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***In vitro* total phenolic content and antioxidant activities of green and roasted coffee bean extracts used in eye shadow formulation**

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Abstract

The two species of coffee are grown in Thailand, specifically arabica and robusta. Coffee bean contains phenolic compounds which have antioxidant activity. The aims of this study were to explore the consequence of coffee bean varieties, degrees of roasting, and drying procedures on total phenolic content and antioxidant activities. The formulation, properties, and stability of eye shadow which formulated from coffee bean extract were considered. The arabica and robusta coffee beans were contemplated. The four degrees of roasting of coffee beans were investigated (green beans, light roast, medium roast, and dark roast). The coffee beans were extracted by a boiling methodology and concentrate dried by free evaporating and spray drying techniques. The total phenolic content of the extracts were measured by the Folin-Ciocalteu procedure. Antioxidant activities were determined by 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and Ferric reducing antioxidant power (FRAP) assay. The eye shadow from coffee bean extract was formulated. The properties of the eye shadow and stability by heating-cooling cycle were observed. The results demonstrated that the robusta green coffee bean extract which dried by spray drying, had the highest of total phenolic content and antioxidant activities. The total phenolic content, 50% inhibitory concentration (IC₅₀) by DPPH assay, and ascorbic acid (vitamin C) equivalent antioxidant capacity (VEAC) by FRAP assay were $236.7 \pm 0.82 \mu\text{g GAE mg}^{-1}$ of extract, $1.16 \pm 0.07 \text{ mg mL}^{-1}$, and $1340.12 \pm 4.44 \mu\text{mol VEAC mg}^{-1}$ of extract, respectively. Accordingly, the green bean of robusta coffee extract which dried by spray drying, was formulated into the eye shadow. The sixth formula of eye shadow was appropriate physical properties. The eye shadow had a white pearly shine and fine soft texture. It was easy to apply on the skin. The hardness was suitable and the impact resistance was highest. After heating-cooling cycle, the eye shadow had the good physical and chemical stability. The total phenolic content was no statistically significant difference ($p > 0.05$). The green bean of robusta coffee extract, which dried by spray drying, has the potential and efficiency to be developed into eye shadow.

Keywords: Coffee bean extracts, Spray dry, Total phenolic content, Antioxidant activities, Eye shadow

1. Introduction

Eye shadow is an eye make up cosmetic that is applied on the eyelids and beneath the eyebrows. It is usually accustomed build the eyes illustrious or look a lot of luscious. Numerous individuals utilized eye shadow as a cosmetic merely to boost their look. However, the addition of natural antioxidant within the eye shadow formulation as a cosmeceutical is useful.

The coffee tree belongs to the Rubiaceae family, genus *Coffea*. The two main economically important coffee bean varieties are *Coffea arabica* (arabica) and *Coffea canephora* (robusta) [1-2]. The constituents of coffee include phenolic compounds (caffeic, chlorogenic, coumaric, ferrulic acid) [3-4] that probably have antioxidant activities [5-7]. Ground roast coffees are commercially accessible in several colors or roasting degrees that

fluctuate from light to dark. Roasting defines sensory characteristics and affects their chemical composition [2]. During roasting, the green beans are heated at 200-240 °C for 10-15 min depending on the degree of roasting required, which is typically assessed by shading [8]. The degree of roasting (light, medium, high) influences the phenolic compound composition of the coffee [9-10] and furthermore its antioxidant capacity [8,11].

Spray drying could be a technique accustomed to prepare stuff utilized in dry pressing processes. The granules from spray dry process are spherical in shape, have high flowability, great rearrangement and compaction density [12], that are thought of to be brilliant tableting properties as eye shadow. Great flowability is a vital necessity for a direct compression material. A material produced for direct compression process ought to possess an adequate level of flowability once blending with different ingredients in a very formulation to confirm a consistent die filling of a powder mix throughout compression [12-13].

The aims of this work were to study the effects of coffee bean varieties (arabica and robusta), degree of roasting (green, light, medium, and dark), and drying method (free evaporation and spray drying) on the total phenolic content and antioxidant activities. The formulation, properties, and stability of eye shadow from coffee bean extract were investigated.

