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Effect of wheat protein isolate addition on the quality of grape powder added wheat flour extrudates

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RESEARCH ARTICLE

Abstract

Addition of bioactive ingredients into cereal products is reported to have some detrimental effects on the product quality. However, sometimes those negative effects are known to be compensated by protein sources. In this study, the single and combined effects of both Concord grape extract powder (CGEP) and wheat protein isolate (WPI) on quality parameters of hard wheat flour extrudates were investigated. Beside the physical quality and colour parameters of extrudates, their pasting and thermal properties were also evaluated at three different die temperatures. Results revealed that CGEP substitution (7%), even with WPI (6.5 and 13%), was not so much effective on quality of extrudates extruded at 90 and 120 °C die temperatures. The only parameter effective on quality was the die temperature; especially at 150 °C, differences between formulations were more distinct for (diametric expansion, bulk density) when compared to lower die temperatures (90 and 120 °C). However, pasting properties were detrimentally affected by the single addition of CGEP (about 55.4% loss in final viscosity) with respect to single WPI substitution (about 66.7% loss in final viscosity). Presence of both CGEP and WPI made the pastes even weaker (34.1 to 61.1% decrease in peak viscosity), on the other hand, decreased the extent of delay (in gelatinisation (about 2 to 9 °C) provided by the sole use of CGEP. Besides no distinct effect on retrogradation was observed neither by CGEP nor with WPI. Therefore, in extrudates, CGEP substitution and related decrease in protein content did not exert a significant quality loss and even by increasing the protein level no improvement in quality parameters was obtained.

Keywords: concord grape, extrusion, pasting properties, thermal properties

1. Introduction

Extrusion has become a widely used operation for processing increasing numbers of food products over the years. However, it is still considered a complicated procedure with 'multiple input-output' variables. During the process, protein and starch interact with each other under high temperature and pressure conditions within the barrel. Protein and starch available in cereal products are thermo-mechanically transformed into new food products in the extruder barrel with a number of textural advantages, such as increased expansion and crispiness, in addition to extrusion's economic advantages of high productivity, low operating costs, and energy and time savings (Brennan *et al.*, 2011).

In recent years, increased consumer interest in functional foods, especially ones rich in antioxidants, has driven extrusion research in how to more effectively incorporate functional ingredients into extrudate formulations. Grapes are good sources of these compounds, particularly anthocyanins.

Fruits have been added into extrudate formulations as either fresh, pomace or powder. Use of fruit powders such as blueberry, cranberry, Concord grape and raspberry (Camire *et al.*, 2007); or apple, banana, strawberry and tangerine (Potter *et al.*, 2013) in extrusion feed is an easy way of incorporating fruit into extrusion processing. Some studies have mainly focused on the effects of extrusion conditions on the properties and quality of functional extrudates (Altan *et al.*, 2009; Karkle *et al.*, 2012; Yağcı and Göğüş, 2008, 2009). These studies investigated considerable increases in substitution levels of health-promoting substances; however the combined effects of adding fruit sources and basic cereal components, particularly proteins, were not evaluated.

Behaviour of cereal proteins under various extrusion conditions warrants further research as generally proteins are highly affected by processing conditions. Temperature may be considered one of the most important factors as high temperature has irreversible effects on proteins, including protein unfolding and loss of functionality, as a result of aggregation and/or covalent bond breaking (Leon *et al.*, 2003). In contrast, the effect of high pressure on protein denaturation is different as it leaves some parts of the molecule unchanged (Knorr *et al.*, 2006). However, as proteins are exposed simultaneously to multiple extreme conditions, such as high temperature, high pressure, and mechanical shear during extrusion, changes occur in proteins, including conformational disassembling and reassembling through different interactions of disulfide, hydrogen and non-covalent bonds. This results in extrusion products that are completely different in comparison to their raw materials. Protein based sources (legumes and soy products as blends with cereals, being the most common) were generally added into extruded products to help nutritional balance (Bhattacharya, 2012).

Starch is another cereal macro molecule having a significant role during extrusion. Moreover, amylose is known to have a role as a protective barrier in terms of retention of fruit anthocyanins during extrusion, as suggested in a previous study (White *et al.*, 2010). Also the former study by our group (Tacer-Caba *et al.*, 2014) investigated the effect of amylose on the quality of extrudate pertaining Concord grape extract powder (CGEP) as the anthocyanin source. However, for the effect of proteins no such information is available.

Therefore, detailed investigation of interactions is required during extrusion between health-promoting substances from fruit-based ingredients and basic cereal components, such as protein, and more importantly exploring combined effects on the quality of the extruded products.

The objective of the present study is to investigate the changes in the quality parameters of grape powder enriched wheat flour extrudates that are substituted with wheat protein isolate (WPI) and to see if WPI has any interaction with CGEP that is effective on extrudate quality. Besides, the effects of different extrusion die temperatures on the quality of protein and grape powder substituted wheat flour extrudates are also evaluated.

2. Materials and methods

Materials

Hard wheat bread flour used in this study was donated by Mennel Milling Company (Fostoria, OH, USA). WPI supplied by MGP Ingredients Inc. (Atchison, KS, USA) was used. CGEP from Milne Fruit Products (Prosser, WA, USA) was selected as fruit based ingredient. CGEP was claimed as being 100% all-natural after extraction process with no carriers, fillers or solvents in the company product specification.

Preparation of extrudate formulations

In total, six extrudate formulations were prepared in order to evaluate the effects and interactions of CGEP and WPI (Table 1). Each formulation was prepared in duplicate.

Extrusion

For extrusion, a laboratory scale twin-screw extruder (Model MP 19T2-25; APV Baker, Grand Rapids, MI, USA) was used. Constant feed moisture content and screw speed were 25% and 360 rpm, respectively. The following screw configuration was applied, where screw diameter is 19.0 mm (1 D) and one kneading paddle is 1/4 D: 8 D twin lead screws, 7 × 30° forward kneading elements; 8 D twin lead screws; 3 × 60° forward kneading elements; 3 × 30° reverse kneading elements; 2 D single lead screws; 4 × 60° forward kneading elements; 3 × 30° reverse kneading elements; 2 D single lead screws; 7 × 90° kneading elements; 2 D single lead screws. Two replicates of extrusion process were made for each treatment of formulation and process temperature combination.

The exit die had an opening of 3 mm, barrel diameter was 19 mm, and barrel length to diameter ratio (L/D) was 25:1. Moisture content of sample flour mixes changed between

Table 1. Extrudate formulations.[1]

Sample	Bread flour (%, w/w)	WPI (%, w/w)	CGEP (%, w/w)
F-0 (control)	100	–	–
F-6.5	93.5	6.5	–
F-13	87.0	13.0	–
F-0-C	93.0	–	7.0
F-6.5-C	86.5	6.5	7.0
F-13-C	80.0	13.0	7.0

[1] WPI = wheat protein isolate; CGEP = concord grape extract powder.

11.0-12.0% before extrusion and distilled water was injected with an E2 Metripump positive displacement-metering pump (Bran + Luebbe, Northampton, UK) to maintain feed moisture of 25%. Samples were extruded at three different processing temperature profiles at a 2 kg/h feed rate. Temperature profiles of the five zones of the extruder barrel, from feed port towards exit die, were: 40-60-60-70-90 °C, 40-60-80-100-120 °C, and 40-60-100-140-150 °C. Hereafter, the three processing temperatures will be referred to as 90, 120, and 150 °C, respectively.

The extruded samples were cut into 100 mm lengths, cooled down to room temperature, and dried in an air oven at 50 °C for 16 h. A portion of each dried extrudate sample was milled using a laboratory grinder (Model 4 3375-E45; Thomas Scientific, Willey Lab. Mill, Swedesbono, NJ, USA), sieved through a 500-μm sieve, and stored in a zipped plastic bag at -20 °C for further analysis.

