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Original Research Article

Stability of corn cobs extract in powdered and concentrated form

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ABSTRACT

An attempt to utilize the agricultural by-products is getting interested from many researchers and factory owners. Corn cob is one of an agricultural by-product that can be found generally in Thailand and contains valuable phenolic acids such as ferulic acid and coumaric acid. Extraction of phenolic compounds for food application is an alternative way to add value of the agricultural by-products due to its physiological functions on human health and can be used as functional ingredient. However, a research paper related with stability of corn cobs extract in different forms during storage time is limited. Therefore, this research was to study on the stability of corn cobs extract in powdered form (by freeze drying) and concentrated form (by vacuum evaporation) during storage for 12 weeks at 4°C. Corn cobs were extracted using alkaline treatment prior to lignin and hemicellulose precipitation by using ethanol. The samples were taken every 2 weeks to analyze the physical and chemical properties including color measurement, antioxidant activity using DPPH method, and total phenolic content using Folin-Ciocalteu method. The results showed that all properties of those corn cobs extract in powdered and concentrated form significantly changed during storage ($P \leq 0.05$). Compare to an initial week (week 0), total phenolic content started changing at week 2 which the powdered sample decreased about 2.26% (ranged from 2.26 to 12.58% until week 12) and concentrated sample decreased about 3.95% (ranged from 3.95 to 6.50% until week 12). In terms of the antioxidant activity, IC_{50} started changing at week 4 which the powdered sample decreased about 21.41% (ranged from 20.75 to 36.08% until week 12) and concentrated sample decreased about 14.39% (ranged from 13.68 to 24.03% until week 12). It can be concluded that the decreasing trend during 12 weeks of storage of the powdered sample showed higher than that of concentrated form. Therefore, for further use of the corn cobs extract as a functional ingredient, it was no need to make the extract solution in the powdered form.

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INTRODUCTION

Thailand is one of developing country that has various agricultural sources due to its suitable planting area. Many processed food factories have been grown rapidly in Thailand for decades. By-products during the food processing occurred unavoidably. An attempt to utilize the agricultural by-products is getting interested from many researchers and factory owners in these days. Currently, an increased attention has been focused on the industrial waste for search of natural antioxidants (Sultana et al., 2007). Corn cobs is one of an important agricultural by-product that can be found generally in Thailand and contains valuable phenolic acids such as ferulic acid and coumaric acid (Torre et al., 2008). Many by-products from the corn industry such as starch, sweet corn processing, corn oil, alcohols, etc. were observed: for every 100 kg of corn grain approximately 18 kg of corn cobs are produced (Torre et al., 2008). To add value of the agricultural by-products especially for corn cobs, an extraction process was normally applied. Phenolic compound is a target compound after the extraction process because it can be used as functional ingredient for the food application. Moreover, their physiological functions on the human health were also reported in many academic articles from the researchers (Pandey and Rizvi, 2009; Vauzour et al., 2010; Seetharaman and McSweeney, 2015). By-products from the plants generally considered of the beneficial from their phenolics contain. The major phenolic compounds identified in both primary and secondary cell walls of the graminaceous plants, and of cereals, are cinnamic acids such as ferulic acid and p-coumaric acid (Pan et al., 1998). In 2008, Torre et al. (2008) reported about using an alkaline extraction treatment in the corn cobs for the phenolic extraction purpose. In addition, solvent extraction techniques including ethanol and methanol extraction have also been found to an alternative extraction way (Sultana et al., 2007; Kapcum and Uriyapongson, 2017). Because the corn cobs contain remains unused as lignocelluloses waste (Torre et al., 2008) which can be removed by the alkaline treatment by dissolving lignin by cleavage of ester linkages in lignin-polysaccharide complexes, thus releasing phenolic acids (Kondo et al., 1992) to further use as functional ingredient. Generally, the extracted solution after the extraction process was kept whether in powder or aqueous forms. However, a research paper related with stability of corn cobs extract in different forms during storage time is limited.

Therefore, the aim of this research was to study on the stability of the corn cobs extract in powdered form (by freeze drying) and concentrated form (by vacuum evaporation) during storage at 4°C for 12 weeks to investigate whether making the corn cobs extract in powdered form is necessary or just keep it in aqueous form after the extraction.

