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Evaluation of high pressure treatment for improvement of physicochemical and functional qualities in purple corncobs

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Abstract

Preparation of purple corncob (PCC) using high pressure treatment (200-600 MPa, pH 3-7) and their effects on physical properties, physiological functions, and antioxidant activities of PCC were investigated. The color of treated PCC powders became an intense red. Treated PCC powders showed porous structure which was related to a significant decrease in bulk density property. High pressure induced an increase in total and soluble dietary fibre and also modified the functional properties of PCC. The water holding capacity, glucose retardation index, alphaamylase inhibition, and bile salt binding capacity were significantly increased. However, the oil holding capacity was decreased regardless of the pressure level. Bioactive compounds contents and antioxidant activities of treated powders were slightly decreased compared to the control, especially in those treated at pH 7. PCC properties can be improved by high pressure treatments with pH adjustments, and can be used as a novel functional ingredient in food products.

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Introduction

Nowadays the increasing demand for functional ingredients to improve health and wellness has boosted the food industry to develop novel foods with health benefits. However, when we consider the investment costs, availability of raw materials as well as concern about environmental pollution, the utilization of wastes from agricultural sources is much of interest. Purple corn (Zea mays L.) has recently attracted consumers' attention because of its possible benefits for health. Purple corncob is a by-product from corn processing contains high amount of dietary fibre and bioactive compounds. Phenolic compounds and anthocyanins that remain in PCC exhibit antioxidant capacity and therapeutic benefits such as reduced risk of coronary heart disease, reduced risk of stroke, anticancer, and antiinflammatory properties (Saura-Calixto, 2010). In addition, dietary fibres can reduce risk of coronary heart disease, hyperlipidemia, diabetes, obesity, and some types of cancer (Johnson, 2012). The properties of a dietary fibre depend on its chemical composition, physical and structural properties. For example, soluble fibres increase water binding and contribute to the viscosity of food system. Thus they can reduce cholesterol and improve blood glucose regulation. Insoluble fibres have been linked to water absorption

and intestinal regulation and it has been reported that they are effective to increase volume, hardness and lower the glycemic index value of food products (Elleuch *et al.*, 2011). Modification of dietary fibres can improve their functional, bioavailability, and antioxidant activity as well as the quality of food products. Several studies have demonstrated that mechanical treatments such as extrusion, microwave, micronization, and high pressure treatment cause the added energy to break the chemical bonds in dietary fibre which may change its functional properties (Daou and Zhang, 2012).

High pressure causes intense heat energy and forces that can damage the cell wall matrix, inducing the bond breaking. This technique has shown to be more effective in reducing particle size, altering the microstructure and thus affecting the physicochemical and functional properties of dietary fibres from several byproducts such as carrot pomace, okara soybean, peach and oat pulp, and orange pulp (Chau et al., 2007; Meteos et al., 2010; Chen et al., 2013; Buggenhout et al., 2015). Most recently, researchers have examined the effects of different levels of high pressure or combined high pressure and temperature treatment on improving the properties of insoluble dietary portion. They have found that those processes can increase the soluble fibre and improve physiological properties of the fibres.

PCC, an agro by-product residue rich-in insoluble fibre and antioxidant compounds such as anthocyanins which is still underutilized (Yang and Zhai, 2010). Since stability of anthocyanins is affected by pH variation (Giusti and Wrolstad, 2001), the present work studies the effect of high pressure and pH treatments on the dietary compositions, physicochemical and physiological properties, as well as on antioxidant compounds and their activities in PCC. The relationship between the evaluated properties and potential applications of PCC powder as functional additive in food industry will be discussed.

Material and Methods

Chemicals and reagents

All chemicals and solvents were analytical grade, alpha-amylase (type VI-B from porcine pancreas, A3176), were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). Glucose assay kit (liquicolor; Ref No. 10121) was purchased from Human GmbH (Wiesbaden, Germany).

Plant material and sample preparation

Fresh purple corncobs (*Zea mays*) were collected from a local market (Chaiyaphum, Thailand). The corncobs were separated from fresh corns, ground into small pieces, and dried on a tray dryer at 50° C for 12h. Dried powders were ground and passed through a mesh ($\leq 80 \mu m$), yielding a PCC powder.