Specific mechanical energy

Specific mechanical energy (SME) values of the extrudates were calculated according to the following equation (Hu *et al.*, 1993):

$$SME\ (kWh/kg) = \frac{screw\ speed \times power\ (kW) \times torque\ (\%)}{maximum\ screw\ speed \times throughput\ (kg/h) \times 100} \quad (1)$$

Diametric expansion

Diametric expansion ratios were calculated as the cross-sectional diameter of a cooled (3-4 h at room temperature) extrudate piece divided by the diameter of the extruder die opening (3 mm) (Camire *et al.*, 2007). Twenty pieces from each extrusion run were measured with a caliper and an average value was reported.

Bulk density

Bulk density measurements of cooled extrudate samples (3-4 h at room temperature) were made similar to the method of Alvarez-Martinez *et al.* (1988) and calculated according to Equation 2, where m is the mass, d is the extrudate diameter and L is the length:

$$Bulk\ density\ (kg/m^3) = 4m/2\pi d^2 L \quad (2)$$

Textural properties

Hardness values of extrudates were measured using a TA-HDi texture analyser (Stable Microsystems, Godalming, UK) fitted with a 50-kg load cell and coupled with Texture Expert Software (Version 1.22; Stable Micro Systems). Fresh extrudate from each sample was cooled down to room temperature for 3-4 h, and a 100 mm length was compressed between a 3-point bending rig (HDP/3PB) and blade (90 mm long and 3 mm thick) at a crosshead speed of 2 mm/s (Stojceska *et al.*, 2009). The highest value of force (N) in the force-time (distance) curve was taken as the value of hardness. Ten measurements were made for each extrudate sample.

Water absorption

Water absorption capacity of the ground extruded samples was determined according to AACCI Method 56-30 (AACCI, 2000) with some modifications. Ground sample (1 g) was weighed in a 50 ml centrifuge tube, 30 ml of distilled water added, and the tube was vortexed for 10 s. The tubes were left at room temperature for 30 min, with intermittent vortexing for 5 s and then centrifuged at 2,000×*g* for 15 min at room temperature. After centrifuging, the supernatant was decanted and discarded; tubes were then left upside down for 10 min to drain excess water. The drained residues were weighed and the level of water absorption was calculated.

Colour

Colour measurements of the ground extruded samples were measured using a colorimeter (CR-400; Konica Minolta Holdings Inc., Tokyo, Japan). Hunter Lab values were measured. The instrument was calibrated with a standard white reflector plate prior to the measurements. The values reported are the mean values of 5 measurements made on each ground extrudate sample. In addition, the total colour change was calculated according to the following equation:

$$\Delta E = \sqrt{(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2} \quad (3)$$

Where, subscript 'o' indicates colour values of samples without WPI or CGEP (F-0) that were extruded at the one of three distinct process temperatures.

Scanning electron microscope

Extrudate powders and their respective non-extruded flour mixes were fixed on aluminium pin-type stubs using carbon paste. Excess powder was removed using high-pressure air. Samples were coated with gold-palladium by a mini sputter coater (Quorum SC7620; Quorum Technologies Ltd, Laughton, UK). The samples were viewed at 1,500× resolution with a scanning electron microscope (FEI Quanta 250 Company, Hillsboro, OR, USA).

Pasting properties

For determining the viscoelastic properties of the flour mixes and ground extrudates, a Rapid Visco Analyzer (RVA-4; Newport Scientific, Warriewood, Australia) was used by following AACCI Method 76-21 (AACCI, 2000): 3.5 g of sample (14% moisture basis) were mixed with 25 ml of distilled water using the standard 1 heating cycle of 50-95-50 °C. Sample was equilibrated at 50 °C for approximately 1 min, heated to 95 °C in 3.75 min, then held at 95 °C for 2.5 min, cooled down to 50 °C in another 3.75 min, and held at 50 °C for 2 min, for a total heating cycle of 13 min. Pasting data was acquired using Thermocline version 1.2 software (Newport Scientific).

Thermal properties

Thermal properties (transition onset temperature, T_o; transition peak temperature, T_p; and transition enthalpy, ΔH) of extruded powder samples were investigated using a differential scanning calorimeter (DSC; Model Q10; TA Instruments Inc., New Castle, DE, USA). Universal Analysis 2000 Version 4.5A (TA Instruments Inc.) software was utilised. The calibration was made using an indium standard. According to the modified method of Kim *et al.* (2006), samples of about 3 mg of ground extrudates were weighed into aluminium pans (TA Instruments Inc.) and 12 μl of distilled water added to each pan using a micro-syringe (Kim *et al.*, 2006). The sample pans were hermetically sealed and allowed to equilibrate for 2 h at room temperature. Samples were heated from 20 to 160 °C at a rate of 10 °C/min. A sealed empty pan was used as a reference. Gelatinisation was evaluated by searching for the presence of any transition around 60 °C.

For determining retrogradation, scanned samples in their sealed pans were stored at 4 °C for seven days. The samples were then re-run in the DSC by heating from 20 to 180 °C at a rate of 10 °C/min, and retrogradation was evaluated by searching for the presence of any transition around 40 to 50 °C (Jane *et al.*, 1999).

Statistical analyses

Factors of exit die temperature (3 levels) × CGEP addition (2 levels) × WPI addition (3 levels) were tested statistically. The differences among extrusion die temperatures and the formulations were each investigated by one-way analysis of variance (ANOVA) ($P<0.05$). Detailed examinations for significant differences were made using Duncan's new multiple range test. Pearson's correlation matrix was applied to for all three extrusion temperatures individually to determine the correlation coefficients between parameters measured. SPSS 16.0 statistical software (SPSS Inc., Chicago, IL, USA) was used in all statistical analyses. Correlation coefficients matrix was produced using Pearson's correlation coefficients.

3. Results

Specific mechanical energy

In the present study, SME values measured for all samples (Table 2). For samples without CGEP, the decreases in SME due to a rise in temperature were not significant ($P>0.05$). However, in samples with CGEP, SME values decreased significantly ($P<0.05$) at 9.3, 25.7 and 13.5% levels, for F-0-C, F-6.5-C and F-13-C samples, respectively, as die temperature increased from 90 to 120 °C.

Diametric expansion

Expansion indices increased gradually for all samples with increases in exit die temperatures (Table 3). Addition of WPI decreased the extent of expansion only at 120 °C, therefore differences among products that are extruded at 90, 120 and 150 °C die temperatures were found to be statistically significant, while for samples without WPI (F-0 and F-0-C), differences were insignificant ($P>0.05$). CGEP addition had also no effect on expansion ratio; as extruded samples with CGEP and their counterparts without CGEP were found to be similar in their expansion characteristics. Diametric expansion was found to correlate significantly with water absorption (r=0.797) at 120 °C die temperature, and with bulk density (r=-0.838) at 150 °C die temperature ($P<0.01$).

Table 2. Specific mechanical energy (SME) of samples extruded at three different temperatures.[1,2]

Sample	SME (kWh/kg)		
	90 °C	120 °C	150 °C
F-0	0.200±0.001xa	0.205±0.000xbc	0.204±0.001xa
F-6.5	0.208±0.007xa	0.188±0.008xc	0.190±0.001xbc
F-13	0.226±0.021xa	0.196±0.006xbc	0.182±0.004xc
F-0-C	0.270±0.001xa	0.245±0.000ya	0.170±0.002zd
F-6.5-C	0.288±0.063xa	0.214±0.012yb	0.196±0.006yab
F-13-C	0.223±0.003xa	0.193±0.003ybc	0.188±0.003ybc

[1] The mean value ± standard deviation of duplicate analyses are given. Values with different letters (a-d) within the same column and different letters (x-z) within the same row differ significantly ($P<0.05$).
[2] See Table 1 for sample descriptions.

