Development of Functional Chocolate with Spices and Lemon Peel Powder by using Response Surface Method: Development of Functional Chocolate

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ABSTRACT

Functional foods are a new category of products that promise consumers improvements in targeted physiological functions. Aniseed, ginger and lemon peel powders which are known to have some antimicrobial effects, have been added to the chocolate mix prior to conching. The amount of additive was determined by an experimental design in response surface methodology. After optimization of additive, dark chocolate mass was conched with these additives. The effect of these additives on the moisture, color, melting properties, and total polyphenol content of dark chocolate was investigated. Variation in the moisture and color was observed to be highest in the cinnamon-chocolate. Total polyphenol content of dark chocolate (with no additive), aniseed, ginger, cinnamon and lemon chocolate were measured to be 283.3, 284.8, 284.6, 287.6 and 283.2 mg GAE/L while their melting points were 25.26, 24.71, 25.81 24.58 and 24.57 respectively. As seen, ginger, cinnamon and aniseed powders increased the total polyphenol content while they decreased the melting properties of dark chocolate after conching.

Key Words: Dark chocolate, Ginger, Aniseed, Cinnamon, Lemon peel powders.

Yüzey Tepki Metodu Kullanılarak Baharat ve Limon Kabuğu Tozuyla Foksiyonel Çikolata Tasarımı

ÖZET

Foksiyonel gıdalar fizyolojik gelişmeyi hedefleyen yeni bir ürün kategorisidir. Antimikrobiyel etkisi bilinen anoson, zencefil, tarçın ve limon kabuğu konçlama işleminden önce çikolataya eklenmiştir. Bu maddelerin mikaları yüzey tepki metodunda bir deney tasarımı ile belirlenmiştir. Optimizasyondan sonra çikolata kütlesi bu katkı maddeleriyle birlikte konçlanmıştır. Bu katkı maddelerinin nem, renk, erime noktası ve toplam polifenol üzerindeki etkisi araştırılmıştır. Çikolatanın renginde ve neminde en fazla değişime tarçın neden olmuştur. Toplam polifenol miktarı kontrol numunesinde (bitter çikolata), anasonlu, zencefilli, tarçınlı ve limon kabuğu tozlu çikolatada sırasıyla 283.3, 284.8, 284.6, 287.6 ve 283.2 mg GAE/L olarak ölçülürken erime noktası 25.26, 24.71, 25.81, 24.58 ve 24.57 ölçülmüştür. Zencefil, tarçın ve anason çikolatanın toplam polifenol miktarını artırırken erime noktasında düşüşe neden olmuştur.

Anahtar Kelimeler: Bitter çikolata, Zencefil, Anason, Tarçın, Limon kabuğu tozu.

INTRODUCTION

Functional foods differ from conventional foods in several ways. Firstly, conventional 'healthy' foods are typically presented as types of foods contributing to a healthy diet, e.g. low-fat products, high-fibre products, or vegetables, without emphasizing the role of any single product. In functional foods, particular components are

directly connected with well-defined physiological effects and the health benefit is linked to a single product [1]. Usually scientifically proved substantiation about the health effect is required when manufacturers develop specific, functional products. This is one reason why the development and marketing of functional foods is expensive and exceptionally risky.

Secondly, functionality creates a novelty aspect on the food without necessarily changing the sensory quality of the product. Consumers have to trust the information concerning the functional effect as the functional and conventional product can appear to be identical when used. The base product in which the health effect is added can affect the credibility, too [2].

Thirdly, the manufacture of functional foods often requires modern food technology since a constituent needs to be added, removed or modified. This means that there is a risk that functional products are perceived as being less natural than conventional products and are thus avoided by those who value naturalness in food choices [3].

The consumption of functional foods has increased due to the tendency of consumer choices towards healthier products. Since chocolate is known to be one the mostly consumed foods, it seems to be natural to make it as "functional" as possible. For this purpose, various spices such as aniseed, ginger, and lemon peel powders, which are known to have some antimicrobial activity, were added to the dark chocolate prior to conching to see their effect on some quality parameters like moisture, color, melting point, and total polyphenol content. A detailed literature survey revealed that there was no sufficient work relevant with the concept. The present study was, therefore, carried out to fill this gap.