PCC powders were mixed with phosphate buffer (pH 3 and pH 7) at ratio 1:7.5 (w/v) in seal bags and subjected to high pressure treatment (S-FL-850-9-W model, Stansted Fluid Power Ltd, Essex, UK) at 200, 400 and 600 MPa for a holding time of 15 min at 25°C. After the treatment, the treated powders were dried in a tray dryer at 50°C for 6 h, and then stored at -20°C until analysis. PCC powder that was not treated with high pressure was used as a control in each analysis.

Proximate compositions and physical properties

Proximate compositions of untreated PCC powder including moisture content, crude protein, crude fat, and ash were analyzed according to AOAC method. Total (TDF), soluble (SDF), and insoluble dietary fibre (IDF) contents in samples were determined using a fibre assay kit (Sigma TDF-100A, St. Louis, MO, USA). Color values were measured in the CIELAB system (L* a* b*) with a ColorFlex EZ spectrophotometer (HunterLab Inc., USA). The color difference, ΔE, was calculated to assess the difference in color due to high pressure

treatment. The PCC powder images were observed using a scanning electron microscope (S-3400N, Hitachi, Japan). Bulk density was expressed as the weight per volume of powder.

Functional properties

The hydration properties of PCC powders were determined according to the method described by Garau *et al.* (2007) with some modifications. The water-holding capacity (WHC) and oil-holding capacity (OHC) were determined by mixing dried sample with distilled water or sunflower oil (1:30, w/v) for 18h at room temperature, and then it was centrifuged at 1500 x g for 10 min. For WHC, the supernatant was decanted and the hydrated sample was weighted and dried at 105°C for 2 h to obtain its dry weight. WHC and OHC were determined as: WHC (g/g) = (Hydrated weight after centrifugation - Dry weight)/Dry weight. OHC (g/g) = (Residue weight - Dry weight)/Dry weight.

Physiological properties

Glucose dialysis retardation index (GDRI) of samples was carried out with a slightly modification method of Chau *et al.* (2004). A mixture solution prepared by mixing 0.5 g of PCC powder in 25 mL of glucose solution (50 mmol/L) was dialyzed against 50 mL of distilled water at 37°C using a dialysis membrane (MW cut off value of 12,000 D). After incubation time (0-180 min), the glucose content in the dialysate was determined using glucose assay kit. A control test was done without the addition of PCC powder. The GDRI was calculated as follows: GDRI (%) = 100 - [(total glucose diffused from PCC sample / total glucose diffused from control sample) x 100].

Sodium cholate binding capacity was performed using the method described by Kahlon *et al.* (2015) with some modifications. One gram of PCC powder was mixed with 10 mM sodium cholate in 100 mL of phosphate buffer (pH 7.0). The mixture was incubated at 37°C for 2h with continuous mild agitation. The mixture was then centrifuged at 2000 x g for 20 min and the concentration of sodium cholate in the supernatant was determined by the colorimetric assay, where 2.5 mL sample was mixed with 0.5 mL 70% sulfuric acid and left for 2h before measuring the absorbance of sample at 510 nm.

Inhibition of α -amylase activity by action of PCC powders was determined as described by Chau *et al.* (2004) with slight modifications. A mixture containing 0.25 g of PCC powder and 1 g of α -amylase in 10 mL of corn starch solution (4 g/100 mL) was incubated at 37°C. After 60 min, starch digestion was stopped

by addition of absolute ethanol (15 mL). Then it was centrifuged at 2000 x g for 15 min and the supernatant was analyzed for glucose content by glucose assay kit. A control test was done without the addition of PCC powder. The amylase inhibitory activity (%) was defined as the percent decrease in the glucose production rate over the control.

Determination of bioactive compounds and antioxidant activity

To determine anthocyanin and phenolic content as well as antioxidant activity of the samples, extraction of bioactive compounds from PCC powders was performed. A 1 g PCC powder was extracted with 10 mL of 85% ethanol: 1 N HCl (85:15 v/v). The suspension was agitated for 30 min, and centrifuged at 12000 x g for 10 min before the supernatant was collected. The residue was re-extracted twice and then all supernatant was stored at -20°C until analysis.