2. Materials and methods

2.1 Chemicals and raw materials

Green and roasted arabica and robusta coffee beans were kindly provided by the native coffee manufacturer in Thailand. Standards of gallic acid and ascorbic acid were purchased from Sigma Aldrich (USA). All of the reagents were associate degree analytical grade. The eye shadow was formulated from cosmetic grade reagents.

2.2 Preparation of green and roasted coffee extracts

The green and roasted of arabica and robusta coffee beans were studied. The 3 degrees of roasting of coffee beans were light, medium, and dark roast. The coffee beans were ground using an electronic coffee grinder. Ground coffee was boiled in distilled water (ratio 1:17) at 90 °C for 15 min and cooled at room temperature for 3 hours. The filtrate was collected.

2.3 Drying method

2.3.1 Free evaporation method

The filtrate was dried in an evaporating dish by indirect heat on a water bath at 80 °C.

2.3.2 Spray drying method

The filtrate was dried by using spray dryer (Labplant®, model SD-6 basic, UK). The condition of drying was 190 °C of inlet temperature, 89-90 °C of outlet temperature, 5 ml min⁻¹ of feed rate, 2.5 bar of inlet air pressure, medium of deblocker rate. The diameter of the nozzle was 0.5 mm.

2.4 Determination of total phenolic contents

The total phenolic was determined using the Folin-Ciocalteu assay [14]. Briefly, 5 µL of coffee bean extract was placed in 96 well microplates. 60 µL of 7.5 %W/V sodium carbonate, 15 µL of Folin-Ciocalteu reagent, and 200 µL of distilled water were added and mixed. The sample was incubated at 60 °C for 5 min and cooled at room temperature. The total phenolic content was spectrophotometrically determined at 700 nm with a UV-VIS spectrophotometer (SPECTROstar BMG LABTECH, Germany). The total phenolic content was expressed as microgram gallic acid equivalent per dry weight extract (µg of GAE mg⁻¹ extract).

2.5 Determination of antioxidant activities

2.5.1 Free radical scavenging activity on 2,2 Diphenyl-1-picrylhydrazyl assay (DPPH assay)

The DPPH assay was performed as previously described by Bravo et al. [15] with slight modifications. The standard solution of 6.1x10⁻⁵ M DPPH methanolic solution was freshly prepared. The 4 mg mL⁻¹ of the sample were prepared and diluted for two-fold dilution (concentrations between 0.004-2 mg mL⁻¹). 5 µl of coffee bean extract was placed in a 96 well microplates. 195 µL of DPPH solution was added and mixed. The samples were kept at room temperature for 18 min and furthermore, the absorbance was measured at 515 nm utilizing UV-vis Spectrophotometer. Aqueous solutions of ascorbic acid (concentration between 8 - 250 mM) were used for

standard calibration. The results were expressed as the percentage of radical scavenging. Following these concerns, the % radical scavenging could likewise be composed as:

$$\% \text{ radical scavenging} = [(Abs_{\text{control}} - Abs_{\text{sample}}) / Abs_{\text{control}}] * 100 \quad (1)$$

Where, Abs_{control} is the absorbance of the control reaction and Abs_{sample} is the absorbance of the test sample.

2.5.2 Ferric reducing antioxidant power assay (FRAP assay)

The FRAP assay was performed with minor modifications of the strategy of Mussatto et al. [14]. 10 μL of coffee bean extract and 290 μL of FRAP reagent were placed and mixed in a 96 well microplate. The sample was incubated at 37 $^{\circ}\text{C}$ for 15 min and the absorbance estimated utilizing UV-vis spectroscopy at 595 nm. Aqueous solutions of ascorbic acid (concentration between 8 - 250 mM) were utilized as the standard. The results were reported as ascorbic acid (vitamin C) equivalent antioxidant capacity ($\mu\text{mol VEAC mg}^{-1}$ extract).

2.6 Eye shadow formulation

All of the ingredients were passed through sieve no. 200. The material was mixed by geometric dilution technique in a closed container. The eye shadow composition is shown in Table 1. The mixer was compressed by a hydraulic tableting machine with 22 mm of diameter at 7 kilonewton (kN).

Table 1 The composition of eye shadow formula.

