

Effect of Extrusion on the Antioxidant Capacity and Color Attributes of Expanded Extrudates Prepared from Purple Potato and Yellow Pea Flour Mixes

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Abstract: Foods with antioxidant capacity provide protection against cardio-vascular, certain forms of cancers, and Alzheimer's diseases caused by oxidative damages and contribute health benefits. The effect of extrusion cooking on the antioxidant capacity and color attributes of extruded products prepared from 3 selected formulations of purple potato and yellow pea flours using a co-rotating twin screw extruder were studied. Expansion ratios of the extruded products varied from 3.93 to 4.75. The total antioxidant capacities (TAC) of the extruded products, using DPPH assay, were 3769 to 4116 μg trolox equivalent/g dry weight sample and not significantly different ($P > 0.05$) from their respective raw formulations. The total phenolic contents (TP) of the extruded products varied from 2088 to 3766 μg of gallic acid equivalent/g dry weight sample and retained 73% to 83% of the TP from the raw formulations after extrusion. The total anthocyanins contents (TA) in the extrudates were 0.116 to 0.228 mg of malvidin-3-glucosides/g dry weight sample. Compared with their raw formulations, significant losses (60% to 70%) of the TA in the extruded products occurred due to extrusion cooking. Browning indices and color attributes such as brightness, chroma, and hue angle agreed with degradation of anthocyanins in the extruded products. However, extrusion cooking retained antioxidant capacities of the raw formulations in the extruded products either in their natural forms or degraded products with radical scavenging activity. This study demonstrated the potential for the production of puffed extruded food products with the improved antioxidant content from colored potatoes and pulse formulations.

Keywords: anthocyanins, antioxidant capacity, colored potato, extrusion, phenolics, yellow pea

Introduction

Colored potatoes are rich in anthocyanins, which are known for providing natural color to fruits and vegetables and for exhibiting antioxidant properties (Nayak and others 2011; Cevallos-Casals and Cisneros-Zevallos 2003). Antioxidants play an important role to protect against diseases by reacting with and quenching the oxidative free radicals, reducing peroxides, chelating transition metals, and stimulating anti-oxidative defense enzyme activities (Velioglu and others 1998). Rice-Evans and others (1995) reported that anthocyanins are more effective antioxidants *in vitro* than ascorbic acid and vitamin E. Pulses (such as yellow pea) are packed with high content of protein, dietary fibers, complex carbohydrates, isoflavones, and folate and are low in fat and sodium (Madar and Stark 2002). Most of these components are also associated with health benefits such as protein with hypocholesterolemic effects (Pusztai and others 1998), and isoflavones help in prevention of osteoporosis and certain cancers (Messina 1999). Therefore, it would be desirable to make commodities such as colored potato and yellow pea into functional foods

in the form of extruded snacks and breakfast cereal-type food products.

Extrusion cooking is a high temperature, short-time process in which food materials are plasticized and cooked by the combination of temperature under pressure and mechanical shear, resulting in molecular transformation and chemical reactions. This technology uses a continuous process with high productivity, significant retention of nutritional quality (Singh and others 2007), natural color, and flavor of food (Bhandari and others 2001).

Expansion ratio is one of the important characteristics of puffed extruded products. Extrusion cooking of legumes restricts the expansion ratio of the extruded product, but addition of starch-containing ingredients, such as potato flours, could improve puffing of the extrudates for the development of expanded-type foods.

Some studies have demonstrated that it is possible to puff pulse flours and pulse-based formulations into potentially commercial nutritious snack and breakfast cereal-type products (Berrios and others 2002, 2004, 2010; Patil and others 2007). The goals of this study were to (1) produce puffed extrudates from mixes of colored potatoes and yellow pea flours, and (2) evaluate the effect of extrusion conditions on antioxidant capacities, color attributes, and some physical characteristics of the extrudates.

Materials and Methods

Materials

Fresh Purple Majesty potato cultivars were purchased from SLV Research Center, Colorado State Univ., Colo., U.S.A.

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White (Russet) cultivars were purchased from a local store. The potatoes were stored at 4 °C and 80% relative humidity for a couple of weeks, before processing. Split yellow pea was purchased from Giusto's specialty food, South San Francisco, Calif., U.S.A. The peas were pin milled into fine flour and stored at room temperature until use.

Production of potato flours

The purple and white potatoes were peeled using an abrasive peeler and sliced to 6 mm thickness. Potato slices were blanched in a steam blancher for 8 min to ensure peroxidase inactivation, since 98% reduction in peroxidase activity was previously achieved in approximately 5 mm thickness red- and purple-fleshed potatoes slices blanched for 3 min. (Reyes and Cisneros-Zevallos 2007). Blanched potato slices were immediately cooled in ice-water for 8 min, drained, and then pureed using a mixer (The Hobart Mfg Co., Troy, Ohio, U.S.A.). Water (half of the weight of the blanched potatoes) was added to the puree to make it of uniform consistency, before applying it to a drum dryer. A 15.24 × 20.32 cm pilot-scale, counter-rotating twin-drum dryer (Blaw Knox Food & Chemical Equipment Co., Buffalo, N.Y., U.S.A.) was used to dehydrate the puree. The surface temperature of the drums was maintained at 135 to 138 °C. The gap between the drums, rotating at 1.13 rpm (revolution per minute), was maintained at 0.3 mm. The dehydrated flakes prepared by drum drying were pin milled into flour and stored at -30 °C until further use. The mean particle sizes of pin milled purple potato flour (PPF) and white potato flour (WPF) were 225 and 220 μm, respectively, as analyzed on a laser scattering particle size distribution analyzer (Horiba LA-900, Horiba Instruments Inc., Irvine, Calif., U.S.A.).

Sample preparation

A mix of 35/65 (w/w) WPF and split yellow pea flours (SYPF) were prepared in a mixing bowl under continuous mixing for 10 min. A total of 3 formulations with ratio of 35/65, 50/50, 65/35 (w/w), PPF, and SYPF, respectively, were prepared in a similar way. These formulations will be referred from here on as 35%, 50%, and 65% PPF. All the formulations were kept in polyethylene bags and stored at room temperature overnight before extrusion processing.

