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Original Article

Antimutagenicity and anthocyanin of purple hybrid corn during storage in selected packaging materials

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Abstract

This study was conducted to determine the antimutagenicity and anthocyanin content of extracts from "purple corn strain KPSC903", both whole kernel (WG) and corn grit (CG), during six-month storage in nylon bags (N) or aluminum foil bags (F) under vacuum at 25ºC. Ethanol extract of WG showed 87% and 81% inhibitions of the mutagens Trp-P-1 and 2-aminoanthracene, respectively, whereas that from CG showed 84% and 64% inhibitions, respectively. Diethylether and water extracts showed less inhibition. During storage, both WG and CG decreased in antimutagenicity, while anthocyanin increased from 48.81 to 66.04 mg/100 g in WG and from 26.54 to 33.01 mg/100 g in CG. Correlation coefficients between % inhibition of Trp-P-1 and anthocyanin were -0.898, -0.869, -0.956, and -0.667 for WG in N, WG in F, CG in N, and CG in F. F retarded the loss of antimutagenicity of CG better than N.

Keywords: hybrid corn, purple corn, antimutagenicity, anthocyanin, vacuum packaging

1. Introduction

According to the Thailand national report on health status and health problems, the mortality of the Thai people attributable to cancer is increasing every year. The identification of active ingredients, especially natural ones, that can prevent, inhibit, and eliminate the growth or development of cancer cells is a highly active field of research. Among these ingredients are the anthocyanins, that have several health benefits on account of their antioxidant activity. Antioxidants benefit health by neutralizing free radicals, which otherwise contribute to oxidative stress and can cause diseases such as vascular disease, cancer, the decay of nerves, and aging (Lazze *et al.*, 2004). In Japan, the Ayamurasaki cultivar of purple sweet potato has been promoted for cultivation and for the production of processed food and health products. Similarly, purple corn powder is produced at an industrial level in Peru.

Purple corn is flour corn (*Zea mays* L.) that in addition to anthocyanins contains more starch in its kernels than yellow or white corn, and more lysine than yellow corn. Purple corn is an important source of anthocyanins and phenolic compounds; it is used by the beverages and sweet food industries as raw material and a coloring material (Jing & Giusti, 2007) for example in tortilla chips in the United States and in nutraceutical products in Japan. Additionally, a colored extract from purple corn is now widely available in Asia, South America, and Europe. The cobs and kernels of purple corn contain 54.6% and 45.4% anthocyanins, respectively. In various parts of purple corn, anthocyanin content is correlated with the total content of phenolic compounds (Cevallos-Casals & Cisneros-Zevallos, 2003). Anthocyanin content and phenolic compounds in Peruvian purple corn were 1,642 mg/100 g and 1,756 mg/100 g, respectively; this was more than in fresh blueberries containing 138-385 mg anthocyanin/100 g and phenolic content of 292-672 mg/100 g. The highest quantity of anthocyanins was found in this corn peel, at 45% of total volume. Gautam, Saxena, & Kumar (2016) stated that fruits and vegetables naturally contain antimutagens and referred to the World Health Organization quote that antimutagenic diet could be an effective solution to prevent cancer. In addition, anthocyanins have antimutagenic and chemopreventive activities (Jing & Giusti, 2007). They are effective in inhibiting free radicals and preventing cancer,

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arthritis, and cardiovascular diseases. Anthocyanin also has anti-inflammatory and antiulcer activities, and can decrease blood sugar in diabetes patients (Motohashi & Sakagami, 2008). Against the mutagenic heterocyclic amines 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole (Trp-P-1), 3-amino-1-methyl-5H-pyrido[4,3-b]indole (Trp-P-2), and 2-amino-3-methylimidazo-[4,5-f]quinoline (IQ), the Ayamurasaki potato has a high 41% inhibition activity (Yoshimoto *et al.*, 1999). Pedreshi & Cisneros-Zevallos (2006) found that different extracts from purple corn had different antimutagenic activities. Extract with 850 µg phenolics/plate inhibited 96% of mutations while the total phenolic fraction inhibited 97.2% of mutations; water extracts containing high levels of anthocyanins exhibited 92.1% inhibition; and the ethyl acetate fraction provided 100% inhibition. High levels of anthocyanins extracted from purple corn better prevented proliferation of colon cancer cells than did anthocyanin-containing extracts from chokeberry, bilberry, purple carrots, grape, purple daikon, and elderberry (Jing *et al.*, 2008). The Chinese purple corn hybrid coded EZPC demonstrated antiproliferative capacity against the HT-29 colon cancer cell line on account of its high anthocyanin content, especially cyanidin derivatives (Zhao *et al.*, 2009).