Ingredients	Content (%)							
	1	2	3	4	5	6	7	8
Coffee bean extract	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Talcum	55	52.5	50	47.5	40	35	37.5	32.5
Kaolin	15	15	15	15	15	15	15	15
Zinc oxide	15	15	20	20	20	20	20	20
Titanium dioxide	5	7.5	5	7.5	5	5	7.5	7.5
Zinc stearate	-	-	-	-	10	15	10	15
Mica	9.8	9.8	9.8	9.8	9.8	9.8	9.8	9.8

The 1.74 IC_{50} of coffee bean extract by DPPH assay was added in an eye shadow formula (0.2% of the formula). Talcum and zinc stearate were used for slip properties. Kaolin was used as an absorbent. Zinc oxide and titanium dioxide were opacity.

2.7 The properties and stability of eye shadow

2.7.1 Physical and chemical properties

The physical properties like texture, color uniformity, and easy application of eye shadow were determined. The hardness was investigated by digital force gauge hardness tester (INSPEX[®], UK). The impact resistance was performed by raising the eye shadow and allowing it to drop under its own weight a specified distance at 10 inch. The tapping was repeated until the eye shadow was broken. The time was counted.

2.7.2 Heating-cooling cycle

The heating-cooling cycle was used as a stability test. The eye shadows were kept at 4 $^{\circ}\text{C}$ for 12 h followed by storage at 40 $^{\circ}\text{C}$ for 12 h to complete one cycle. Six cycles were performed. The total phenolic content using Folin-Ciocalteu was investigated as chemical stability.

2.8 Statistical analysis

All the experiments were done in triplicate and data were showed as mean \pm standard deviation (SD). One-way analysis of variance (ANOVA) was carried out to determine the significant difference of the data between the green and roasted coffee bean extracts at the level of p-value < 0.05 using software SPSS (Version 16.0).

3. Results and discussion

3.1 Coffee bean extracts

The green and light, medium, or darkly roasted arabica and robusta coffee beans were extracted. The extracts were dried using the free evaporation and spray drying methods. The result of the percentage of yield of coffee bean extracts is shown in Table 2.

Table 2 The percentage of yield of coffee bean extract.

Variety	Degree of roasting	Method of drying	% Yield
Arabica	Green bean	Free evaporation	8.67
		Spray dry	7.15
	Light	Free evaporation	12.50
		Spray dry	11.81
	Medium	Free evaporation	12.00
		Spray dry	7.84
	Dark	Free evaporation	12.03
		Spray dry	9.67
Robusta	Green bean	Free evaporation	9.35
		Spray dry	8.31
	Light	Free evaporation	15.78
		Spray dry	9.01
	Medium	Free evaporation	14.80
		Spray dry	7.23
	Dark	Free evaporation	15.24
		Spray dry	11.29

The percentage of yield of coffee bean extract which had been dried by the free evaporation methodology was higher than that dried by the spray dry methodology. However, the outcomes demonstrated that the products which were dried by free evaporation methodology were a flaky shape whereas that dried by the spray dry methodology was a spherical shape. As a result of the spray dried powder particles are homogeneous, approximately spherical in shape and almost uniform in size and being uniform in size and spherical shape, the particles possess great flowability [16]. Thus, the spray dried materials are reachable for direct compression formulations like eye shadows.

3.2 Determination of total phenolic content

The total phenolic content of coffee bean extracts was investigated using Folin-Ciocalteu method. The total phenolic content of coffee bean extracts was reported as μg of gallic acid equivalent (GAE) per 1 mg of extract. The results of total phenolic content were shown in Table 3.

Table 3 The total phenolic content of coffee bean extracts.