Extrusion conditions

A co-rotating twin-screw extruder (Micro 18, American Leistritz Extruder Corp., N.J., U.S.A.) was used to process the different extrudates under study. The extruder was equipped with 5 independently controlled heating zones that were electrically heated and water cooled by means of a water cooling system. The temperature of the feeding zone was maintained constant at 80 °C. The detail temperature profile in the heating zones, feed moisture and screw speeds with experimental design is given in Table 1. Barrel wall and die temperatures were monitored by respective thermocouples attached to the top and bottom of the 5 heating/cooling zones and the die. A pressure transducer was also attached at the die to monitor the operating pressure. Raw formulations were fed at a constant rate of 45 g/min using a volumetric twin-screw feeder (K-Tron Process Group, Pitman, N.J., U.S.A.). A Bran Luebbe metering pump (Pumps & Process Equipment Inc., Ill., U.S.A.) connected to the feeding zone was used to add water to the feed during processing. The feed moisture content was maintained by changing the water flow rate while keeping the feed rate constant. Extrusion parameters were displayed on an in-built monitor to the extruder and the data were auto-saved on a

personal computer. Extruded samples were collected once the operation reached a steady state condition, that is, the drift in torque was minimal for at least 5 min. The samples were collected for 3 min, cooled at room temperature under natural convection conditions, double packed in polyethylene bags, flushed with nitrogen, and stored at -30 °C for further analysis.

Experimental design

Extrusion cooking of the WPF and SYPF formulations was designed following the procedures of Box and Behnken (1960) (Table 1). Total of 3 independent extrusion parameters, namely feed moisture content (% wet basis), screw speed (rpm), and die temperature (°C) were considered for a 3-level (+1, 0, -1) design. The effects of these parameters on the expansion ratio of the extrudates were investigated using response surface methodology (RSM). A total 15 experiments were designed according to $N = k^2 + k + q$, where N is the total number of experiments, k is the factor number, and q is the number of replicates at the central point. Total of 3 factors with 3 levels and 3 replicates at the central point were considered for the experimental design. The optimum extrusion conditions to obtain acceptable expansion ratios were considered for producing extruded products from PPF and SYPF.

Determination of expansion ratio

Total of 5 randomly chosen extruded rods per extrusion run were considered for measurement. Total of 5 readings at the nodes and space between the nodes of the rods were taken with a caliper for calculating the mean diameter of the extrudates. The expansion ratio was calculated as the ratio of mean cross-sectional diameter of an extrudate to diameter of the die (Camire and others 2007).

Moisture content determination

Moisture contents of the raw PPF, SYPF, and extruded samples were determined using the standard procedures of AACC moisture-air-oven method number 44-45A (AACC 2000).

Pasting profile of potato flours

Pasting profile of WPF and PPF was evaluated using a Rapid Visco Analyzer (RVA) following the procedures of Batey and others (1997). Total of 3 g (dry basis) of pin milled WPF or PPF was weighed into an RVA canister followed by adding deionized water to a final net weight of 28 g and analyzed with continuous stirring at 160 rpm. The mixture was initially held at 60 °C for 2 min, followed by 4 min of heating to 95 °C at 5.83 °C/min and held for 4 min. The mixture was then cooled to 50 °C in 4 min at 11.25 °C/min and held for 4 min for a total run time of 20 min. The parameters such as time-to-peak viscosity, peak viscosity (the maximum hot paste viscosity), trough viscosity (the trough at the minimum hot paste viscosity), and final viscosity (the viscosity at the end of the test) were measured. Breakdown and total set back associated with the degree of collapse of swollen starch granules corresponding to release of solubilized starch capable of re-association during cooling were calculated as following:

$$\begin{aligned} \text{Breakdown} &= \text{Peak viscosity} - \text{Trough viscosity} \\ \text{Total setback} &= \text{Final viscosity} - \text{Trough viscosity} \end{aligned}$$

Color evaluation

Extruded samples were ground into flour using a food processor to pass through US nr 35 sieve (0.5 mm), whereas raw flour formulations required no further preparation for evaluation of color

attributes. Color attributes were determined using a computer vision system (CVS) following the procedures of Pandit and others (2007). Briefly, the CVS included a digital camera (Nikon D70 model) with 18 to 70 mm zoom lens providing 6.1 megapixel resolutions, a lighting system, and a personal computer. Flour samples were kept in a cylindrical container and placed on a white plate inside a shooting tent. Images of the samples were taken with the digital camera mounted downwards on the top of the tent at 50 cm above the sample plate. The images were downloaded to a PC and analyzed using Adobe Photoshop CS2 software (version 8.0, Adobe Systems Inc, San Jose, Calif., U.S.A.) to determine the color parameters (CIE L^* , a^* and b^*). Since color values in Photoshop software are encoded from 0 to 255, standard scaling values were determined using the method of Briones and Aguilera (2005):

$$L^* = \frac{L}{2.5} \quad (1)$$

$$a^* = \frac{240a}{255} - 120 \quad (2)$$

$$b^* = \frac{240b}{255} - 120 \quad (3)$$

where L , a and b values are from Photoshop and L^* , a^* and b^* values are standardized values depicting brightness, greenness/redness, and blue/yellowness, respectively. The hue angle h° and Chromaticity C^* were computed from a^* and b^* using the following equations:

$$h^\circ = \tan^{-1}(b^*/a^*) \quad (4)$$

$$C^* = \sqrt{(a^*)^2 + (b^*)^2} \quad (5)$$

Hue angle (h°), the angular representation of color, is often described as "red," "blue," and so on, whereas chromaticity describes the purity (saturation) of color. The reference values for h°

at 0/360°, 90°, 180°, 270° are magenta red, yellow, bluish-green, and blue, respectively. Color differences between extruded samples and their respective raw formulations were expressed as ΔE^* where,

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (6)$$

Chemical analyses

Folin–Ciocalteu reagents, potassium chloride, sodium acetate, trolox(6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), DPPH (2, 2-diphenyl-1-picrylhydrazyl), and gallic acid were purchased from Sigma-Aldrich (St. Louis, Mo., U.S.A.). Laboratory grade methanol and ethanol were used in extraction and preparation of samples.

Total antioxidant capacity (TAC)

Ten grams of flour from the raw or extruded samples were blended with aqueous methanol (50:50, v/v) under constant stirring for 2 min at room temperature. The final volume of the mixture was brought to 100 mL and kept for 90 min at 4 °C, before centrifuged at 30000 × g at 4 °C for 20 min. The supernatants were collected and stored at –20 °C for further analysis.

The TAC of the raw and extruded samples was determined using DPPH assay following the procedures of Brandwilliams and others (1995). DPPH (a stable, deep purple color radical) is reduced in the presence of antioxidants decolorizing the solution. Loss of color results in a decrease in the absorbance intensity, which can be monitored spectrophotometrically at 515 nm, provides the basis for measurement of the antioxidant capacity of the extracts. Stored supernatants were brought to room temperature and DPPH assay was prepared by adding 0.05 mL of supernatants to 1.95 mL of freshly prepared DPPH solution (6×10^{-5} M) in a cuvette. The cuvettes were covered with parafilm and thoroughly mixed before taking readings at 515 nm using a UV/visible spectrophotometer (Ultraspec 4000, Pharmacia Biotech, Cambridge, England) until a plateau was reached (Brandwilliams and others 1995). The absorbance at 2 h was considered optimum for determining the TAC of samples as there was little change in the reading toward the end of the indicated time. Methanol was used as a blank, whereas

Table 1—Structured matrices and experimental results for expansion ratios of extrudates prepared from white potato flour (WPF) and split yellow pea flour (SYPF) using a twin screw extruder at a feed rate of 45 g/min. Factors and levels of experiments are: (A) moisture content (–1, 0, +1 corresponding to 17%, 21%, and 25% wet basis, respectively); (B) screw speed (–1, 0, +1 corresponding to 200, 250, and 300 rpm, respectively); (C) die temperature (–1, 0, +1 corresponding to 120, 130, and 140 °C, respectively).

