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Pigmented edible bean coats as natural sources of polyphenols with antioxidant and antibacterial effects

Ren-You Gan [a], Zi-Qing Deng [a], Ai-Xin Yan [a], Nagendra Prasad Shah [a], Wing-Yee Lui [a], Chak-Lun Chan [a], Harold Corke [a, b, *]

[a] School of Biological Sciences, The University of Hong Kong, Pokfulam Road, Hong Kong, China
[b] Department of Food Science and Engineering, Shanghai Jiao Tong University, Shanghai, China

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ABSTRACT

Food preservatives are important for the storage of foods, and natural polyphenols with antioxidant and antibacterial effects are often effective as food preservatives. In this study, we systemically investigated the phenolic composition, antioxidant and antibacterial effects of 28 pigmented edible bean coat extracts. Most of these bean coat extracts had a relatively high level of total phenolic content, mainly flavonoids and proanthocyanidins, and they also exhibited much higher antioxidant effect than most common fruits, vegetables and cereal grains. In addition, most of them showed antibacterial effects, mainly against gram-positive bacteria, higher than the effect of phenol. Correlation analysis and principal component analysis indicated that flavonoids and proanthocyanidins were mostly responsible for the antioxidant and antibacterial effects of these bean coat extracts. Therefore, the pigmented bean coats rich in polyphenols with antioxidant and antibacterial effects are potential candidates as food preservatives.

1. Introduction

Food safety is an important health concern all over the world, and food poisoning is the most common food safety event. Lipid oxidation and foodborne pathogenic bacteria growth in foods are two major causes of food spoilage (Aliakbarlu, Mohammadi, & Khalili, 2014), which can lead to food poisoning. In order to inhibit lipid oxidation and bacterial growth in foods, many synthetic food preservatives are widely used in raw and processed food (Frankel, 1993). However, some synthetic food preservatives, such as butylated hydroxyanisole (BHA), have been reported to have side effects and may be harmful to human health (Jeong, Kim, Kang, Ku, & Cho, 2005; Maeura & Williams, 1984). In addition, there are growing concerns among people about the safety of foods containing synthetic chemicals. Therefore, exploring natural food preservatives with antioxidant and antibacterial effects has attracted increasing interest for both food industry and scientists (Aliakbarlu et al., 2014; Shan, Cai, Brooks, & Corke, 2007).

Edible beans are an important component of human diets and many edible beans are consumed by people together with their bean coats. We previously investigated the antioxidant effect and total phenolic content of 42 edible beans and found pigmented edible beans generally had much higher antioxidant effect and total phenolic content than non-pigmented ones (our unpublished data). Therefore, it was speculated that pigmented bean coats could be rich in antioxidant polyphenols and might be a potential natural source of food preservatives. To test this hypothesis, the phenolic composition, antioxidant and antibacterial effects of 28 pigmented edible bean coat extracts were systemically investigated. The results can be useful for the food industry and scientists to explore new natural food preservatives.

2. Materials and methods

2.1. Chemicals and reagents

2,2′-azinobis(3-ethylbenothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), ampicillin, ciprofloxacin, ferric chloride anhydrous, ferrous sulfate heptahydrate, phenol, potassium persulfate, sodium acetate, sodium carbonate, sodium nitrite, sodium hydroxide and vanillin were obtained from Sigma-Aldrich (St. Louis, MO, USA). 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was from

* Corresponding author. School of Biological Sciences, The University of Hong Kong, Pokfulam Road, Hong Kong, China.
E-mail address: hcorke@yahoo.com (H. Corke).

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Fluka Chemie AG (Buchs, Switzerland). Acetic acid, aluminum chloride hexahydrate, dimethyl sulfoxide (DMSO), Folin-Ciocalteu reagent and formic acid were from BDH (Dorset, U.K.). Ethanol, methanol (for extraction) and sulfuric acid were from Merck KGaA (Darmstadt, Germany). HPLC-grade methanol was from International Laboratory USA (South San Francisco, CA, USA). Lysogeny broth (LB) agar and broth were purchased from Difco (Sparks, MD, USA). Authentic standards, including catechin, gallic acid, kaempferol, quercetin, quercitrin and rutin were purchased from Sigma-Aldrich (St. Louis, MO, USA), methyl gallate was purchased from Yuanye Bio-tech (Shanghai, China), vitexin and isovitexin were purchased from Biopurify Phytochemicals (Chengdu, China), and cyanidin-3-galactoside, malvidin-3-glucoside, cyanidin-3-glucoside and peonidin-3-glucoside were obtained from Extrasynthese (Genay, France). Deionized water was used for all the experiments.

