Influence of Supercritical Fluid Extract of *Cinnamomum zeylanicum* Bark on Physical, Bioactive and Sensory Properties of Innovative Cinnamaldehyde-Enriched Chocolates

MUHAMMAD AHMAR JAHANGIR1*, AAMIR SHEHZAD1, MASOOD SADIQ BUTT1 and MUHAMMAD SHAHID2

1National Institute of Food Science and Technology and 2Department of Biochemistry, University of Agriculture, Faisalabad

*Corresponding author: majahangir03@gmail.com

Abstract


The potential utility of cinnamaldehyde obtained from cinnamon (*Cinnamomum zeylanicum*) bark powder in enhancing the antioxidant capacity of dark chocolates was evaluated. To this end, the effects of conventional solvent extracts (CSE) and supercritical fluid extracts (SFE) on total phenolic contents, physical and sensory attributes of cinnamaldehyde-enriched chocolates were determined over the course of 60 days of storage. The addition of 0.5% nutraceutical SFE and 1% nutraceutical CSE extracts significantly increased the total phenolic contents of the chocolates to 15.62 ± 0.35 and 13.31 ± 0.26 mg GAE/g in T2 and T1, respectively. In terms of texture (hardness), 0.5% extract resulted in softer chocolates (64.87 ± 2.41 N) as compared to the control (70.91 ± 2.83 N). At storage termination, colorimetric results revealed a whitening of the chocolate surface that resulted in incremental increases in $L^*$ and $b^*$, whereas $a^*$ exhibited a declining trend. Moreover, the sensory results showed a better hedonic response for the enriched products. Taken together, cinnamaldehyde enrichment is beneficial for both the functional as well as oxidative stability of chocolates. We used cinnamaldehyde and maltitol (sugar replacer) to prepare functional chocolates with a special emphasis on diabetic patients, an approach that will open new horizons for innovative product development. We also optimised and characterised the extraction conditions for bioactive components using green extraction technology, i.e. a supercritical fluid extraction technique which is cost-effective and environmentally friendly.

Keywords: cinnamaldehyde; enrichment; oxidative stability; sensorial; maltitol

Supported by Higher Education Commission (HEC), Government of Pakistan
In chocolate manufacturing, the key ingredients include cocoa butter, cocoa solids, sugar and lecithin. Nevertheless, a wide range of products available on the market incorporate various ingredients like fruits, nuts and cereals. Cocoa solids, one of the major ingredients in chocolate products, are a rich source of polyphenols that provide protection to the vascular system and to health in general through their antioxidant activity (Thamke et al. 2009).

Cinnamon (Cinnamomum zeylanicum) belongs to the family Lauraceae and has been utilised as a potential therapeutic agent in various cultures for centuries. Historically, cinnamon bark is amongst the oldest known spices used against gastrointestinal complaints, chronic bronchitis and inflammation of the eyes; it has been used in ayurvedic medicine for over 6000 years (Sangal 2011). The German Commission E and the European Scientific Cooperative on Phytotherapy (ESCP) have approved two medicinal herbs of the genus Cinnamomum: C. zeylanicum, and C. cassia (European Scientific Cooperative on Phytotherapy 2003).

Cinnamaldehyde (3-phenyl-2-propanal) represents the main constituent of cinnamon bark oil and constitutes 49.9–62.8% of the total (Simi et al. 2004; El-Baroty et al. 2010). It provides protection against metabolic syndromes like cardiovascular complications and diabetes. It also improves the functionality of insulin receptors by virtue of activation of the enzyme (insulin receptor kinase) which is responsible for the binding of insulin to cells. Moreover, it is responsible for inhibiting the activity of the enzyme that impedes this process (insulin receptor phosphatase), ultimately leading to the maximum phosphorylation of insulin receptor, which is associated with improved insulin sensitivity. It also increases glucose tolerance by enhancing the activity of hexokinase and mobilising glycogen stores in the liver and skeletal muscle. Cinnamon polyphenols contribute to the regulation of various proteins like glucose transporter 4 (GLUT4), insulin receptor β and tristetraprolin involved in the insulin signal transduction pathway (Maheouz et al. 2010). Furthermore, it exhibits good antioxidant and hypolipidaemic properties, which reduce the concentration of total cholesterol and triglyceride and curb intestinal α-glycosidase activity (Al-Jamal 2009). To the best of our knowledge, insufficient research has been conducted with respect to polyphenol-enriched chocolates. The enrichment of cinnamaldehyde as an antioxidant from cinnamon bark at different concentrations enhances the bioactive profile of enriched chocolates and contributes to further improving the attractive flavour of the product. Consequently, the aim of this study was, for the first time, to evaluate the bioactive profile and hedonic response of chocolates enriched with cinnamaldehyde.

