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Alternative application approach on black bean: hair coloring product

Chiraporn Inman¹, Nattaya Lourith^{1,2*} and Mayuree Kanlayavattanakul^{1,2}

Abstract

Background: Black bean is one of the important beans as the second most consumed bean globally after soybean. It has been reported as a sustainable source of anthocyanins, important natural colorants used in food and consumer products. However, industrial uses of black bean anthocyanins in pharmaceutics and cosmetics have rarely been explored. Thus, here, a black bean anthocyanin extract was prepared, quality controlled and developed into an efficient hair coloring product.

Results: Black bean was macerated in hydrochloric ethanol to produce a dark-reddish $1.85 \pm 0.04\%$ dry extract with total anthocyanin content (TAC) of 1.24 ± 0.48 µg/ml cyanidin-3-glucoside. The extract was readily dissolved in propylene glycol (25%), giving a reddish to red-brownish solution at pH of 4.00-7.00. The addition of antioxidant significantly (p = 0.000) enhanced the stability of the extract in an accelerated stability test (90.34 ± 5.32 from $67.83 \pm 3.45\%$ TAC remaining). Three base hair gels containing different thickeners were developed to yield clear gels with a viscosity of 101,666.67-447,333.33 cps and a pH of 4.80-4.97. The gel base with the best hair application properties was chosen to mix with 8, 10 and 12% black bean extract to produce similar visually assessed red-brown gels ($L^* = 33.25-33.76$, $a^* = 0.06-0.09$, $b^* = -0.45-0.38$ and $b^* = 277.54-283.31$) at 73,733.33-99,666.67 cps and a pH of 5.00-5.01. All of the black bean hair coloring gels were stable under the accelerated test. The 10% hair gel was tested for its hair staining efficacy. The black bean hair coloring gel was found to efficiently color hair, and the color was maintained through four washings.

Conclusions: An alternative application approach of using black bean as a natural colorant is presented here, including its basic requirements for preparation and quality control from an industrial aspect. The efficient semi-permanent hair coloring product containing black bean is an innovative natural product and is considered as one progress towards a sustainable bio-based economy using a readily available industrial crop, black bean.

Keywords: Black bean, Hair coloring, Hair dyeing, Anthocyanins

Background

Black bean (*Phaseolus mungo*) is one of the important beans as the second most consumed bean globally after soybean. Furthermore, it has been reported to be a sustainable source of anthocyanins, which could be applied as a natural colorant in food, pharmaceutic and cosmetic products. Natural anthocyanins are known to be

successfully prepared by maceration in hydrochloric ethanol. Black bean with a total anthocyanin content (TAC) of 213 ± 2 mg/100 g was characterized to mostly consisted of delphinidin 3-glucoside, petunidin 3-glucoside and malvidin 3-glucoside [1], and the TAC of black bean is higher than that of red cabbage, one of the important known sources of natural anthocyanins [2]. Thus, black bean could be an economical and feasible source of natural colorant that could be used in a variety of product applications.

Anthocyanins are important natural colorant agents found in food and consumer products due to their

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various health benefits [3, 4]. Although they tend to lack stability, the addition of antioxidant was reported to prolong their shelf life [5]. Industrial approaches to the use of black bean anthocyanins have been extensively explored in food [2, 6, 7], but only sparsely for pharmaceutic and cosmetic products. Notably, consumers' awareness of and preferences for bio-based or natural products have been increasing recently.

Natural hair care products are of increasing interest among the consumers and researchers [8] as alternatives to synthetic products. Hair dyeing or hair coloring products generate enormous revenues for industries, among which professional salon and home use demands are increasing year by year [9]. Hair coloring products not only include colorants to change the natural hair color shade, but also are applicable for covering grey hair [10]. Hair coloring products are therefore classified into temporary, semi-permanent and permanent products based on the dyeing mechanism of the colorant molecules. The natural colorants dye hair on temporary or semi-permanent basis. The acidic colorant molecules coat the epicuticle, cuticle and the outermost cortex along the hair shaft. On the other hand, the permanent dyes penetrate and diffuse deeper into hair shaft than the temporary and semi-permanent hair dyes. Later, oxidization and polymerization of the colorant molecules in the hair cortex result in coloring on the hair [11, 12]. Accordingly, oxidation dyes have better hair coloring abilities than natural hair dyes [12], but there are issues that have been reported concerning the safety of synthetic hair dyes. Thus, preference for natural dyes is increasing [9] as well as consumers' expectations of their safety [10, 13]. Thus, available industrial crops are sources that can be developed into highly efficient, sustainable products using low-cost technologies for selective applications as multipurpose products that are feasible for agricultural and personal care industrial practices.