Storage conditions, particularly pH, temperature, and ultraviolet light, may affect the stability of anthocyanins (de Pascual-Teresa & Sanchez-Ballesta, 2008). In general, postharvest storage of plant seeds avoids moist and high-temperature conditions that provide an opportunity for aflatoxin or mycotoxin production by mold, which would result in loss of commercial value. Additionally, temperatures of 22-34°C are suitable for insect growth and possible destruction of seeds by them. Lower storage temperatures have been found in many studies to increase the anthocyanin content of many fruits (Gonc¸alves *et al.*, 2004; Lo Piero, Puglisi, Rapisarda, & Petrone, 2005; Sanchez-Ballesta *et al.*, 2007). The stored seeds are usually packed in a sealed plastic bag that provides a water barrier and protection against ultraviolet light. The bag contains high carbon dioxide and low oxygen atmosphere (Casini, Juliana, & Audicio, 2009). Giorni, Battilani, Pietri, & Magan (2008) reported that 25% CO_2 level inhibited the growth of *A. flavus*, while 50% CO_2 can inhibit aflatoxin formation in high-moisture corn seeds. However, controlled atmospheres with modified CO_2 content can inhibit the increase of anthocyanin during storage (Arte's-Herna'ndez, Artes, & Tomas-Barberan, 2003; Remon, Ferrer, Lopez-Buesa, & Oria, 2004). Meanwhile, although oxygen can reduce anthocyanin levels by oxidizing the anthocyanin compounds both directly and indirectly, varying oxygen contents (0%, 5%, and 10%) during storage did not affect the anthocyanin content of Hom Nil rice or Red Hom Mali rice (Htwe *et al.*, 2010). However, anthocyanins stored under vacuum or in nitrogen atmosphere were more stable than those under oxygen. Therefore, to prevent anthocyanin reduction during storage and distribution, products containing anthocyanins should be packaged in material that serves as an oxygen barrier, and the headspace should be reduced.

To support the utilization of KPSC903 purple corn, a single corn hybrid containing high starch and anthocyanin contents bred by the National Corn Sorghum Research Center, Kasetsart University, this research aimed to investigate the antimutagenicity and anthocyanin content of KPSC903 corn kernels and corn grit during storage. The relationship between antimutagenicity and anthocyanin content was also assessed. The results obtained from this research will benefit the utilization of this corn variety to solve health problems in Thai society, including disease prevention and rehabilitation of the people's health.

2. Materials and Methods

2.1 Determination of antimutagenicity of high anthocyanin hybrid corn

2.1.1 Preparation of KPSC903 hybrid corn

Whole-grain corn (WG) was supplied by Dr. Chokechai Aekatasanawan of the National Corn Sorghum Research Center and corn grit (CG) was prepared by Mrs. Chulalak Charunuch of the Institute of Food Research and Product Development. Both WG and CG were ground using a blender and sieved through a 12-mesh screen. Five grams of the appropriate powder were extracted using a Soxhlet apparatus for 10 h with 250 mL of ethanol or diethyl ether. For water extraction, 15 g of powder was extracted with 150 mL water in an Erlenmeyer flask sealed with a screw cap. The flask was shaken at 250 rpm for 20 h in a water bath (Memmert SV1422, Germany) and then filtered through No.4 filter paper. Each extract was evaporated to dryness using a rotary evaporator under vacuum, the yield was calculated, and the evaporated crude extract was stored at -20°C in a closed container until antimutagenicity determination.

