



Effect of extrusion cooking on the physicochemical properties, resistant starch, phenolic content and antioxidant capacities of green banana flour



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ABSTRACT

Green banana flour was extruded through a co-rotating twin-screw extruder with constant barrel temperature. The objectives of this study were to determine the effect of extrusion cooking variables (feed moisture, FM, 20% and 50%; screw speed, SS, 200 and 400 rpm) and storing of the extruded flours at 4 °C for 24 h on the physicochemical properties, resistant starch (RS), pasting properties and antioxidant capacities. Extrusion cooking at higher FM and lower SS increased the amylose content, which was expressed in highest RS content. Water adsorption index (WAI) and pasting properties were increased, while water solubility index (WSI), total phenolic content (TPC) and antioxidant activities (FRAP, ABTS.⁺, DPPH) in free and bound phenolics were decreased compared to the other extruded samples. Storing the extruded flours at 4 °C for 24 h prior to oven drying was the main factor leading to a further increase in the content of amylose, RS, TPC and WSI values, as well as pasting properties – in particular peak viscosity. Compared to native banana flour, extrusion cooking caused significant changes in all studied properties of the extruded flours, except for soluble DF and antioxidant capacity (ABTS.⁺ and DPPH) of bound phenolics.

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1. Introduction

According to the FAO, banana (*Musa* spp.) is amongst the world's leading crops, after rice, wheat and maize. The worldwide production of banana tends to increase and was 102 million tonnes in 2010. Over 130 countries produce banana. Ecuador, Colombia, Philippines and Costa Rica are the leading exporters (FAOSTAT, 2011). Banana is usually consumed as a sweet or dessert fruit. When banana is green or unripe, it is very rich in indigestible carbohydrates (up to 60–80% dm), which is composed of cellulose, hemicelluloses, lignin, starch, dietary fibre, and resistant starch (RS2) (Da Mota, Lajolo, Ciacco, & Cordenunsi, 2000; Haslinda, Cheng, Chong, & Noor Aziah, 2009; Menezes et al., 2011; Ramli, Alkarkhi, Yong, & Easa, 2009). Large quantities of green banana rejection such as below-grade fruit or fruit with skin appearance defects are produced into flour in the export industry (Aurore, Parfait, & Fahrassmane, 2009). Nowadays, the industrial flour production from green banana is of interest in view of its nutritional value, especially its high quantity of RS (approximately 40.9–58.5%)

(Tribess et al., 2009) and dietary fibre (6.0–15.5%) (Da Mota et al., 2000), as well as bioactive compounds like phenolic acids (Aurore et al., 2009). Banana pulp contains several classes of free and bound phenolics including anthocyanidins (Bennett et al., 2010). Others reported that green banana flour was low in total polyphenol contents, but antioxidant activity was moderate (Menezes et al., 2011). Most studies on the phenolic content and antioxidant activities of native or extruded flours reported only the content of total free phenolics but not of bound phenolics (Haslinda et al., 2009; Menezes et al., 2011). Only limited literature is available which report on free, bound, and total phenolic contents and antioxidant activities from native and extruded banana flours.

Extrusion cooking involves high heat, pressure, and shear forces, which causes clear changes in all physicochemical and functional properties of the flour, including polyphenolic compounds and their antioxidant activity, depending on type of raw material and extrusion cooking variables such as feeding rate, feed moisture, screw speed and configuration, die geometry, temperature and time (Brennan, Brennan, Derbyshire, & Tiwari, 2011; Singh, Gamlath, & Wakeling, 2007). RS content is often decreased after extrusion cooking. In the study performed by Gonzalez-Soto et al. (2006) RS content was decreased in banana flours after extrusion cooking, but was still higher compared to mango and corn.

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The objectives of this study were to study the effects of (1) extrusion cooking process variables (feed moisture FM and screw speed SS) and (2) storing of the extruded samples at 4 °C for 24 h on the physicochemical properties (WAI, WSI, pasting properties), the content of amylose, RS, dietary fibre (TDF, IDF and SDF), as well as total phenolic content and their antioxidant activities (TPC, FRAP, ABTS⁺, DPPH of free and bound phenolics) of green banana flour, in order to promote the utilisation of extruded green banana flour for further food use.

2. Materials and methods

2.1. Materials

Green banana flour was obtained from Chiquita Brands International Inc, Costa Rica. The proximate composition of green banana flour was 8.30% moisture, 3.70% protein (N × 5.32), 1.86% fat, 2.43% ash, and 83.70% carbohydrate.

The Folin–Ciocalteu reagent, 2,4,6-tripyridyl-s-triazine (TPTZ), 2,2'-azino-(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), potassium persulphate, sodium hydroxide, hydrochloric acid, ethyl acetate, sodium acetate trihydrate, glacial acetic acid, and phenolic acid standards (gallic acid) were purchased from Sigma–Aldrich (Vienna, Austria) and of analytical grade. Methanol and ethanol were of HPLC grade and purchased from Carl Roth GmbH + Co.KG (Graz, Austria).