Thus, a black bean anthocyanin extract was prepared. The color at different pH conditions applicable for cosmetic products was examined as a basic information requirement for developing cosmetics. Thereafter, hair coloring gels containing black bean colorant extract were developed and evaluated for their dyeing performance. The developed efficient semi-permanent hair coloring product containing black bean is an innovative natural product and considered as an achievement suitable for a sustainable bio-based economy using an available industrial crop, black bean.

Methods

Materials

The solvents used for extraction and quality control were all analytical grade. Those of cosmetic grade were applied for hair coloring gel development.

Black bean colorant extract preparation

Black bean colorant extract was prepared by a procedure modified from a previously described method [1, 7] to fit into industrial practice. In brief, black bean (Makro, Thailand) was ground into a fine powder (sieved with 35 mesh) and then macerated in 0.5% HCl in 95% ethanol (1:4, w/v) at 4 °C for 24 h. The colorant solution was filtered (Whatman no. 1) and concentrated to dryness under vacuo. The extractive yield of the black bean colorant extract was calculated. Preparation of the colorant extract was undertaken 3 times.

Quality control of the colorant extract

Physicochemical properties in terms of color (CIELAB system); L*, a*, b* and h*, were determined (CM-700d, Konica Minolta, Japan). Solubility of the extract in water, 95% ethanol and propylene glycol, the common diluents used for cosmetic and personal care products, was examined. Briefly, the solvent (20 µl) was stepwise added into the extract (5 mg) until a clear solution was obtained. The solubility was calculated and the pH (B200, Qis, Germany) of the solution was examined. The coloration of black bean extract at different pH conditions was monitored [10, 14]. Its active content in terms of TAC was analyzed by pH differential method. In short, the colorant extract (1000 µg/ml) in each buffer (KCl, 0.2 M, pH 1.0; and C₂H₃O₂Na, 1 M, pH 4.5) was UV-vis spectroscopically determined at 520 and 700 nm (Genesys 10S, Thermo-Fisher, USA). TAC was calculated with the extinction coefficient for cyanidin-3-glucoside as follows:

Absorbance =
$$(A_{520\text{nm}} - A_{700\text{nm}})$$
pH1.0
- $(A_{520\text{nm}} - A_{700\text{nm}})$ pH4.5

and expressed as $\mu g/ml$ cyanidin-3-glucoside [15, 16]. The assay was undertaken in triplicate.

Stability of the black bean colorant extract with or without antioxidant, vitamin E acetate [5], was assessed under an accelerated stability test (4 °C, and 45 °C, 24 h each) for 4 cycles. Color and TAC following the accelerated test were re-examined [10, 14].

Hair coloring gel preparation Hair gel base formula development

Hair gel base formulas containing DI water, ethanol, propylene glycol, carbomer, vitamin E acetate, disodium EDTA, triethanolamine, phenoxyethanol/ethylhexylglycerin and either xanthan gum, hydroxyethyl cellulose (HEC) or hydroxypropyl methylcellulose (HPMC) were formulated. The appearance, pH, viscosity and color of the gel bases were recorded.

The stabilities of the gel bases were tested with an accelerated condition similar to that used to test the black bean

extract. A stable gel base with a good texture was selected to be developed into black bean hair coloring gel.

The development of a hair coloring gel containing black bean extract

The selected gel base was mixed with the black bean colorant extract at different concentrations based on the colorant's solubility and the expected color shade. The formulated back bean hair coloring gels were then assessed for their physicochemical properties and stability as above. The most stable developed product was selected for the wash fastness analysis [10].