Variety	Degree of roasting	Method of drying	Total phenolic content (μg GAE mg^{-1} extract)
Arabica	Green bean	Free evaporation	145.6 \pm 0.01
		Spray dry	171.4 \pm 1.30
	Light	Free evaporation	139.1 \pm 3.60
		Spray dry	141.2 \pm 2.41
	Medium	Free evaporation	135.3 \pm 0.60
		Spray dry	113.9 \pm 1.54
	Dark	Free evaporation	113.0 \pm 1.15
		Spray dry	110.8 \pm 2.52
Robusta	Green bean	Free evaporation	184.1 \pm 3.61
		Spray dry	236.7 \pm 0.82
	Light	Free evaporation	160.4 \pm 1.01
		Spray dry	155.3 \pm 2.01
	Medium	Free evaporation	153.9 \pm 1.64
		Spray dry	138.0 \pm 1.71
	Dark	Free evaporation	141.5 \pm 2.02
		Spray dry	113.0 \pm 1.32

The greatest of total phenolic content was found inside the green coffee bean of robusta dried by a spray drier, $236.7 \pm 0.82 \mu\text{g GAE mg}^{-1}$ of extract ($p < 0.05$). The result was supported by Hecimovic et al. [17] and Cuong et al. [10], which found that a green coffee, a variety of *Coffea canephora* exhibited the highest overall content of total phenols. Based on the results, which are in accordance with the very fact that polyphenolic compounds are highly thermolabile compounds that are easily decomposed under the effect of high temperature (above 80°C) [18]. Numerous complex physical and chemical changes happen throughout roasting, including the obvious change in color from green to brown. The major compositional changes occurring are decreases in protein, amino acid, sucrose, chlorogenic acid, and water, and therefore the formation of melanoidins. Several of changes are due to the Maillard reaction [19]. The phenolic compounds were incorporated into melanoidins primarily at early stages of the roasting process, being thereafter partly oxidized to dihydrocaffeic acid, and degraded [20]. The spray drying involves rapid evaporation, consequently, the evaporation time is shortened. The product is not subject to heat degradation. When the spray comes into contact with the drying air, evaporation takes place within the droplets until the moisture content becomes too low to diffuse through the dried droplet surface. Finally, the recovery of the spherical shape of dried powder is distributed [21]. The total amount of phenolic acids decreased with the increasing degree of roasting the coffee beans [22]. Additionally, the robusta treatment resulted in additional total phenolic content than arabica treatment at the identical degree of roasting. Similar results were obtained within the previous study by Chotanakoon and Naradosorn [23]. As a result of robusta contains concerning double the concentration of chlorogenic acid over arabica.

3.3 Antioxidant activities

3.3.1 Free radical scavenging activity on DPPH assay

The free radical scavenging activity on DPPH assay was expressed as 50% inhibitory concentration (IC_{50}) as shown in Table 4.

Table 4 The antioxidant activity of coffee bean extracts evaluated by DPPH assay.

Variety	Degree of roasting	Method of drying	IC_{50} (mg mL^{-1})
Arabica	Green bean	Free evaporation	1.79 ± 0.01
		Spray dry	1.65 ± 0.05
	Light	Free evaporation	1.97 ± 0.04
		Spray dry	2.13 ± 0.04
	Medium	Free evaporation	2.02 ± 0.03
		Spray dry	2.46 ± 0.05
	Dark	Free evaporation	2.37 ± 0.01
		Spray dry	2.57 ± 0.02
Robusta	Green bean	Free evaporation	1.50 ± 0.03
		Spray dry	1.16 ± 0.07
	Light	Free evaporation	1.70 ± 0.03
		Spray dry	1.82 ± 0.03
	Medium	Free evaporation	1.84 ± 0.03
		Spray dry	2.02 ± 0.01
	Dark	Free evaporation	1.99 ± 0.02
		Spray dry	2.11 ± 0.02

The consequences of free radical scavenging activity were expressed as IC_{50} value. Lower IC_{50} value indicated greater antioxidant activity. The IC_{50} of the ascorbic acid standard was $0.21 \pm 0.01 \text{ mg mL}^{-1}$. The lowest IC_{50} value was determined within the green coffee bean of robusta which had been dried by a spray drier ($p < 0.05$). The IC_{50} of the green coffee bean of robusta which dried by spray drier was $1.16 \pm 0.07 \text{ mg mL}^{-1}$. Its associated with highest of free radical scavenging activity. This result was supported by Perez-Hernandez et al. [11] which recommended that roasting decreases antioxidant activity. Additionally, the IC_{50} value of robusta treatment indicated a higher potency of free radical scavenging activity than arabica treatment when contrasted with a similar degree of roasting. The finding is in accordance with recently considered by Budryn and Nebesny [24] and Chotanakoon Naradosorn [23]. They found that extracts of robusta coffee beans demonstrated a stronger antioxidant capacity than extract obtained from arabica coffee beans. The correlation between total phenolic content and antioxidant activity was found amid this outcome.

