Food Chemistry 196 (2016) 925–934

Contents lists available at ScienceDirect

Food Chemistry

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

Stability of phenolic compounds, antioxidant activity and colour through natural sweeteners addition during storage of sour cherry puree

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ARTICLE INFO

Article history:
Received 19 June 2015
Received in revised form 4 October 2015
Accepted 6 October 2015
Available online 9 October 2015

Keywords:
LC–MS-QTof
UPLC–PDA–FL
Sucrose, palm sugar, erythritol, xylitol, steviol glycoside, Luo Han Kuo, inulin
Storage time

ABSTRACT

The aim of this study was to describe the changes in phenolic compounds, antioxidant activity and colour of sour cherry puree supplemented with different natural sweeteners (sucrose, palm sugar, erythritol, xylitol, steviol glycoside, Luo Han Kuo), and natural prebiotic (inulin). A total of 18 types of polyphenolic compounds were assessed in the following sour cherry puree by LC–MS-QTof analysis, before and after 6 months of storage at 4 °C and 30 °C. Total phenolics determined by UPLC–PDA–FL was 1179.6 mg/100 g dm. In samples with addition of sweeteners the content of phenolic compounds ranged from 1133.1 (puree with steviol glycoside) to 725.6 mg/100 g dm (puree with erythritol), and the content of these compounds strongly affected on antioxidant activity. After 6-month storage, protective effects of some additives (palm sugar, erythritol, steviol glycoside, xylitol and inulin) on the polyphenol content, especially on anthocyanins and consequently on colour, and antioxidant activity were noticed. The results showed that some natural sweeteners might be interesting from a nutritional as well as commercial and pharmaceutical perspective.

1. Introduction

Chronic non-communicable diseases such as cardiovascular disease, cancer, obesity, and diabetes are the leading causes of death around the world. Their occurrence is largely diet-dependent. Dietary habits, the number of meals per day, and diet composition can both prevent and increase the incidence of these diseases. Therefore, one way to effectively prevent them can be varied and balanced diet, in which fruit consumption will play an important role (Chung, Oh, & Lee, 2012).

Scientific reports have validated advantageous interaction between fruit consumption and prevention and treatment of obesity, diabetes or cardiovascular disease. Fruit are characterised by high content of polyphenolic compounds and high antioxidant activity. Furthermore, they stimulate insulin secretion, reduce blood glucose level, and lower blood pressure, serum cholesterol and triglycerides. One of attractive fruit, in terms of both sensory and health-promoting properties, is sour cherry (Damar & Ekşi, 2012; Nowicka & Wojdyło, 2015; Wojdyło, Figiel, Lech, Nowicka, & Oszmiański, 2014).

Sour cherry (*Prunus cerasus* L.) is a stone fruit commonly cultivated throughout the world. It is suitable for direct consumption, but makes also an excellent processing material. Sour cherry is a good source of phytochemicals that improve its quality and contribute to its nutritional value (Damar & Ekşi, 2012; Wojdyło, Nowicka, Laskowski, & Oszmiański, 2014). Among various bioactive compounds, phenolics are one of the main groups of phytochemicals present in sour cherry fruit that display a broad spectrum of health promoting benefits. The main fraction of polyphenols present in sour cherries are anthocyanins that impart red colour to the raw material (Damar & Ekşi, 2012; Kirakosyan, Seymour, Urcuyo Llanes, Kaufman, & Bolling, 2009). They exhibit antioxidant, anti-inflammatory, antibacterial, and antidiabetic properties (Halvorsen et al., 2002). Organic acids present in sour cherry fruit also exert a favourable impact on the human body. They stimulate secretion of digestive enzymes and regulate the course of chemical reactions in the body, and they also confer the sour taste of the fruit (Seymour et al., 2008).

Tart taste of sour cherry is not always accepted by consumers, and therefore sucrose is added during its industrial processing (Nowicka & Wojdyło, 2015). This way, there are different sour cherry products like wines, jams, juices or dried products with high content of sucrose and low levels of bioactive compounds (Damar & Ekşi, 2012; Kim & Padilla-Zakour, 2004). Therefore, they are not intended for people with chronic, non-communicable diseases, especially diabetes and obesity. In addition, greater consumer awareness concerning healthy eating, as well as its impact on

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http://dx.doi.org/10.1016/j.foodchem.2015.10.019
0308-8146/

proper functioning of the human body, mean that the consumers begin to look for healthy products with high nutritional value. As a consequence, the food industry is looking for new solutions to produce healthy and low-calorie products. A good idea to increase customer satisfaction may be replacing sucrose with other sweet substitutes (Nowicka & Wojdyło, 2015). There are many alternatives in the form of natural and synthetic sweeteners. They are characterised by different degree of sweetness, but mostly have lower energy value and much lower glycemic index than sucrose (Hossen et al., 2005; Ishak, Sapuan, Leman, Rahman, & Anwa, 2012; Koyama et al., 2003; Nowicka & Wojdyło, 2015). Our previous study (Nowicka & Wojdyło, 2015) showed that the use of different kinds of natural sweeteners in sour cherry puree had a positive effect on the sensory appeal of the final product. However, the study did not investigate their impact on either the polyphenol content or the antioxidant activity and colour of sour cherry products during storage.

Therefore, the aim of this study was to investigate how the addition of natural sweeteners (sucrose, palm sugar, xylitol, erythritol, steviol glycoside, Luo Han Kuo fruit) and natural prebiotic (inulin) affects the content of polyphenol compounds (anthocyanins, phenolic acid, flavonols and flavanols), antioxidant activity and colour of sour cherry puree (SCP), immediately after processing and after storing in different conditions (6 months at 4 °C and 30 °C).