It has been reported that different varieties of purple corn contain the predominant cyanidin-3-glucoside (C3G) (Harakotr *et al.*, 2014; Pascual-Teresa *et al.*, 2002). Total anthocyanin contents in this work were measured using a spectrophotometric pH differential method (Giusti and Wrolstad, 2001) and expressed as milligrams of C3G per gram of the dried sample. The total phenolic contents were estimated using the Folin-Ciocalteau colorimetric method (Singleton *et al.*, 1999). A standard curve of total phenolic was developed using gallic acid and the concentration was expressed as milligrams of gallic acid equivalent (GAE) per gram of the dried sample.

Antioxidant activities were determined using DPPH and FRAP assays, as described by Yang and Zhai (2010). Regarding DPPH assay, an aliquot of PCC extract was mixed with DPPH reagent and absorbance was determined at 516 nm. In terms of FRAP assay, an aliquot of PCC extract was mixed FRAP reagent, and the absorbance of the sample was determined at 593 nm against blank. The results were expressed as mg Trolox equivalents per gram of dried sample (mg TE/g).

Statistical analysis

All analyses were conducted in triplicate. Data were expressed as mean \pm SD. The significant differences among samples were determined by analysis of variance and Duncan's multiple-range test (p \leq 0.05) using SPSS software version 13.

Results and Discussion

Proximate composition and physical properties of PCC powders

PCC powder was found an excellent source of TDF (71.82 \pm 1.96%, dw) containing significant lower values of protein (0.45 \pm 0.06%), fat (0.89 \pm 0.13%), and ash (1.88 \pm 0.10%). This was in agreement with previous results indicating that normal corncob had high content of TDF (90%) with minor protein (2.85%), fat (0.59%), and ash (1.50%) (Aniola *et al.*, 2009).

The dietary fibre compositions of PCC powders are presented in Table 1. The results showed that IDF is the largest fraction of PCC powders in all treatments, which is consistent with the results reported in corncob research in which cellulose and hemicellulose were the main composition, followed by lignin, and other compounds (Ma et al., 2015). The content of soluble and insoluble dietary fibre in PCC samples was affected by pressure and pH treatment. The samples treated at high pressure showed an increase in SDF, but the IDF content decreased regardless the pH levels. The most remarkable increase in SDF was found in all samples treated at pH 7 (p \leq 0.05). The redistribution from insoluble to soluble fibre fractions as a result of high pressure has been reported (Mateos et al., 2010; Chen et al., 2013; Buggenhout et al., 2015). When pressure was applied, some cell walls were disrupted, and the non-starch polysaccharide components in the cell walls such as cellulose and hemicellulose can be degraded to smaller fragments, resulting in more solvable. In this work, the increase in SDF was an evident in all samples treated at high pressure combined with pH 7, as SDF: TDF increased (5-8 folds) compare to the control. This may because acid can hydrolyze hemicelluloses into oligosaccharides and monosaccharides (Martínez et al., 2015). Therefore, samples treated at neutral pH could retain high amounts of lignocellulosic parts that can expose dietary fibre properties. The IDF and SDF fractions are important for dietary, biological, and functional properties, thus modification the ratio of IDF and SDF by this technique could improve the physiological and physicochemical properties of dietary fibre. Application of high pressure to PCC powders resulted in increasing of TDF. This is corresponding to the increase in SDF content.

Color values and images of the PCC powders are presented in Table 1. In this study, a low temperature $(25^{\circ}C)$ was maintained during a high pressure treatment in order to stabilize anthocyanins, the main bioactive pigment in PCC powder. Compared to the control (ΔE value), the color of treated powders