2.2. Extrusion cooking

Green banana flour was extruded in duplicate using a laboratory scale co-rotating twin-screw extruder (Model MPF19-25, APV Baker Ltd., Grand Rapids, MI, USA) with a 19 mm barrel diameter, a ratio of barrel length to diameter (L/D) of 25:1 and a die with one opening of 2 mm diameter. The barrel temperature profile in the five barrel zones from the feeder to the die zone were set constant during the study at 40, 60, 80, 100 and 130 °C, respectively. Low shear configuration was used in this study. The flour was fed constantly into the extruder (2 kg/h). According to preliminary trials, 15% FM content was the minimum needed for these flours, otherwise the torque increased above 100%. The moisture content of banana flour was determined prior to extrusion cooking (AACC method 44-15A, AACC, 2000) and later adjusted by adding water during extrusion cooking at two different levels (20% and 50% FM). Screw speeds were 200 and 400 rpm. After extrusion cooking the samples were cooled to room temperature. As a further possible approach to improve the RS content in the extruded samples storing at 4 °C for 24 h was applied as cool storage is known to increase RS content to some extent (Kim, Tanhehco, & Ng, 2006). For this aim, one batch of sample was stored at 4 °C for 24 h before drying at 50 °C for 24 h, the second batch was directly dried. The dried samples were milled using a Wiley mill (Arthur H. Thomas Co., Philadelphia, PA, USA) to pass through a US Standard sieve No. 40 (425 µm). The milled samples were packed in plastic bags, kept in an airtight container and stored at room temperature (25 °C) until analyses.

2.3. Chemical and physical analyses

Proximate composition analyses were done for native and extruded flours. Amylose, RS (AOAC method 2002.02), Total (TDF), Insoluble (IDF) and Soluble dietary fibre (SDF) (AOAC Method 991.43) were determined using Megazyme kits (Megazyme Int. Ireland Ltd., Wicklow, Ireland). All determinations were performed in triplicate.

Water adsorption index (WAI) and water solubility index (WSI) were determined according to the method of Anderson, Conway, Pfeifer, and Griffin (1969), in triplicate.

The pasting properties were determined using the Brabender Viscograph E (Type 802520, Duisburg, Germany) in triplicate. Starch suspensions of 10% (db) were heated from 30 to 95 °C at a heating rate of 1.5 °C/min and a bowl speed of 75 rpm, held at 95 °C for 5 min and then cooled to 30 °C at the same rate. Parameters recorded were peak viscosity, hot paste viscosity (start of cooling period), and cold paste viscosity (end of cooling period). Breakdown was calculated as difference peak viscosity-hot paste viscosity, setback as difference cold paste viscosity – hot paste viscosity).

Extraction of free and bound phenolic compounds of the native and extruded flours was performed according to the methods described by Adom, Sorrells, and Liu (2003) and Mattila, Pihlava, and Hellstrom (2005). Total phenolic content (TPC) was determined using Folin–Ciocalteu reagent (FCR) according to the method of Singleton, Orthofer, and Lamuela-Raventos (1999), and was expressed as milligram of gallic acid equivalent (GAE) per 100 gram db (mg GAE/100 g db).

FRAP assay was performed according to a modified method reported by Benzie and Strain (1999). Results were expressed as mmol of Fe(II) equivalents per 100 g db (mmol Fe(II)/100 g db).

ABTS⁺ scavenging capacity was measured using the modified method described by Pellegrini, Del Rio, Colombi, Bianchi, and Brighenti (2003) and Moore et al. (2005). Results were expressed as trolox equivalents antioxidant capacity (TEAC) in mmol of trolox equivalents per 100 g db (mmol TEAC/100 g db).

The method for determination of DPPH radical scavenging ability is based on measurement of the loss of DPPH colour at 515 nm after reaction with sample extract and was performed as described by Brand-Williams, Cuvelier, and Berset (1995). Trolox was used as standard antioxidant. Results were expressed as Trolox equivalents antioxidant capacity (TEAC) in mmol of Trolox equivalents per 100 g db (mmol TEAC/100 g db). Determination of TPC, FRAP, ABTS, DPPH were performed for the free as well as bound phenolic extracts. All analyses were performed in triplicate.

2.4. Experimental design and statistical analyses

The experimental design was carried out for two independent variables, feed moisture (FM, 20% and 50%) and screw speed (SS, 200 and 400 rpm) by applying a 2 × 2 factorial design. The storage period of the extrudates at 4 °C (0 and 24 h) was designed as a block. Based on pre-trials (results not presented) and according to the findings of Kim et al. (2006) some variable extruder parameters were set constant (feed rate and barrel temperature), while screw speed and feed moisture, having the highest influence on extrusion cooking properties were selected as variable parameters for the experimental design. A total of 8 treatments were conducted with one replication of all data points. All statistical analyses of data were carried out using SPSS (Statistical Package for Social Science) software version 15.0, for analysis of variance (ANOVA). Significant differences between means were determined by Duncan's Multiple Range Tests. *P*-values <0.05 were regarded as significant. Correlation coefficients amongst data obtained were calculated using Pearson's correlation coefficients (*r*).