Tresses of a certain length and weight ($10 \text{ cm} \times 1.5 \text{ g}$) were bleached with H_2O_2 (12% for 30 min) and washed with tap water (6 cycles). The bleached tress was color recorded [17] before a slow application (1 min) of the developed black bean hair coloring gel (1.5 g) and then blow-dried (Philips Voyager Twist 1400, Thailand) at a distance of 5 cm from the tress with heat of level 2, followed by color recording. The dried and dyed tress was rinsed with 0.1 g Kodomo Baby Shampoo (Lion, Thailand) in tap water (300 ml), blow-dried and color recorded. This wash fastness cycle was repeated until the determined L^* , a^* , b^* and b^* were insignificantly different from the bleached tress.

Statistical analysis

Data are presented as the mean \pm SD. Statistical analysis was performed using SPSS program version 16.0 for Windows. The parameters were compared and analyzed using one-sample t test and ANOVA test with a significance level of p < 0.05.

Results and discussion

Preparation and quality control of black bean colorant extract

Black bean, a common bean purchased from a grocery store, was macerated in hydrochloric alcohol because it has been reported to be a better solvent than water for an efficient anthocyanin extraction [1, 7]. Ethanol was therefore used instead of methanol due to its greater safety and suitability for the food and cosmetic industries [18, 19]. The black been colorant extract was obtained at an extractive yield of $1.85 \pm 0.04\%$. A pH differential method, an accurate and substantive method that is an alternative to HPLC [16], was applied for TAC quantification in the unit of cyanidin-3-glucoside. This quality control procedure will therefore be feasible as a routine practice for the industry as it is a widely commercially adopted quantification method [20]. The TAC of the black bean colorant extract in this study was shown to be 1.24 ± 0.48 µg/ml cyanidin-3-glucoside, that is, higher than the average range previously reported for water extraction [7]. Color recorded in terms of L* (0-100; black to white), a* (-60 to +60; green to red), b* (-60 to +60; blue to yellow) and hue (h*), the important values used to describe the shade of anthocyanins, were recorded. The reddish-pink (L*= 42.86 ± 1.10 , a*= 8.82 ± 0.71 and b*= -0.76 ± 0.12) black bean anthocyanin colorant extract was stronger in color than found by a previous report [7] and more blueish with regard to h* (355.04 ± 0.73) . The strength of the color conformed to its TAC.

The back bean colorant extract was then further examined with regard to its solubility. The solubility of an extract is one of the basic characteristics required for stability assessment and formulation design. The solubility of the black bean colorant extract was examined in water, 95% ethanol and propylene glycol, which are used as the most common solvents in cosmetics. The extract dissolved well in 95% ethanol and propylene glycol (25 and 12.5%, respectively), but was undissolved in water (0%). These 2 solvents were then used during the design of the hair coloring product. The CIELAB color parameters of the black bean colorant extract in these 2 solvents were also examined. Although the black bean colorants extract dissolved less well in propylene glycol, the color was a deeper red shade (L*= 48.94 ± 0.51 , a*= 10.02 ± 0.51 , $b^* = 1.09 \pm 0.10$ and $h^*=6.24\pm0.90$) than that of 95% ethanol $(L^* = 52.44 \pm 0.13,$ $a^* = 8.69 \pm 0.04$ $b^* = 1.05 \pm 0.01$ and $h^* = 6.91 \pm 0.09$) according to a^* .

The coloration of the black bean extract at pH ranges suitable for cosmetic applications was consequently monitored [14]. Black bean extract in 95% ethanol and propylene glycol (5000 μ g/ml, pH 2.7) was adjusted to pH 4–7 using 0.5% NaOH and their color was monitored (Fig. 1). The examined color shade in this pH ranges were used for the design of a grey hair covering product design.

The stability of the black bean anthocyanin extract was evaluated using an accelerated stability test. A reduction of a* is responsible for TAC as per ΔE . In addition, storage of the black bean colorant extract in a dried powder form better maintained the extract's quality. Furthermore, the addition of vitamin E acetate at 0.04% obviously enhanced this natural colorant extract's stability (Table 1). Thus, vitamin E acetate was therefore included in the hair coloring product development.