**MATERIAL AND METHODS**

**Procurement of raw material.** The dried cinnamon (Cinnamomum zeylanicum) bark was procured from a local market and was ground to obtain particle sizes in the range of 300–500 µm for further analysis. The cocoa liquor, cocoa butter, soy lecithin and maltitol were purchased from a local supplier. The reagents (analytical and HPLC grade) and standards were purchased from Merck KGaA (Germany) and Sigma-Aldrich (Japan).

**Conventional solvent extraction (CSE).** The extraction of cinnamaldehyde was performed using aqueous ethanol (50% v/v) as a solvent. Thirty grams of cinnamon (Cinnamomum zeylanicum) bark powder were poured into the solvent and extracted for 60 min under a constant temperature of 50°C following the guidelines of Mariod et al. (2010). The resulting solvent extracts were filtered and recovered using a rotary evaporator (Eyela, Japan).

**Supercritical fluid extraction (SFE).** Cinnamon powder was subjected to a supercritical fluid extraction system (SFT-150) to obtain supercritical fluid extracts using 99.8% pure CO2. Accordingly, samples were placed in a 100-ml extraction vessel followed by optimisation of CO2 at 5000 psi pressure while maintaining time and temperature conditions constant (Marongiu et al. 2007).

**Preparation of cinnamaldehyde-enriched chocolates.** In the product development steps, three types of functional chocolates were prepared. T1 contained nutraceutical CSE, whilst T2 was prepared using nutraceutical SFE supplementation. T0 acted as a control (Table 1). Briefly, to produce cinnamaldehyde-enriched chocolates, cocoa butter was pre-melted by heating at 60°C to achieve 25% total fat content and added to cocoa liquor, maltitol (sucrose alternative) and vanillin followed by mixing for 10 minutes. Afterwards, the chocolate mixtures were conched for 6 h with the addition of lecithin as an emulsifier at the end of the conching process. The resulting functional chocolates were stored at 18°C after tempering.
Data thus obtained were used to calculate chroma of 0, 15, 30, 45, and 60 days. Contents and sensory response at storage intervals were examined for physical parameters, phenolic addition of bioactive ingredient. The same procedure was followed only without the addition of bioactive ingredient.

Preparation of chocolate extract. Chocolate extracts were prepared using the protocols published by Belščak-Cvitanović et al. (2015). Frozen chocolate (2 g) was subjected to extraction three times with 10 ml of n-hexane in order to obtain fat free samples; this was followed by 24 h of air drying to remove residual organic solvents. Afterwards, extraction of phenolic compounds was performed using 10 ml aqueous methanol (70% v/v) for 30 min and the mixture was centrifuged at 3000 g for 10 minutes. The supernatants were removed, filtered and then combined to make 20 ml of extract.

Colour analysis. Colour is a critical discriminating feature of a given product and is used for rational selection by consumers. It also constitutes the basis of raw material assessment and quality product development. The colour of cinnamaldehyde-enriched chocolate was measured using a CIELAB colour system that includes \( L^* \) for lightness, \( a^* \) for redness, and \( b^* \) for yellowness. The mean \( L^* \) of chocolates are presented in Table 2. The \( L^* \) for control chocolates (T0), cinnamaldehyde-enriched chocolates with nutraceutical CSE (T1), and cinnamaldehyde-enriched chocolates with nutraceutical SFE (T2) were recorded as 21.19 \( \pm \) 0.57, 17.36 \( \pm \) 0.45, and 19.82 \( \pm \) 0.48, respectively. Storage resulted in a significant increase in this trait, from 18.69 \( \pm \) 0.58 (day 0) to 20.21 \( \pm \) 0.43 (day 60). Similarly, \( a^* \) were also significantly affected by treatments. The highest value for this trait, 8.48 \( \pm \) 0.23, was observed in T2 followed by 8.13 \( \pm \) 0.25 in T1. The lowest value, 7.78 \( \pm \) 0.19, was noted in T0. Over the course of storage, values for \( a^* \) decreased non-significantly, from 8.42 \( \pm \) 0.24 at the initiation of the study to 7.88 \( \pm \) 0.20 at its end (Table 2).

Texture analysis. The triple beam snap (three-point break) technique of a texture analyser (TA-HDi; Stable Microsystems, UK) was used to determine the texture of prepared chocolates. A penetration test was conducted by using a 2-mm cylinder probe with a load cell of 5 kg. The chocolate samples were penetrated with a crosshead speed of 0.5 mm/s at room temperature. Force (N) required for penetration was noted and average values were calculated according to the prescribed methods (Lara et al. 2010; Belščak-Cvitanović et al. 2012).