3.3.2 FRAP assay

FRAP assay has been used to determine antioxidant activity. The results of the FRAP assay were reported in Table 5.

Table 5 The VEAC of coffee bean extract.

Variety	Degree of roasting	Method of drying	VEAC (μmol ascorbic acid mg^{-1} of extract)
Arabica	Green bean	Free evaporation	956.40 \pm 3.91
		Spray dry	984.60 \pm 1.48
	Light	Free evaporation	945.18 \pm 0.88
		Spray dry	868.26 \pm 0.89
	Medium	Free evaporation	795.95 \pm 0.88
		Spray dry	689.28 \pm 3.87
	Dark	Free evaporation	702.62 \pm 1.54
		Spray dry	666.21 \pm 4.70
Robusta	Green bean	Free evaporation	1106.81 \pm 4.44
		Spray dry	1340.12 \pm 4.44
	Light	Free evaporation	1033.39 \pm 1.54
		Spray dry	1005.69 \pm 5.33
	Medium	Free evaporation	979.03 \pm 0.88
		Spray dry	851.33 \pm 3.87
	Dark	Free evaporation	899.54 \pm 1.53
		Spray dry	758.51 \pm 0.89

The results indicated that VEAC values were highest within the green coffee bean of robusta which had been dried by a spray drier ($p < 0.05$). The VEAC value of green coffee bean of robusta which dried by spray drier was 1340.12 \pm 4.44 μmol ascorbic acid mg^{-1} of extract. The robusta treatments had higher amounts of VEAC value than arabica treatment when compared to the identical degree of roasting. The result indicated a correlation between total phenolic content and antioxidant activities.

It has been previously established that phenolic antioxidants occurring in coffee are lost during the roasting process. This decline of phenolic compounds is related to the degradation of chlorogenic acid [10], which influences the antioxidant capacity of the roasted coffee [25-26]. The spray drying method, the moisture content of the feed is removed terribly quickly. This method could likewise be appropriate for the drying of heat-sensitive materials, similar to phenolic compounds [27] in coffee bean extracts.

3.4 Properties and stability of eye shadow

The physical properties of eye shadow were reported in Table 6.

Table 6 The physical properties of eye shadow.

Formula	Physical properties		Hardness (Newton)	Impact resistance (Time)
	Texture	Color		
1			9.8 \pm 0.8	3.7 \pm 0.6
2	fine and soft texture touch skin was not rough		13.7 \pm 0.2	2.7 \pm 0.6
3			13.3 \pm 1.3	2.3 \pm 0.6
4		white pearly shine	12.7 \pm 0.4	2.7 \pm 0.6
5	fine texture touch skin was rough	color uniformity was good	16.6 \pm 1.3	5.7 \pm 0.6
6	fine and soft texture touch skin was not rough		26.1 \pm 1.6	6.3 \pm 0.6
7			19.6 \pm 0.5	5.7 \pm 1.2
8			33.3 \pm 0.4	5.3 \pm 0.6

The sixth formula of eye shadow had sensible physical properties. The formula had a white pearly shine and fine soft texture. It was appropriated to use on the skin. The hardness was suitable and the impact resistance was highest. It is durable during transport and use. After heating-cooling cycle, the eye shadow still had the good physical and chemical properties. The total phenolic content of eye shadow before and after heating-cooling cycle were 0.0372 \pm 0.0019 and 0.0341 \pm 0.0002 mg GAE mg^{-1} extract, respectively. However, the total phenolic content were no statistically significant differences ($p > 0.05$).

4. Conclusions

The results indicated that the green coffee bean of robusta which had been dried by spray drier had the highest the total phenolic content, IC₅₀, and VEAC value which related to highest of antioxidant activities when compared to the other treatments. The sixth formula of eye shadow had the good properties and stability. The green coffee bean of robusta which had been dried by spray drier has outstanding benefit and efficiency to be used into eye shadow.

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