Table 3. Physical properties of extrudates produced at three different temperatures.[1,2]

Sample	Diametric expansion ratio			Bulk density (kg/m^3)		
	90 °C	120 °C	150 °C	90 °C	120 °C	150 °C
F-0	1.16±0.01ya	1.78±0.27xya	2.45±0.06xab	1,282.2±4.7xa	966.5±196.9xa	397.3±12.8ybc
F-6.5	1.19±0.01za	1.37±0.01ya	2.31±0.00xbc	1,267.3±5.2xa	1,239.4±4.1xa	469.4±27.6ya
F-13	1.27±0.05ya	1.39±0.01ya	2.09±0.07xd	1,184.9±40.1xb	1,158.8±27.2xa	478.3±19.8ya
F-0-C	1.25±0.05ya	1.74±0.30xya	2.52±0.01xa	1,286.1±0.7xa	1,033.4±279.7xya	333.0±16.6yc
F-6.5-C	1.22±0.03za	1.38±0.01ya	2.36±0.05xb	1,249.4±11.4xa	1,215.2±7.4xa	387.7±11.5yc
F-13-C	1.23±0.00za	1.34±0.00ya	2.17±0.00xcd	1,242.2±0.0xa	1,225.2±4.6xa	456.8±2.3yab
	Hardness (N)			**Water absorption (ml water/g sample)**		
	90 °C	120 °C	150 °C	90 °C	120 °C	150 °C
F-0	0.696±0.01xyab	0.912±0.08xa	0.440±0.12ya	1.50±0.02yb	3.79±0.69xa	5.30±0.00xab
F-6.5	0.659±0.01yab	0.939±0.03xa	0.537±0.07ya	1.58±0.10zb	2.71±0.12ybc	5.51±0.51xa
F-13	0.525±0.06xb	0.712±0.06xb	0.498±0.09xa	1.48±0.11zb	3.47±0.17yab	4.71±0.11xab
F-0-C	0.750±0.07ya	0.987±0.02xa	0.496±0.04za	2.11±0.22za	3.50±0.31yab	5.62±0.35xa
F-6.5-C	0.614±0.04yab	0.944±0.01xa	0.438±0.01za	1.65±0.22zb	2.30±0.04yc	4.43±0.03xb
F-13-C	0.586±0.07yab	0.913±0.02xa	0.439±0.04ya	1.36±0.04zb	2.42±0.16yc	5.43±0.14xa

[1] The mean value ± standard deviation of duplicate analyses are given. Values with different letters (a-d) within the same column and different letters (x-z) within the same row differ significantly (P<0.05).
[2] See Table 1 for sample descriptions.

Bulk density

Die temperature was one of the main factors affecting bulk density of extrudates (Table 3). For each formulation, bulk density decreased with increasing die temperature, showing a statistically significant drop at 150 °C in comparison to 120 °C die temperature. For bulk density values of samples without CGEP about 58.7-62.1% decreases, on the other hand for samples with CGEP little higher (about 62.7-68.1%) decreases were calculated. Neither WPI- nor CGEP-substitution levels had significant effects on bulk density results, with the only exception for samples with CGEP which are extruded at 150 °C; for which increase in protein levels significantly increased the extrudate bulk density (P<0.05).

Textural properties

Statistically significant changes in hardness values were detected for samples with CGEP extruded at different die temperatures (P<0.05). When the die temperature increased from 90 to 120 °C, extrudate hardness values for all samples increased; however from 120 to 150 °C hardness values decreased (Table 3). All extrudates at 150 °C were less hard than their respective counterpart samples produced at lower extrusion die temperatures (P<0.05). Hardness significantly correlated significantly (r=0.837) with bulk density only at 90 °C die temperature (P<0.01).

Water absorption

Water absorption values of extrudates were highly affected by temperature and increased by the increase in extrusion temperature for each sample tested (Table 3). Water absorption of extrudates decreased with the increasing WPI substitution level, especially from 0 to 6.5% WPI substitution, only in samples with CGEP.

Colour

Colour parameters such as darkness/lightness (L* values), redness (a* values), and yellowness (b* values) in addition to total colour change (ΔE) were compared among the formulations within each extrusion die temperature and among the die temperatures within each formulation (Table 4). Samples with CGEP were found to be significantly different from samples without CGEP, in all three individual colour parameters measured. Therefore, ΔE values were also significantly different between samples with CGEP and samples without CGEP (P<0.05). When each parameter was evaluated individually for samples without CGEP produced at each temperature, it was observed that changes in colour

Table 4. Hunter Lab colour measurements of flour samples extruded at three different temperatures.[1,2]

Sample	ΔE			L*		
	90 °C	120 °C	150 °C	90 °C	120 °C	150 °C
F-0	0	0	0	84.58±0.26xa	84.35±0.78xa	81.70±0.04ya
F-6.5	1.50±0.17xc	2.48±0.76xc	0.78±0.09xc	85.55±0.07xa	82.09±1.37xya	81.16±0.00ya
F-13	1.13±0.06xc	0.77±0.03yd	0.65±0.22yc	85.35±0.16xa	83.23±0.06ya	81.54±0.45za
F-0-C	41.07±1.74xa	40.02±0.63xa	38.33±1.33xa	45.44±2.09xc	46.23±1.35xc	45.34±1.54xc
F-6.5-C	37.53±0.77xb	37.97±0.08xb	32.72±2.65xb	49.13±0.56xb	48.33±0.52xbc	48.50±2.56xb
F-13-C	35.69±0.55xb	36.87±0.46xb	34.19±1.44xab	51.09±0.27xb	49.47±0.18xb	49.67±1.40xb
	a*			b*		
	90 °C	120 °C	150 °C	90 °C	120 °C	150 °C
F-0	-0.05±0.01zd	0.22±0.01yb	0.62±0.07xb	11.33±0.12ya	11.48±0.29xya	12.43±0.25xa
F-6.5	-0.05±0.01yd	0.46±0.13xb	0.52±0.21xb	10.19±0.18xb	10.97±0.68xa	11.97±0.05xa
F-13	-0.03±0.00yd	0.27±0.03xb	0.41±0.09xb	10.56±0.07yb	11.48±0.40xya	12.30±0.57xa
F-0-C	5.94±0.13xya	5.62±0.00ya	6.48±0.29xa	0.45±0.05yc	0.57±0.01yb	1.82±0.20xb
F-6.5-C	5.50±0.03xb	5.62±0.30xa	6.32±0.78xa	0.32±0.15yc	0.75±0.12xyb	2.05±0.99xb
F-13-C	5.12±0.10xc	5.55±0.03xa	5.85±0.52xa	0.14±0.00yc	0.80±0.06yb	1.68±0.25xb

[1] The mean value ± standard deviation of duplicate analyses are given. Among each of L*, a* and b*, values with different letters (a-d) within the same column and different letters (x-z) within the same row differ significantly (P<0.05).
[2] See Table 1 for sample descriptions.

parameters and also ΔE values among different formulations were generally not significant among samples (P>0.05), indicating that protein substitution level did not seem to affect neither colour parameters nor the total colour change. However, for samples with CGEP, yellowness (b* values) was found to decrease, whereas darkness (lower L* values) and redness (a* values) increased significantly (P<0.05) when compared to values for the counterpart samples without CGEP. On the other hand, for samples with CGEP, increases in protein substitution levels increased lightness (higher L* value) of extrudates while not effecting a* and b* values (except for a* values for samples extruded at 90 °C). Similarly, ΔE values significantly decreased with the WPI substitution in samples with CGEP (P<0.05). When the effects of different extrusion temperatures are evaluated, samples without CGEP and extruded at 150 °C were found to have higher darkness, redness and yellowness than all other extrudates without CGEP obtained at lower temperatures (P<0.05). On the other hand, same samples had significantly higher ΔE values when extruded at 90 °C (P<0.05), in comparison to higher temperatures. However, for samples with CGEP and extruded at 150 °C, only yellowness was higher (P<0.05) whereas darkness and redness were not different than values of all other CGEP-containing samples extruded at other temperatures (P>0.05). ΔE values were not measured as different, statistically (P>0.05). Colour parameters (L*, a*, b*) correlated significantly among each other at all die temperatures (P<0.01).