			pH3			pH 7	
Properties	control	200 MPa	400 MPa	600 MPa	200 MPa	400 MPa	600 MPa
TDF (%dw)	71.82±1.31 ^{ce}	68.91±0.14 [†]	70.64±0.14 ^{er}	76.19±2.18°	85.97±0.16 ^a	75.80±2.63°	74.35±1.60°
SDF (%dw)	1.24±0.75 ^e	2.10±0.02 ^{ae}	2.26±0.02°	2.20±0.28 ^e	4.95±0.00°	6.23±0.43 ^a	5.95±1.11°
IDF (%dw)	70.59±0.94°	66.81±0.12°	68.38±0.16°	74.99±1.90°	81.02±0.16 ^a	69.57±0.19°	68.40±0.49°
SDF:TDF*	1.73	3.05	3.20	2.89	5.76	8.22	7.97
L*	43.96±0.19 ^a	33.60±0.45°	36.26±0.30°	36.13±0.24°	37.16±0.58°	36.89±0.61°C	36.73±0.26°C
a*	18.48±0.12°	23.67±0.12 ^a	22.81±0.09°	22.59±0.07°	11.62±0.01 ^e	11.53±0.01 ^{er}	11.41±0.051
b*	4.66±0.03 ¹	7.07±0.05°e	6.95±0.12 ^e	7.14±0.06°	9.56±0.06 ^a	9.22±0.10°	9.08±0.10 ^c
ΔΕ	0	11.84±0.34	9.12±0.29	9.18±0.21	10.84±0.34	10.92±0.35	11.03±0.18
Bulk density (g/cm ⁴)	0.20±0.00 ^a	0.07±0.00 ^{ca}	0.07±0.01°	0.06±0.00°	0.06±0.00°	0.07±0.01°C	0.08±0.00°
WHC (g/g)	4.66±0.16°	7.95±0.10°	8.43±0.23°	8.27±0.20°	10.77±0.29 ^a	9.82±0.95°	9.30±0.56°
OHC (g/g)	7.81±0.26ª	3.64±0.07°	7.76±0.05 ^a	6.36±0.11°	7.25±0.70°	7.16±0.17°	7.56±0.21 ^a

Table 1. Dietary fiber composition and physicochemical properties of PCC powders

Values with different letters within one row are significantly different (p≤0.05)

were altered. L* value of PCC powders after being subjected to high pressure treatment shows slightly lower values than that of the control. Values of a* for the samples treated at pH 7 were lower than those of the control, while the b* value significantly increased $(p \le 0.05)$. Both a^* and b^* values of the samples treated at pH 3 were higher than that of the control. This means that the PCC powders prepared at neural pH were less red color, suggesting some degradation of anthocyanins, while the powders prepared at low pH showed brighter red color. This is because anthocyanins are stable and color is strong red at low pH. When increasing pH, anthocyanin in the form of flavilium cation which presents red color decrease, and the structure change into carbinol or pserdobase form which is colorless (Giusti and Wrolstad, 2001).

Physicochemical properties including bulk density, water holding capacity, and oil holding capacity of PCC powders are also presented in Table 1. The results show that high pressure combined with pH treatment could significantly decrease the bulk density ($p \le 0.05$). High pressure treatment significant decreased the bulk density of fibres because high pressure induced the increasing of porosity as well as the surface area of the samples (Chau *et al.*, 2007). The results found in this work are in line with that of the microstructure pictures shown in Figure 1.

WHC of fibre refers to its ability to retain water within its matrix. Fibre with strong hydration properties could increase stool weight and can enhance viscosity of the added food. WHCs of treated powders were much higher compared to control ($p \le 0.05$). Related to increasing SDF of PCC as shown in Table 1, water then can be held on the hydrophilic sites of the PCC powders. In addition, water may be held within porosity of powders as shown in SEM images (Figure 1). The modification of the matrix could open the PCC structures and free hydroxyl

groups from hemicellulose and cellulose available to bind water in this work. WHCs of PCC powders in this study were in the range of 4.66-10.77 g/g (db) which were comparable with those of reported dietary fibre from other byproducts sources, e.g., citrus pulp (7.0 g/g), grapefruit peel (7.0-9.3 g/g), and passion fruit albedo (13.00 g/g) (Lario et al., 2004; Crizel et al., 2013; Lopez et al., 2013). A slightly lower OHC value of all treated samples compared to control was related to the increased amount of SDF after high pressure treatment and result in less hydrophobic sites to absorb oil. Other factors that affected the OHC property are surface properties, overall charge density, and thickness of fibre particles (Fernández et al., 2009). OHC of treated PCC powders were in the range of 3.64-7.76 g/g, which were similar to the results reported by Meteos et al. (2010) for okara from soybean (3.78-7.97 g/g), or by Femenia et al. (2009) for fibre from ripe kiwifruit peel (6 g/g). The results indicate that PCC powders could be used as functional dietary fibre to reduce calories in fried food because of their low OHC property and be used in food products which require hydration, viscosity development, and freshness retention. Additionally, WHC properties are associated with intestinal tract function such as reduce transit of digested food and trap toxic substances, thus treated PCC powders can reduce the risk of gastrointestinal disorders.