2.2. Microorganisms and culture

Four common foodborne pathogenic bacteria, including *Bacillus cereus*, *Straphylococcus aureus*, *Escherichia coli* and *Salmonella typhimurium*, were used in this study. To prepare the inoculum, single colony of the bacterial grown on LB agar plate was picked up and cultured in LB solution overnight in a rotary incubator at 37 °C and 250 rpm. The bacterial suspension was adjusted to about 1×10^6 colony-forming unit (cfu)/mL for the following antibacterial experiments.

2.3. Sample preparation

Pigmented edible beans (28 varieties) were bought from markets in China (Table 1) and their bean coats were directly removed from dry beans or harvested after soaking beans in deionized water for about 2–6 h at room temperature (21 ± 1 °C), and dried at 40 °C in a ventilated oven for 24 h. Dry bean coats were ground into fine powders and stored at 4 °C. The methanol extract of bean coat powder was prepared as we previously reported (Shan et al., 2007) with some modifications. Briefly, the bean coat powder (2.0 g) was extracted with 50 mL of 80% methanol for 24 h at room temperature (21 ± 1 °C) in a water bath shaker (150 rpm, in dark), and then the solution was centrifuged (2370 × g, 30 min, 4 °C). The supernatant was adjusted to 50 mL, and an aliquot of 10 mL was used for the color measurement, phenolic composition and antioxidant analysis, and the rest was concentrated by rotary evaporation, frozen in liquid-nitrogen and then freeze-dried for antibacterial assays. The supernatants and freeze-dried powders were stored at -20 °C for use. Extraction was performed in triplicate.

2.4. Color measurement of bean coat extracts

The three chromatic coordinates L^*, a^*, and b^* of the methanol extracts of bean coats were measured by a colorimeter (Chroma Meter CT-310, Minolta Co., Osaka, Japan). Briefly, 5 mL of sample or 80% methanol (blank) was added into the glass cell (CT-A21, 10 mm in width), and calibration and measurements were performed according to the manual of this colorimeter. The hue angle (H°) was calculated according to the equation: $H^\circ = \text{arctangent}(b^*/a^*) \times 360/(2 \times 3.14)$.

2.5. Determination of antioxidant capacity

The ferric-reducing antioxidant power (FRAP) assay and ABTS free radical scavenging assay were carried out as we previously described (Gan et al., 2010). The FRAP values were expressed as

Table 1
Background information of 28 edible beans and the color measurement of their pigmented coat extracts.

