LWT - Food Science and Technology 66 (2016) 252–259

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

Influence of processing conditions on procyanidin profiles and antioxidant capacity of chocolates: Optimization of dark chocolate manufacturing by response surface methodology

Mine Gültekin-Özgüven, İjlal Berktaş, Beraat Özçelik*

Department of Food Engineering, Faculty of Chemical and Metallurgical Engineering, Istanbul Technical University, Maslak, TR-34469, Istanbul, Turkey

ARTICLE INFO

Article history:
Received 8 May 2015
Received in revised form
15 October 2015
Accepted 19 October 2015
Available online 21 October 2015

Keywords:
Dark chocolate
Polyphenols
Process
Antioxidant
RSM

ABSTRACT

Dark chocolate is a good source of dietary flavonoids, mainly comprised of (+)-catechin, (−)-epicatechin, and their oligomeric and polymeric procyanidins. Nevertheless, flavonoid content and antioxidant capacity are affected during chocolate manufacturing. In this study, the influence of manufacturing process of dark chocolate, particularly, roasting of cocoa beans (115–135 °C), conching (60–80 °C), and alkalization treatment (pH 7–9) was studied and the process conditions were optimized via Response Surface Methodology (RSM). Validation of the model accomplished applying the conditions generated by RSM. Considering the data obtained from the model; chocolate manufacturing process, particularly increasing alkalization degree and roasting temperature significantly reduced phenolics and related antioxidant capacity ($p < 0.05$). Nevertheless, increasing conching temperature was insignificant ($p > 0.05$) since higher temperature leads to a shorter required processing time.

1. Introduction

Cocoa bean, and its derivative products (dark chocolate and cocoa powder) are rich polyphenol sources which have beneficial effects for health such as anti-ulcer, anti-carcinogenic, anti-thrombotic, anti-microbial effects and they exhibit antioxidant activity (Jonfia-Essien, West, Alderson, & Tucker, 2008). Cocoa bean polyphenols consist of catechins or flavan-3-ols (37%), proanthocyanidins (58%) and anthocyanins (4%) (Cooper et al., 2007; Miller et al., 2008; Wollgast & Anklam, 2000). However, phenolic content of cocoa beans depends on botanical variety, genetic and agronomical factors such as postharvest handling, fermentation and drying conditions (Tomas-Barberan et al., 2007).

Fermentation, drying, and roasting are essential for characteristic flavour development in cocoa beans. During fermentation, polyphenols undergo oxidation to condensed high molecular insoluble tannins catalysed by polyphenol oxidase enzyme. Then, cocoa beans are dried to decrease the moisture content below 8% which is the critical moisture content for mould growth (Wollgast & Anklam, 2000). Meanwhile, water activity of cocoa beans must be below 0.70 for microbiological stability (Sandoval & Barreiro, 2002). After drying, beans are roasted at 100–150 °C for maximum 120 min to develop further typical chocolate flavour. The final nib (broken beans) is ground to obtain “chocolate liquor” by releasing cocoa butter from the broken-down cell walls (Wollgast & Anklam, 2000).

Chocolate liquor and cocoa butter are the main ingredients of chocolate manufacturing with others such as sugar and emulsifiers. After mixing all ingredients in mixers, obtained chocolate paste is refined by roll refiners. For the final texture and flavour, refined chocolate paste is conched with varied temperature-time conditions. During conching, moisture is reduced, volatile substances are removed and all particles are dispersed in continuous fat phase. Meanwhile, phenolic content is expected to be reduced due to high temperature and oxygen in roasting and conching steps. Moreover, if alkalization treatment (Dutching) which relies on neutralization of acetic acid is present, phenolic content decreases sharply (McShea et al., 2008). In Dutching, colour and flavour are modified by raising pH from 5.5 up to 8.2 with potassium or sodium bicarbonate. Finally, melted chocolate is cooled by agitation in tempering step where small stable cocoa butter crystals occur and give glossy appearance, snap, good texture, bloom resistance and contraction to the chocolate (Afoakwa, Paterson, Fowler, & Vieira, 2008; Tanabe & Hofberger, 2006).

* Corresponding author.
E-mail address: ozcelik@itu.edu.tr (B. Özçelik).

http://dx.doi.org/10.1016/j.lwt.2015.10.047
0023-6438/

In the literature, some researchers studied phenolics and antioxidant capacity of cocoa beans (Arlorio et al., 2008; de Brito et al., 2000; Jonfia-Essien et al., 2008; Niemenak, Rohsius, Elwers, Omokolo Ndoumou, & Lieberei, 2006; Othman, Ismail, Ghani, & Adenan, 2007) cocoa powder (Lee, Kim, Lee, & Lee, 2003; Miller et al., 2008), different types of chocolates (Pimentel, Nitzke, Klipel, & de Jong, 2010; Sulistyowati & Misnawi, 2008; Wollgast & Anklam, 2000), and cocoa liquor (Jinap, Jamilah, & Nazamid, 2005). Considering their findings, it is possible to say that choosing cocoa beans rich in phenolics, reducing temperature and/or time in roasting or conching may conserve polyphenols.