2.1.2 Determination of antimutagenicity by Ames method

2.1.2.1 Bacterial culture

The culture media for *Salmonella typhimurium* strains TA98 (TA98) and strain TA100 (TA100) consisted of two parts:

1) An agar plate (minimal agar) with 30 mL minimal glucose agar medium containing 1.5% Bacto-Difco agar + 2% glucose in Vogel-Bonner medium E (1 L stock prepared from 670 mL distilled water, 10 g magnesium sulfate, 100 g citric acid, 500 g monohydrate dibasic potassium phosphate, and 175 g sodium ammonium phosphate); and

2) A top agar plate with 0.6% agar and 0.5% NaCl dissolved in water. The agar solution was sterilized and stored at room temperature in a screw cap bottle. When preparing the top plate, the solution was melted by immersing in boiling water, and to it was added 10 mL sterile 0.5 M L-histidine.HCl/ 0.5 mM biotin per 100 mL top agar.

2.1.2.2 Mutagenicity determination

The pre-incubation method, an improvement to Ames method, was used to activate bacteria in thorough contact with chemicals and for an extended period. The standard mutagens 3-amino-1,4-dimethyl-5H-pyrido[4,3-6]indole (Trp-P-1) at a concentration of 50 ng/plate and 2-aminoanthracene (2-AA) at a concentration of 5 µg/plate were

mixed into culture media appropriate for TA98 and TA100, respectively. The mutagens were activated by the addition of 0.7 mL phosphate buffer pH 7.0 and 0.1 mL S-9 mix enzyme. Next, 0.05 mL of corn extract (from Section 1.1) and 0.1 mL of *S. typhimurium* bacteria were added, and the mixture was immersed in the shaking water bath at 37°C for 20 min. The top agar, containing histidine and biotin, was poured into a glucose agar plate that had been prepared at least three days before. The plate was incubated at 37°C for 48 h, after which colonies were counted and the inhibition percentage calculated. Two plates were prepared for each treatment, and two replicate experiments were performed.

$$\text{Percent inhibition} = 100 \times (C_0 - S) / (C_0 - C_{100})$$

C_0: number of colonies of mutant bacteria activated by standard mutagen without extract sample (positive control)

C_{100}: number of colonies of natural mutated bacteria (negative control)

S: number of colonies of mutated bacteria activated by standard mutagen with an extract sample

2.2 Determination of antimutagenicity and anthocyanin content of high anthocyanin hybrid corn during storage

WG or CG (200 g) was packed under vacuum in two types of containers: aluminum foil laminated bags (F) and nylon bags (N). The samples were stored for six months at 25°C, with once-a-month determinations of antimutagenicity by Ames' test and anthocyanin content by water extraction (Jing & Giusti, 2007) and the pH differential method (Giusti & Wrolstad, 2001). Determining anthocyanin in aqueous solution showed less solvent effect than in acidic ethanol or methanol when using pH differential method (Wrolstad, Durst, & Lee, 2005).

2.2.1 Antimutagenicity determination by Ames' method

Antimutagenicity of the sample was determined using the same method in 2.1.2.

2.2.2 Anthocyanin content determination by pH differential method

2.2.2.1 Sample extraction

A mixture of 1 g of WG or CG powder and 25 mL deionized water was shaken in a 50°C water bath (Heto SBD50, Denmark) for 1 h at 80 rpm. The resulting mixture was filtered by Whatman No.1 filter paper and the filtrate was transferred to 25 mL test tube.

2.2.2.2 Anthocyanin measurement

Two dilutions of the sample were prepared, one with 0.025 M potassium chloride buffer, pH 1.0, and the other with 0.4 M sodium acetate buffer, pH 4.5 (diluting each by the previously determined dilution factor). After the dilutions were equilibrated for 15 min, the absorbance of each dilution was measured at 510 nm and at 700 nm (to correct for haze) with distilled water as a blank using Spectrophotometer UV-1601 (Shimadzu Corporation, Japan). The total monomeric anthocyanins were calculated as cyanidin-3-glucoside, using the extinction coefficient of 26900 L/(cm)(mg) and a molecular weight of 449.2 g/L. Cuvettes of 1-cm path length were used. The absorbance of the diluted sample (A) was calculated as follows:

$$A = (A_{510} - A_{700})_{pH1} - (A_{510} - A_{700})_{pH4.5}$$

Anthocyanin (mg/L) = (A x MW x Dilution factor x 1000)/ 26900 x Path length

2.2.3 Correlation between anthocyanin content and % inhibition

The Pearson correlation over time between anthocyanin content and % inhibition of Trp-P-1 at the highest concentration of the corn extract was calculated.

