JAASP *Quality evaluation for health product development*

SHORT COMMUNICATION

Quality evaluation of purple waxy corn cobs for health use

Sucharat Limsitthichaikoon[1], Bhattaranitch Khampaenjiraroch[1], Kedsarin Saodaeng[1], Thithima Rimdusit[2] and Suthasinee Thapphasaraphong[2]

[1]Division of Pharmaceutical Technology, Faculty of Pharmaceutical Science, Khon Kaen University, Khon Kaen, 40002, Thailand and [2]Division of Pharmaceutical Chemistry, Faculty of Pharmaceutical Science, Khon Kaen University, Khon Kaen, 40002, Thailand

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Correspondence
Sucharat Limsitthichaikoon
Faculty of Pharmaceutical Science
Khon Kaen University
123 Mittraphap Road
Khon Kaen 40002
Thailand

E-mail
lsucharat@gmail.com

Abstract

Purple waxy corn cob is an anthocyanin-containing agricultural waste used as a test sample herein in quality evaluation as it is highly interested for health use. Its implementations require some critical quality concerns. This study aims to assess purple waxy corn cobs for some physicochemical characteristics, antioxidative activity and hazardous contamination. Aqueous extracts of the cobs were found to contain 12.3 and 68.7 mg/g of total anthocyanins and total phenolic contents, respectively. Activation energies of about 20-30 kJ/mol were dependent of pH of the aqueous solutions. Its aqueous and ethanolic extracts gave IC_{50} of about 77.8 and 96.5 ppb, respectively. Total aflatoxins of the cob samples was determined by ELISA to be more than 8.5 ppb. Heavy metals such as lead, cadmium, arsenic and mercury are less than the allowance limits of WHO. Thus, purple waxy corn cobs, under the storage conditions, were shown to be safe and potential for health use.

Introduction

Purple corn (*Zea mays* L.) contains various bioactive phenolic compounds, such as anthocyanins, ferulic acid, rutin, quercetin, naringenin, kaempferol, with antioxidative activity and capability to promote endogenous antioxidant en-zymes in isolated mouse organs (Ramos - Escudero et al, 2012). Agricultural wastes from purple corns with

pigments may possess valuable potentials in health product Industries as they also contain the bioactive compounds. Public concerns in environmental protection raise innovative utilization of agricultural wastes, thus, cobs of purple corns have been used as one of the anthocyanin resources (Yang *et al*, 2008). Purple waxy corn (*Zea mays* L. *ceritina* Kulesh.), locally known as 'Kao Kum', from an open-pollinated variety, is edible with high anthocyanin contents (Harakotr *et al*, 2014), in comparable to cops from Chinese purple corns (Yang *et al*., 2010). However, anthocyanins from Chinese purple corn cobs undergo rapid thermal degradation with an activation energy (E_a), the minimum energy used to activate degradation, of 18 kJ/mol (Yang *et al*, 2008). Cyanidin, delphinidin, pelagonidin, 3 of the mostly found anthocyanins, were reported to be the major anthocyanins in purple waxy corn (Harakotr *et al*, 2014). These emphasize some concerns of purple corns in related to physicochemical characteristics.

Antioxidative activities can be screened by various methods including an assay by 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Tirzitis and Bartosz, 2010). DPPH reacts with free radicals such as superoxide anion, hydroxyl or peroxyl radicals. IC_{50} of purple corns extracted by various solvents was reported to be in the range of 50 – 70 ppb (Ramos-Escudero et al, 2012). Screening of antioxidative activity should be one of the quality evaluation of the cobs.

Contaminations with microbes and heavy metals can become hazardous. Aflatoxins, one of the most potent carcinogens, are secondary metabolites of *Aspergillus* spp. These fungi produce the toxins while infecting food, feed and dairy products which produce from animals that had been fed with the contaminated feeds (Weaver and Truckess, 2010). Aflatoxin may produce any time of the harvesting if moisture contents in the crops and environment reach the level which facilitates mold growth. Invasion of insects or rodents to the storage sites can introduce the molds to the crop as well. Protection of mold growth and close storage is currently the best approach to prevent aflatoxin contamination. However, it is not to totally eliminate aflatoxins, thus, each country has set its own regulation on allowance on some specific aflatoxins (Trafton, 2010), for example the US Food and Drug Administration limits total aflatoxins (B1, B2, G1 and G2) at 20 ppb in all foods while 0.5 ppb of aflatoxin M1 being limited for milk and dairy products (Lawley, 2007). Enzyme-linked immunosorbent assay (ELISA), a sensitive analysis for aflatoxins using antibodies or antigen coupled to an enzyme to form detectable colored solution in the presence of aflatoxins, has been used to screen aflatoxin in agricultural samples because it is easy, convenient and rapid (Agudelo, 2014).