Nattress et al. [4] studied influence of hazelnut paste on the sensory properties and shelf life of dark chocolate. They concluded that, addition of hazel nut paste increased the shelf life and positively affected the sensory properties of milk chocolate. Vieira et al. [5]

investigated the impact of limonene on the physical properties of milk chocolate with reduced fat. They found that it decreased its viscosity and hardness. Chemical and sensory evaluation of dark chocolate with the addition of quinoa was studied by Schumacher et al. [6]. They concluded that quinoa addition increased its protein content. In a PhD thesis by Özgen [7] the effect of rosemary and grape seed particles on crystallization, rheological, shelf life and antioxidant activities of chocolate was studied. They found that there was no effect of these additives on the crystallization and rheological properties of chocolate while they increased its shelf life and antioxidant capacity. In a PhD thesis by Çağındı [8] some physical, chemical and sensory properties of white and dark chocolates with enriched milk, sunflower seed, flax seed, oat and dried damson plum particle was investigated. It was concluded that these ingredients increased the nutritional value of chocolate and changed some physical, chemical and sensory properties during the storage period.

MATERIALS and METHOD

Conching Process

Refined cocoa mass, sugar, lecithin and cocoa butter were supplied from a local plant in Gaziantep. Aniseed, cinnamon and ginger were purschased from a local store in Gaziantep. Aniseed, ginger, cinnamon and lemon peel, which were added at the beginning of conching process, were dried, powdered and screened to 20μm mesh prior to conching.

A 5 kg-capacity, laboratory-scale conching machine (ELK'olino single-shaft conche, Bühler, AG, Switzerland) with temperature and speed control was utilized. The process was composed of three phases; dry phase: 2 hours at 50°C, pasty phase: 4 hours at 80°C and final phase: 1 hour with linear decrease in temperature from 80°C to 45°C. The rotation speed of the conching machine was in the range of 600-1500 rpm. The formulations of enriched and dark chocolates are shown in Table1.

Table 1. Formulations of enriched and dark chocolates

Ingredients (%)	Dark chocolate	Aniseed Chocolate	Ginger chocolate	Cinnamon chocolate	Lemon peel chocolate
Cocoa mass	45.07	43.47	42.57	40.87	43.07
Sugar	39.14	39.14	39.14	39.14	39.14
Added cocoa butter	15.45	15.45	15.45	15.45	15.45
Lecithin	0.34	0.34	0.34	0.34	0.34
Additive	-	1.6	2.5	4.2	2

Composition Analysis

The moisture, fat, protein and ash contents were analyzed according to the standard methods [9].The percent carbohydrate was then determined by difference. The analyses were carried out in triplicate and the values were averaged. The composition of chocolate with and without additives is shown in Table 2.

Optimization of Amount of Additives

An experimental design was performed in accordance with response surface methodology. D-optimal design feature of Design-Expert version 6.01.0 (Stat- Ease, Inc Minneapolis, MN) was used to observe the effects of the process variable (amount of additive) over change in viscosity, total color, moisture and hardness of dark chocolate.

The data were fitted to a second order polynomial. The significance of terms in the model was found by analysis of variance (ANOVA) for each response. Significance was judged by determining the probability level.

Response surface methodology was used to determine optimum condition that yield minimum viscosity, color, hardness and moisture change.

Table 2. Composition of dark chocolate with and without additives (%)

Chocolate type	Moisture	Fat	Protein	Ash	Carbohydrates
Dark chocolate	0.52±0.09	34.25±0.29	8.42±0.33	1.73±0.07	55.08
Cinnamon chocolate	0.54±0.05	30.70±0.21	8.73±0.4	2.15±0.09	57.80
Aniseed chocolate	0.96±0.34	35.16±0.21	9.47±0.48	1.92±0.005	52.49
Ginger chocolate	0.66±0.18	34.71±0.35	9.14±0.06	1.92±0.02	53.57
Lemon chocolate	0.54±0.02	33.45±0.15	9.16±0.09	1.92±0.09	54.93

Viscosity Determinations

Rheological behaviors of the samples were characterized using a RheoStress RS1 (Haake) controlled stress rheometer equipped with a TCP/P peltier temperature controller unit and a thermostat. A cone and plate configuration with 3.5cm diameter and $2°$ angle was used. The shear rate range was 0–300 s^{-1}.

