as natural and safe antioxidants by the food industry (Bastida et al., 2009). The most of the observed biological effects of carob pod extract are associated with the high content of certain polyphenolic compounds (Avallone, Cosenza, Farina, Baraldi, & Baraldi, 2002; Corsi et al., 2002). Therefore, when optimizing the carob processing (roasting) conditions for such purposes, the targeted characteristics of the final product (carob powder) are not so much associated with the taste, colour or aroma as for the use in the traditional food industry but more with antioxidant activity and the content of bioactive compounds (polyphenols and Maillard reaction products).

Therefore, the main goal of our work was to propose the roasting conditions optimal for obtaining high polyphenol yields and strong antioxidant activity and at the same time the lowest possible formation of certain Maillard reaction products that are known for their toxic properties such as HMF, furfural, AGEs, etc.

Additionally, we used the simulation of gastrointestinal step for the extraction of targeted compounds to provide novel data on the solubility polyphenols and MRPs formed during carob roasting or their antioxidant activity after undergoing gastrointestinal digestion. Namely in order to exert their biological effects, positive or negative, bioactive food ingredients must be released from food matrix during the digestion process. Moreover, under gastrointestinal conditions the chemistry of released compounds can be additionally modified (Rufian-Henares & Delgado-Andrade, 2009) and they can differ significantly from those obtained by chemical extraction employed in most studies, in terms of both, quantity and biological activity.

2. Materials and methods

2.1. Instruments

The HPLC analysis was performed on an Agilent 1100 Series LC system (Agilent Technologies, Waldbronn, Germany). HPLC column was obtained from Waters, (Milford, MA, USA). Cellulose nitrate filters were from Sartorius (Goettingen, Germany) and Acrodisc GHP filters were from Gelman, (Ann Arbor, USA). Spectrophotometrical measurements were preformed on Unicam UVVIS spectrometer UV4, and fluorescence intensity was measured on Perkin Elmer 3000 spectrofluorometer. (Waltham, MA, USA). Shaking water bath was from GFL Gaselschaft Labortechnik, (Burgwedel, Germany) and centrifuge was from Thermo Fisher Scientific Inc. (Waltham, MA, USA).

2.2. Chemicals

Pancreatin, pepsin, bile salts, furfural, 6-hydroxy-2,5,7,8-tetra methylchroman-2-carboxylic acid (Trolox), 5-hydroxymethyl-2-furaldehyde (HMF), phosphate saline buffer and 2,2′-azobis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) were from Sigma (St. Louis, MO, USA). 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), Rhodamine B and amylase were from Fluka Chemicals (Buchs, Switzerland). Acetonitrile and formic acid were gradient grade for liquid chromatography (Merck, Darmstadt, Germany). Ultra pure water was obtained by Milli-Q Water Purification System (Merck Millipore, Billerica, MA, USA). All other chemicals were purchased from Kemika (Zagreb, Croatia).

2.3. Samples

Carob pods were obtained from the local grower. After removing the seeds, pods were kibbled, milled and roasted in drying oven at different time/temperature conditions: at 130 °C (5, 10, 15, 30 and 45 min); at 150 °C (5, 10, 15 and 30 min); at 165 °C (5, 10 and 15 min). Thickness of the carob powder layer during roasting was 0.5 cm. Longer roasting at higher temperatures was impossible due to partial carbonization and formation of undesirable organoleptic characteristics of carob powder. After roasting, samples were stored in dry containers at 4 °C until analysis.

2.4. Isolation of soluble and insoluble carob fraction

In order to assess antioxidant activity and soluble fraction of MRPs and polyphenols, simulation of *in vitro* digestion of carob samples was conducted according to previously described method (Vitali, Vedrina Dragojević, & Šebečić, 2008) with slight modifications. Shortly, during the simulation of gastric digestion 1.2 g of sample was dispersed in 19 mL of distilled water and 1 mL of pepsin (20 g/L in 0.02 mol/L HCl), pH was adjusted to 2 and the mixture was incubated at 37 °C for three hours in a shaking water bath at 100 oscillations/minute. For the simulation of intestinal digestion pH was adjusted to 7.5 using 60 g/L/$NaHCO_3$; 5 mL of NaCl/KCl solution (120 mmol/L NaCl and 5 mmol/L KCl) and 5 mL of the pancreatin-amylase-bile salt solution was added (12 g/L of bile salt, 2 g/L of pancreatin and 1 g/L of α-amylase in 0.1 M $NaHCO_3$) to digestion solutions, the volume of obtained solutions was adjusted to 30 mL using distilled water and simulation of intestinal digestion was continued for additional two hours. Digestive enzymes were then inactivated by heat treatment (5 min, 100 °C in boiling water bath), samples were cooled, and centrifuged at 4 °C and 3500 g for 40 min to separate digestible and indigestible fraction. Soluble MRPs and antioxidant activity were measured in obtained supernatants. Insoluble residue was submitted to 60 min reflux cooking; after cooling and centrifugation obtained aqueous supernatants were used for the estimation of insoluble MRPs, polyphenolic content and antioxidant activity. Solubility (%) was calculated as [(soluble content)/(soluble content + insoluble content)] × 100. Experimental design is schematically presented in Fig. 1.

2.5. Fluorescence and browning index determination

Browning index (BI) was determined spectophotometrically at 420 nm in adequately diluted extracts (1 g/L) obtained after enzymatic digestion (soluble) or water extraction of indigestible residue (insoluble). Results were expressed as the absorbance at 420 nm.

Determination of free fluorescent compounds was conducted in clear supernatants obtained after the simulation of gastrointestinal digestion (soluble) and after the extraction from indigestible carob fraction (insoluble) according to the method of Ferrer, Alegria, Farre, Clemente, and Calvo (2005). Prior to measurements, proteins were precipitated from sample solutions by addition of the same volume of 200 g/L trichloroacetic acid. Samples were vortexed, left at −4 °C for 30 min and centrifugated (3500 *g*, 4 °C,