Black bean hair coloring gel development

Hair coloring formulations are classified into temporary, semi-permanent and permanent colorings with regard to rinse-off persistence and dyeing duration. Natural colorants mostly function as temporary hair dyes by cohesion and adhesion of color molecules onto the epicuticle of the hair shaft. This mechanism is therefore known as a color coating effect. Generally, large colorant molecules with

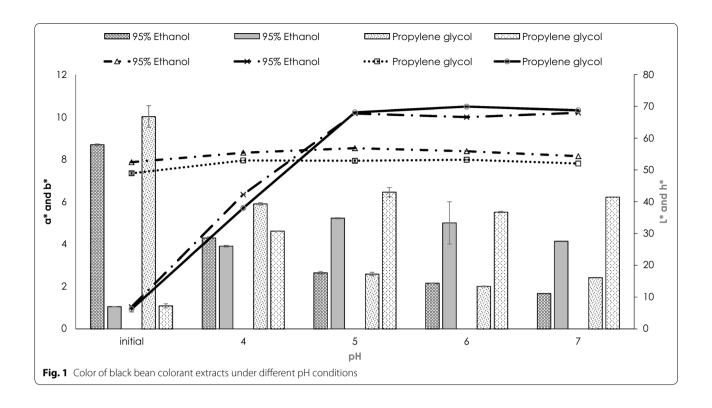


Table 1 Physicochemical stability of black bean anthocyanins extract

	Initial	Accelerated stability test			
		Without antioxidant		With antioxidant	
		Powder	Solution	Powder	Solution
Color parameter					
L*	57.02 ± 0.34	52.53 ± 0.09	54.48 ± 0.64	52.46 ± 0.10	58.80 ± 0.01
a*	3.71 ± 0.01	1.07 ± 0.07	0.43 ± 0.02	1.05 ± 0.01	0.28 ± 0.01
b*	-0.41 ± 0.03	3.88 ± 0.03	1.10 ± 0.07	3.70 ± 0.02	1.53 ± 0.01
h*	353.61 ± 0.38	74.53 ± 0.03	68.63 ± 0.68	74.14 ± 0.22	79.73 ± 0.49
ΔΕ	-	9.02 ± 0.23	3.74 ± 0.41	6.70 ± 0.32	4.33 ± 0.14
Visual observed color	Reddish pink	Brown	Pinkish brown	Brown	Pinkish brown
Precipitation*	_	+	-	+	-
TAC (µg/ml cyanidin-3-glucoside)	1.24 ± 0.48	0.85 ± 0.03	0.48 ± 0.03	1.14 ± 0.04	0.50 ± 0.00
Reduction of TAC (%)	_	67.83 ± 3.45	46.24 ± 2.86	90.34 ± 5.32	51.64 ± 1.15

Remark*: (-) none, (+) slightly, (++) moderate, (+++) high

an acidic nature dye hair by this mechanism. Semi-permanent hair dyes with smaller acidic molecules permeate or diffuse onto the cuticle and some parts of the cortex of the hair shaft by Vander Waals forces and partially ionic bonds [11, 12]. Thus, hair coloring product design can enhance the color staining efficacy of the natural colorant extract from temporary to a semi-permanent hair dye. Hair coloring products can be prepared in the forms of mousse, stick, gel, cream or spray [12]. Although the natural colorant in temporary dyes for hair is based on its

colorant molecule size, the acidic nature of the black bean anthocyanins extract might possibly enable it to function as a semi-permanent dye. Thus, a dosage form of gel that is capable of loading the colorant at a high quantity is of interest and is a challenge to overcome in this study.