All samples were incubated at 50°C for 75 minutes for complete melting. Shear stress was measured at 40 °C as a function of shear rate from 5 to 300 s^{-1}. 60 measurements were taken in 300 s. The mean value and standard deviation of the duplicate readings were recorded.

Casson plastic viscosity and Casson yield values were calculated from the data by interpolation using ThermoHaake RheoWin Pro Data Manager, Version 2.64 Copyright 1997software.

Texture Measurements

The hardness of solid chocolate samples was measured by a TA-XT plus Texture Analyser with a penetration probe (needle P/6) attached to an extension bar. The maximum penetration force through the samples with 4.5 cm diameter and 6 mm thickness was determined as triplicates at a pre-speed of 3 mm/s, a test speed of 1mm/s, post-speed of 10 mm/s, and trigger force of 1g, penetrating 1mm at room temperature. The mean values was converted into

hardness data using TEE 32 Exponent Microsystem Version 4.09.0 (2007) Software.

Differential Scanning Calorimetry

Differential scanning calorimeter (Perkin Elmer FC100 ped2 27603) was used for the determination of melting point of cinnamon chocolate. A- 9.36 mg sample was placed into a pan, which was sealed with lids using a sample press. The pans were heated from -5°C to 65°C in a N_2 stream. The onset temperature (T_{onset}), peak temperature (T_{peak}), and the end temperature (T_{end}) were calculated automatically by the software (Pyris software for windows version 7).

Moisture Determination

Moisture was determined in a drying oven (W.C. Heraeus Hanau; RT 500 (Hanau, Germany) at 105°C for 6 hours until a constant weight obtained [10].

Color Determination

Variation in color was followed with Hunter Lab Colorimeter (Colorflex /A60-1010-615 Model Calorimeter, Reston, VA) in terms of L, a, b values as measures of lightness, redness and yellowness, respectively. The equipment was calibrated with a white tile standard (L=93.01, a=-1.10, b=1.29). For each sample, three measurements were taken and averaged. The results were expressed as total color difference (ΔE) between the reference (dark chocolate) and samples according to the following equation:

$$\Delta E = \sqrt{(L_{sample} - L_{dark})^2 + (a_{sample} - a_{dark})^2 + (b_{sample} - b_{dark})^2}$$

* dark: dark chocolate as reference sample

Melting Point Determination

A differential scanning calorimeter (Perkin Elmer FC100 ped2 27603) was used for the determination of melting points of the chocolate samples. 9 mg of each sample was placed into a pan, which was sealed with lids using a sample press. The pans were heated from -5°C to 65°C in a N_2 stream. The onset temperature (T_{onset}), peak temperature (T_{peak}), and the end temperature (T_{end})

were calculated automatically by the software (Pyris software for windows version 7).

Total Polyphenol Determination

Total polyphenol was determined spectrophotometrically using the modification method of Singleton and Rossi (1965). 250 milligrams of defatted chocolate was solved in 40 ml of 80% acetone solution. The solution was sonicated in a beaker fitted inside an ultrasound bath

(Model B-2200 E4 Blason, Power output 60W, Frequency 47 Hz, Danbury, CT) for 30 minutes at 0℃. Sonication was preferred over shearing as an aid in solubilizing polyphenol since shearing promotes browning of the polyphenol extract by oxidation**.** The solution was then filtered through Whatman no.1 filter paper under vacuum and thus a clear solution was obtained. The residue and Erlenmeyer were washed with 80% acetone solution and total volume was completed to 100 ml. 1 ml of the extract was placed in a flask and diluted with 70 ml of distilled water. 5 ml of 2N Folin-Ciocalteau's reagent was added and kept for 2 minutes. 15 ml of saturated Na_2CO_3 solution was then added to stabilize the color within 2 hours. The absorbance was measured at 760 nm spectrophotometrically. Gallic acid of nine known concentrations, ranging from 1 to 9 mg/L, was used as the standard curve. The results were expressed as mg of gallic acid equivalent per liter (GAE/L).

