LWT - Food Science and Technology 57 (2014) 1–8

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

Sucrose supplementation during traditional carob syrup processing affected its chemical characteristics and biological activities

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ARTICLE INFO

Article history:
Received 13 November 2013
Received in revised form
15 January 2014
Accepted 18 January 2014

Keywords:
Carob syrup
Cell cytotoxicity
Sucrose supplementation
Chromatographic analysis
Polyphenols

ABSTRACT

The addition of sucrose is optional during carob syrups traditional processing. In this work, the polyphenolic profiles of carob syrups supplemented (CSS) or not with sugar (CS) were analyzed using RP-HPLC–ESI-MS. Quantitative data showed that adding of sucrose significantly ($p < 0.05$) decreased the polyphenolic amounts of about 58.6%, from 4.5 ± 0.32 to 1.86 ± 0.26 mg/g of CS and CSS, respectively. Gallic acid was the most abundant compound in both syrups representing 60.93% (CS) and 69.26% (CSS) of the total phenolics. Moreover, the adding of sugar decreased ($p < 0.05$) the antiradical potentials of 28% and 74%, as determined, respectively, by the ABTS and DPPH assays. CS was found to exhibit stronger antibacterial and antifungal activities than CSS. *Bacillus cereus* was the most sensitive strain to the extracts with CMI ≈ 500 (CS) and 622 (CSS) μg/ml. Both syrup extracts were cytotoxic to human neuroblastoma (SH-SY5Y) and fibroblast (3T3) cell lines as well as to mouse embryonic stem cells (D3). Tumoral SH-SY5Y cells were the most susceptible to the extracts with IC50 $= 311.7 \pm 23.65$ (CS) and 390.6 ± 34.97 μg/ml (CSS). This study provides, for the first time, new analytical insights into traditionally made carob syrups and highlights the negative effect of sugar supplementation during processing.

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1. Introduction

The carob tree (*Ceratonia siliqua* L.) is a worldwide evergreen species. Particularly, the Mediterranean region has been one of its domestication centers (Sidina et al., 2009). Carob has been cultivated for millenaries not only as a forage crop or as food for human consumption but also to cure various diseases (Sidina et al., 2009). Recently, this species attracted great attention and became socio-economically important for its multipurpose uses (Sandolo, Coviello, Matricardi, & Alhaique, 2007). Pods and seeds are used as row material in food, pharmaceutical and cosmetic industries, especially for gum extraction (Barracosa, Osorio, & Cravador, 2007). Bark and leaves have been used in folkloric medicine as laxative, diuretic, antidiarrhoeal and for the treatment of gastroenteritis in lactating babies and children (Kivçak, Mert, & Ozturk, 2002). The experimental and clinical studies performed on *C. siliqua* showed that most of its pharmacological actions were attributed to the antioxidant activity, which scavenges free radicals and/or inhibits lipid peroxidation (Custodio et al., 2011).

Processed syrups are generally used for softening and conserving seasonal fruits or for the preparation of cakes, cookies and homemade confectionery. These food byproducts are widely consumed in Tunisia, known locally as “Rub”, especially during the cold periods of the year for its high energetic sugar content (Dhaouadi et al., 2011, 2013). This is also the case for many North African and Arabic countries where these fruit syrups are poured on cooked dough (“asseeda”) on specific occasions, such as the celebration of religious festivities e.g. the Muhamed Prophet’s birthday (FAO, 2004). In these countries, the main fruit derived syrup products are prepared from date, barbary-fig and carob pods. Optionally, sucrose is supplemented to the fruit juice to prepare sweet syrup and reduce water activity for conservative purposes. Despite the high sucrose content of the carob pods, sugar is sometimes added as a traditional practice.

During the last decades, food trends are focused on healthful products. Thus, many scientific reports attempted to emphasize the advantages and benefits of particular foods, especially traditional

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http://dx.doi.org/10.1016/j.lwt.2014.01.025

and folkloric ones. Accordingly, these nutritional products are more and more drawing local and international market tendencies. Nowadays, the consumption of fruit and vegetables is regarded as important and good for health. Indeed, recent scientific studies indicated that a high intake of fruit and vegetables is associated with reduced risk for a number of chronic and degenerative diseases (Mansouri, Embarekb, Kokkalouc, & Kefalasa, 2005). In this respect, the recent explosion of interest in phytochemicals is attributed to the potential health benefits of plant antioxidants, especially, polyphenols. These latters exhibit several biological activities acting as antimicrobials, anti-carcinogenic, anti-inflammatories, antivirals, anti-allergic and immune-stimulators (Parr & Bolwell, 2000). The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. In addition, they have a metal chelation potential (Atoui, Mansouri, Boskou, & Kefalas, 2005).

To our knowledge, there is no report available on chemical composition of carob syrups and their biological activities. Thus, the aim of this work was to investigate traditionally made carob syrups and determine the effect of sucrose addition, a common practice during processing, on some functional properties, particularly (i) polyphenolic quantitative and qualitative content, (ii) antioxidant and antimicrobial capacities, and (iii) cytotoxic potentials using tumoral as well as non-tumoral human and mouse cell lines. This study should bring new knowledge about the adding of sugar during carob syrup preparation in order to support or oppose to this practice.