Hair coloring gel containing the black bean anthocyanin extract was therefore designed and developed based on its basic characteristics and with the goal of improving the dyeing performance of this natural colorant extract. Hair gel bases containing the ingredients listed in Table 2

Table 2 Development of base hair coloring gel

Ingredients (% w/w)	Formula				
	A	В	С		
DI water					
Ethanol	66.32	66.32	66.32		
Propylene glycol	\downarrow	\downarrow	↓		
Vitamin E acetate	0.04	0.04	0.04		
Disodium EDTA	↑	\uparrow	↑		
Purple spirit	1.04	1.04	1.04		
Triethanolamine	↓	\downarrow	↓		
Carbomer	0.50	0.50	0.50		
Xanthan gum	0.30	_	=		
HEC	_	0.30	=		
HPMC	_	_	0.30		
Phenoxyethanol/ethylhexylglycerin	1.00	1.00	1.00		
Initial					
рН	4.97 ± 0.02	4.97 ± 0.01	4.97 ± 0.02		
Viscosity (cps)	$123,000.00 \pm 13,228.76$	$304,333.33 \pm 12,423.10$	$410,666.67 \pm 30,022.21$		
L*	37.24 ± 0.01	35.76 ± 0.01	34.71 ± 0.01		
a*	-0.10 ± 0.01	0.06 ± 0.02	0.04 ± 0.01		
b*	0.42 ± 0.01	-0.47 ± 0.01	-0.28 ± 0.01		
h*	103.36 ± 1.03	276.98 ± 2.15	277.45 ± 0.15		
Visual observed color	Clear light yellow gel	Opaque colorless gel	Opaque colorless gel		
Precipitation/separation	None	None	None		
Accelerated stability test					
рН	4.87 ± 0.01	4.84 ± 0.01	4.80 ± 0.03		
Viscosity (cps)	$357,333.00 \pm 17,243.00$	$584,333.00 \pm 16,258.00$	$575,000.00 \pm 9643.00$		
L*	33.25 ± 0.02	31.96 ± 0.02	31.59 ± 0.28		
a*	-0.02 ± 0.03	-0.03 ± 0.06	-1.08 ± 0.52		
b*	0.37 ± 0.01	-0.51 ± 0.01	-0.54 ± 0.42		
h*	92.78 ± 3.83	266.41 ± 6.34	203.61 ± 13.24		
ΔΕ	3.99 ± 0.03	3.81 ± 0.02	3.36 ± 0.42		
Visual observed color	Clear light yellow gel	Opaque colorless gel	Opaque colorless gel		
Precipitation/separation	None	None	None		

were firstly developed. Water and ethanol function as diluent and solubilizer while propylene glycol works as a humectant in addition to performing its solvent task. Although black bean anthocyanins extracts have been reported to exhibit antioxidant activity [21], the addition of an antioxidant will enhance its stability [5]. In this present study, vitamin E acetate at 0.04% was found to enhance the stability of this natural colorant extract as described above. Thus, this antioxidant was selected and included in the gel base development. Furthermore, the pH of the preparations was adjusted to 5 using triethanolamine to minimize shifting of the black bean color. Three gelling agents were tested in order to enhance the texture of the base preparations.

These bases were examined for their physicochemical properties and tested with regard to their stability. All the

formulated bases were stable. The texture of each base was then examined. Base A had the best texture, whereas base B and C containing HEC and HPMC, respectively, were greasier due to their viscosities. Hair gel base A was therefore selected to be developed into the black bean hair coloring gel.

The black bean anthocyanin extract was incorporated into the selected base at 8, 10 and 12% (A8, A10 and A12), selected after taking into account the compatibility of the colorant extract and the preparation ingredients as well as the shade of color as visually assessed. A greater concentration of colorant extract reduced viscosity of the gel due to the acidic nature of the black bean anthocyanin extract. The viscosity of the preparation was governed by carbomer, for which the pH of the system affects its hydrogel network. The visual observed colors of the

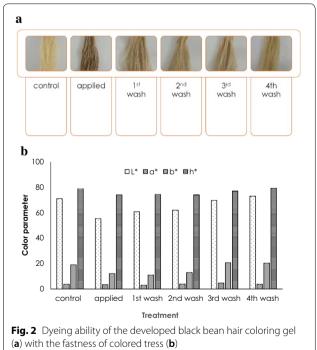
Table 3 Development of black bean hair coloring gel