2.3 Data analysis

Statistical analyses were carried out using SPCC 16 software (SPSS Inc., Chicago, IL, USA). The significance of differences between mean values was determined by Duncan's Multiple Range and Least Significant Difference tests with results considered significant at the 5% level ($p<0.05$).

3. Results and Discussion

3.1 Antimutagenicity of high anthocyanin hybrid corn

The yields of extraction of KPSC903 corn with the three solvents ethanol, diethyl ether, and water, are given in Table 1. Ethanol extracts gave the highest yield, followed by water extracts and diethyl ether extracts. The yield percentages of WG extracts were significantly different, whereas for CG samples they were not ($p>0.05$).

In antimutagenicity tests, TA98 was used as the indicator for Trp-P-1 and TA100 for 2-AA. The Trp-P-1 is activated in TA98 to form NO-acetyl-Trp-P-1, which can damage DNA by forming DNA adducts that result in genetic changes. It can additionally produce reactive oxygen species that can cause oxidative damage to DNA (Wakata *et al.*, 1985). In TA100, the 2-AA reacts with the S-9 mix enzyme to produce N-hydroxy-2-aminoanthracene, which damages DNA. In both cases, corn extract was added at a concentration of 5 mg/plate. The ethanol extracts of WG and CG showed the highest Trp-P-1 inhibition percentages, at 87% and 84%, respectively. Diethyl ether extracts and water extracts demonstrated lesser inhibitory effects. Against 2-AA, the ethanol extracts showed the highest inhibitions at 81% and 64% for WG and CG, respectively, followed by water and then diethyl ether extracts. Thus, ethanol was selected as the solvent for sample extraction in further experiments.

Table 1. Yield and inhibition percentages of KPSC903 hybrid corn extracts using three solvents

Sample	Solvent	% Yield	% Inhibition	
			Trp-P-1	2-AA
Whole grain (WG)	Ethanol	$13.13^{a} \pm 1.12$	$87^{a} \pm 5$	$81^{a} \pm 5$
	Diethyl ether	$4.51^{b} \pm 1.33$	$76^{a} \pm 6$	$47^{b} \pm 2$
	Water	$8.39^{c} \pm 0.34$	$54^{b} \pm 6$	$57^{b} \pm 7$
Corn grit (CG)	Ethanol	$7.59^{A} \pm 2.17$	$84^{A} \pm 6$	$64^{A} \pm 6$
	Diethyl ether	$2.73^{A} \pm 1.37$	$64^{B} \pm 4$	$39^{B} \pm 3$
	Water	$5.08^{A} \pm 1.73$	$51^{B} \pm 4$	$47^{B} \pm 4$

[a, A] Means in a column of each sample with different superscripts are significantly different ($p \leq 0.05$).

The inhibitory activity of KPSC903 corn extracts against the Trp-P-1 was tested at four concentrations: 1.013, 2.026, 5.065, and 10.13 mg/plate (Figures 1 and 2). Percent inhibition was found to correlate with extract dose; higher concentrations of extract gave stronger inhibition. Overall, WG extracts exhibited higher inhibition than the CG extracts. At the beginning of storage (month 0), the highest concentrations (10.13 mg/plate) of the WG extract and the CG extract resulted in 90% and 75% inhibition, respectively. Against 2-AA, inhibition again correlated with dose (Figure 3 and 4), and WG extracts resulted in significantly greater inhibition than did the CG extracts. At the beginning of storage, the highest concentrations (10.13 mg/plate) of WG extracts and CG extracts resulted in 81% and 53% inhibition, respectively. The lower antimutagenic activity of the CG extracts can be explained by loss of the seed coat, which is a purple part that contains anthocyanins, during the germ separation process.

The KPSC903 corn extracts showed antimutagenic effects on both mutagens: Trp-P-1 and 2-AA, of which Trp-P-1 is active on TA98. The N-hydroxy formation of Trp-P-1 resulted in NO-acetyl-Trp-P-1 occurrence that can damage DNA by forming DNA-adducts and leading to genetic changes. It can produce reactive oxygen species (ROS) that can cause oxidative reactions that damage DNA (Wakata *et al.*, 1985). The 2-AA is active on TA100. After reacting with the S-9 mix enzyme, N-hydroxy-2-aminoanthracene was produced that is ready to damage DNA. The results showed that WG extract had higher antimutagenicity than the CG extract. This due to the CG loss the seed coat, which is a purple part that contains anthocyanins in the germ separation process.