It is aimed to investigate for setting qualitative and some quantitative values for purple waxy corn cobs by determination of total anthocyanin and phenolic contents with antioxidative activity screening, as well as determi-nation of some potential hazardous contaminants.

Materials and Methods

Collection and extraction: Locally harvested dried cobs of purple waxy corn (*Zea mays* L. *ceritina* Kulesh.) from an open-pollinated variety (Kao Kum) were ground twice with a

hammer mill and then a blender to obtain cob powder. The cob powder was extracted with hot water and freeze dried to obtain dark purple powder, abbreviated as CC.

Activation energy: Triplicate batches of CC was dissolved into 10 mg/mL in buffered solutions at pH 4.0 and 7.0. Air-tight and light-protected containers were used to store the samples and kept 65±2 % relative humidity at 30, 50 or 70°C. At predetermined time, samples (n=3 each) were taken from each batch for determination of total anthocyanin content (TA) by pH differential method (Lee, 2005). Degradation rate constants (k) of each were obtained from linear regression analysis of semi-log plots of TA and storage time. Activation energies (E_a) of the stored CC extracts were obtained from a linear regression plot of storage temperatures and k, in accordance with the Arrhenius equation, as follows:

$$\ln k = \ln k_0 - E_a / RT \quad (1)$$

k = rate constant (h^{-1}), k_0 = frequency factor (h^{-1}), E_a = activation energy (kJ/mol), R = universal gas constant (8.314 J/mol·K) and T = absolute temperature (K).

Thermal analysis: Differential scan-ning calorimetry (DSC) and thermal gravimetric analysis (TGA) to determine melting or transition temperatures (exothermic or endothermic) and weight loss by heat were conducted. CC powder (3-5 mg each) were placed in a pierced aluminum pans and heated at a scanning rate of 5°C/min from 25°C to 200°C under air atmosphere using blank aluminum pan as a reference by DSC (DSC_{822^e}, Mettler Toledo, U.S.A) and TGA ($TGA/SDTA_{851^e}$, Mettler Toledo, U.S.A) with cooling machine in a nitrogen bath (40 ml/min).

Determination of total phenolic content: Folin–Ciocalteu reagent (Sigma-Aldrich, U.S.A.) using gallic acid (Sigma-Aldrich, U.S.A.) as the standard equivalence was used. Samples of CC were mixed with Folin–Ciocalteu reagent for 4 min followed by adding 7.5% sodium carbonate. The mixture was incubated at 45°C for 15 min then its UV absorbance was determined using UV-Visible spectro-photometer (1240 model, Shimadzu, Tokyo, Japan) at 765 nm. Deionized water was used as the blank.

Determination of total anthocyanin content: Total anthocyanin content was analyzed by the pH differential method (Lee, 2005). In brief, the samples were mixed with KCl-HCl buffer (pH 1) and acetate buffer (pH 4.5) and the absorbance at 520 nm and 700 nm were measured using a microplate absorbance reader (Sunrise, Switzer-land). Total anthocyanin content (TA) as cyanidin-3-glucoside equivalent being calculated as follows:

$$\text{TA (mg/L)} = \frac{A \times MW \times DF \times 10^3}{\varepsilon \times 1} \quad (2)$$

$A = (A_{520}\text{-}A_{700})_{pH1.0} - (A_{520}\text{-}A_{700})_{pH4.5}$, MW = molecular weight (449.2 g/mol for cyanidin-3-glucoside), DF = dilution factor, 10^3 = factor for conversion from g to mg, ε = molar extinction coefficient (26900) in L mol^{-1} cm^{-1}, and 1 = path length in cm.