Scanning electron microscope

Micrographs of some ingredients and extruded samples are presented in Figure 1. When scanning electron microscope (SEM) images of ingredients are evaluated, dense protein matrix was seen in WPI, whereas for CGEP more ordered crystal-like structure was observed. In flour blends; starch granules, protein matrix and protein adhesion to starch granules were noticed. For flour samples containing WPI (F-13 and F-13-C), a more dense protein matrix in which all other components were embedded was a characteristic micro-structure.

Pasting properties

Main pasting parameters of both non-extruded and extruded samples were evaluated (Table 5). RVA profiles of samples were shown in detail in Figure 2. The peak viscosity and final viscosity values of non-extruded samples were generally at least two-fold of the values for respective counterpart extruded samples. Peak viscosity and final viscosity values of non-extruded samples both of with CGEP

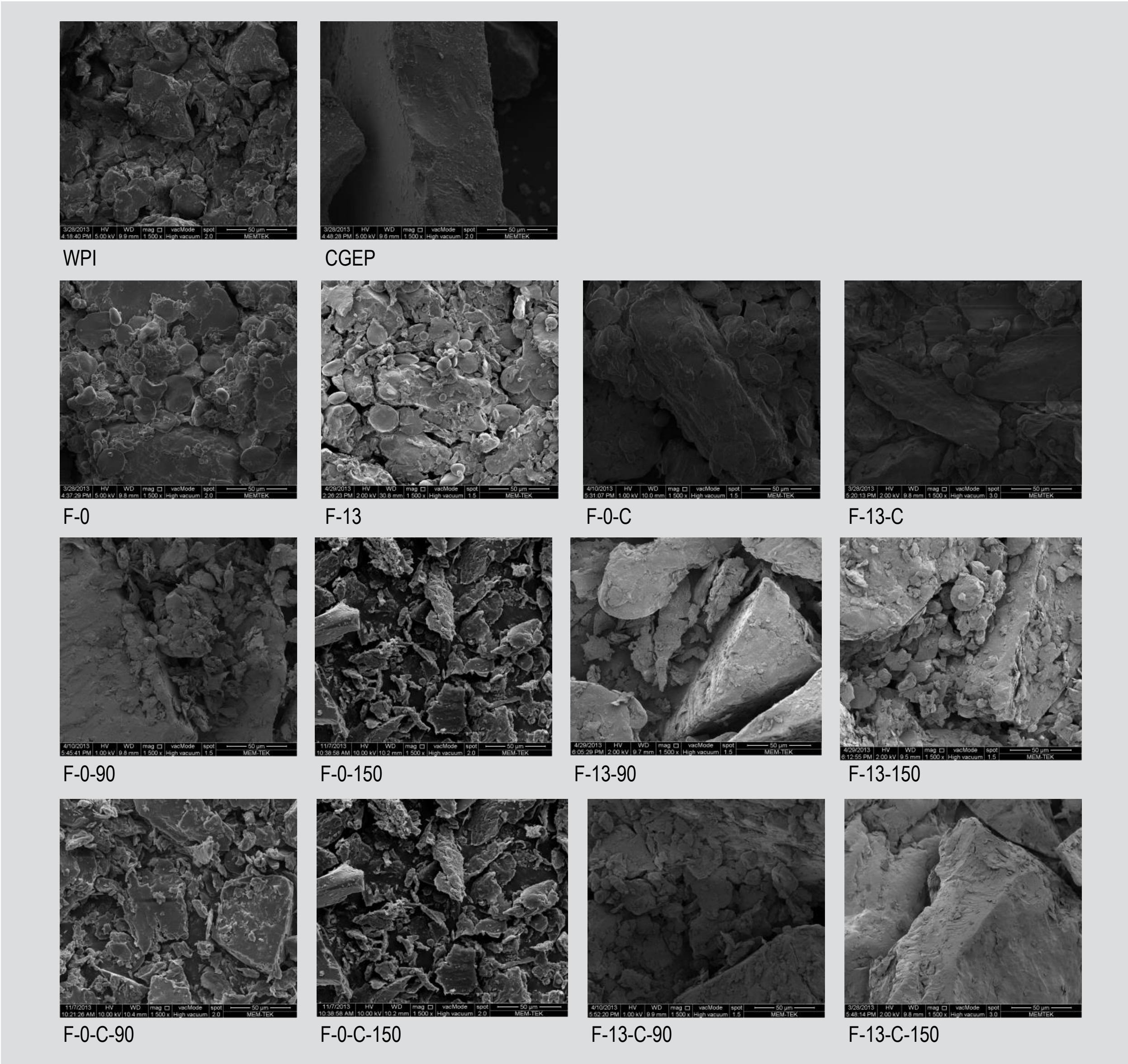

Figure 1. Scanning electron microscope (SEM) micrographs of selected raw and extruded materials. Magnification bars in the SEM micrographs represents 50 μm. WPI = wheat protein isolate; CGEP = concord grape extract powder; F-0 = bread flour; F-13 = bread flour substituted with 13% WPI; F-0-C = bread flour substituted with 7% CGEP; F-13-C = bread flour substituted with 13% WPI and 7% CGEP. 90 and 150 indicate the extrusion temperature in °C of the extruded samples.

(F-0-C, F-6.5-C, F-13-C) and without CGEP (F-0, F-6.5, and F-13) were found to decrease significantly as the level of protein substitution increased. The lowest peak and final viscosity values among all were measured by samples extruded at 150 °C. They were significantly lower than the values obtained for samples extruded at the lower die temperatures 120 and 90 °C (P<0.05). The level of protein substitution had a significant effect on final viscosity values of samples without CGEP (P<0.05). For all samples, except for F-0-C, pasting temperatures were significantly lower for samples extruded at 150 °C (around 50 °C difference) with respect to those extruded at 90 or 120 °C (around 80-90 °C difference). Within each extrusion temperature studied, neither the level of protein substitution nor the CGEP substitution made significant differences (P>0.05) among extruded samples (except for F-0-C at 120 °C).