MATERIALS AND METHODS

Chemicals and reagents

Folin-Ciocalteu's phenol reagent, DPPH (2,2-diphenyl-1-picryhydrazyl), gallic acid (≥ 99%), were purchased from Sigma-Aldrich (Steinheim, Germany). Ethanol (95%) was purchased from Labscan (Bangkok, Thailand). All other chemicals in this study were of analytical grade.

Plant materials

The plant materials, corn cobs from 'Super sweet corn's variety, used for this study were collected locally from Nakhon Nayok province (Thailand) in July 2017. Corn cobs were cut for size reducing and firstly dried in hot air oven at 50°C for 48 h, then ground into a small particle using a commercial blender (ground to pass a 28-mesh sieve; 600 micron). The plant material samples were then dried again until the moisture content was about 5-7%. Corn cobs powder was vacuum packed and kept at -20°C for further extraction purpose.

Determination of moisture content

Corn cobs samples (after grinding) was weight to the nearest 5.000±0.001 g and placed in a moisture can and incubated in an oven at 105±2°C for at least 16 h to constant weight. The percent moisture content was then calculated following the method of AOAC, 2000.

Preparation of corn cobs extract

Two gram of dried corn cobs sample was saponified with NaOH (2 M, 60 mL) at room temperature, 150 rpm for 24 h (Tilay et al., 2008). The extracts were separated from the residue by vacuum filtering and then neutralized to pH about 7.00±0.50 using hydrochloric 6 M. To precipitate lignin and hemicelluloses, ethanol 95% was added to the filtrate about 3 times volume and stand at room temperature for 10 min. The murky solution was filtered using vacuum filter (Whatman no.1) and the ethanol was removed using vacuum rotary which controlled the temperature at 35-40°C. Evaporation process was finished after the corn cobs extract reached 15°Brix and stored in amble bottle at 4°C for further study.

Preparation of corn cobs extract in powdered form

Corn cobs extract in powdered form was done by freeze-drying process. 15°Brix of corn cobs extract solution was firstly frozen at -70°C for 24 h and then dried by methods of freeze-drying. Freeze-drying conditions with CoolSafe Freeze dryer (Coolsafe 90-80A, Denmark) were set as drying at -40°C for 72 h. Corn cobs extract powder was ground into a fine particle and kept at 4°C in aluminum foil bag with vacuum packing for further study.

Stability testing

Corn cobs extract were packed individually in 10 mL ample bottle for concentrated form and aluminum foil bag (vacuum packed) for powdered form, and then stored in refrigerator at 4°C for 12 weeks. The samples were taken every 2 weeks to analyze the physical and chemical properties including color measurement, total phenolic content using Folin-Ciocalteu method, and antioxidant activity using DPPH method.

Color measurement of corn cobs extract

Objective color measurements were obtained with a HunterLab ColorQuest XE (Reston, Virginia, USA). The color was measured by using CIE (L*a*b*) color space. The sample was diluted to 10% w/w with distilled water before measuring. All samples were performed in triplicate.

Determination of total phenolic content (TPC)

The total phenolic content (TPC) was quantified using the Folin-Ciocalteu method developed from the method by Ingkasupart et al. (2015), using gallic acid as a standard. Powdered sample was diluted to 10 mg/mL and concentrated sample was diluted to 100 mg/mL with distilled water. Pipette the aliquots 0.4 mL and mixed with 2 mL of Folin-Ciocalteu reagent (10% v/v diluted in distilled water). The mixture was incubated for 2 min, and then added 1.5 mL of sodium carbonate solution (7.5% w/v), mixed and left the mixture stand at room temperature for 2 h in the dark. Absorbance was measured at 760 nm. The total phenolic content was derived from a standard curve of gallic acid and expressed as mg of GA/mL.