Antioxidative activity by DPPH: Various solvents were used to compare the antioxidative activity of

the cobs. DPPH (0.1 mM) was used to react with the extracts of CC in comparison to Trolox (2 mM) and L-ascorbic acid (8 mM). All of the standards and samples were diluted into 96 well-plate and incubated in the dark for 30 min for absorbance reading at 517 nm using a microplate reader (Bio Rad680, Japan). Then, IC_{50} was calculated.

Quantification of total aflatoxins: The ELISA method was used in this study in accordance to the AOAC 990.32. In brief, 70% methanol was used to extract free alflatoxin from the cob samples after adjusting the pH to 6-8. The samples and controls were competed with enzyme-labeled toxin (conjugate) for the antibody binding sites in the microwells. After washing step, substrate reacts with the bound enzyme to produce blue color. Result-ant solutions were transferred to a microplate reader (Bio Rad680, Japan) to read the absorbance at 650 nm. Standard curves (n = 4) were validated (with correlation coefficients of higher than 0.99) for use in the determination of the samples.

Quantification of heavy metals: The heavy metal assay used in this study was followed AOAC 999.10. Standard range of each metal parameter was followed by Thai herbal pharmacopeia (Supplement to Thai Herbal Pharmacopoeia 2011).

Statistical analysis: Data were expressed as mean ± standard deviation (SD). Coefficients of variation (CV) were calculated for determination of precision of data and methods. Statistical significance was determined by one-way analysis of variance (ANOVA). The significant level was considered at $p < 0.05$.

Results and Discussion

Aqueous extracts of purple cobs of *Z. mays* L. *ceritina* Kulesh. appeared as the dark red-blue solutions with no smell and taste. Freeze drying of the aqueous extracts resulted in dark purple powder (designated herein as CC).

Table 1 Physicochemical characteristics of aqueous extracts of *Z. mays* L. *ceritina* Kulesh.

	Items	value
Chemical analysis	• Delphinidin[1] (mg/g)	153 ±19.9
	• Cyanidin[1] (mg/g)	6.2 ±0.05
	Total contents of	
	• Anthocyanins (C3G[2] mg/g)	12.3 ±1.6
	• Phenolic (GAE[3] mg/g)	68.7 ±2.7
Thermal analysis	• calories (mJ)	4401.7
	• onset (°C)	136.83
	• %residue	78.22
	• Melting temp (°C)	138.03
	Activation energy (kJ/ mol)	
	• pH4	28.8
	• pH7	18.0

[1]HPLC results from Khampaenjiraroch *et al*, 2014)
[2]Cyanidin-3-glucoside equivalence
[3]Gallic acid equivalence

Summary of the physicochemical characteristics of *CC* was shown in Table 1. In terms of chemical analysis, our previous report using an isocratic HPLC to separately analyze the CC detected the level of delphinidin at about 2.5 times higher than that of cyanidin (Khampaen-jiraroch *et al*, 2014).

Total anthocyanins extracted from our purple waxy corn cobs yielded 12.3±1.6 mg/g dried weight which was about 2 times higher than 6 mg/g from the Chinese purple corn cobs as previously reported (Yang *et al*, 2008). High yield could partially be a result of pH of the extraction conditions with the highest yield from purple waxy corn cobs at pH 6 (Priprem *et al*, 2014). Total phenolic content of CC of 68.70±2.67 mg/g dried weight was obtained.