Viscosity parameters were found to correlate significantly among each other and also with colour parameters (P<0.01). However they varied in three different die temperatures. At 90 °C, peak viscosity correlated with final viscosity (r=0.957), setback viscosity (r=0.894), peak time (r=0.876) and colour parameters of L* (r=0.844), a* (r=-0.850), and b* (r=0.888) (P<0.01). At 120 °C die temperature, on the other hand, although a significant correlation between peak viscosity and final (r=0.781) and setback viscosities

Table 5. Rapid Visco Analyzer pasting parameters of flour samples and their extrudates produced a three different temperatures.[1,2]

Sample	Non-extruded samples		
	Peak viscosity	Final viscosity	Pasting temperature (°)
F-0	213.2±4.6a	243.6±4.5a	68.0±0.2b
F-6.5	183.5±6.2b	206.8±4.6b	68.8±0.6b
F-13	152.9±1.6d	174.3±1.3c	68.6±0.0b
F-0-C	167.8±1.9c	184.7±1.0c	76.7±7.3ab
F-6.5-C	129.2±2.2e	139.7±2.9d	84.6±1.8a
F-13-C	117.0±1.9e	128.7±1.6e	84.4±0.4a

Sample	Extruded samples								
	Peak viscosity			Final viscosity			Pasting temperature (°C)		
	90 °C	120 °C	150 °C	90 °C	120 °C	150 °C	90 °C	120 °C	150 °C
F-0	114.9±0.3xa	106.3±5.5xa	92.4±3.4ya	165.9±3.6xa	130.2±7.8ya	74.0±0.8za	91.3±0.8xa	88.5±0.4ya	50.1±0.1za
F-6.5	92.6±2.4xyb	106.5±5.0xa	77.7±2.2yb	136.59±2.3xb	137.9±0.3xa	63.8±1.0yb	91.0±0.8xa	89.7±1.6xa	50.3±0.0ya
F-13	90.4±0.8xb	88.3±4.2xb	54.5±1.0yc	129.1±1.4xb	112.3±0.5yb	47.7±5.4zc	90.9±0.0xa	90.1±0.0xa	50.2±0.1ya
F-0-C	74.2±7.9xyc	90.7±8.3xab	43.2±4.2yd	102.3±4.5xc	91.9±9.0xcd	28.7±1.2yd	80.8±9.3xa	62.9±12.7xb	50.1±0.0xa
F-6.5-C	65.8±2.8xyc	79.6±4.2xbc	50.3±4.1ycd	84.2±3.0yd	105.5±4.1xbc	35.9±4.0zd	89.5±1.0xa	91.7±0.4xa	50.2±0.0ya
F-13-C	66.3±1.9xc	65.6±0.7xc	39.9±1.5yd	87.4±1.5xd	84.9±0.2xd	28.7±0.9yd	91.5±1.0xa	91.9±0.6xa	50.2±0.1ya

[1] The mean value ± standard deviation of triplicate analyses are given. Values with different letters (a-e) within the same column and different letters (x-z) within the same row differ significantly (*P*<0.05).
[2] See Table 1 for sample descriptions.

(r=0.838) were identified; no correlation was found with the pasting temperature and peak time. Pasting temperature only correlated significantly with peak time (r=0.852) and moisture content (r=-0.764) ($P<0.01$). Peak viscosity did not correlate with any of colour parameters significantly ($P<0.01$). At 150 °C, peak viscosity correlated with final viscosity (r=0.967), setback viscosity (r=0.873), peak time (r=0.769), and also colour parameters of L* (r=0.810), a* (r=-0.774), and b* (r=0.780) (p<0.01).

Peak viscosity, final viscosity, setback viscosity and peak time also correlated with colour parameters at 90 °C whereas, at 120 °C only final viscosity and setback viscosity values correlated significantly with colour parameters, and at 150 °C peak viscosity and final viscosity at $P<0.01$

Thermal properties

T_o, T_p and ΔH for extruded samples were found to be in the ranges of 60.74-69.36 °C, 65.56-74.28 °C and 0.02-6.09 J/g, respectively (Table 6). T_o values for gelatinisation increased significantly ($P<0.05$) both in samples with and without CGEP as the die temperature was raised from 90 to 120 °C. For samples extruded at 150 °C, no gelatinisation and retrogradation peaks were observed, indicating that all samples were fully gelatinised during the extrusion process at that temperature. Samples with 13% WPI (both with and without CGEP) extruded at 120 °C were also found to be fully gelatinised. No significant changes were observed in T_p values of gelatinisation with an increase in extrusion temperature from 90 to 120 °C for the wheat flour only (F-0) and wheat flour with CGEP (F-0-C) samples. However, for extrudates containing 6.5% WPI, both with and without CGEP, T_p values increased significantly for the same die temperatures mentioned.

The presence of CGEP in flour (F-0-C) increased T_o and T_p of extrudates in comparison to the counterpart extruded samples without CGEP (F-0). This effect with CGEP substitution was evident at both die temperatures of 90 and 120 °C. Samples with WPI, on the other hand, behaved differently at the two different die temperatures. At 90 °C, there were no significant differences ($P<0.05$) among the T_o values or the T_p values of samples without CGEP (with WPI, F-6.5 and F-13) and the F-0 samples (no WPI, no CGEP). Moreover, the shifting effect of CGEP to

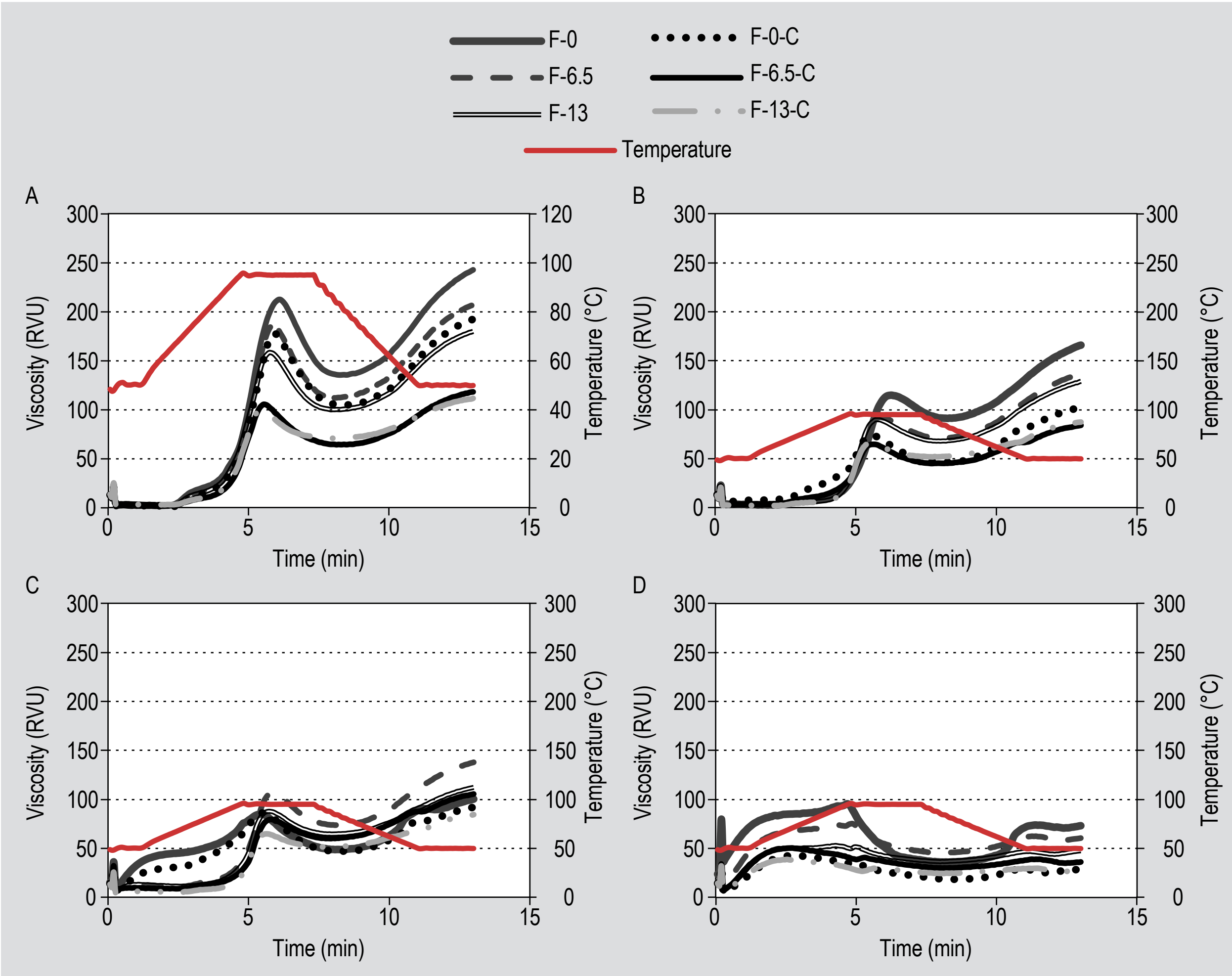

Figure 2. Pasting profiles of unextruded and extruded samples. (A) Pasting profiles of unextruded samples; (B) Pasting profiles of samples extruded at 90 °C; (C) Pasting profiles of samples extruded at 120 °C; (D) Pasting profiles of samples extruded at 150 °C. See Table 1 for sample descriptions; RVU: Rapid Visco Units.

higher temperatures, detected in F-0-C vs F-0 samples, became less pronounced by WPI substitution at samples with CGEP and WPI (F-6.5-C and F-13-C).