Randomized order	Run order	Feed moisture (% wb)	Screw speed (rpm)	Die temp ^A (°C)	Expansion ratio
1	1	+1	0	+1	2.93 ± 0.23 ^d
13	2	0	–1	+1	3.45 ± 0.19 ^c
9	3	0	+1	+1	4.49 ± 0.32 ^b
15	4	–1	0	+1	5.18 ± 0.10 ^a
14	5	+1	–1	–1	1.79 ± 0.15 ^f
11	6	+1	+1	–1	1.97 ± 0.15 ^f
7	7	0	0	–1	1.81 ± 0.19 ^f
2	8	–1	–1	–1	2.61 ± 0.56 ^{de}
8	9	0	0	–1	2.05 ± 0.41 ^{ef}
10	10	–1	+1	–1	4.36 ± 0.94 ^{abc}
6	11	0	0	–1	2.39 ± 0.30 ^e
3	12	+1	0	0	2.46 ± 0.11 ^e
5	13	0	+1	0	2.77 ± 0.23 ^d
12	14	0	–1	0	2.36 ± 0.18 ^e
4	15	–1	0	0	4.12 ± 0.36 ^b

^ABarrel zones set for die temperatures at 120, 130, and 140 °C: 80/90/100/110/120, 80/100/110/120/130, and 80/100/115/130/140 °C, respectively. Significant differences within the values in the same column are indicated by different superscript letters ($P < 0.05$, Tukey's pairwise comparison test). Temp = temperature; wb = wet basis; rpm = revolution per minute.

DPPH without sample was taken as control. For each measured sample, the percentage of DPPH remaining was calculated as:

$$(\text{DPPH})_{\text{remaining}} = \frac{\text{DPPH}_{\text{absorbance@Time=t}}}{\text{DPPH}_{\text{absorbance@Time=0}}} \times 100 \quad (7)$$

where $\text{DPPH}_{\text{absorbance@Time=t}}$ is the absorbance of DPPH at time t min and $\text{DPPH}_{\text{absorbance@Time=0}}$ is the absorbance of DPPH measured at zero min. The TAC was quantified from a trolox standard curve and expressed as micrograms of trolox equivalent per gram of dry weight sample ($\mu\text{g TE/g DW}$). Total of 3 replicates were considered for determination of the TAC.

Total phenolics (TP)

Extracts from the total antioxidant assay were used for determining the TP content of the raw and extruded samples using Folin–Ciocalteu colorimetric method, following the procedures of Swain and Hillis (1959) and Singleton and Rossi (1965). Briefly, in presence of phenolates, the Folin–Ciocalteu reagents is reduced to produce molybdenum–tungsten blue, which can be measured with a spectrophotometer. Extracted supernatants were brought to room temperature before diluting them 4 times with 50% aqueous methanol. To 0.5 mL supernatants, 8 mL of deionized water were added followed by 0.5 mL of 0.25 N Folin–Ciocalteu reagents. The samples were mixed thoroughly and equilibrated for 3 min at room temperature. After 3 to 4 min, 1 mL of 1 N sodium carbonate was added to the mixture and mixed. Sodium carbonate raises the pH of the phenols to be oxidized rapidly in an alkaline medium to form phenolates. The aliquots were kept at room temperature for 2 h before taking readings at 725 nm. A total of 0.5 mL methanol was treated in the same way as the diluted samples and used as blank. The TP content was quantified from a gallic acid standard curve and expressed as micrograms gallic acid equivalent per gram of dry weight sample ($\mu\text{g GAE/g DW}$). A total of 3 replicates were considered for determination of the TP.

Total anthocyanins (TA)

Determination of TA on the raw and extruded samples was carried out following the procedures of Fuleki and Francis (1968) with modifications. A total of 5 g of flour from the raw or extruded samples was blended with aqueous acidified ethanol (50/50 water/acidified ethanol, v/v) and thoroughly stirred for 2 min. Acidified ethanol was prepared from 95% ethanol/1.5 N HCl (85/15, v/v). The final volume of the mixture was brought to 100 mL with aqueous acidified ethanol. The mixture was covered with parafilm and kept for 90 min at 4 °C to equilibrate, before centrifuging at $23000 \times g$ at 4 °C for 15 min. The supernatants were collected and stored at –20 °C for further analysis.

The TA contents were quantified using the pH differential method, following the procedure of Giusti and Wrolstad (2001). Anthocyanin assays were prepared by adding 0.2 mL of supernatant to 1.8 mL of KCl buffer (pH 1) or sodium acetate buffer (pH 4.5). The cuvettes containing aliquots were covered with parafilm, thoroughly mixed and equilibrated for 15 min at room temperature, before reading absorbances. Malvidin–3–glucoside was considered as the major anthocyanin in PPF (Lewis and others 1998; Han and others 2006; Jansen and Flamme 2006) detected at the maximum wavelength (λ_{max}) of 535 nm with molecular weight (MW) of 718.5 g/mol and molar extinction coefficient of 30, 200 $\text{L}^{-1}\text{cm}^{-1}\text{mol}^{-1}$. Absorbance readings at 535 nm (λ_{max}) and 700 nm (for correcting turbidity) (Reyes and Cisneros–Zevallos 2003) were taken using a UV/visible spectrophotometer, previously

blanked with distilled water. The TA contents of the raw formulations and the extrudates were calculated according to the following formula (Giusti and Wrolstad 2001):

$$C(\text{mg/l}) = \frac{A * \text{MW} * \text{DF}}{e * d} \quad (8)$$

where A = Absorbance of the sample given by $A = (A_{\lambda_{\text{max}}} - A_{700})_{pH1.0} - (A_{\lambda_{\text{max}}} - A_{700})_{pH4.5}$, MW = molecular weight of malvidin–3–glucoside, DF = dilution factor, e = molar extinction coefficient, and d = path length of the cuvette (1 cm). TA contents were expressed as milligram of malvidin–3–glucosides per gram of DW sample (mg mv-3-glu/g DW). Individual anthocyanins were not identified or calculated.