Determination of total phenolic contents (TPC). Total phenolic contents of all chocolates were estimated following the method of Lachman et al. (1998).

Sensory evaluation. The end products were evaluated with quantitative descriptive analysis by a panel of judges in accordance to ISO standards using a 9-point hedonic scale system as mentioned by Sim et al. (2016).

Statistical analysis. The resulting data were examined using completely randomised design (CRD) in Cohort v6.1 (Costat 2003). Moreover, significance was determined using analysis of variance (ANOVA) following the principles outlined by Steel et al. (1997).

RESULTS AND DISCUSSION

Colour. Colour is a critical discriminating feature of a given product and is used for rational selection by consumers. It also constitutes the basis of raw material assessment and quality product development.

Texture. The triple beam snap (three-point break) technique of a texture analyser (TA-HDi; Stable Microsystems, UK) was used to determine the texture of prepared chocolates. A penetration test was conducted by using a 2-mm cylinder probe with a load cell of 5 kg. The chocolate samples were penetrated with a crosshead speed of 0.5 mm/s at room temperature. Force (N) required for penetration was noted and average values were calculated according to the prescribed methods (Lara et al. 2010; Belščak-Cvitanović et al. 2012).

Determination of total phenolic contents (TPC). Total phenolic contents of all chocolates were estimated following the method of Lachman et al. (1998).

Sensory evaluation. The end products were evaluated with quantitative descriptive analysis by a panel of judges in accordance to ISO standards using a 9-point hedonic scale system as mentioned by Sim et al. (2016).

Statistical analysis. The resulting data were examined using completely randomised design (CRD) in Cohort v6.1 (Costat 2003). Moreover, significance was determined using analysis of variance (ANOVA) following the principles outlined by Steel et al. (1997).
The recorded values for $b^*$ were $8.89 \pm 0.32, 9.40 \pm 0.29$, and $9.93 \pm 0.44$, respectively at days 0, 30, and 60. Regarding chroma, substantial increases up to $11.99 \pm 0.45, 12.99 \pm 0.47$, and $13.08 \pm 0.63$ were detected in T1, T0, and T2, respectively (Table 2). In contrast, storage resulted in non-significant increase in chroma values at day 60 compared to day 0, i.e., $12.69 \pm 0.48$ vs. $12.26 \pm 0.42$. The hue angle underwent a significant increase in response to treatments. The highest value for this trait was noted in
T₀ (0.91 ± 0.022) followed by T₂ (0.85 ± 0.017), and T₁ (0.81 ± 0.013). Furthermore, at initiation and termination of the study, hue angle presented values of 0.81 ± 0.024 and 0.90 ± 0.021, respectively, that differed non-significantly from each other (Table 2). The findings are in agreement with the results of Rossini et al. (2011) who investigated the impact of synthetic and natural casein peptide antioxidants on non-enzymatic browning and lipid oxidation of milk chocolate stored at 20 and 28°C for 10 months. They observed a rise in the red colour intensity of milk chocolates as evidenced by increasing $a^*$. They also reported the negative effects of antioxidants on yellow colour development: $b^*$ in antioxidant-enriched chocolates increased to a lesser degree compared to samples without antioxidants.

The results regarding the functional/nutraceutical chocolates are also supported by the work of Briones and Aguilera (2005), who examined the effect of temperature fluctuations between 16 and 28°C on the colour of milk chocolate tablets over the course of 52 days of storage. They witnessed an increasing trend in the whiteness index (WI) and in $L^*$ after 36 days of storage. This change in colour might be due to the migration of fat to the chocolate surface resulting in a non-uniform colour pattern. Similarly, Afoakwa et al. (2008) observed a higher $L^*$ of 80.60 ± 1.26 in under-tempered dark chocolate compared with 43.43 ± 1.02 in over-tempered and 44.79 ± 1.16 in optimally tempered samples using the same 25-µm particle size. They were of the view that the tempering process results in the occurrence of blooming which affects the colour of dark chocolate samples during storage.

The colorimetric results of Nightingale et al. (2009) revealed the variations in colour, i.e., $L^*$, $a^*$, and $b^*$ in dark chocolate stored at various temperatures and degrees of relative humidity. They concluded that chocolates stored at high temperatures with fluctuations were substantially lighter in colour with a 70% increase in the whiteness index over the course of eight weeks of storage. Mexis et al. (2010) reported that chocolate packaged under a vacuum illustrated the smallest increase in the $L^*$, from 32.89 to 49.00, and that the highest increase, from 32.89 to 56.12, occurred in commercially packaged chocolates. They concluded that lightness in chocolates is related to the incidence of white spots on the surface during storage.