Determination of DPPH radical scavenging activity (DPPH assay)

Corn cobs extract in powdered form was diluted to 10 mg/mL and concentrated form was diluted to 100 mg/mL with distilled water. The method of DPPH assay was developed from Ingkasupart et al. (2015) with some modifications. The reaction mixture contained 0.4 mL of aliquot sample and 3.6 mL of 150 µM DPPH (2,2-diphenyl-1-picrylhydrazy) in 95% ethanol. The mixture was incubated in the dark at room temperature for 30 min and measured the absorbance at 517 nm. The DPPH radical scavenging activity was expressed and recorded as $IC_{50.}$

Statistical analysis

Corn cobs extract samples were analyzed in triplicate and expressed as means ± standard deviation. Different between the sample at week 0 and others were evaluated by the analysis of variance (ANOVA) and Duncan's multiple-range test, using the SPSS program (version 13; IBM, Armonk, NY, USA).

RESULTS AND DISCUSSION

Effect of storage time on the color stability of corn cobs extract in powdered and concentrated form

The color changes during storage of corn cobs extract in powdered and concentrated form at 4°C for 12 weeks were shown in Table 1. Freeze-drying process was applied to make the powdered sample while vacuum evaporation was used for the concentrated one. Water content of the sample from these 2 processes is a main cause of color difference. The lightness value (L^*) of the concentrated sample showed higher than that of powdered sample due to its water content. On the other hand, water in the powdered sample has been removed almost 95% during freeze-drying process (Ciurzynska and Lenart, 2011; Shukla, 2011). Thus, the concentrated sample had more lightness value as compared to the powdered one. L*a*b* value of those powdered and concentrated sample showed significantly different from week 0 ($P \leq 0.05$). However, not much difference was found. Porto et al. (2017) studied about the physicochemical stability in beet and orange mixed juice and found that the color parameters during storage period had a slightly higher of L* and a* value than the newer ones (day 0) and had a decrease in the parameter b*. The alterations in the color parameters were probably caused by the oxidative and non-oxidative reactions of polyphenols, resulting in colored condensation products. These results are consistent with the work reported by Özkan et al. (2003) who studied on the effect of moisture content on CIE color values in dried apricots. They confirmed that as the moisture content increased, the L* color values increased while the a* value decreased.

Effect of storage time on retained total phenolic content of corn cobs extract in powdered and concentrated form

Phenolics and polyphenolics were main active components in vegetables, fruits, grains, and so on (Velioglu et al., 1998; Dong et al., 2014). TPC were determined by using Folin-Ciocalteu reagent. The Folin-Ciocalteu reagent react nonspecifically with phenolic compounds as it can be reduced by a number of nonphenolic compounds e.g., vitamin C, Cu (II), etc. (Sultana et al., 2007). The results showed that significant different of total phenolic content of both powdered and concentrated sample started from week 4 until week 12 was found ($P \leq 0.05$) (Table 2 and Table 3). Percent decreasing rate at initial storage (week 2, 4, and 6) as compared to week 0 of those of powdered and concentrated sample was not much different; powdered samples (ranged from 2.26 to 7.56% until week 12) (Table 2) and concentrated samples (ranged from 3.95 to 6.50% until week 12) (Table 3). Interestingly, percent decreasing rate of the powdered sample after 6 weeks storage until week 12 showed almost 2-time higher than that of concentrated sample. Al-sanabani et al. (2016) reported that total phenolics and flavonoids content in freeze-dried treated seeds were significantly lower than those of the frozen or fresh seeds. It might be explained that during freeze-drying treatment, there may be a chance of decline in the content of antioxidants due to degradation of certain compounds (Marques et al., 2006). In addition, Moser et al. (2017) studied about the storage stability of phenolic compounds in microencapsulated grape juice and reported that percent decreasing rate of hydroxycinnamic acid derivative of microencapsulated grape juice (stored at 5ºC for 150 days) was about 23% which showed a similar decreasing trend related with this work.

Effect of storage time on retained DPPH antioxidant activity of corn cobs extract in powdered and concentrated form

Oxidation is universally existent and has deleterious effects on both food quality and human health. DPPH is a stable free radical, which has been widely used for studying the free radical-scavenging activities of natural antioxidants (Dong et al., 2014). DPPH radical

Table 1. Stability of color of corn cobs extract in powdered and concentrated form at 4°C for 12 weeks.