RESULTS and DISCUSSION

Optimization of Amount of Additives

D-optimal design was used to optimize of amount of additives which was used in dark chocolate. For this purpose change in the four parameters, describing the chocolate quality, (moisture, viscosity, hardness and total color) was followed depending on additive amount. These data was assessed in response surface methodology and found optimum amount of additive without modifying dark chocolate. Optimum amount of additives was shown in Figure 1 and Table 1.
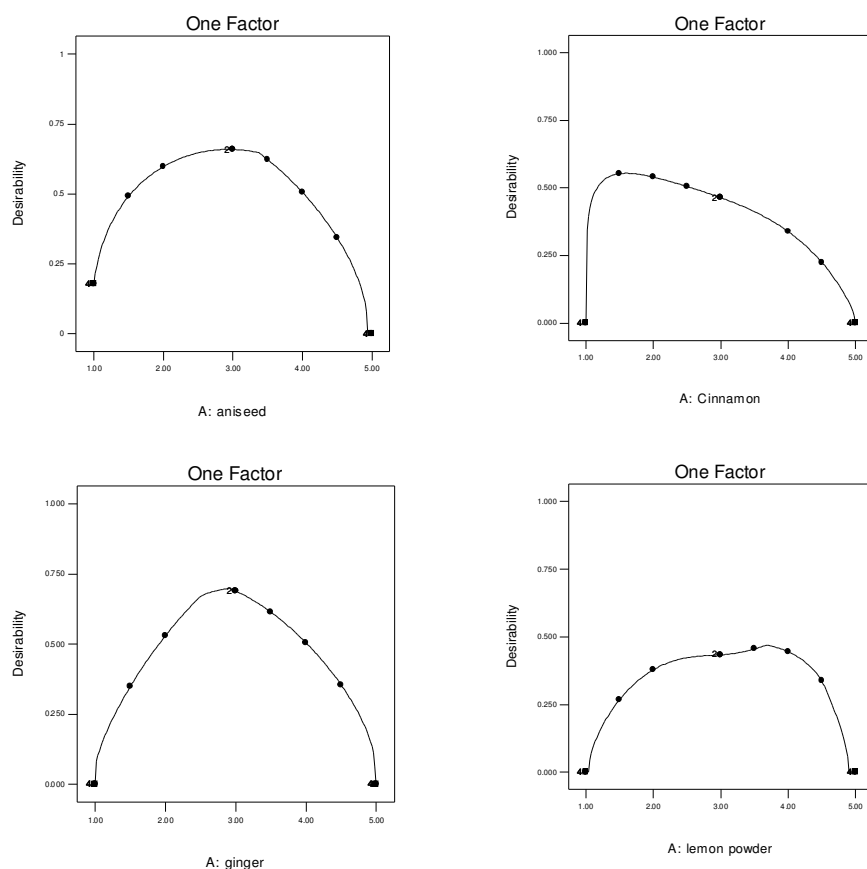

Figure 1. Optimization of additive by using D-optimal desing

In this study, the impact of aniseed, cinnamon ginger, and lemon peel powders on some quality parameters such as moisture, color, melting properties and total polyphenol content of dark chocolate was investigated. Dark chocolate was used as the reference sample. Chocolate moisture generally changes in the range of 0.5-1.5%. Above these values, difficulties in chocolate flow are observed because of sugar agglomeration leading to gritty lumps and increasing the apparent viscosity. As shown in Figure 2, aniseed chocolate caused the highest increase in the moisture content. Chevalley [11] stated that increase in the chocolate moisture causes an increase in its viscosity and the yield value.
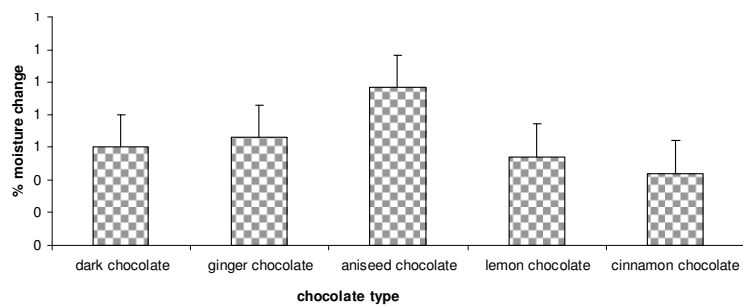
Figure 2. Moisture change of enriched chocolates and dark chocolate

The moisture content of aniseed, ginger, cinnamon and lemon peel powders were determined to be 9.81, 9.21, 9.43 and 11.37 % in wet basis, respectively. In spite of its higher moisture content, lemon peel powder did not affect the chocolate's moisture content as other powders. This may be attributed to the lower fat content of lemon peel compared to aniseed powder. Figure 2 shows that the moisture content of ginger and lemon chocolate is close to that of the dark chocolate.