3.2 Antimutagenicity and anthocyanin content of high anthocyanin hybrid corn during storage

During six-month storage, corn extract antimutagenicity against Trp-P-1 decreased significantly (Figures 1 and 2). Inhibition percentage was not significantly different ($p>0.05$) between storage bag types (N or F) for either WG or CG. During the first three-month storage, there was no difference in the mutagenic inhibition effect against 2-AA for WG in F and N ($p>0.05$), but in months 4-6 the inhibition significantly decreased (Figures 3 and 4). For CG, inhibition decreased more rapidly when stored in N than when in F.

Meanwhile, the anthocyanin content of WG significantly increased during storage, from 48.81 mg/100 g (dry basis, db) to 65.85 and 66.04 mg/100 g db in F and N, respectively (Figure 5). For CG, anthocyanin content significantly increased from 26.54 to 33.01 mg/100 g db when packed in F but not when packed in N (32.52 mg/100 g db, ($p>0.05$). When comparing the two container types, the difference in anthocyanin content was not significant ($p>0.05$). During storage, the moisture contents of WG and CG were 9.98-11.35% and 9.88-11.90%, respectively (data not shown). The change of moisture did not have any pattern, showing both significant and not significant differences. The anthocyanin increase of KPSC903 corn during storage at 25°C was similar to the anthocyanin content of wild blueberry powder stored at 25°C with less reduction than when stored at other temperatures (Fracassetti *et al.*, 2013). Azuma, Yakushiji & Sato (2019) recently reported that anthocyanin content in grapes increased during storage in light at 15-25°C. In contrast, they reported that darkness inhibited anthocyanin accumulation and also concluded that light was a major factor for anthocyanin accumulation and had a synergistic effect on anthocyanin accumulation in grapes with an appropriate temperature. The KPSC903 corn was stored in an opaque refrigerator, in the dark. The reason for the anthocyanin increase found in the stored KPSC903 corn is not clearly understood, but it was similar to the anthocyanin increase of stored raspberry (Palonen & Weber, 2019). Thus, Saure's sentence in 1990, "the role of temperature seems to be ambivalent for anthocyanin formation", that Ratanamarno, Uthaibutra & Saengnil (2005) referred to is still valid.

Percent inhibition of Trp-P-1 and anthocyanin content of corn extract were found to be negatively correlated during storage, with $r = -0.898$ for WG packed in N, -0.869 for WG packed with F, -0.956 for CG packed in N, and -0.667 for CG packed in F ($p \leq 0.01$). Similarly, % inhibition of 2-AA and anthocyanin content of corn were negatively correlated, with $r = -0.873$ for WG packed in N, -0.710 for WG packed in F, -0.927 for CG packed in N, and -0.677 for CG packed in F ($p \leq 0.01$). It can be concluded that antimutagenicity in this high anthocyanin hybrid corn is not solely the result of anthocyanin. It has been demonstrated that the purple coloring agent (anthocyanin) in purple corn can inhibit the mutagenic activity of Trp-P-1 (Pedreschi & Cisneros-Zevallos, 2006) and 2-AA (Mendoza-Díaz *et al.*, 2012) by entering an electrophile (a nimble medium with DNA capture) before getting into the DNA. However, many other phenolic compounds have antimutagenic effects, such as chlorogenic acid, ferulic acid, caffeic acid; such compounds usually accumulate in the leaves and seeds of monocotyledon plants, especially of the cereal group, which harbor these types of acids in large quantities.