For each formulation studied, the increase in extrusion die temperature from 90 to 120 °C significantly decreased the measured enthalpy values (P<0.05). Samples with CGEP had significantly lower enthalpy values than their counterpart samples without CGEP for extrudates produced at 90 °C.

Onset and peak temperatures of retrogradation, for all samples produced at 90 and 120 °C, were lower than all T_o and T_p values of gelatinisation (Table 6). There were no significant changes in T_o or T_p of retrogradation for any of the extrudates with the increase in die temperature from 90 to 120 °C, except for the T_o value of F-0-C.

Correlation coefficients between the physical properties of extrudates

The Pearson's correlation coefficients between the physical properties of extrudates are shown in Table 7. Moisture content of the extrudate products changed between 4.2 and 10.4%. Generally diametric expansion, bulk density, and hardness presented significant correlations with other parameters at different die temperatures. On the other hand, viscosity parameters and color parameters were the most distinct properties as they significantly correlated with each other at all die temperatures (P<0.01).

Table 6. Thermal properties of extrudates determined by differential scanning calorimeter.[1,2]

	Thermal properties of gelatinisation					
	T_o (°C)		T_p (°C)		ΔH (J/g)	
Extrusion temperature	90 °C	120 °C	90 °C	120 °C	90 °C	120 °C
F-0	61.61±0.52ybc	63.40±0.02xc	65.75±0.36xb	66.48±0.04xb	3.52±0.08xb	0.02±0.00yc
F-6.5	62.07±0.98ybc	69.00±0.09xab	65.56±0.47yb	72.59±0.56xa	4.06±0.59xab	0.08±0.02yc
F-13	60.74±0.71c	n.d.	65.96±0.16b	n.d.	6.09±1.44a	n.d.
F-0-C	66.89±1.52ya	69.36±0.43xa	74.28±3.70xa	72.92±0.82xa	1.05±0.41xc	0.43±0.09yb
F-6.5-C	63.59±0.74yb	68.49±0.26xab	67.00±0.67yb	72.41±1.11xa	2.58±0.64xbc	0.90±0.06ya
F-13-C	62.41±0.20bc	n.d.	66.54±0.09b	n.d.	3.33±1.15b	n.d.
	Thermal properties of retrogradation					
	T_o (°C)		T_p (°C)		ΔH (J/g)	
F-0	43.01±0.05xc	43.12±0.37xb	51.22±0.08yb	53.07±0.09yab	0.38±0.01xbc	0.19±0.01yb
F-6.5	47.00±0.05xb	43.11±0.31xb	54.82±0.05xab	55.75±1.40xab	0.10±0.01yd	0.53±0.00xa
F-13	47.26±2.08ab	n.d	55.08±1.10ab	n.d.	0.49±0.01b	n.d.
F-0-C	47.42±0.76xab	41.78±0.85yb	53.70±0.30xab	50.43±0.23xb	0.25±0.08ycd	0.45±0.02xab
F-6.5-C	51.22±1.27xa	43.37±0.86xa	57.65±0.53xa	56.09±0.7xa	0.27±0.13xcd	0.36±0.22xa
F-13-C	42.88±1.85c	n.d.	56.22±1.31a	n.d.	0.81±0.07a	n.d.

[1] The mean value ± standard deviation of triplicate analyses are given. Values with different letters (a-d) within the same column and different letters (x-y) within the same row differ significantly (P<0.05).
[2] See Table 1 for sample descriptions; To = onset temperature; Tp = peak temperature; ΔH = enthalpy of gelatinisation; n.d. = not detected.

4. Discussion

Specific mechanical energy

SME is a common measure of the work input from the extruder to the extrusion material and is an indicator of the effect of molecular breakdown or degradation and enables a good characterisation of the final product (Guerrero *et al.*, 2012). It is considered to be highly related to the extrusion process parameters of screw speed, die temperature, feed moisture, and feed composition, all of which affect the viscosity and flow of the extrusion melt (Schaich and Rebello, 1999).

The general decreasing trend of SME values with increasing extrusion temperature was similar to that noted in previous studies (Fischer, 2004). When samples at each extrusion temperature were evaluated, it was noticed that among samples without CGEP, SME values were significantly different only for 150 °C extrudates. A significant decreasing trend detected for samples up to a certain level of WPI substitution (from 0 to 6.5%) was similar to that of Zhu *et al.* (2010) who found that change in the level of protein addition from 0 to 10% resulted in a sharp decrease in extrusion SME by means of viscosity decrease, although further increases in protein made almost no more significant changes in SME in their study. For the samples with CGEP, SME values were significantly different only among 120 and 150 °C samples. Other researchers have reported a decreasing trend or even no differences in SME values (Camire *et al.*, 2007) for corn flour extrudates substituted with fruit powder (Potter *et al.*, 2013) or apple pomace (Karkle *et al.*, 2012), similar to the results in the present study obtained for 150 °C samples (F-0 vs F-0-C). The significant decrease in SME values between F-0 and F-0-C samples extruded at 150 °C could be related to the lubricative roles played by sugar and fiber present in the fruit ingredients (Potter *et al.*, 2013) which is CGEP in the present study. Another reason for this significant change in SME values among samples extruded at 150 °C with CGEP could be related to WPI substitution in the formulations, since the decreasing effect of CGEP (observed in samples F-0 vs F-0-C) which was not statistically significant (P>0.05) was lost by the addition of WPI (F-6.5 vs F-6.5-C and F-13 vs F-13-C) and the differences in SME values between F-6.5 vs F-6.5-C and between F-13 vs F-13-C were not found to be statistically significant (P>0.05).