Color is an important attribute to the food industry. Consumers frequently look at a product and make a judgment decision largely based on overall appearance including color. A suitable color model is necessary to fully describe the visual appearance of a food material. CIE L*, a*, b* scale are commonly used for determination of color difference in food industry. This system is dependent on the measurement of $L, a,$ and b. The L value represents lightness and change from 0 (black) to 100 (white). The value a change from $-a$ (greenness) to $+a$(redness) while b values is from $-b$ (blueness) to $+b$ (yellowness). ΔE is an equally weighed combination of the coordinate (L^*, a^*, b^*) differences. It represents the magnitude of difference in color but does not indicate direction of color. The total color change of chocolate with the mentioned additives is shown in Figure 3.
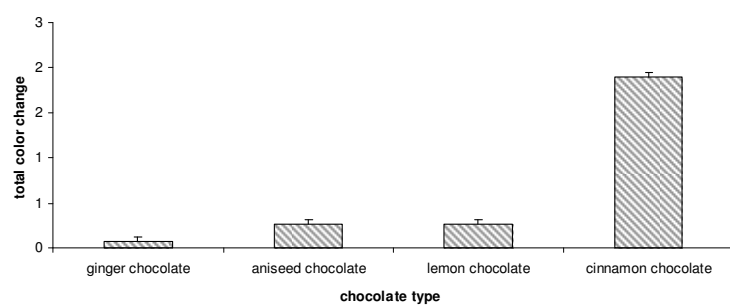

Figure 3. Total color change of enriched chocolate with additives

Dark chocolate was used as a reference in calculating of total color variation. The most significant color change occurred in cinnamon chocolate due to color and amount of these additives. It is seen in Figure 3 that, ginger did not affect the color of dark chocolate appreciably.

The thermal behavior of chocolate, with additives in question, was studied in DSC in order to see their effect on the melting point characteristics. Figure 4 shows a typical DSC melting curve for dark chocolate with and without additives. It is seen that, the fat melting profile corresponding to each additive is different. It is known that, the peak temperature for melting is the average of melting point of chocolate and the onset of melt indicates the time when the fat just starts to melt.

Melting properties of chocolate are important because of their contribution to the taste and sensation in the mouth. Marangoni, and McGauley [12] stated that the structure of fat in a food product is an important property that strongly influence its perceived mechanical and melting properties. The melting point was observed to be 25.26 °C for the control sample (dark chocolate without additive) while it was 24.71, 25.81 24.58 and 24.57°C for aniseed, lemon, cinnamon and ginger chocolate respectively. These results show that, ginger and aniseed affected the melting point of the dark chocolate within the proportion they added. Especially ginger was observed to be more effective in increasing the melting point in comparison with lemon peel and aniseed powders probably due to its higher fiber content.