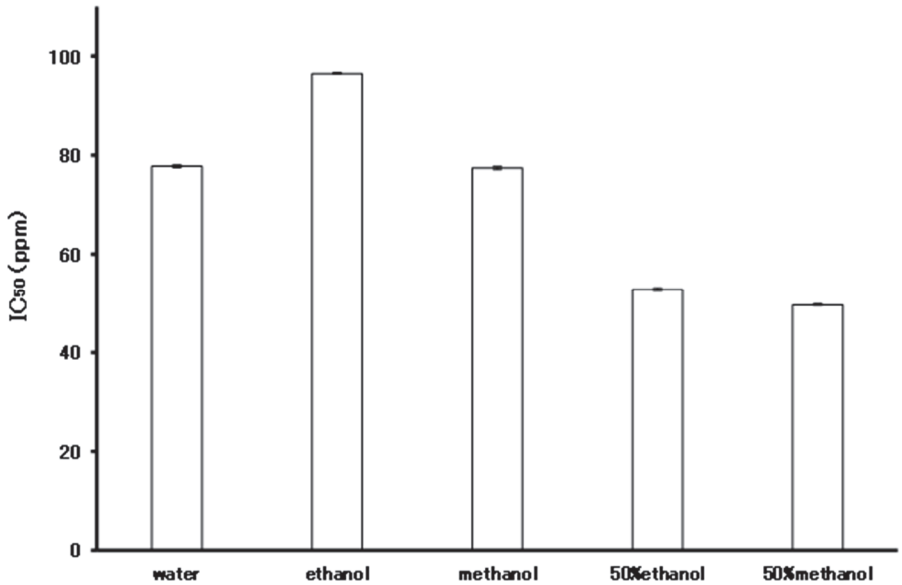

Figure 1 Comparison of extraction solvents on IC_{50} (ppb) of cobs of *Z. mays* L. *ceritina* Kulesh. by DPPH assay.

Table 2 Average (± SD) total aflatoxins detected from purple waxy corn cobs (n = 4).

Sampling site*	Aflatoxin (ppb)
Top	2.59 ± 0.0
Bottom	1.53 ± 0.4
Middle left	8.55 ± 2.2
Middle right	7.48 ± 1.6

*storage box about 15 ×23 × 15 inch.

Anti-oxidant activity of CC extracted by various solvents was compared using DPPH assay, as shown in Figure 1. It was remarkably notified that the extracts from water, absolute ethanol and methanol showed low anti-oxidant activities (the ethanolic extract provided the lowest activity with an IC_{50} of 96.5 ppb, the water and methanolic extracts provided the moderate activity with IC_{50} of about 78 ppb), while 50% of both ethanol and methanol showed more appreciable capacity. These results are in line with the studies of Ramos-Escudero et al (2012) and Harakotr et al (2014).

Total aflatoxins present in the cob samples and 0-50 ppb of standards were conjugated with the antibody to give a detectable color as a result from substrate conjugation. Standards (n=4) show linearity (r > 0.99) with an average slope of -

0.0141 and y-intercept of 0.862 were used to calculate the aflatoxin content in the raw materials of the stored corn cobs. The results showed that the stored purple waxy corn cobs for 8 months (sampled from top, middle and bottom) of the storage containers were in the range of 1.5-8.5 ppb, as shown in Table 2. It was observed that the middle portion of the stored cobs produced significantly higher levels of total aflatoxins ($p < 0.05$). There has been no sample which gave extractable aflatoxins of higher than 20 ppb.

Table 3 Heavy metals detected in samples of purple waxy corn cobs.

Metals	Limit (mg/kg)	Results (mg/kg)
Arsenic	<0.2	Not detected
Cadmium	< 2.0	0.012
Lead	< 1	<0.050
Mercury	<0.5	0.008

Table 3 shows the detected amounts of prohibited heavy metals in herbs. Cadmium was the highest content found in the cob samples. Lead was the second highest while lead and arsenic are the least found. Based on the Thai Herbal Pharmacopoeia (2005), the purple waxy corn cobs did not contain prohibited heavy metals beyond the allowance limits.

Conclusion

Purple waxy corn cobs which were collected from the 2013 harvesting was shown to contain anthocyanins, particularly delphinidin and cyanidin which are reported to be beneficial to health. Aqueous extracts of the cobs was optimal in terms of total anthocyanins, total phenolic and anti-oxidative activity. Thermal stability of aqueous extract of the cobs was reasonably in line with the Chinese purple corn cobs. DSC/TGA suggested transition tem-perature of aqueous extracts of the cobs of <130°C. In terms of hazardous contaminants, aflatoxins and prohibited heavy metals (arsenic, cadmium, lead and mercury), the stored cobs did not substantially contaminate with all indicated items. Thus, aqueous extracts from purple waxy corn cobs were shown to have potential valuable contents for health use. Using the cobs with care handling technique, it is expectable that this type of agricultural waste can be kept with little or no hazardous production during storage. Therefore, purple waxy corn cob is one of the interesting agricultural wastes for health products.

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