Physicochemical properties	Black bean hair coloring gel				
	A8	A10	A12		
Initial					
рН	5.00 ± 0.01	5.10 ± 0.01	5.00 ± 0.01		
Viscosity (cps)	$111,333.33 \pm 0.02$	$87,366.67 \pm 4845.21$	$74,800.00 \pm 1200.00$		
L*	33.76 ± 0.02	33.25 ± 0.07	33.55 ± 0.02		
a*	0.09 ± 0.02	0.06 ± 0.00	0.07 ± 0.01		
b*	-0.38 ± 0.02	-0.45 ± 0.01	-0.38 ± 0.01		
h*	283.31 ± 2.30	277.54 ± 0.47	279.80 ± 0.23		
Visual observed color	Brownish red	Brownish red	Brownish red		
Precipitation/separation	None	None	None		
Accelerated stability test					
рН	4.47 ± 0.01	4.38 ± 0.00	4.43 ± 0.01		
Viscosity (cps)	$119,666.67 \pm 5686.24$	$98,066.67 \pm 8533.07$	83,733.33 ± 4772.14		
L*	31.21 ± 0.05	30.36 ± 0.01	31.31 ± 0.05		
a*	0.50 ± 0.06	0.09 ± 0.02	0.12 ± 0.01		
b*	-0.38 ± 0.10	-1.36 ± 0.08	-0.44 ± 0.77		
h*	323.48 ± 4.13	273.63 ± 1.06	285.14 ± 1.61		
ΔΕ	2.60 ± 0.06	2.96 ± 0.03	2.25 ± 0.04		
Visual observed color	Brownish red	Brownish red	Brownish red		
Precipitation/separation	None	None	None		

preparations were similar, although A10 was remarkably different in b*, being more bluish and in harmony with h*, referring to the anthocyanin coloration. All the black bean hair coloring gels were then tested for stability and were found to be stable (Table 3). The A10 gel was selected as the candidate for dyeing efficacy evaluation due to its coloring properties and economic feasibility.

The bleached tress with its analyzed color parameters was used as the reference. The black bean hair coloring gel was then applied to the bleached tress. Color in terms of L*, a* and b* was used as the international standard method, with grey hair having a higher L* (13-83) and a lower a* (2-7) and b* (0-13) [17]. In addition, h* was recorded based on the nature of the anthocyanin colorant extract. The black bean hair coloring gel was able to visually darken grey hair (Fig. 2) in accordance with L*, a* b*, and h*.

Wash fastness was then tested. Washing cycles were repeated until the monitored color parameters were insignificantly different from the control bleached tress. The black bean hair coloring gel was able to persist through shampooing at least 4 times. Despite the natural anthocyanin property of only temporarily dyeing hair, this developed black bean hair coloring gel could be classified as a semi-permanent coloring gel based on its color staining ability. Adhesion of the coloring gel on the hair enhances the contact time of the black bean anthocyanin



extract, enabling penetration of the colorant molecules into the hair cuticle and partially diffusing throughout the cortex.

Conclusions

Black bean can be used as a multipurpose industrial crop for anthocyanin extracts that are applicable for cosmetic preparations. A natural hair coloring product containing black bean colorant extract was shown to be compatible with commonly available ingredients used for topical products. In addition, basic information required for the preparation and quality evaluation of this black bean colorants extract and the developed hair coloring products are presented and are consistent with use in industrial practice. The developed black bean hair coloring gel was shown to be an efficient semi-permanent coloring product for hair. The black bean hair coloring product is an alternative application of this common crop.

Abbreviations

CIE: Commission Internationale de l'Eclairage; DI water: deionized water; EDTA: ethylenediaminetetraacetic acid; HEC: hydroxyethyl cellulose; HPMC: hydroxypropyl methylcellulose; TAC: total anthocyanins content.

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Authors' contributions

CI (M.Sc. student) contributed in all the experimental works, analyzed the data. NL contributed to project planning, study design and manuscript drafting. MK contributed to co-study design and critical reviewing of the manuscript. All authors read and approved the final manuscript.

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