Physiological properties

The retardation in glucose diffusion by fibre is related to an ability to delay glucose absorption in gastrointestinal tract and it was expressed by value of GDRI (Figure 2). GDRI was affected by high pressure and pH treatment. At the first 60 min, GDRI of all treated powders were higher than those of the control. PCC powders treated at pH 7 showed greater ability to delay glucose diffusion. This may

A Dietary fibre ratio (%) is SDF: TDF = (SDF/TDF) x100

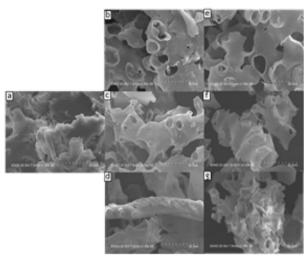


Figure 1. SEM of treated and untreated PCC powders. a) Control, b) pH 3 at 200 MPa, c) pH 3 at 400 MPa, d) pH 3 at 600 MPa, e) pH 7 at 200 MPa, f) pH 7 at 400 MPa, g) pH 7 at 600 MPa

be because the high portion of SDF in these samples could increase viscosity of solution, and then retard the glucose diffusion. Overall, GDRI of all samples was decreased by time because the gradual increase in viscosity of the solutions and the saturation of absorption of glucose in the porosity of treated PCC powders. Several studies also reported similar findings in different sources of IDF, e.g., carrot pomace (Chau *et al.*, 2007), and peach and oat (Chen *et al.*, 2013).

The inhibition of digestive enzymes, i.e., α -amylase could imply the ability of fibre to reduce the rate of glucose absorption in intestinal tract since this enzyme hydrolyzes the starch into glucose. All PCC powders showed a great ability to inhibit α -amylase activity as shown in Figure 3. Ou et al. (2001) and Sreerma et al. (2012) reported that hydroxyl groups of polyphenols could interact with the binding site of α -amylase leading to the loss of enzyme activity. As PCC is a good source of polyphenols, untreated PCC powder can reduce amylase activity by its natural component. The superior inhibition ability of all treated PCC powders (almost 100%) may be attributed to both the presence of the phytochemicals and the physical structures, since they exerted more surface area and contain porous that allow enzyme and starch to be entrapped, causing reduced accessibility between enzyme and substrate, and consequently lower blood-glucose response (Bravo et al., 1995; Ou et al., 2001).

One role of dietary fibre is to bind bile acids. Therefore, it could increase the loss of these acids with feces, increasing the demand for cholesterol to synthesize bile acids in liver, resulting in lowering of serum cholesterol (Kahlon *et al.*, 2005). In this study,

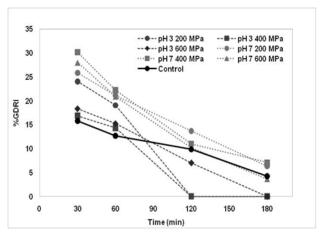


Figure 2. Glucose diffusion retardation index of treated and untreated PCC powders

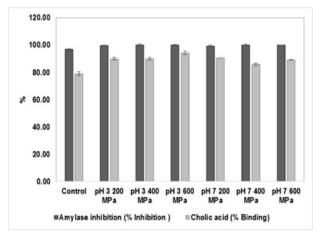


Figure 3. Amylase inhibition and bile acid binding capacity of treated and untreated PCC powders

sodium cholate binding capacities of all treated PCC powders were significantly higher than the control (p \leq 0.05) as shown in Figure 3. It has been found that bile acid binding of dietary fibre was affected by both SDF and IDF portions. SDF attributed the increasing the viscosity of the solutions in gastrointestinal tract; thus, it could bind bile acids and help excretion. IDF could bind bile acids via hydrophobic interaction (Mudgil and Barak, 2013). Here, treated PPC powders showed higher ability to bind bile acids than those of the control due to the increasing of SDF portion after high pressure treatment. It is suggested that PCC powders may have pronounced impact on hypoglycemic and hypocholesterolemic mechanism as they showed ability to adsorb glucose, slow down glucose diffusion, inhibit the α -amylase activity, and bind the bile salts in vitro test.