2. Materials and methods

2.1. Chemicals

Quercetin and keampferol-3-*O*-glucoside, cyanidin-3-*O*-rutinoside, -3-*O*-glucoside, -3-*O*-sophoroside, peonidin-3-*O*-rutinoside, pelargonidin-3-*O*-glucoside, *p*-coumaric acid, (+)-catechin, and (−)-epicatechin, procyanidin B2 and C1 were purchased from Extrasynthese (Lyon Nord, France). Chlorogenic, neochlorogenic and cryptochlorogenic acids were supplied by TRANS MIT GmbH (Giessen, Germany). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), 2,2′-azinobis-(3-ethyl benzthiazoline-6-sulfonic acid) (ABTS), 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH), fluorescein disodium (FL), potassium persulfate, acetic acid, TPTZ (2,4,6-tripyridyl-1,3,5-triazine), $FeCl_3$, phloroglucinol, ascorbic acid, acetonitrile, and methanol were purchased from Sigma–Aldrich (Steinheim, Germany).

2.2. Plant material

Samples of sour cherry (*P. cerasus* L.) cv. 'Turgieniewka' were harvested at the Research Station for Cultivar Testing in Zybiszów near Wrocław (Poland), at processing maturity in June 2013. Then, the fruit were immediately de-pitted and processed.

2.3. Puree manufacturing

Pitted sour cherries were ground and heated at 80 °C in a Thermomix device (Vorwerk, Wuppertal, Germany) and mashed in a blender (Symbio, Zelmer, Rzeszów, Poland). To prevent enzymatic browning of the fruit, 10 mL of ascorbic acid solution (10%) per 1 kg of fruit were added to the sour cherry pulp.

After grounding and mashing, the puree samples (200 g) were mixed with natural sweeteners – sucrose (7%; in the crystals form, produced by the Diamant company in Poland), palm sugar (7%; in the crystals form, produced by Cock Brand Marque Deposee in Thailand), erythritol (7%; 100% erythritol in the powder form, produced by Intenson company in Poland), xylitol (7%; 100% xylitol in the powder form, produced by Danisco Sweeteners company in Finland), steviol glycosides (0.2%; 98.5% of steviol glycosides, produced by the PlantaDulce company), and Luo Han Kuo fruit (1%; produced in China, imported by Sin Wah Foods, Nederland, purchased in the form of dried fruit), and a natural prebiotic – inulin (7%; in the powder form, produced by SENSUS, Roosendaal, Nederland). The sweetness of SCP with sucrose was determined according to the preferences of consumers, but the doses of the other natural sweeteners were calculated on the basis of literature data (Hossen et al., 2005; Ishak et al., 2012; Koyama et al., 2003; Periche, Koutsidis, & Escriche, 2014; Nowicka & Wojdyło, 2015), so as to achieve the same intensity of sweet taste. Then, the products were heated up to 100 °C and put into glass jars, pasteurised for 10 min and finally cooled to 20 °C. As a result, eight different puree products were obtained. Each SCP sample was prepared in two replicates. The purees were subjected to analyses directly after processing and after 6 months of storage at 4 °C and 30 °C.

2.4. Identification of polyphenols by LC–PDA-MS method

The solvent for identification (LC/MS QTOF) and quantitative (UPLC–PDA–FL) analysis of polyphenols (anthocyanin, flavan-3-ol, flavonol, and phenolic acid) were performed as described previously by Wojdyło, Nowicka, et al. (2014). All measurements were repeated three times. The results were expressed as mg per 100 g dry matter (dm).

2.5. Analysis of procyanidins by phloroglucinolysis method

An analysis of polymeric procyanidins by phloroglucinol method was performed according to the protocol described previously by Kennedy and Jones (2001). All measurements were repeated three times. The results were expressed as mg per 100 g dm.

2.6. Determination of antioxidant activity

A solvent for the analysis of polyphenols was prepared as described previously by Wojdyło, Nowicka, et al. (2014). The ORAC, ABTS and FRAP assays were prepared as previously described by Ou, Huang, Hampsch-Woodill, Flanagan, and Deemer (2002), Re et al. (1999) and Benzie and Strain (1996), respectively. The antioxidant activity was expressed as millimoles of Trolox per 100 g dm. ORAC assay was carried out on RF-5301 PC spectrofluorometer (Shimadzu, Kyoto, Japan). Measurements by means of ABTS and FRAP methods involved UV-2401 PC spectrophotometer (Shimadzu, Kyoto, Japan).

2.7. Colour measurement

Colour properties (L^*, a^*, b^*) of SCP were determined using A5 Chroma-Meter (Minolta CR300, Osaka, Japan), referring to colour space CIE $L^*a^*b^*$. The colour coordinates of the samples were determined using Illuminant D65 and 10° observer angle, and the samples were measured against a white ceramic reference plate ($L^* = 93.92$; $a^* = 1.03$; $b^* = 0.52$). The data were mean of five measurements. Total change in SCP colour (ΔE^*) was calculated according to Wojdyło, Figiel, et al. (2014).

2.8. Statistical analysis

Statistical analysis was conducted using Statistica version 9.0 (StatSoft, Krakow, Poland). Significant differences ($p \leqslant 0.05$) between means were evaluated by one-way ANOVA and Duncan's multiple range test. Pearson's correlations were determined using Microsoft Excel 2010. All analyses were performed in triplicates.