Week	Powdered sample			Concentrated sample		
	L^*	a^*	b^*	L^*	a^*	b^*
0	71.97±0.01^{b}	10.20±0.01^{e}	73.89±0.02^{b}	98.72±0.00^{c}	-2.11±0.01^{a}	8.19±0.01^{g}
2	72.42±0.01^{f}	10.15±0.00^{c}	73.84±0.02^{b}	98.75±0.00^{e}	-1.37±0.00^{f}	6.32±0.01^{a}
4	72.50±0.00^{g}	9.60±0.02^{a}	73.48±0.09^{a}	98.48±0.00^{a}	-1.66±0.01^{c}	7.10±0.01^{e}
6	71.88±0.01^{a}	10.65±0.01^{f}	75.07±0.03^{e}	98.71±0.00^{b}	-1.51±0.01^{e}	6.70±0.00^{b}
8	72.38±0.01^{e}	10.15±0.01^{c}	74.63±0.02^{d}	99.17±0.00^{f}	-1.69±0.01^{b}	6.84±0.01^{d}
10	72.22±0.00^{c}	10.17±0.01^{d}	74.19±0.08^{c}	98.75±0.01^{e}	-1.67±0.01^{c}	7.14±0.01^{f}
12	72.36±0.01^{d}	10.07±0.00^{b}	74.59±0.03^{d}	98.73±0.01^{d}	-1.59±0.01^{d}	6.82±0.02^{c}

Values are expressed as means ± SD of triplicate experiments (n=3). Significant difference at 95% level of confidence based on Duncan's multiple range test. Means denoted with different letters within the same column were observed to differ significantly.

is commonly used for the assessment of antioxidant of antioxidant activity in vitro and is foreign to biological systems (Zhou and Yu, 2004; Sultana et al., 2007). As the concentration of phenolic compounds or degree of hydroxylation of the phenolic compounds increase their DPPH radical scavenging activity also increase and can be defined as antioxidant activity (Sanchez-Moreno et al. 1999; Sultana et al., 2007). This work, antioxidant activity using DPPH method was expressed as IC_{50}. Compared to an initial week (week 0), a significant difference of those samples (powdered and concentrated form) was found at week 4 of storage ($P \leq 0.05$) (Table 4 and Table 5). The antioxidant activity and total phenolic content of corn cobs extract in powdered and concentrated form showed similarly decreasing rate trend. At week 4, percent decreasing rate was 21.41% for the powdered sample which ranged from 21.41 to 36.08% until storage for 12 weeks (Table 4) and percent decreasing rate of the concentrated sample was 14.39% which ranged from 14.39 to 24.03% until storage for 12 weeks (Table 5). Results from Al-sanabani et al. (2016) stated that the freeze-drying process caused a significant decrease in antioxidant capacity measured by DPPH assay of pomegranate seeds as compared to that for fresh and frozen seeds. It might be indicated that some of the compounds present in the extracts were electron donors and could react with free radicals to terminate radical chain reactions and therefore, were able to boost the natural antioxidant defense mechanism (Xu and Chang, 2008).

Table 2. Stability of total phenolic content of corn cobs extract in powdered form at 4°C for 12 weeks.

Week	µg gallic acid/mL	Decreasing rate (%)
0	161.16±1.36^{e}	-
2	157.52±0.40^{d}	2.26
4	149.46±1.07^{c}	7.26
6	148.97±1.37^{c}	7.56
8	140.88±0.53^{a}	12.58
10	142.27±0.30^{ab}	11.72
12	143.05±0.86^{b}	11.24

Values are expressed as means ± SD of triplicate experiments (n=3). Significant difference at 95% level of confidence based on Duncan's multiple range test. Means denoted with different letters within the same column were observed to differ significantly.

Table 3. Stability of total phenolic content of corn cobs extract in concentrated form at 4°C for 12 weeks.