Antioxidant compounds and activities

Antioxidant compounds such as anthocyanins and total phenolic contents of treated and untreated PCC powder were analyzed and the results as shown in

Treatments	AC	TPC	DPPH	FRAP
	(mg C3G/g)	(mg GAE/g)	(mg TE/g)	(mg TE/g)
Control	1.57±0.07 ^a	9.29±0.74 ^a	84.17±6.98 ^a	36.75±4.72 ^a
pH3 200 MPa	1.40±0.11 ^{eo}	8.98±0.83 ^{ao}	78.79±1.25 ^{eo}	32.33±5.27 ^{eo}
pH3 400 MPa	1.29±0.21 ^{oc}	9.52±0.57 ^a	79.70±2.13 ^{eo}	32.91±1.08 ^{eo}
pH3 600 MPa	1.16±0.15 ^c	9.30±0.44 ^a	73.93±2.90°c	28.79±3.48°c
pH 7 200 MPa	0.49±0.02°	8.42±0.18°	70.82±1.37 ^c	22.84±2.76 ^c
pH 7 400 MPa	0.45±0.04°	8.35±0.43°	70.72±0.36°	22.74±3.05°
pH 7 600 MPa	0.44±0.04°	7.45±0.34 ^c	69.02±2.54°	22.73±1.20°

Table 2. Anthocyanin content (AC), total phenolic content (TPC) and antioxidant capacity of treated and untreated PCC powders

Values with different letters within one column are significantly different (p≤0.05)

Table 2. Control PCC powder had the highest level of anthocyanins. This results were in line with a* values (Table 1), which showed anthocyanin contents were lower in samples treated at pH 7, but it was higher in sample treated at pH 3. This is because anthocyanin stability decreases towards neutrality as previously discussed. Anthocyanin contents in control and pH 3-treated samples were in the range of 1.16-1.57 mg/g, which was higher than that reported in Chinese purple corn (0.56-0.92 mg/g), but the values were comparable to those found in Canadian purple corn (1.28 mg/g) (Yang and Zhai, 2010). This was due to the different varieties, growth conditions and extraction methods. At the same pH treatment, no significant difference of the anthocyanin contents was found between different pressure levels indicating that the impact of pH on the anthocyanin of PCC powders was more pronounced than that of the pressure. High pressure treatment was reported to have a minimal effect on the degradation of anthocyanin contents. Yu et al. (2013) reported that anthocyanin of Chinese bayberry juice remained almost constant after high pressure treatment at 400-600 MPa. Similar results were observed in strawberry puree and blood orange juice (Patras et al., 2009; Torres et al., 2011).

There was no significant difference between TPC of treated PCC powders at pH 3 and of the control. The sample treated at pH 7 showed slightly lower TPC ($p \le 0.05$) (Table 2). High pressure may have no effect, increase, or decrease TPC of the samples because the distribution and aggregation of phenolic compounds depended on the treatment pressure, time, and type of phenolic compounds (Chen *et al.*, 2015). The increase in TPC was related to the extractability of some phenolic hydroxyl group after high pressure treatment, while the decrease in TPC may result from aggregation of those free compounds. Phenolic compounds presented in the purple corn corresponded to ferulic acid and p-coumaric acid and the TPC ranged found in this study (7.45-9.52 mg GAE/g)

was corresponded to result of Cuevas et al. (2011).

The antioxidant activity values of PCC powders are presented in Table 2. DPPH radical is scavenged by polyphenols and anthocyanins through the donation of hydrogen from hydroxyl groups, forming the reduced DPPH-H*. Control exhibited highest antioxidant activity but was not significantly different from the samples treated at pH 3, while samples treated at pH 7 showed the lowest antioxidant activity (p \leq 0.05). This result is in consistent with the content of total anthocyanin and TPC in each treatment. A similar trend of results was observed in ferric reducing power (FRAP) assay. Both the control and pH 3-treated PCC powders showed significantly stronger ferric ion-reducing activities than that of the pH 7-treated samples. This result showed that all PCC powders showed great potential as antioxidant and could be used as functional ingredients to promote health. Additionally, incorporation of PCC powders in food products may retard the lipid oxidation and extend the shelf life.

Conclusion

High pressure treatment with adjusting pH could improve the functional and physiological properties, i.e., water holding capacity, oil holding capacity, glucose retardation ability, as well as bile acid binding capacity. However, PCC powders treated at a low pH level could retain more natural red color and anthocyanin contents. After the treatment, the anthocyanins and total phenolic compounds still remained in high content which contributed the antioxidant activity. These results provided useful information for potential use of purple corncob as a novel functional ingredient which could benefit the development of food products with health promoting functions and could improve the color appearance of food products.

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