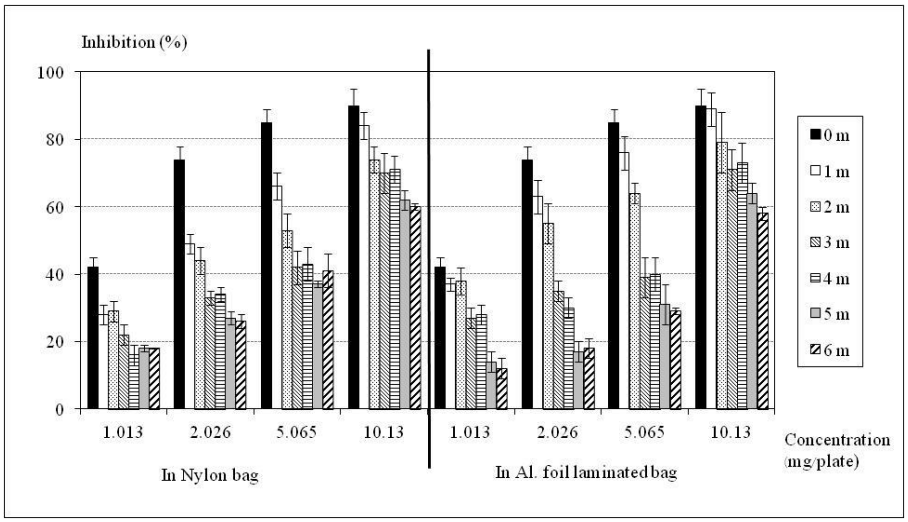

Figure 1. Inhibition percentage of whole grain extract on Trp-P-1 during six-month storage

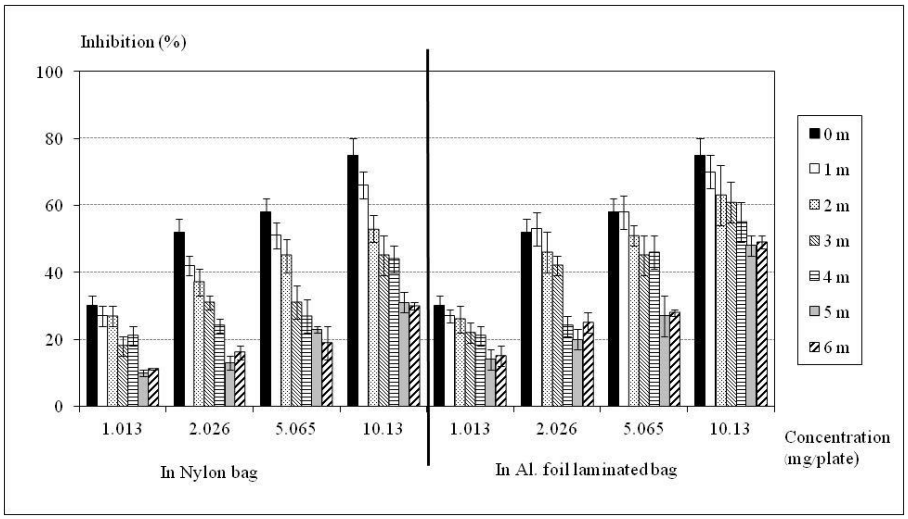

Figure 2. Inhibition percentage of corn grit extract on Trp-P-1 during six-month storage

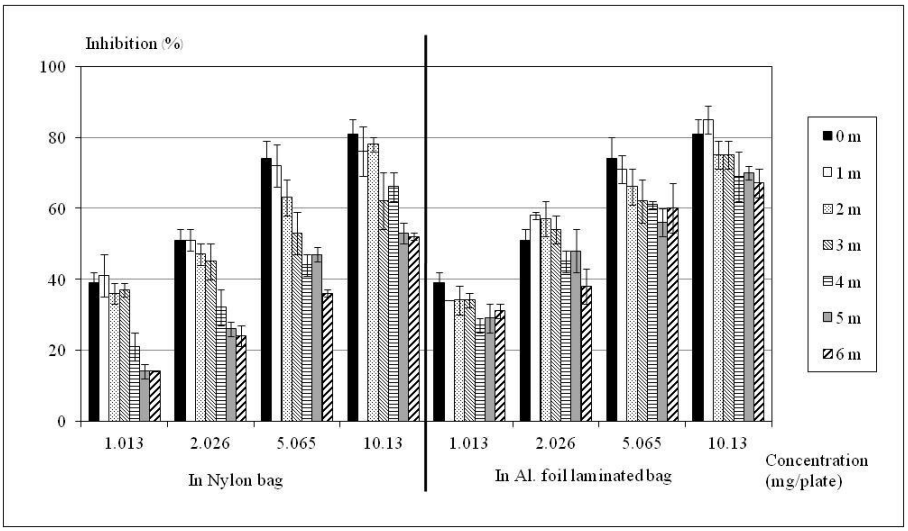

Figure 3. Inhibition percentage of whole grain extract on 2-AA during six-month storage