Browning index (BI)

Degradation of anthocyanins and formation of brown Maillard reaction products (MRP) were assessed based on the BI of the products. Extracts from the total anthocyanins were used to determine the BI in the raw and the extruded samples, following the procedures of Jackman and others (1987). Absorbance readings of 2 mL of supernatants were taken at 535 (λ_{max}), 420, and 700 nm for calculating Browning Index of the samples as:

$$BI = \frac{A_{420} - A_{700}}{A_{535} - A_{700}} \quad (9)$$

where A_{420} , A_{535} , and A_{700} were absorbances at 420, 535, and 700 nm, respectively.

Statistical analysis

All physical and chemical data obtained from the raw formulations and extruded samples were collected and analyzed with SAS (version 9.1, SAS Inst. Inc., Cary, N.C., U.S.A.) using analysis of variance (ANOVA). Tukey's pair-wise comparison at 95% confidence level was used to identify statistical significant differences ($P < 0.05$). All the data were expressed as mean \pm standard deviation.

Results and Discussion

Expansion ratio (ER)

The use of response surface methodology allowed determining how the ER of the extrudates produced from WPF and SYPF formulations varied under the influence of the selected extrusion parameters of screw speed (200, 250, and 300 rpm), and die temperature (120, 130, and 140 °C). At the selected extrusion parameters, the ER of the extrudates produced from the 35/65 (w/w) WPF and SYPF formulation ranged from 1.78 to 5.18 (Figure 1). We had a very limited supply of PPF. Therefore, experimental designs for the extrusion of the PPF and SYPF formulations were based on the optimized conditions for desired ER of extrudates produced using WPF and SYPF formulations. Expansion ratio of about 5.0 was selected from the optimized extrusion conditions with 17% (wb) feed moisture, 250 rpm screw speed, and 140 °C. However, the actual extrusion processing of PPF and SYPF formulations were conducted at screw speed of 300 rpm and temperature at 130 °C in addition to 140 °C, to reduce the residence time and exposure to heat of the mix in the extruder barrel, to achieve maximum color retention in the final extrudates.

In the present study, expansion ratios of the extrudates prepared from the PPF and SYPF formulations at 130 and 140 °C die

temperatures varied from 3.93 to 4.75 depending on the proportion of raw ingredients (Table 2). In contrast, Camire and others (2007) reported relatively lower diametric expansions of 1.90 to 1.93 in extruded products prepared from 84.3% cornmeal, 14.3% sucrose, 0.4% citric acid, and 1% of dehydrated blueberry or cranberry or raspberry or Concord grape powders) processed at extrusion conditions of 175 rpm screw speed, 163 °C die temperature, and a feed rate of 255 g/min. Differences in the expansion ratios of the extrudates in our study could be due to the quantity of potato flour used as well as the structure of potato starch containing higher content of phosphate groups on amylopectin compared to corn starch with no phosphate groups. The ER of the extrudates produced from formulations containing 50% and 65% PPF were not significantly different ($P > 0.05$) at 130 and 140 °C. This tended to indicate that a difference in processing temperatures of 10 °C might not be sufficient to promote a significant increase ($P < 0.05$) on ER of the extrudates. However, the ER of extrudates produced from formulations containing 35% PPF were significantly smaller ($P < 0.05$) than those produced from formulations containing 50% and 65% PPF, at 130 and 140 °C. It is known that starch has a positive effect on increasing expansion, while fiber and/or protein have a negative and lowering effect on expansion of extrudates (Conway 1971a, 1971b). These information goes along with the results on ER reported in this study, as high content of potato flour (source of starch) and low content of dry pea flour (source of protein and fiber), in the extrudates produced from formulations containing 50% and 65% PPF, presented the extrudates with the highest values of ER. Conversely, low concentration of

potato flour and high concentration of dry pea flour, in the extrudates produced from formulations containing 35% PPF, resulted in extrudates with significant ($P < 0.05$) lower values of ER. Additionally, extrudates produced from formulations containing 35% PPF showed large variability on diameter (used for calculation of ER) among the different extrudates. Therefore, the difference ($P < 0.05$) on ER observed between extrudates produced at 130 and 140 °C is attributed to this indicated variability.

Pasting behavior of potato flours

Differences and/or similarities in the pasting behavior of WPF and PPF can be explained based on their RVA's pasting curves. The pasting behaviors of both the WPF and PPF followed similar pattern with regard to times to attain their peak, trough and final viscosities (data not shown). Breakdown and total setback were 433 and 601 cP for WPF, and 314 and 629 cP for PPF. The similar proximate composition presented in WPF and PPF may be responsible for the similar pattern on pasting profile displayed by the 2 flours. Most importantly, higher content of phosphate-monoesters found mainly on amylopectin of both potato starches confers enhanced paste clarity, high peak consistency, and significant shear thinning (Hoover 2001). Similar observation was previously reported on pasting profile of white and color potato flours (Hoover 2001). Use of PPF was justified and used with SYPF for further analyses with the optimized extrusion process parameters.

Color attributes

The brightness (L^*) or color lightness among different raw formulations prepared from the PPF and SYPF varied significantly ($P < 0.05$) from 77 to 88 in a scale of 0 to 100. The raw formulation containing 35% of PPF was significantly ($P < 0.05$) brighter than the formulation containing 50% PPF, which was significantly ($P < 0.05$) brighter than the formulation containing 65% PPF. The values of chroma and hue followed the same trend as those observed for the brightness. That is a decreased in chroma and hue values with increase of PPF in the formulations (Table 3). The raw SYPF flour had a whitish color while the potato flour had a purplish color, due to their anthocyanins content. Therefore, as the amount of PPF increased in the formulations, the brightness, chroma, and hue significantly ($P < 0.05$) decreased.

When comparing the brightness values (L^*) of different raw formulations with those of their extrudates it was observed that extrusion processing, at die temperatures of 130 and 140 °C caused a significant ($P < 0.05$) decrease in the brightness, chroma, and hue, at all levels of PPF addition. It is known that reducing sugars and proteins (amino acids) in foods can react under high processing

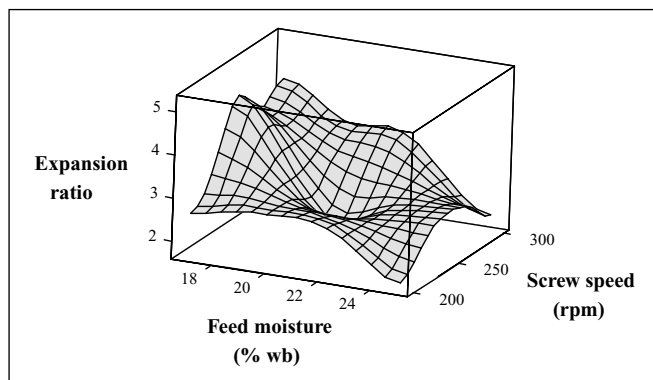


Figure 1—Response surface graph for expansion ratios of extrudates as a function of extruder screw speed (200 to 300 rpm) and feed moisture (17% to 25% wb) prepared from WPF and yellow pea flour using a co-rotating twin screw extruder with feed rate of 45 g/min and barrel zones set at 80/100/115/130/140 °C. wb = wet basis; rpm = revolution per minute.