**Texture.** Means of the hardness of cinnamaldehyde-enriched chocolates in response to treatments and storage are shown in Figures 1 and 2, respectively. Amongst treatments, T₀ (control chocolate) exhibited the highest hardness of 70.91 ± 2.83 N followed by 67.20 ± 2.94 and 64.87 ± 2.41 N in cinnamaldehyde-enriched chocolate with nutraceutical$_{CSE}$ (T₁) and cinnamaldehyde-enriched chocolate with nutraceutical$_{SFE}$ (T₂), respectively. Nevertheless, during storage, there was a non-significant reduction in hardness which fell from 67.96 ± 2.65 N at the beginning to 67.62 ± 2.49 and 67.37 ± 2.36 N on days 30 and 60, respectively. Recently, Belščak-Cvitanović et al. (2015) investigated the influence of natural sweeteners on the texture and particle size distribution of chocolate. They showed that a combination of fructose, maltitol and stevia leaves leads to increased hardness (86.60 ± 0.06 N) as compared to control (56.82 ± 8.63 N) chocolate, which was attributed to an increased particle size median. Moreover, these...
authors suggested that the particle size of the mixtures significantly affected the hardness of chocolates.

Previously, Ali et al. (2001) reported that the increased migration of the palm mid fraction (PMF) into the chocolate layer stored at higher temperatures causes polymorphic transformation and blooming. Chocolates stored at 18°C exhibited very slow PMF and DCN migration, which resulted in minimal changes in glossiness, hardness, and polymorphic stability. The current findings are also in agreement with the work of Do et al. (2007) who estimated the effect of particle size distribution on the textural properties of chocolate. They reported optimising PSD and fat content resulted in a reduction in hardness from 55 to 42 N. Mechanistically, a decline in hardness is due to inadequate contact between particles in the suspension. In a more recent study, Lee et al. (2009) replaced cocoa butter with β-glucan-rich hydrocolloid and evaluated its impact on the rheological and textural properties of chocolate. The results revealed that the addition of 15% C-trim30 (β-glucan-rich hydrocolloid) resulted in hardness dropping to 27 N compared to 40 N in control. Replacement of cocoa butter with β-glucan hydrocolloid influences the tempering process which results in soft-textured chocolates.

The current results relating to the cinnamaldehyde enrichment of dark chocolates are in harmony with the findings of Belščak-Cvitanović et al. (2012) who evaluated the enrichment of 1 and 3% concentrated and 1% freeze-dried red raspberry leaf extract. They found the lowest hardness of 13.06 ± 0.31 N for chocolates enriched with freeze-dried extract, whereas, amongst dark chocolates, addition of 3% concentrated extract resulted in an increased hardness of 37.08 ± 0.41 N. Furthermore, they concluded that the addition of red raspberry leaf extract significantly reduced the hardness of chocolates.

**Total phenolic contents (TPC).** It is evident from Figure 3 that the means for total phenolic contents (TPC) of cinnamaldehyde-enriched chocolates showed the highest value of 15.62 ± 0.35 mg GAE/g in T2 (cinnamaldehyde-enriched chocolate with nutraceutical CSE) followed by 13.31 ± 0.26 mg GAE/g in T1 (cinnamaldehyde-enriched chocolate with nutraceutical CSE), while the lowest TPC value of 9.86 ± 0.29 mg GAE/g was measured in control chocolate (T0). Likewise, during storage, the values for TPC varied from 13.27 ± 0.33 mg GAE/g (day 0) to 12.56 ± 0.22 mg GAE/g (day 60) representing a significant decrease (Figure 4). The increases in TPC values caused by cinnamaldehyde enrichment are in agreement with the results of Sim et al. (2016) who assessed the rise in total phenolic contents in response to the addition of mangosteen pericarp powder in dark (DC) and compound chocolates (CC). The research findings showed 13 and 50% increases in DC3% and CC3%, respectively, for this trait when compared with their control counterparts.

Komes et al. (2013) determined the polyphenolic contents and antioxidant capacity of milk and bitter chocolates enriched with various dried fruits. Their findings are in agreement with ours and they discovered bitter chocolate enriched with the acetone extract of dried prunes evinced higher total phe-nolic contents (6.08 mg GAE/g) than plain chocolate (4.60 mg GAE/g). Belščak-Cvitanović et al. (2012) described the impact of *Rubus idaeus* L. polyphenol...
enrichment on semisweet chocolate, milk chocolate and dark chocolate. They found that enrichment with 3% concentrated red raspberry extract increased the total phenolic contents (TPC) from 0.64 mg GAE/g in milk chocolate to 1.79 mg GAE/g in semisweet chocolates.