(a) aniseed-dark chocolate

(b) ginger-dark chocolate

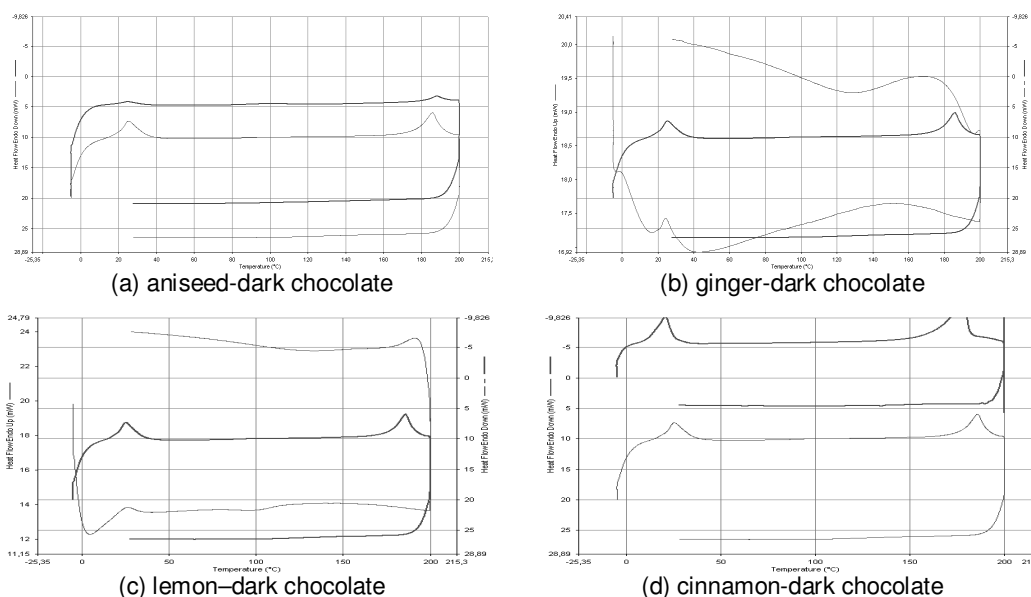(c) lemon–dark chocolate

(d) cinnamon-dark chocolate

Figure 4. DSC thermograms showing the fat melting profiles of dark chocolate with and without additives for each case

Table 3. Melting properties of dark chocolate with and without additives

Chocolate type	T_0 (°C)	T_{end} (°C)	T_{peak} (°C)	ΔH_{melt}(J/g)
Dark chocolate	19.29±0.07	34.13±0.08	25.26±0.05	20.66±0.04
Aniseed chocolate	17.36±0.08	34.32±0.09	24.71±0.1	19.74±0.05
Lemon chocolate	19.81±0.2	33.75±0.5	24.57±0.06	19.68±0.07
Ginger chocolate	20.59±0.3	31.13±0.6	25.81±0.05	19.79±0.04
Cinnamon chocolate	17.47±0.04	27.89±0.05	24.89±0.06	20.07±0.07

Polyphenols are known to affect the color of plant-based foods and to increase their antioxidant properties which are considered to have anti-carcinogenic. The antimicrobial activity, on the other hand, may essentially be attributed to the essential oil content of ginger, aniseed and lemon peel. In the present work, the total polyphenol content of aniseed, ginger cinnamon and lemon chocolate were found to be 284.8, 284.6 287.6 and 283.2 mg/L respectively as compared to 283.3 mg/L for dark (the control sample) chocolate as shown in Figure 5. These figures reveal that cinnamon powders increased the total polyphenol content of dark chocolate

by about 1.5%, while lemon peel powder did not have a significant effect.

Karen et al. [13] studied the strength of the relationship between polyphenol-rich non-fat cocoa solid content (NFCS) and polyphenol content. They found that in the dark chocolate NFCS is linearly related to total polyphenols and also extra ingredients such as milk, or butter might influence polyphenol content. Steinberg et al. [14] declared that most of antioxidant activity in the chocolate came from its polyphenol content. All fractions of cocoa bean polyphenols had been identified to have some antioxidant properties.
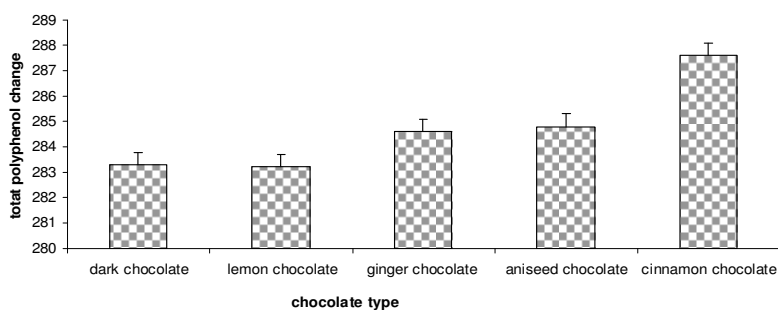

Figure 5. Total polyphenol content of dark, aniseed, lemon and ginger chocolate

CONCLUSION

Variation in the color and moisture was observed to occur mostly in aniseed and cinnamon chocolates. Ginger was the most influential additive on the melting point and total polyphenol content of dark chocolate. It was inferred from the results that, ginger chocolate was close to dark chocolate in terms of the color and moisture content and also cinnamon had higher polyphenol content in comparison with dark chocolate.

Acknowledgements

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