Table 2—Moisture contents and expansion ratios of the extrudates prepared from split yellow pea flour (SYPF) and purple potato flour (PPF) using a co-rotating twin screw extruder at 45 g/min feed rate, 300 rpm screw speed, 17% (wet basis) feed moisture, and barrel zones set at 80/100/110/120/130 °C (die temperature at 130 °C) and 80/100/115/130/140 °C (die temperature at 140 °C) ($n = 3$).

Formulations (potato/pea)	Treatment	Moisture content (% wet basis)	Expansion ratio
35/65, w/w	Raw Formulation	9.32	0
	Extruded @130 °C	7.03	4.28 ± 0.11 ^b
	Extruded @140 °C	6.97	3.93 ± 0.22 ^c
50/50, w/w	Raw Formulation	9.01	0
	Extruded @130 °C	7.26	4.74 ± 0.10 ^a
	Extruded @140 °C	7.39	4.48 ± 0.12 ^a
65/35, w/w	Raw Formulation	8.69	0
	Extruded @130 °C	7.43	4.75 ± 0.27 ^a
	Extruded @140 °C	7.19	4.53 ± 0.26 ^a

Significant differences within the values in the same column are indicated by different superscript letters ($P < 0.05$, Tukey's pairwise comparison test). @ = at.

temperatures to promote nonenzymatic browning (Maillard reaction), which results in darkening of the final product. Potatoes are high in sugars and dry peas high in protein (amino acids). Therefore, the observed decrease in brightness may be attributed to the Maillard reaction, as a consequence of extrusion processing. Similarly, previous researchers have observed that extrusion of the whey protein concentrate and corn starch gave higher color differences with the increase in the amylose content (Matthey and Hanna 1997). Additionally, degradation of purple anthocyanins due to extrusion temperatures could have generated Maillard reaction products that promoted the changes in the brightness, chroma, and hue values, as observed in the unprocessed (raw) and extruded products.

Color difference (ΔE^*) was used to represent the color change between the unprocessed and processed flours (effect of processing). In this study, the values of ΔE^* for all the extrudates increased significantly ($P < 0.05$) as the processing temperature increased from 130 to 140 °C (Table 3). The values of ΔE^* for the extrudates formulated with 35% PPF were similar to that of 50% PPF, at both indicated processing temperatures. However, extrudates formulated with 65% PPF were significantly ($P < 0.05$) greater. These results indicated that the color in the PPF had, along with processing temperatures, a direct effect on the values of ΔE^* . Additionally, the results obtained for ΔE^* support those obtained for the brightness, chroma, and hue values. Berrios and others (2004) reported that there are not established threshold or cut-off values for color development of an acceptable legume-based snack, due to the lack of this type of products in the market place. Therefore, the color data in this study may have important value for future product development of pulse-based snack type products.

Total antioxidant capacity

The TAC of the raw formulations and extrudates were determined using DPPH assay and expressed as $\mu\text{g TE/g DW}$ (Figure 2). The TAC of the raw formulations with 35%, 50%, and 65% PPF were 3936 ± 71 , 4025 ± 35 , and $4083 \pm 37 \mu\text{g TE/g DW}$, respectively. While these values were not statistically different ($P > 0.05$) the data clearly showed that the TAC increased with increase in PPF in the formulations. Those increases represented 2.21% and 1.42%, from 35 to 50 and from 50% to 65% PPF in the formulations, respectively. On the other hand, when we determined the TAC from 0% to 35% PPF addition, this represented 60.5% increase (data not shown in Figure 2). This significant ($P > 0.05$) increase in TAC can be attributed to the purple color in the PPF. Studies on the TAC of colorful fruits and vegetables at the Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts Univ. revealed that a large group of color

compounds are flavonoids (including anthocyanins) with potent antioxidant protection against peroxy radicals (Wang and others 1997). When compared the TAC of different raw formulations with those of their extrudates it was observed that, even though the TAC of the extrudates were lower, they were not statistically ($P < 0.05$) different. Similar contents in the TAC of the raw formulations and extruded products could be attributable to the effect of extrusion on (1) breaking complex polyphenols into low molecular weight phenolic compounds with scavenging activity, (2) interaction of the phenolics with protein under heat treatment, and (3) formation of Maillard reaction products. On the other hand, with the exception of extrudates formulated with 50% PPF, the TAC of those formulations extruded at die temperatures of 140 °C showed significantly ($P < 0.05$) lower TAC than their raw counterpart. High temperature extrusion promote the Maillard reaction and formation of brown compounds that may have had an effect on the TAC of the extrudates (Anese and others 1999). Additionally, the potential binding of phenolic compounds to the protein matrix may account for the decreased in the TAC observed on those formulations extruded at die temperatures of 140 °C compared to their raw samples. At high-protein concentration, complex interactions and cross-linking of different protein molecules with phenolic compounds forms hydrophobic surface (Mcmanus and others 1985). No significant effect ($P > 0.05$) as result of extrusion temperatures at 130 and 140 °C was observed on the TAC of extruded products formulated with 35%, 50%, and 65% PPF, respectively. This result may indicate that to see an effect on the TAC, an increase in the die temperature greater than 10 °C needs to be used. Camire and others (2007) reported no significant change ($P > 0.05$) in the antioxidant activity of the control and cranberry extruded products prepared with corn at substitution level of 1% and extrusion temperature of 165 °C.

Total phenolics

The TP content of the raw formulations and extruded products were determined using FC reagents method and expressed as $\mu\text{g of GAE/g DW}$ (Figure 3). Comparing different raw formulations it was observed that the TP content of the 65% PPF formulation ($4548 \pm 117 \mu\text{g of GAE/g DW}$) was significantly higher ($P < 0.05$) than 50% PPF formulation ($3838 \pm 286 \mu\text{g of GAE/g DW}$); and this one, significantly higher ($P < 0.05$) than 35% PPF formulation ($2818 \pm 46 \mu\text{g of GAE/g DW}$). Previous researchers have demonstrated that purple-flesh potato cultivars had higher phenolic contents than white-flesh cultivars (Nayak and others 2011; Stushnoff and others 2008) and yellow peas (Xu and Chang 2008). These reports support the results obtained in the present study that purple-flesh potatoes has higher content of

Table 3—Color attributes of the extruded products prepared from split yellow pea flour (SYPF) and purple potato flour (PPF) using a co-rotating twin screw extruder at 45 g/min feed rate, 300 rpm screw speed, 17% (wet basis) feed moisture, and barrel zones set at 80/100/110/120/130 °C (die temperature at 130 °C) and 80/100/115/130/140 °C (die temperature at 140 °C) ($n = 3$).