Week	µg gallic acid/mL	Decreasing rate (%)
0	113.81±0.62^{c}	-
2	109.31±0.65^{b}	3.95
4	106.41±2.52^{a}	6.50
6	108.51±0.88^{ab}	4.66
8	106.56±0.95^{a}	6.37
10	106.90±0.89^{a}	6.07
12	107.70±0.33^{ab}	5.37

Values are expressed as means ± SD of triplicate experiments (n=3). Significant difference at 95% level of confidence based on Duncan's multiple range test. Means denoted with different letters within the same column were observed to differ significantly.

Table 4. Stability of DPPH antioxidant activity (IC_{50}) of corn cobs extract in powdered form at 4°C for 12 weeks.

Week	IC_{50}	Decreasing rate (%)
0	19.57±0.87^{a}	-
2	20.73±1.32^{a}	-
4	23.76±0.45^{b}	21.41
6	23.63±2.11^{b}	20.75
8	24.81±1.17^{bc}	26.78
10	26.63±1.90^{c}	36.08
12	26.45±0.24^{c}	35.16

Values are expressed as means ± SD of triplicate experiments (n=3). Significant difference at 95% level of confidence based on Duncan's multiple range test. Means denoted with different letters within the same column were observed to differ significantly.

Table 5. Stability of DPPH antioxidant activity (IC_{50}) of corn cobs extract in concentrated form at 4°C for 12 weeks.

Week	IC_{50}	Decreasing rate (%)
0	225.80±8.62^{a}	-
2	222.73±7.17^{a}	-
4	258.30 ± 9.51^{b}	14.39
6	276.66±7.85^{bc}	22.52
8	256.68±9.25^{b}	13.68
10	266.83±3.70^{bc}	18.17
12	280.06±7.85^{c}	24.03

Values are expressed as means ± SD of triplicate experiments (n=3). Significant difference at 95% level of confidence based on Duncan's multiple range test. Means denoted with different letters within the same column were observed to differ significantly.

The observed stability trend of antioxidant activity of corn cobs extract in powdered and concentrated form during storage was unclear. Similar results were reported by Kallithraka et al. (2009) and Rocha-Parra et al., (2016) for white and red wine. They found that content of most phenolics diminished with time, but the antioxidant activity increased with storage and stated that although one would expect oxidation of antioxidants to yield a lower antioxidant capacity, reactions between oxidized phenolics may produce formation of new antioxidants.

CONCLUSIONS

Corn cob is a by-product from corn processing which normally use for animal feed due to its high fiber and nutrition. Extraction of phenolic compounds of this agricultural by-product to use as functional ingredient is getting more interested from many researchers and corn processing factory owners in these days. However, a research paper related on the stability of corn cobs extract in different forms during storage time is limited. This study worked on the stability of the corn cobs extract in powdered form by freeze drying and concentrated form by vacuum evaporation which stored at 4°C for 12 weeks. The results can be concluded that the decreasing trend during 12 weeks of storage of the powdered sample showed higher than that of concentrated form. Therefore, for further use of the corn cobs extract as a functional ingredient, it was no need to make the extract solution in the powdered form.

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REFERENCES

Al-Sanabani, A. S., Youssef, K. M., Shatta, A. A., El-Samahy, S. K. 2016. Impact of freeze-drying processes on color, phytochemical contents and antioxidant capacity of pomegranate seeds. Journal of Food Sciences; Suez Canal University 3: 27-34.

AOAC. 2000. Official methods of analysis. 18th eds. The Association of Official Analytical Chemists Arlington, Virginia.

Ciurzynska, A. and Lenart, A. 2011. Freeze-drying – Application in food processing and biotechnology – A review. Polish Journal of Food and Nutrition Science 61: 165-171.

Dong, J., Cai, L., Zhu, X., Huang, X., Yin, T., Fang, H., Ding, Z. 2014. Antioxidant activities and phenolic compounds of cornhusk, corncob and stigma maydis. Journal of the Brazilian Chemical Society 25: 1956-1964.

Ingkasupart, P., Manochai, B., Song, W.T., Hong, J.H. 2015. Antioxidant activities and lutein content of 11 marigold cultivars (*Tagetes* spp.) grown in Thailand. Food Science and Technology, Campinas 35: 380-385.

Kallithraka, S., Salacha, M. I., Tzourou, I. 2009. Changes in phenolic composition and antioxidant activity of white wine during bottle storage: accelerated browning test versus bottle storage. Food Chemistry 113: 500-505.