2. Material and methods

2.1. Carob syrup preparation

Carob (*C. siliqua* L.) fruit samples were purchased from local markets. The fruits were immediately washed with water and dried in the oven for 30 min at 40 °C. The fruits are then stored in dry environment at room temperature until used. The carob syrup is prepared according to the traditional method used by Tunisian families (Fig. 1). Pods pulps were ground using a blender (MOLINEX A 327R1/APO-2210R) and filtration was achieved through cloth. The obtained final syrups were divided into two batches (50 g, each) and kept in dark bottles at room temperature similarly to traditional practice.

2.2. Polyphenols extraction

The syrup (10 g) was mixed and extracted with distilled water (1:3, w:v) in a sonicator apparatus for 30 min. Following a centrifugation at 10,000×*g* for 15 min, cold acetone (−20 °C) was added to the supernatant (ratio 7:3, v:v). The precipitate was discarded following a centrifugation at 12,000×*g* for 10 min. The supernatant was collected and concentrated using a rotary evaporator (60 °C). The extracts were then stored at −20 °C until use. Immediately before the *in vitro* or cellular assays, samples were sterile-filtered through 0.22 μm filters.

2.3. Total phenolic quantitative estimation

Total phenolic content (TPC) was quantified following the method previously described by Li, Wong, Cheng, and Chen (2008) with slight modifications. One hundred microliter of syrup extract (diluted 20 times in water) was mixed with 400 μl of 10% Folin–Ciocalteu reagent (Sigma–Aldrich, France). Following incubation for 15 min in the dark at room temperature, 500 μl of 7.5% sodium bicarbonate (Na_2CO_3) were added to the mixture. After incubation for 30 min, the absorbance at 765 nm was recorded and gallic acid (Sigma–Aldrich, France) was used as a standard. TPC was expressed as gallic acid equivalent (GAE) per 1 g of syrup.

2.4. HPLC-DAD–MS instrumentation and conditions

The different extracts were analyzed and quantitated using an Agilent LC1100 series (Agilent Technologies, Inc., Palo Alto, CA, USA) controlled by the Chemstation software and equipped with pump, autosampler, column oven and UV–vis diode array detector. The HPLC instrument was coupled to an Esquire 3000+ (BrukerDaltonics, GmbH, Germany) mass spectrometer equipped with an ESI source and ion-trap mass analyzer, and controlled by Esquire Control and Data Analysis software. Analytical assays were performed using Merck Lichrospher 100 RP-18 (5 μm, 250 × 4 mm) column. The mobile phase comprised 0.5% formic acid in water (A) and methanol (B). Phenolic compounds separation was achieved using the previously described liquid chromatography technique (Fattouch et al., 2007) with minor modifications. The solvent gradient started at 95% A and 5% B, reaching 35% B at 20 min, 50% B at 25 min, 95% B at 40 min, 5% B at 42 min, and 5 more minutes for re-equilibration. The flow rate was 0.5 ml/min, and the injection volume was 10 μl. Diode-array detection was set at 280, 320, 340 and 360 nm. Mass spectrometry operating conditions were optimized in order to sensitivity values. The ESI source was operated in negative mode to generate [M−H] ions using the following conditions: desolvation temperature at 250 °C and vaporizer temperature at 400 °C, dry gas (nitrogen) was set at 4.5 l/min, probe voltage 4.5 kV, fragmentor voltage 20 V. The MS data were acquired as full scan mass spectra at 50–800 *m*/*z* by using 200 ms for collection of the ions in the trap. Identification of the main compounds was performed by HPLC-DAD analysis, comparing the retention time, UV spectra and MS data of the peaks in the samples with those of authentic standards or data reported in the literature. Quantitation of *p*-coumaric acid, epigallocatechin gallate, epigallocatechin, kaempferol, catechin, syringic acid, quercetin, caffeic acid, gallic acid, catechin gallate, myricetin and cinnamic acid was performed using commercial standards (Sigma–Aldrich, France). The software Chemstation for LC 3D (Agilent Technologies Life Sciences and Chemical Analysis, Waldbronn, Germany) was used for quantitation purposes. Quantitative evaluation of the compounds was performed by means of a five points regression curve ($R^2 > 0.996$) in a concentration range between 25 μg/ml and 1.2 mg/ml, using external standards and evaluated at 280 nm, which is the monitoring wavelength.

2.5. In vitro antioxidant activity

2.5.1. DPPH assay

The DPPH free radical-scavenging activity of each sample was determined according to the method described by Yang et al. (2012) with slight modifications. Briefly, 50 μl of sample solution or ascorbic acid standard at different concentrations were added to 2 ml of 40 μM DPPH (Sigma–Aldrich, France) in methanol. The mixture was shaken vigorously and left to stand for 1 h at room temperature in the dark. The radical-scavenging activity was calculated as follows:

$$\text{Inhibition}(\%) = [1 - (\text{Ai} - \text{Aj})/\ \text{A0}] \times 100$$

where, A0 is the absorbance of the blank sample, Ai is the absorbance in the presence of the test compound at different concentrations and Aj is the absorbance of the blank reagent. The IC50 (concentration providing 50% inhibition) was calculated graphically

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