Table 7. Correlation coefficients between the physical properties of extrudates.[1,2]

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Extruded at 90 °C														
1	1.000													
2	0.161	1.000												
3	0.293	-0.667*	1.000											
4	0.347	-0.339	0.837**	1.000										
5	0.625*	0.529	-0.128	-0.091	1.000									
6	-0.205	-0.348	0.114	0.189	-0.500	1.000								
7	-0.153	-0.487	0.138	0.153	-0.599*	0.957**	1.000							
8	0.101	-0.414	0.207	0.273	-0.466	0.894**	0.954**	1.000						
9	-0.187	-0.590*	0.206	0.211	-0.609*	0.876**	0.931**	0.852**	1.000					
10	-0.363	-0.480	-0.256	-0.563	-0.370	0.093	0.270	0.104	0.345	1.000				
11	0.619*	0.211	0.303	0.488	0.376	-0.379	-0.362	-0.161	-0.233	-0.539	1.000			
12	-0.388	-0.266	-0.227	-0.174	-0.605*	0.844**	0.870**	0.789**	0.734**	0.374	-0.600*	1.000		
13	0.375	0.282	0.216	0.175	0.606*	-0.850**	-0.881**	-0.801**	-0.746**	-0.388	0.600*	-0.999**	1.000	
14	-0.291	-0.293	-0.165	-0.106	-0.571	0.888**	0.919**	0.861**	0.790**	0.343	-0.522	0.990**	-0.992**	1.000
Extruded at 120 °C														
1	1.000													
2	0.797**	1.000												
3	-0.666*	-0.692*	1.000											
4	-0.320	0.164	-0.305	1.000										
5	0.292	0.470	-0.660*	0.385	1.000									
6	0.532	0.477	-0.406	0.088	-0.008	1.000								
7	0.171	-0.025	0.005	-0.116	-0.434	0.781**	1.000							
8	0.295	0.151	-0.250	0.009	-0.222	0.838**	0.953**	1.000						
9	-0.423	-0.628*	0.528	-0.143	-0.596*	0.027	0.607*	0.433	1.000					
10	-0.441	-0.643*	0.626*	-0.368	-0.764**	-0.227	0.387	0.184	0.852**	1.000				
11	0.216	0.480	-0.419	0.366	0.586*	0.270	-0.236	-0.074	-0.706*	-0.874**	1.000			
12	0.408	0.045	-0.004	-0.475	-0.551	0.692*	0.824**	0.751**	0.350	0.340	-0.300	1.000		
13	-0.433	-0.054	0.027	0.483	0.509	-0.702*	-0.827**	-0.763**	-0.345	-0.315	0.291	-0.997**	1.000	
14	0.439	0.063	-0.020	-0.484	-0.523	0.700*	0.829**	0.759**	0.358	0.324	-0.294	0.997**	-0.998**	1.000
Extruded at 150 °C														
1	1.000													
2	0.337	1.000												
3	-0.296	-0.838**	1.000											
4	-0.144	-0.066	0.205	1.000										
5	-0.300	0.097	0.182	-0.331	1.000									
6	-0.127	0.292	0.134	0.083	0.658*	1.000								
7	-0.193	0.107	0.287	0.098	0.600*	0.967**	1.000							
8	0.167	0.388	-0.091	-0.087	0.533	0.873**	0.861**	1.000						
9	-0.233	-0.170	0.481	0.366	0.247	0.769**	0.862**	0.653*	1.000					
10	-0.284	-0.385	0.500	0.261	0.047	0.082	0.191	-0.078	0.159	1.000				
11	0.002	-0.225	0.099	0.543	-0.299	-0.299	-0.341	-0.378	-0.064	-0.390	1.000			
12	-0.321	-0.259	0.539	0.179	0.387	0.791**	0.875**	0.626*	0.946**	0.225	-0.174	1.000		
13	0.184	0.247	-0.562	-0.220	-0.314	-0.762**	-0.847**	-.610*	-0.938**	-0.249	0.183	-0.971**	1.000	
14	-0.291	-0.201	0.476	0.147	0.354	0.786**	0.862**	0.638*	0.946**	0.124	-0.174	0.992**	-0.959**	1.000

[1] 1 = water absorption; 2 = diametric expansion; 3 = bulk density; 4 = hardness; 5 = SME; 6 = peak viscosity; 7 = final viscosity; 8 = setback viscosity; 9 = peak time; 10= pasting temperature; 11 = moisture %; 12= L*; 13 = a*; 14 = b*.

[2] ** correlation is significant at the 0.01 level (2-tailed); * Correlation is significant at the 0.05 level (2-tailed).

Diametric expansion

Diametric expansion indices of the samples increased gradually with increases in exit die temperature. This trend was in consistent with previous literature findings (Chang and Ng, 2011). Extrudate expansion was expected to decrease with the increase in protein levels in feed materials (Devi *et al.*, 2013; Ding *et al.*, 2006; Linko *et al.*, 1981), however in this study, this decrease was observed only in samples extruded at 150 °C die temperature. Decreases were generally related with the drop in the starch content in feed materials after protein levels were increased. On the other hand, although being statistically insignificant, a reduction at 6.5% WPI substitution followed by an increase at 13% WPI substitution was more remarkable. Similar trend was also detected by Faubion and Hoseney (1982) who noted a decrease in diametric expansion as gluten in extrudates was increased to 11%, followed by an increase in expansion as the protein content increased to 16% (Faubion and Hoseney, 1982). Moreover, CGEP has a total dietary fibre content of 17.1% (data not shown) and fibre content has been reported to be one of the reasons that decrease the expansion in extruded products, as it competes for the free water available in the product (Ravindran *et al.*, 2011).

Bulk density

The decrease in bulk density with increasing die temperature was statistically significant at 150 °C. This condition was due to the fact that during extrusion, by the increase in temperature the degree of superheated water increases, which leads to more bubble formation, decreased melt viscosity, thereby lower bulk density (Ding *et al.*, 2006; Fletcher *et al.*, 1985). Neither WPI- nor CGEP-substitution levels had significant effects on bulk density results, with the only exception for samples with CGEP which are extruded at 150 °C, for which increase in protein levels significantly increased the extrudate bulk density (P<0.05). Increase in protein content and/or the presence of fibre were reported to cause the rupture of cell walls, thus related with the prevention of air bubbles from expanding and increase in the bulk density with a compact texture, previously (Altan *et al.*, 2008; Bisharat *et al.*, 2013).

Textural properties

Temperature was the main parameter to affect the hardness of the extrudates. Similar to the findings by Ding *et al.* (2006), the decrease in sample hardness with increase in extrusion temperature from 120 to 150 °C might be related to the lower melt viscosity that gave rise to bubble growth, increase in expansion ratio (r= -0.512, P<0.01) and decrease in bulk density (r= -0.961, P<0.01) yielding softer extruded samples.

In general, increases in protein content of samples were associated with decreases in hardness values of the extrudates in the present study. Increase in protein content is considered as a weakening factor for the mechanical properties of extruded corn products (Chanvrier *et al.*, 2007). Previous studies linked this weakening and increased brittleness effect to changes in the microstructures of the extrudate, probably resulting from shear effects of the extrusion process on the protein network; as protein content is increased in the formulation, extrudate texture is much more affected by such shearing (Chanvrier *et al.*, 2007; Chaunier *et al.*, 2007). Addition of CGEP seemed to prevent the softening effect exerted by 13% WPI at 120 °C and at 150 °C, as seen from the results of F-13-C sample in the present study (Table 3). This might be related to the previously determined extrudate-hardening effect of fruit-based ingredients which are rich in fibre and sugar (Potter *et al.*, 2013).

Water absorption

The rise in water absorption values by increasing extrusion temperatures are in agreement with previous findings from the literature (Yağcı and Göğüş 2008). Water absorption of extrudates is mostly governed by the starch. Therefore it is widely used as an indirect measurement of starch gelatinisation (Anderson *et al.*, 1969). Water absorption values for samples with only CGEP was higher than the samples with both CGEP and WPI. This might be related with the fibre content of CGEP. Previously, fibre content of extrudates were presented to correlate with water absorption (Sarawong *et al.*, 2014). Water absorption of extrudates generally decreased with the increasing WPI substitution level, especially from 0 to 6.5% WPI substitution. Increase in the formation of gluten network by means of higher WPI substitutions can be related to lower water absorption values. Previous studies revealed that gluten formation restricted starch gelatinisation and by this means decreased the level of water absorption since they are concurrently occurring changes during extrusion (Ding *et al.*, 2006). However, from 6.5 to the 13% WPI substitution level, similar trend was not observed neither among the samples without CGEP nor among the samples with CGEP. Similarly, non-specific trends were reported by previous researchers, when comparing the effect of adding 0 to 20% levels of soy protein concentrate to high amylose corn starch during extrusion (Zhu *et al.*, 2010).