Common name of beans	Scientific name of beans	Chinese name of beans	Color measurement			
			L^*	a^*	b^*	H°
Adzuki bean	*Vigna angularis*	Chi xiao dou	48.3 ± 0.18^{p,q}	47.7 ± 0.52^{c}	41.5 ± 0.45^{q}	41.0 ± 0.01^{t}
Broad bean	*Vicia faba*	Can dou	85.2 ± 0.48^{e}	2.62 ± 0.08^{m}	32.4 ± 0.35^{s}	85.4 ± 0.08^{f}
Climbing bean	*Vigna angularis*	Pa dou	88.6 ± 0.24^{c,d}	−13.3 ± 0.21^{p}	50.9 ± 1.18^{m,n}	105 ± 0.07^{c}
Black cow gram	*Lablab purpureus*	Hei mei dou	23.9 ± 2.04^{s}	56.7 ± 1.29^{a}	28.9 ± 0.26^{t}	27.0 ± 0.46^{u}
Mottled cowpea	*Vigna unguiculata*	Li hua jiang dou	83.0 ± 0.27^{f}	2.08 ± 0.72^{m,n}	62.4 ± 1.02^{h}	88.1 ± 0.48^{e}
Red cowpea	*Vigna unguiculata*	Hua jiang dou	74.4 ± 0.48^{j}	16.9 ± 0.32^{h,i}	50.4 ± 0.45^{n,o}	71.5 ± 0.12^{k}
Black hyacinth bean	*Lablab purpureus*	Hei bian dou	81.2 ± 0.37^{f,g}	0.39 ± 0.07^{n}	46.0 ± 0.30^{p}	89.6 ± 0.07^{d}
Red kidney bean	*Phaseolus vulgaris*	Hong yun dou	57.4 ± 0.61^{m,n}	40.9 ± 0.61^{d}	78.0 ± 0.27^{c}	62.3 ± 0.20^{n}
Big speckled kidney bean	*Phaseolus vulgaris*	Yao xing nai hua dou	73.9 ± 0.30^{j}	15.2 ± 0.12^{i}	71.1 ± 0.36^{e}	78.0 ± 0.03^{h}
Small speckled kidney bean (oval)	*Phaseolus vulgaris*	Yuan xing nai hua dou	71.0 ± 0.15^{k}	20.2 ± 0.09^{g}	64.4 ± 0.09^{g}	72.6 ± 0.04^{j}
Violet red kidney bean	*Phaseolus vulgaris*	Hong yao dou	59.5 ± 0.80^{l,m}	32.1 ± 0.41^{f}	52.5 ± 0.66^{l,m}	58.6 ± 0.06°
Black mung bean	*Vigna radiata*	Hei lv dou	81.6 ± 0.67^{f,g}	−24.7 ± 0.32^{r}	80.4 ± 0.50^{b}	107 ± 0.14^{b}
Green mung bean	*Vigna radiata*	Lv dou	79.5 ± 0.11^{g,h}	−35.8 ± 0.21^{s}	98.7 ± 0.02^{a}	110 ± 0.08^{a}
Panda bean	*Vigna umbellata*	Xiong mao dou	93.6 ± 0.20^{a}	−6.95 ± 0.06°	27.9 ± 0.30^{t}	104 ± 0.10^{c}
Mottled pea	*Pisum sativum*	Ma wan dou	78.7 ± 0.21^{h,i}	12.4 ± 0.30^{j}	38.8 ± 0.61^{r}	72.3 ± 0.10^{j,k}
Pigeon pea	*Cajanus cajan*	Mu dou	60.1 ± 0.48^{l}	33.1 ± 0.52^{f}	70.2 ± 0.24^{e}	64.8 ± 0.21^{m}
Pinto bean	*Phaseolus vulgaris*	Hua yao dou	44.1 ± 0.73^{r}	54.2 ± 0.09^{b}	67.9 ± 0.59^{f}	51.5 ± 0.21^{r}
Big rice bean	*Vigna umbellata*	Da hong dou	71.5 ± 0.59^{k}	19.8 ± 0.44^{g}	54.0 ± 0.78^{k,l}	69.9 ± 0.11^{l}
Small rice bean	*Vigna umbellata*	Xiao hong dou	51.7 ± 0.34°	53.5 ± 0.34^{b}	48.6 ± 0.15°	42.3 ± 0.07^{s}
Big runner bean	*Phaseolus coccineus*	Da hong hua cai dou	76.7 ± 0.47^{i}	15.9 ± 0.52^{i}	49.6 ± 0.79^{n,o}	72.2 ± 0.21^{j,k}
Small runner bean	*Phaseolus coccineus*	Xiao hong hua cai dou	52.1 ± 0.43°	46.9 ± 0.89^{c}	65.1 ± 0.88^{g}	54.3 ± 0.12^{q}
Big black soy bean (green core)	*Glycine max*	Lv xin da hei dou	56.4 ± 0.88^{n}	18.0 ± 0.40^{h}	73.9 ± 0.93^{d}	76.4 ± 0.10^{i}
Big black soy bean (yellow core)	*Glycine max*	Huang xin da hei dou	47.8 ± 0.27^{q}	38.3 ± 1.05^{e}	56.2 ± 0.88^{j}	55.7 ± 0.22^{p}
Small black soy bean (green core)	*Glycine max*	Lv xin xiao hei dou	50.2 ± 1.95°^{,p}	32.6 ± 1.28^{f}	63.5 ± 0.40^{g,h}	62.9 ± 0.70^{n}
Big green soy bean (green core)	*Glycine max*	Lv xin da qing dou	90.9 ± 0.49^{b}	−20.9 ± 0.14^{q}	54.4 ± 0.27^{j,k}	111 ± 0.06^{a}
Brown string bean	*Phaseolus vulgaris*	Zong se si ji dou	89.8 ± 0.22^{b,c}	1.81 ± 0.06^{m,n}	28.3 ± 0.23^{t}	86.4 ± 0.10^{f}
Red sword bean	*Canavalia gladiata*	Hong dao dou	87.6 ± 0.24^{d}	6.61 ± 0.10^{l}	14.3 ± 0.20^{u}	65.2 ± 0.05^{m}
Black sword bean	*Canavalia gladiata*	Hei dao dou	81.0 ± 0.44^{f,g}	10.3 ± 0.88^{k}	60.3 ± 0.56^{i}	80.4 ± 0.69^{g}

Color measurements were performed in triplicate and results were expressed as mean ± SD. L^* indicates black or white ($L^* = 0$ yields black and $L^* = 100$ means white), and a^* means green or red (negative values mean green and positive values mean red), and b^* suggests blue or yellow (negative values suggest blue and positive values suggest yellow). H°, hue angle. Multiple comparison was carried out by one-way analysis of variance (ANOVA) plus *post hoc* Tukey test, with different superscript lowercase letters indicating statistical significance ($p < 0.05$).