On the other hand, there are *in vivo* antioxidant studies related to health benefits of chocolate consumption (Pearson et al., 2002; Rein et al., 2000; Schramm et al., 2001; Serafini et al., 2003). Finally, some studies about probiotic, prebiotic, and synbiotic chocolate development were performed (Cardarelli, Aragon-Alegro, Alegro, de Castro, & Saad et al., 2008; Erdem et al., 2014; Patel, Parekh, & Subhash, 2008). All those studies mentioned the functional properties of cocoa beans, cocoa powder and chocolate. Nevertheless, this study has focused on determination of optimum chocolate manufacturing conditions by using Response Surface Methodology (RSM) since possible modifications in processing conditions regarding roasting, conching and alkalization treatment may reduce the loss of phenolics and antioxidant capacity. To our knowledge, there is limited number of studies investigating the effect of industrial chocolate manufacturing on procyanidin profile, and antioxidant capacity. For this purpose, the aim of the present study was to investigate the changes in procyanidin profile, and antioxidant capacity during roasting, conching, alkalization treatment to optimize the chocolate manufacturing.

2. Materials and methods

2.1. Chemicals and reagents

Epicatechin (EC), epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), 2,2-azobis(2-amidinopropane) dihydrochloride (AAPH), fluorescein, Folin–Ciocalteu reagent were purchased from Sigma–Aldrich Chemie GmbH & Co. KG (Steinheim, Germany). Other chemicals and reagents were of analytical or high-performance liquid chromatography grade. To identify and quantify procyanidins, a composite procyanidin oligomer standard containing monomers through decamers was purified from cocoa beans.

2.2. Chocolate manufacturing

Ingredients such as roasted cocoa beans and alkalized chocolate liquor were provided from Altinmarka Inc. and dark chocolate samples were kindly produced by Nestle Turkey Inc. Recipe of dark chocolate contained cocoa liquor (43.20%), cocoa butter (5.50%), sugar (47.52%), soy lecithin (0.25%), milk fat (3.50%) and vanilla extract (0.03%). Cocoa liquor, sugar and vanilla extract were mixed in a mixer for 5 min. After pre-refining, the batch was fed to the five roller refiner lasting 3 h. After addition of cocoa butter and milk fat to the chocolate paste, conching was performed at 80 °C for 11 h, at 70 °C for 12 h, and at 60 °C for 13 h, respectively. As the last step of conching, lecithin is added to the mass and mixed for 30 min more. Then, chocolate paste was tempered with a continuous process heat exchanger to be ready for deposition. Finally, dark chocolate samples were cooled and demoulded. Chocolate samples were analysed in a week after manufacturing. Until then, they were stored in plastic bucket at room temperature.

2.3. Extraction of phenolics

The samples were extracted following the method described by Wollgast (2004). 1 g of milled samples were defatted twice with 10 ml n-hexane for 5 min in an ultrasonic bath at 30 °C and was subsequently centrifuged for 10 min at 3000× g. Polyphenols were extracted from the air-dried sample with 10 ml of a mixture of methanol and water (80:20 v/v) for 10 min at 30 °C in the ultrasonic bath.

2.4. Extraction of procyanidins

All defatted samples were dissolved in acetone: Milli-Q water: acetic acid (70: 29.5: 0.5, v/v/v) at a ratio of 1: 5. After vortexing for 5 min and centrifuged at 12,000 rpm, the supernatant was filtered through 0.45 μm syringe filters (Adamson et al., 1999).

2.5. Preparation of semi-purified oligomers from cocoa beans

A composite procyanidin oligomer standard containing monomers through decamers was purified from cocoa beans previously described by Adamson et al. (1999) and Gu, House, Wu, Ou, and Prior (2006). The fresh seeds were ground in a high-speed laboratory mill with liquid nitrogen until the particle size was reduced to approximately 90 μm. Lipids were removed from 220 g of the ground seeds by extracting with 1000 mL of hexane three times. The lipid free solids were air-dried to yield approximately 100 g of fat free material. A fraction containing procyanidins was obtained by extraction with 1000 mL of 70 vol% acetone in water. The suspension was centrifuged for 10 min at 1500 *g*. The acetone layer was decanted through a funnel with glass wool. The aqueous acetone was then re-extracted with hexane (75 mL) to remove residual lipids. The hexane layer was discarded, and the aqueous acetone was rotary evaporated under partial vacuum at 40 °C to a final volume of 200 mL. The aqueous extract was freeze-dried to yield approximately 19 g of acetone extract material (Adamson et al., 1999).

Approximately, 2 g of acetone extract was suspended in 10 mL of 70% aqueous methanol and centrifuged at 1500 *g*. The supernatant was semipurified on a Sephadex LH-20 column (6 × 1.5 cm) that had previously been equilibrated with 30% (v/v) aqueous methanol for over 4 h before use. After the sample had been loaded, the column was washed with 40 mL of 30% methanol/water to remove sugars and other phenols. Proanthocyanidins were recovered from the column by elution with 70 mL of 70% (v/v) aqueous acetone (Gu et al., 2006).

Approximately 0.7 g of semipurified acetone extract was dissolved in 7 mL of acetone/water/acetic acid in a ratio by volume of 70: 29.5: 0.5, respectively (Adamson et al., 1999). Semi-purified fractions were analysed by UFLC/MS–MS using the parameters described by Lazarus, Adamson, Hammerstone, and Schmitz (1999).

2.6. Total phenolic and flavonoid content

Total phenolic content (TPC) was measured using Folin–Ciocalteu assay (Wollgast, 2004) while total flavonoid content (TFC) was performed according to the method described by Lee et al. (2003). Results were expressed as mg of catechin equivalents (CE) per kg of defatted samples. All samples were analysed in triplicate.

2.7. UFLC-MS/MS analysis of catechins

Six major catechins were determined [(+)-catechin,