Cervellati et al. (2008) described total phenolic contents of 16 ± 1 in artisan-made rosemary flavoured chocolate and 13.2 ± 0.8 mg GAE/g multinational-made chocolates. This was due to the presence of rich antioxidant compounds in rosemary, i.e., carnosic acid, rosmarinic acid and carnosol. Miller et al. (2006) reported a polyphenol level of 12.97 mg GAE/g in commercially available dark chocolate in the United States. In another investigation, reduced sugar chocolates were produced by incorporating sugar alcohols, syrups, dietary fibres and natural sweeteners. The results indicated that amongst reduced sugar chocolates, the highest TPC (11.27 mg GAE/g) was mainly attributable to the presence of peppermint and stevia leaves (Belščak-Cvitanović et al. 2015).

**Sensory evaluation.** Hedonic response is critical for interpreting consumer response to a product and for determining acceptance and marketability. With this in mind, the functional/nutraceutical chocolates were evaluated for various sensory attributes including appearance, texture, aroma, mouthfeel, aftertaste, and overall acceptability.

Figure 5 presents the sensory evaluation of cinnamaldehyde-enriched chocolates as a function of treatments and storage. It is obvious from Figure 5A that treatments resulted in significant variations in appearance, aroma, mouthfeel, and aftertaste, whilst texture and overall acceptability differed non-significantly. Cinnamaldehyde-enriched chocolate containing 0.5% nutraceutical<sub>SFE</sub> (T<sub>2</sub>) scored higher for appearance, aroma and mouthfeel followed by T<sub>1</sub> (cinnamaldehyde-enriched chocolate with 1% nutraceutical<sub>CSE</sub>) and the T<sub>0</sub> control (without active ingredient). However, the addition of 0.5% and 1% extract negatively altered the aftertaste of chocolate with maximum scores attained by T<sub>1</sub> followed by T<sub>2</sub> and T<sub>0</sub> which got lowest scores for this trait. During storage, all the sensory attributes changed non-significantly except for the mouthfeel of enriched chocolates, which exhibited a significant decline in sensory acceptance for this trait when comparing initiation and termination of storage (Figure 5B).

The current findings relating to the hedonic response of cinnamaldehyde-enriched chocolate are in agreement with the results of Albak and Tekin (2015) who conducted a sensory trial of cinnamon chocolate to evaluate its taste, smell and textural profile. They found significant variations for bitterness, coarseness, thickness and hardness, whilst the overall acceptability of cinnamon chocolate differed non-significantly revealing a high acceptance of the expert panellists of the new product. In a recent investigation, Sim et al. (2016) determined the effect of mangosteen pericarp powder enrichment on sensory and nutritional properties of chocolates. They reported non-significant differences in sensory attributes including sweetness, bitterness, flavour, and aftertaste between plain and enriched chocolates.

Similar findings were documented by Belščak-Cvitanović et al. (2012), which support the sen-

![Figure 5](image-url)

**Figure 5.** Effect of (A) treatments and (B) days of storage on the sensory attributes of chocolates
sory profiling results described here. These authors prepared dark chocolates enriched with freeze-dried (1%) and concentrated (1 and 3%) raspberry leaf extract and evaluated their hedonic response. The dark chocolates enriched with freeze-dried and 1% concentrated extract obtained the highest scores for appearance and texture, whilst chocolates containing 3% extract exhibited the lowest sensory scores for these properties. Likewise, mouthfeel and aftertaste also differed significantly in response to the treatments; the highest scores were observed for chocolates enriched with freeze-dried extract as the low temperature and pressure involved enable a preservation of aroma and taste.

CONCLUSIONS

In the present study, enrichment with a bioactive ingredient, i.e., cinnamaldehyde, imparted significant changes to various sensory attributes (appearance, aroma, mouthfeel, and aftertaste) of developed chocolates. These effects might be due to its intense aroma and strong antioxidant, colouring and flavouring properties. Furthermore, cinnamaldehyde enrichment proved to be efficient during storage and did not exert any detrimental effects on the resulting functional/nutraceutical chocolates. It is worth mentioning that during sensory profiling, all the chocolates attained acceptable scores and achieved superior hedonic responses.

References


Received: 2016–06–22
Accepted after corrections: 2017–07–19
Published online: 2018–01–05