Formulations (potato/pea)	Treatment	Brightness (L^*)	Chroma (C^*)	Hue (h°)	Color difference (ΔE^*)
35/65, w/w	Raw Formulation	88.10 ± 0.4^a	8.60 ± 0.3^f	89.31 ± 0.4^a	0.00
	Extruded @130 °C	73.00 ± 1.6^d	26.48 ± 0.8^d	87.85 ± 1.2^a	23.41 ± 0.8^c
	Extruded @140 °C	73.82 ± 1.5^d	29.87 ± 0.4^b	87.61 ± 1.5^a	25.62 ± 0.7^b
50/50, w/w	Raw Formulation	82.10 ± 1.3^b	5.20 ± 0.3^e	67.83 ± 0.6^c	0.00
	Extruded @130 °C	70.57 ± 0.7^c	22.73 ± 0.8^e	87.71 ± 1.4^a	21.31 ± 1.3^c
	Extruded @140 °C	72.72 ± 1.4^d	28.33 ± 0.4^c	87.47 ± 1.3^a	25.29 ± 0.5^b
65/35, w/w	Raw Formulation	77.03 ± 1.0^c	4.04 ± 0.2^b	21.35 ± 2.4^d	0.00
	Extruded @130 °C	69.46 ± 1.7^c	27.28 ± 0.7^d	83.64 ± 1.6^b	26.75 ± 0.6^b
	Extruded @140 °C	73.80 ± 0.4^d	33.19 ± 0.8^a	84.61 ± 0.3^b	31.74 ± 0.8^a

Significant differences within the values in the same column are indicated by different superscript letters ($P < 0.05$, Tukey's pairwise comparison test). @ = at.

TP than yellow pea. Therefore, those raw formulations containing the highest proportion of PPF had also the highest content of TP. A similar pattern on the TP content was observed in the extruded products. However, significant losses ($P < 0.05$) in the TP content were determined in products processed at die temperature of 130 °C prepared from the formulations containing 50 and 65% PPF, when compared to the TP content of their raw formulations. One exception was observed for extruded products prepared from formulations containing 35% PPF, where the TP content was not

significantly different ($P > 0.05$) from its raw formulation. This could be attributed to high standard deviation values determined on those extruded samples. Similarly, products processed at the die temperature of 140 °C prepared from the formulations containing 35%, 50%, and 65% PPF had significantly ($P < 0.05$) less TP content than their respective raw formulations. Viscidi and others (2004) reported significant loss of total phenolics during extrusion of oat cereals. Additionally, Zadernowski and others (1999) reported losses of up to approximately 60% of phenolic

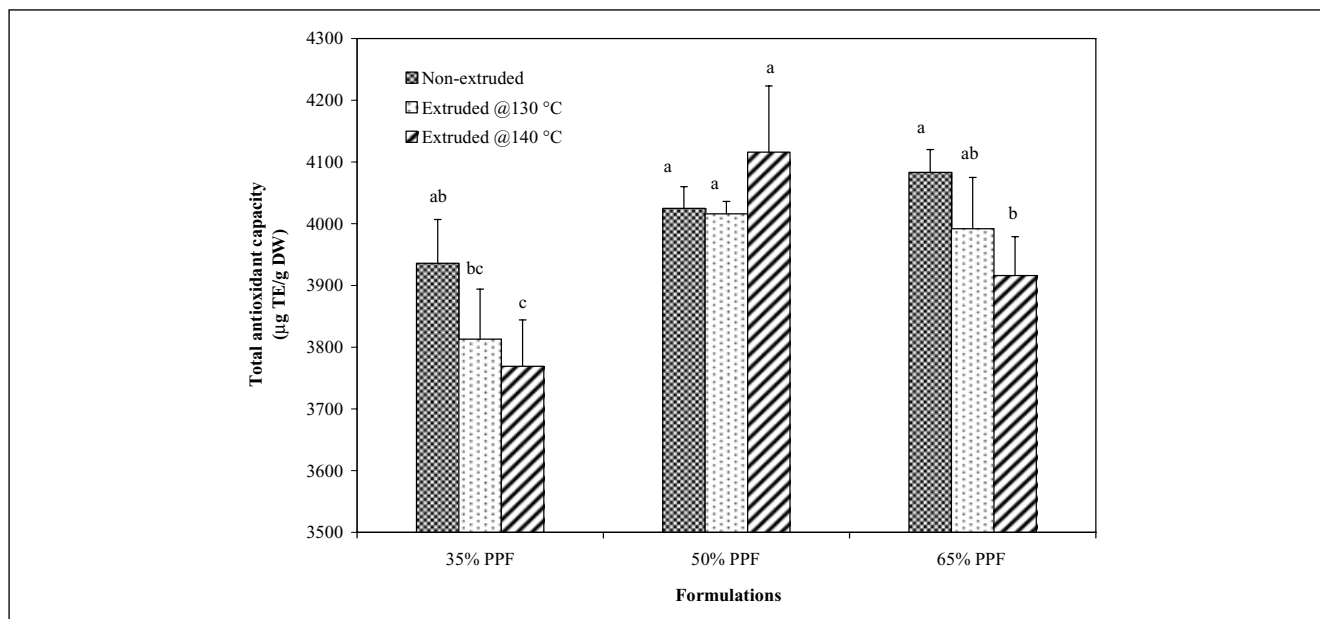


Figure 2—Total antioxidant capacities of the raw formulations and extruded products prepared from purple potato flour (PPF) and split yellow pea flour (SYPF) using a co-rotating twin screw extruder at 45 g/min feed rate, 300 rpm screw speed, 17% (wet basis) feed moisture, and barrel zones set at 80/100/110/120/130 °C (die temperature at 130 °C) and 80/100/115/130/140 °C (die temperature at 140 °C). Selected formulations for preparing extrudates contained x% of PPF and (100 - x)% of SYPF. ^aValues in each bar with no letters in common are significantly different ($P < 0.05$). TE = trolox equivalent; DW = dry weight; @ = at.