Colour

Camire *et al.* (2007) substituted white corn meal with 1% dehydrated Concord grape powder to make extrusion at 163 °C, and reported lower darkness and redness values than those found in the present study, possibly because the CGEP inclusion in their study was lower (1% w/w) than in the present study. Their yellowness values were found to be similar to those found for samples without CGEP in the present study, likely because of lower amount of CGEP substitution. Significant increase in ΔE values in samples with CGEP in comparison to samples without CGEP was similar to the findings of Altan *et al.* (2008) who added grape pomace into extrudates and found that ΔE increased as grape pomace level increased. Similar to present findings at 150 °C, they also obtained a lower value of total colour change at higher extrusion temperatures that may be associated with may be associated with higher destruction of heat sensitive pigments (Altan *et al.*, 2008).

Scanning electron microscope

In the microstructure of extruded samples evaluated by SEM, larger particles observed in the images might be related to swelling and gelatinisation of starch granules during extrusion. For samples extruded at the highest die temperature (150 °C), the voids observed between particles might be the reason for lower hardness and lower bulk density of those extruded samples. For extruded samples with CGEP, CGEP structure was still detectable after extrusion, indicating that the applied extrusion conditions did not completely alter the CGEP structure.

Pasting properties

According to the pasting parameters, the peak viscosity and final viscosity values of non-extruded native flour samples were generally at least two-fold of the values for respective counterpart extruded samples. This finding was consistent with the previous findings (Bouvier *et al.*, 2001) and might be related to the shear forces applied during extrusion which changed the structure of samples. Zaidul *et al.* (2007) stated that the lower final viscosity values of extruded samples were caused by the disruption of starch granules during extrusion.

At the extrusion temperature of 150 °C, peak viscosities of all extruded samples were significantly lower than those of samples extruded at the other two die temperatures (120 and 90 °C). This is probably because the extent of disruption of starch granules was greater, with rapid swelling at 150 °C relative to lower temperatures (Chaunier *et al.*, 2007). When the effect of CGEP presence was evaluated; peak viscosities of samples with CGEP were significantly lower (about 30%) than their counterpart samples without CGEP at each temperature studied. This might be both related to the weakening of protein network and retarding of paste formation in samples (Chaunier *et al.*, 2007; Núñez *et al.*, 2009) which may be attributed to the hygroscopic behaviour of CGEP in formulations. However, the level of protein substitution for samples with CGEP did not have a significant reducing effect as it had for samples without CGEP.

Similar to the present results, gradual increase in protein levels in the formulations is considered as a weakening factor for the gel (Joshi *et al.*, 2014). Each sample without CGEP was significantly higher than its counterpart sample with CGEP, both in peak and final viscosity measurements. This was expected since the starch proportion was lower in samples with CGEP.

Final viscosity values for all samples extruded at 150 °C were significantly lower than the values obtained for samples extruded at the lower die temperatures 120 and 90 °C (P<0.05). Moreover, for samples with CGEP, final viscosity values were about 35% lower than those of the counterpart samples without CGEP. The extent of reduction might be related to both the decreased amount of starch in samples with CGEP and also to the existence of starch-phenol complexes inhibiting the regular alignment of polymer chains which can affect pasting properties (Beta and Corke, 2004).

Decrease in final viscosity values with the increase in WPI levels might be associated with the formation of protein and starch surface complexes (Zaidul *et al.*, 2007). In contrast, final viscosity values of samples with CGEP were not affected significantly by changes in WPI substitution level (P>0.05).

Thermal properties

According to the thermal measurements made with samples it was obvious that WPI substitution had no role on the onset of gelatinisation for samples without CGEP which are extruded at 90 °C. There are some contradictory findings in the literature on the effect of proteins on starch gelatinisation. The present findings at 90 °C were not in agreement with the general increasing effect of protein on gelatinisation T_o and T_p, as reported previously (Eliasson, 1983; Mohamed and Duarte, 2003), but were in accordance with the findings of a study that found no effect of gluten on gelatinisation (Erdogdu *et al.*, 1995). However, on samples with CGEP, it displayed a normalising effect, by preventing the retarding effect of CGEP. For samples extruded at 120 °C, on the other hand, the increasing effect of WPI on T_o and T_p values was significant (P<0.05).

The findings on the enthalpy decreases (P<0.05) with increasing temperatures and presence of CGEP were similar to the findings of Zhu *et al.* (2009), who related phenolic compounds with the smaller enthalpies of gelatinisation in wheat starches. However, for samples extruded at 120 °C, the effect of CGEP was opposite to that at 90 °C, and gelatinisation enthalpies increased significantly (P<0.05) in the presence of CGEP. Increase in gelatinisation enthalpies for WPI-substituted samples (both in samples with and without CGEP) was found insignificant (P>0.05) in present findings. It was previously shown that WPI had a decreasing effect on starch gelatinisation enthalpies (Yağcı and Göğüş, 2008). Moreover, for all parameters of retrogradation T_o, T_p and enthalpy, neither the change in WPI level nor the presence of CGEP had distinct effects (*P*>0.05). Ottenhof and Farhat (2004) revealed that gluten addition (9%) to freshly extruded wheat starch (with 34% water content) did not have any influence on the retrogradation enthalpy of the original sample. But in the present study, 13% WPI added sample with CGEP (F-13-C) had a significantly higher retrogradation enthalpy than its counterpart sample without CGEP (F-13) extruded at 90 °C (0.81 vs 0.49 J/g, respectively). It was reported previously that polyphenols were effective in preventing the retrogradation of starch (Tacer-Caba *et al.*, 2014; Wu *et al.*, 2009). However, this effect was not observed for CGEP in the present study. CGEP and CGEP with WPI retarded retrogradation to higher temperatures up to high levels (13%) in comparison to samples without CGEP. Different types of polyphenols were reported to have different effects on thermal properties as declared by other researchers (Zhu *et al.*, 2009).

Correlation coefficients between the physical properties of extrudates

Significant correlations at 90 °C found between bulk density and hardness (Potter *et al.*, 2013) and, negative correlation between bulk density and diametric expansion at 150 °C were similar to previous studies (Stojceska *et al.*, 2009; Potter *et al.*, 2013). Moreover, negative but significant correlation among colour parameters of L* and a* values was also similar to the findings of other studies worked with extrudates of grape pomace and maize (Altan *et al.*, 2008), while different from the same study (Altan *et al.*, 2008), since parameters of L* and b* correlated positively, while a and b correlated negatively. Correlations detected among pasting parameters were in parallel with the previous findings (Blazek and Copeland, 2008).

5. Conclusions

Present study investigated effects of both CGEP (as the anthocyanin source) alone and together with WPI and only WPI, in an extrusion process of different die temperatures, in order to understand their possible interactions and effects on wheat flour extrudate quality. Results revealed that CGEP substitution (7%), even with WPI (6.5 and 13%), was not so much effective on quality of extrudates extruded at 90 and 120 °C die temperatures. The only parameter effective on quality was the die temperature; especially at 150 °C, differences between formulations were more distinct for (diametric expansion, bulk density) when compared to lower die temperatures (90 and 120 °C). Presence of both CGEP and WPI made the pastes weaker (34.1 to 61.1% decrease in peak viscosity) than single addition of these two ingredients. Besides, no distinct effect on retrogradation was observed neither by CGEP nor with WPI. Therefore, in extrudates, loss of protein content by means of CGEP substitution did not exert a significant quality loss (different from the effect of amylose on CGEP reported in the previous findings) and even by increasing the protein level no improvement in quality parameters was obtained. However, interactions of protein and phenolics in those ingredients require further investigation for evaluating the product's functionality for the future studies.

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