Kapcum, C. and Uriyapongson, J. 2017. Effect of storage conditions on phytochemical and stability of purple corn cob extract powder. Food Science and Technology, Campinas. DOI: http://dx.doi.org/10.1590/1678-457x23217.

Kondo, T., Ohshita, T., Kyuma, T. 1992. Comparison of characteristics of soluble lignins from untreated and ammonia-treated wheat straw. Animal Feed Science and Technology 39: 253-263.

Marques, L. G., Silveira, A. M., Freire, J. T. 2006. Freeze-drying characteristics of tropical fruits. Drying Technology 24: 457-463.

McSweeney, M. and Seetharaman, K. 2015. State of polyphenols in the drying process of fruits and vegetables. Critical Reviews in Food Science and Nutrition 55: 660-669.

Moser, P., Telis, V.R.N., Neves, N.A., Garcia-Romero, E., Gomez-Alonso, S., Hermosin-Gutierrez, I. 2017. Storage stability of phenolic compounds in powdered BRS Violeta grape juice microencapsulated with protein and maltodextrin blends. Food Chemistry 214: 308-318.

Özkan, M., Kirca, A., Cemeroglu, B. 2003. Effect of moisture content on CIE color values in dried apricots. European Food Research and Technology 216: 217-219.

Pan, G.X., Bolton, J.L., Leary, G.J. 1998. Determination of ferulic and *p*-coumaric acids in wheat straw and the amounts released by mild acid and alkaline peroxide treatment. Journal of Agricultural and Food Chemistry 46: 5283-5288.

Pandey, K.B. and Rizvi, S.I. 2009. Plant polyphenols as deietary antioxidants in human health and disease. Oxidative Medicine and Cellular Longevity 2: 270-278.

Porto, M. R., okina, V. S., Pimentel, T. C., Prudencio, S. H. 2017. Physicochemical stability, antioxidant activity, and acceptance of beet and orange mixed juice during refrigerated storage. Beverages 3: 36.

Rocha-Parra, D. F., Lanari, M. C., Zamora, M. C., Chirife, J. 2016. Influence of storage conditions on phenolic compounds stability, antioxidant capacity and colour of freez-dried encapsulated red wine. LWT-Food Science and Technology 70: 162-170.

Sanchez-Moreno, C., Larrauri, J.A., Saura-Calixto, F. 1999. Free radical scavenging capacity and inhibition of lipid oxidation of wines, grape juices and related polyphenolic constituents. Food Research International 32: 407-412.

Shukla, S. 2011. Freeze drying process: A review. International Journal of Pharmaceutical Science and Research 2: 3061-3068.

Sultana, B., Anwar, F., Przybylski, R. 2007. Antioxidant potential of corncob extracts for stabilization of corn oil subjected to microwave heating. Food Chemistry 104: 997-1005.

Tilay, A., Bule, M., Kishenkumar, J., Annapure, U. 2008. Preparation of ferulic acid from agricultural wastes: Its improved extraction and purification. Journal of Agricultural and Food Chemistry 56: 7644-7648.

Torre, P., Aliakbarian, B., Rivas, B., Dominguez, J.M., Converti, A. 2008. Release of ferulic acid from corn cobs by alkaline hydrolysis. Biochemical Engineering Journal 40: 500-506.

Vauzour, D., Rodriguez-Mateos, A., Corona G., Oruna-Concha, M.J., Spencer, J.P.E. 2011. Polyphenols and human health: Prevention of disease and mechanisms of action. Nutrients 2: 1106-1131.

Velioglu, Y.S., Mazza, G., Gao, L., Oomah, B.D. 1998. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. Journal of Agricultural and Food Chemistry 46: 4113-4117.

Xu, B. and Chang, S. K. C. 2008. Effect of soaking, boiling, and steaming on total phenolic content and antioxidant activities of cool season food legumes. Food Chemistry 110: 1-13.

Zhou, K. and Yu, L. 2004. Effects of extraction solvent on the wheat bran antioxidant activity estimation. LWT-Food Science and Technology 37: 717-721.