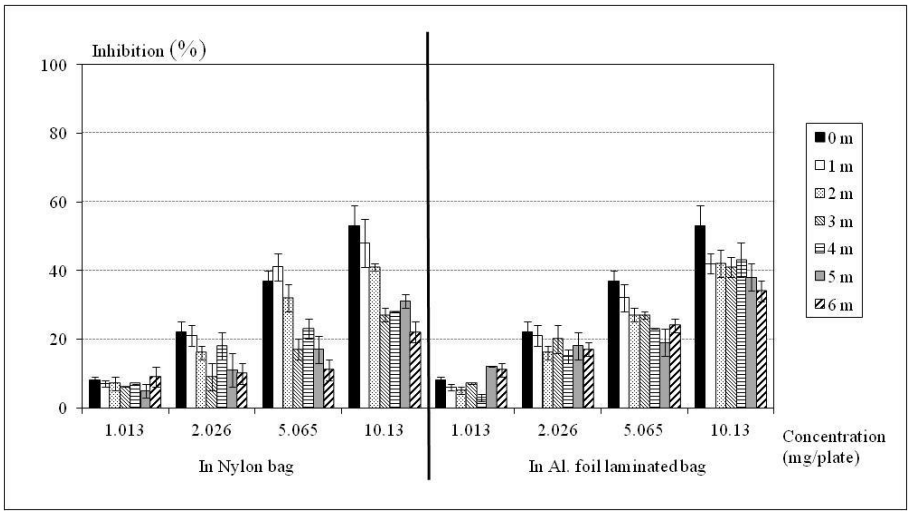

Figure 4. Inhibition percentage of corn grit extract on 2-AA during six-month storage

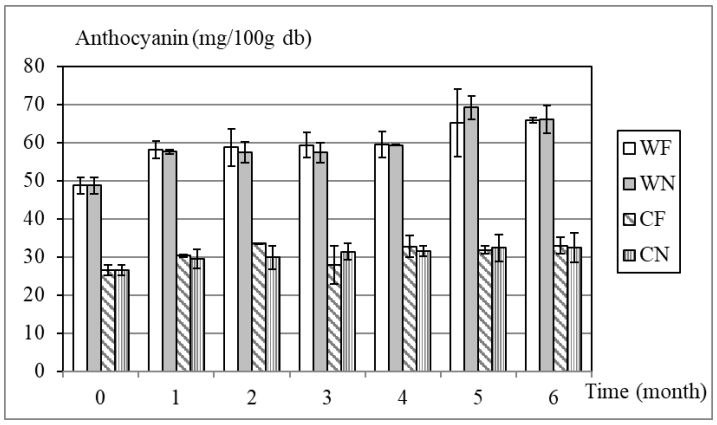

Figure 5. Anthocyanin of whole-grain (W) and corn grit (C) stored in nylon bag (N) and aluminum foil laminate bag (F) at 25 ºC for six months

Such compounds can be dissolved in alcohol, which was used as an extraction solvent in this study. Additionally, linoleic acids in corn may have an antimutagenic effect, as food containing linoleic acid can help reduce cancer (Sauer, Dauchy, & Blask, 2000; Lee, Lee, Cho, & Kim, 2005; Campos-Pérez & Martínez-López, 2015). Finally, it is possible that the anthocyanins analyzed in this study do not comprise the total anthocyanin content of the corn samples; Yang *et al.* (2009) extracted up to 6 mg anthocyanins/g of purple maize using 1 N HCl:95% alcohol at a ratio of 15:85 with heating at 70ºC for 73 min while this study obtained not more than 0.7 mg/g db from whole corn.

4. Conclusions

In conclusion, alcoholic extracts from KPSC903 hybrid corn have the potential to inhibit mutagenicity of Trp-P-1 and 2-AA with WG achieving as much as 87% and 84% inhibitions, while extracts from CG had 84% and 64% inhibitions. Storage of WG for six months in F or N under vacuum at 25ºC decreased antimutagenicity while slightly increasing the anthocyanin content. A negative correlation was observed between % inhibition and corn extract anthocyanin content during storage. The results for stored CG were similar to those for WG. The F slowed the loss of antimutagenicity during storage better than N.

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