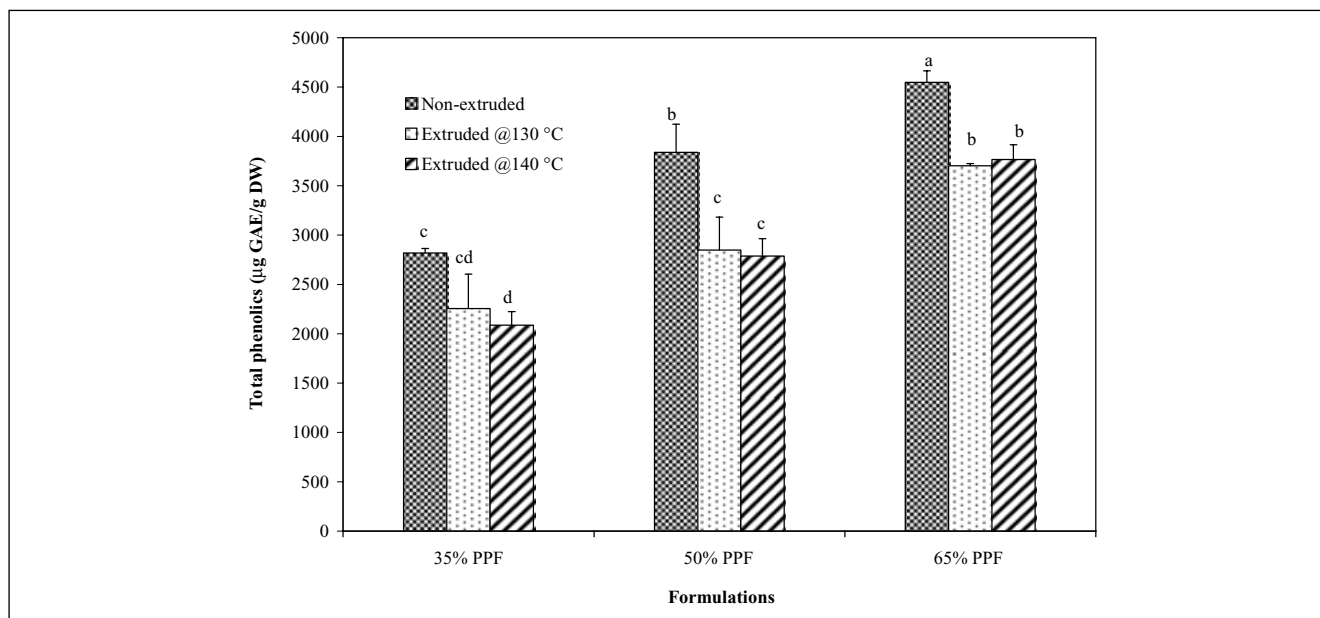


Figure 3—Total phenolics of the raw formulations and extruded products prepared from purple potato flour (PPF) and split yellow pea flour (SYPF) using a co-rotating twin screw extruder at 45 g/min feed rate, 300 rpm screw speed, 17% (wet basis) feed moisture, and barrel zones set at 80/100/110/120/130 °C (die temperature at 130 °C) and 80/100/115/130/140 °C (die temperature at 140 °C). Selected formulations for preparing extrudates contained x% of PPF and (100 - x)% of SYPF. ^aValues in each bar with no letters in common are significantly different ($P < 0.05$). GAE = gallic acid equivalent; DW = dry weight; @ = at.

compounds in extruded oat samples, compared to its respective raw sample. These reports corroborate well with the findings of the present study. Contrary to the previous reports, Camire and others (2007) reported higher content of soluble phenolics, as ferulic acid equivalents, in Concord grape and raspberry extrudates compared to their control samples. Phenolic compounds are heat-labile and can break at the exposure to high temperatures. Therefore, losses in the TP content of the formulations under extrusion are expected to occur, due to break down of complex polyphenols to other phenolic or non-phenolic compounds, at a consequence of high temperatures conditions. However, the effect of extrusion die temperatures, 130 and 140 °C, was not significant ($P > 0.05$) on the TP content of the extrudates (Figure 3). This indicated that, under the extrusion possessing conditions of the study, the die temperature differential of 10 °C had no detrimental effect on TP.

Total anthocyanins

The TA content in the raw formulations and extrudates were determined using the pH differential method and expressed as milligram of mal-3-glu/g DW (Figure 4). Results obtained from different raw formulations demonstrated that the TA content in the 65% PPF formulation (0.729 ± 0.04 mg of mal-3-glu/g DW) was significantly higher ($P < 0.05$) than 50% PPF formulation (0.584 ± 0.05 mg of mal-3-glu/g DW) followed by 35% PPF formulation (0.363 ± 0.01 mg of mal-3-glu/g DW). In general, the TA content in the extruded products prepared from formulations containing 65%, 50%, and 35% PPF and processed at die temperatures of 130 and 140 °C followed same trend as observed for the raw formulations. That is, a significant decrease in the TA content as the percentage of PPF in the formulations decreased from 65% to 35%. Higher concentrations of PPF in the formulations contributed to higher content of TA. Purple color of potato flour is mainly due to the presence of the anthocyanins petunidin and malvidin glucosides present in the flesh and skin of the potato (Stushnoff and others 2008); whereas, preliminary TA results in yellow pea showed negligible values (data not shown). Compared to their raw samples, the extruded ones showed a significant loss in the TA content at all the different levels of PPF in the formulations. The losses in the TA content were more evident on extrudates processed at the highest die temperature of 140 °C. Different from the results obtained previously, where a die temperature differential of 10 °C had not detrimental effect on the content of TP, the extrudates containing 35% and 50% PPF processed at die temperature of 140 °C reflected a significant loss in TA compared to those processed at die temperature of 130 °C. Extrudates containing the highest percentage of 65% PPF, processed at die temperatures of 130 and 140 °C, presented similar TA losses. Stability of anthocyanins is affected by a number of factors such as temperature, pH, light, oxygen, enzymes, ascorbic acid, sulfur dioxide, sugars, metal ions, and so on (Francis 1989). Degradation of anthocyanins in the extruded products could be attributable to the breaking of structures of the anthocyanins at high temperatures. High temperature at the initial step could open either the pyrylium ring of the anthocyanins and form chalcone (Sadilova and others 2007) or hydrolyze the glycosidic moiety and form aglycon (Sadilova and others 2006) providing degradation products as quercetin, phloroglucinaldehyde, and protocatechuic acid. Degradation of anthocyanins in the extruded products might be also due to the formation of browning compounds caused by the Maillard reaction at high temperatures (Nicoli and others 1999). A study on the extrusion cooking

of blueberry and grape anthocyanins, used as breakfast cereal colorants, by Camire and others (2002) reported losses of 90% blueberry anthocyanins and 74% grape anthocyanins, induced by the processing. Similarly, Camire and others (2007) reported almost 90% loss in anthocyanins content on extruded corn products containing fruit powders. These reports support the results of the present study and indicated that when processing food materials that are good source of anthocyanins, special attention should be giving to the processing conditions to avoid significant losses.

Browning index

Results of the BI determined in the raw formulations (Figure 4) showed that formulations with 35% and 50% PPF (0.19 ± 0.0 , 0.20 ± 0.0 , respectively) were not different from ($P > 0.05$) each other. However, the BI of the formulation with 65% PPF (0.15 ± 0.0) was lower ($P < 0.05$) than the former formulations. Since the BI are calculated based on the ratio of absorbances at 420, 535 nm subtracting haziness at 700 nm, higher absorbance value of the 65% PPF formulation at 535 nm (because of higher concentration of PPF than other formulations) contributed to the observed lower BI. The BI of the extrudates processed at 130 and 140 °C were significantly higher ($P < 0.05$) than their respective raw formulations. Additionally, the BI of extrudates prepared at 140 °C (0.34 ± 0.04 , 0.34 ± 0.00 , and 0.49 ± 0.04 for 35, 50% and 65% PPF, respectively) were significantly higher ($P < 0.05$) than those processed at 130 °C (0.29 ± 0.01 , 0.29 ± 0.01 , and 0.33 ± 0.03 for 35%, 50%, and 65% PPF, respectively). Higher BI in the extrudates might be because of the formation of brown compounds by the Maillard reaction of amino acids present in the dry peas and reducing sugar in the potato flour, at high temperature. Degradation of anthocyanins in the extrudates with change in color attributes also agrees with the higher BI in the extrudates. Karel and Labuza (1968) reported that hydrolysis of sucrose giving reducing sugars that has potential for browning in a model system containing sucrose. Browning of extruded products could have related to the feed moisture content in the formulation, concentration of ingredients, and other extrusion parameters. Dominance influence of water on the rate of browning in systems containing carbonyl compounds were reported in the literature (Erlanson and Wrolstad 1972).

Correlation analyses

Correlations (r^2) among the TP, TAC, TA, BI, and color attributes were analyzed using Pearson's correlation coefficients method. The TAC was not strongly correlated with the TP ($r^2 = 0.5281$, $P > 0.05$) or TA ($r^2 = 0.4568$, $P > 0.05$). The correlation coefficients showed that the phenolic compounds including anthocyanins were not solely responsible for antioxidant capacity in the formulations and extruded products. Presence of other secondary metabolites such as volatile oils, carotenoids, and vitamins also might have contributed to the total antioxidant capacity in the raw formulations and extrudates. Strong correlation of the TA with the TP ($r^2 = 0.7622$, $P < 0.05$) agrees the contribution of flavonoids to the phenolic compounds. Contents of the TA in the formulations and extruded products were negatively correlated with the BI ($r^2 = -0.7081$, $P < 0.05$), chroma ($r^2 = -0.8995$, $P < 0.05$), and hue ($r^2 = -0.8650$, $P < 0.05$) but positively correlated with brightness ($r^2 = 0.5161$, $P < 0.05$). Anthocyanins are responsible for the purple color in the PPF. The negative correlations of the TA with the BI, chroma, and hue were attributed to the concentrations of PPF in the formulations and degradation of anthocyanins in the extruded products.

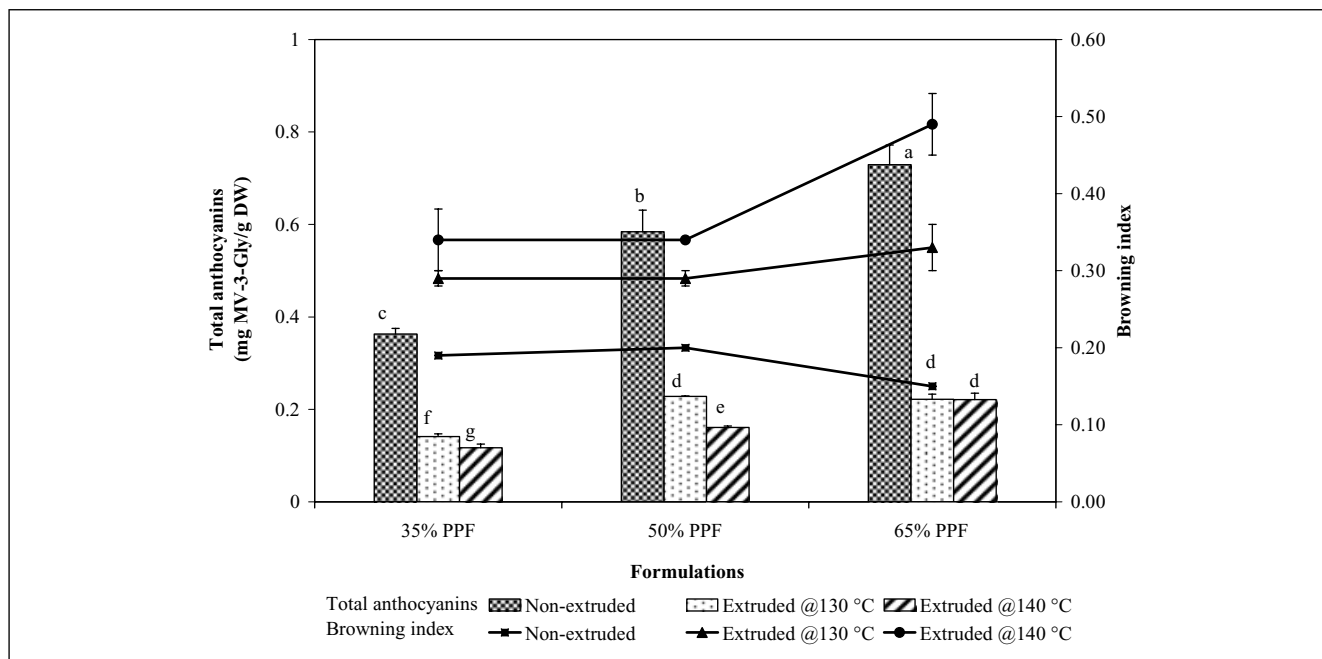


Figure 4—Total anthocyanins and browning indices of the raw formulations and extruded products prepared from purple potato flour (PPF) and split yellow pea flour (SYPF) using a co-rotating twin screw extruder at 45 g/min feed rate, 300 rpm screw speed, 17% (wet basis) feed moisture, and barrel zones set at 80/100/110/120/130 °C (die temperature at 130 °C) and 80/100/115/130/140 °C (die temperature at 140 °C). Selected formulations contained x% of PPF and (100 – x)% of SYPF. ^aValues in each bar with no letters in common are significantly different ($P < 0.05$). MV-3-Gly = Malvidin-3-glucoside; DW = dry weight; @ = at.

Conclusions

Our study demonstrated that natural colored extruded puffed food products rich in antioxidants can be produced from SYPF and PPF using extrusion cooking technology. Although degradation in the total anthocyanins content was observed, some purple color was retained in the final extruded products. This indicated that high temperature–short-time extrusion processing is a suitable process for fabrication of products from antioxidant-rich colored ingredients. The total antioxidant capacities in the extruded products were retained due to the preservation of phenolics, during processing. Addition of PPF to SYPF provided an acceptable expansion ratio to the extruded products. Presence of natural color in the final extrudates could play a major role in the consumer attraction and acceptability, as well as the marketability of the developed extruded food products. More research on the use of extrusion parameters and their effect on the kinetics of anthocyanins are necessary to study the stability of natural color in the final extrudates. Food colored ingredients, such as PPF, has the potential for substituting the use of artificial colors, which are generally added as coating during the downstream processes.

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