3. Results and discussion

3.1. Effect of extrusion cooking on amylose content

Amylose was significantly influenced by FM and SS and there was a significant interaction effect of FM and SS (Table 1). The

amylose content of extruded samples increased with increasing FM by 30.7–43.5% at both SS studied. When increasing SS, it was found that the amylose content of samples extruded at higher FM (50%) decreased by 17.5–21.9%, while at lower FM (20%) there were no significant differences. In general, SS is responsible for the degree to fill the barrel, which directly affects the residence time, and the rate of shear development on the material during extrusion cooking (Karunanithy & Muthukumarappan, 2012). Lower SS relates to a longer residence time thus encouraging prolonged shearing, rendering the release of more starch and resulting in increased amylose content (Anuonye, Badifu, Inyang, & Akpapunam, 2007).

Storing of extruded samples at 4 °C for 24 h prior to drying had a significant effect on amylose content. It was increased by 2.0–9.9%. All extruded samples showed significantly higher content of amylose than native flour, except for the 20% FM/400 SS sample. In contrast, Menegassi, Pilosof, and Arêas (2011) reported that both, mild (15% FM, 120 °C, 158 rpm) and severe (25% FM, 180 °C, 237 rpm) extrusion conditions, did not significantly affect the amylose content of extruded amaranth flours. This might be due to the fact that amaranth contains very low amylose content in the raw material.

3.2. Effect of extrusion cooking on resistant starch (RS)

The RS content of extruded samples was affected by FM content, SS, and storing at 4 °C for 24 h, but no significant interaction effect of FM and SS on RS content was observed. Increasing FM led to higher RS levels. Similar observations were previously reported for extruded phoenix flour from regular barley grain by Vasanthan, Gaosong, Yeung, and Li (2002) and for extruded pastry wheat flour by Kim et al. (2006). Lower SS provided extruded samples with higher RS content compared to samples extruded at higher SS. This result was probably a result of relatively lower starch degradation due to less shear rate at lower SS conditions. Previous studies also reported that lower SS in extruded corn and mango produced higher levels of RS (Gonzalez-Soto et al., 2006). Also the longer residence time at 200 rpm might have been responsible for a higher opportunity in amylose chain association and thus RS formation. Amylose content is generally an important factor that increases RS production during extrusion cooking (Huth, Dongowski, Gebhard, & Flamme, 2000). At high moisture content, RS may be formed and increased at high amylose level, probably by a retrogradation tendency with the formation of strong intermolecular hydrogen bonds in the amylose fraction (Gonzalez-Soto, Mora-Escobedo, Hernández-Sánchez, Sánchez-Rivera, & Bello-Pérez, 2007). A strong correlation between amylose content and RS could be found in this study (Table 3). Storing at 4 °C for 24 h further increased RS by 2.43–17.12%. Also Huth et al. (2000) reported that extrusion cooking parameters and subsequent storage affected the generation of RS content in extruded barley flour. They observed the highest RS formation of up to 6% in extruded barley flours after extrusion cooking at higher FM (approximately 20%), a mass temperature of 150 °C, and SS of 200 rpm, followed by freeze-storage at –18 °C for 7 days.

Compared to native flour, all extruded samples had very low RS content after extrusion cooking. Extrusion cooking caused a significant reduction of RS content by 91.5–98.1%. Any correlations to amylose or any increase in RS content in the extrudates is therefore quite low comparing with its initial amounts. In general, native starch granules from green banana contain very high initial amounts of RS2. However, RS2 is susceptible to be lost by thermal processing (Menezes et al., 2011). Gonzalez-Soto et al. (2007) found that the starch granules of native banana observed by X-ray diffractometry were found in a C-type crystalline pattern, a mixture of the A- and B-type, which makes them easily susceptible to enzymatic hydrolysis after gelatinization. An X-ray pattern of

the extruded samples presented a high amorphous state, while the organised crystalline structure was low. This phenomenon could be due to a destruction of the organised crystalline structure of starch molecules either partially or completely, depending on the amylose–amylopectin ratio and extrusion variables such as moisture and shear, resulting in decreased RS content of extruded samples (Maskan & Altan, 2011).

3.3. Effect of extrusion cooking on dietary fibre

FM significantly increased IDF (by 26.1–28.0%) and TDF (by 17.4–18.7%) but not SDF. This result was similar to that reported by Vasanthan et al. (2002). Changing SS had a small but significant effect on IDF (decreased by 5.8–8.7%) and SDF (increased by 6.4–10.6%), but not on TDF content. This phenomenon could be due to increased mechanical shearing during extrusion cooking at higher SS, resulting in the conversion of some IDF into SDF. There was no significant interaction effect of FM and SS on IDF, SDF, and TDF and no significant effect of storing at 4 °C was observed. Compared to native flour, extrusion cooking significantly increased IDF and TDF contents (11.9–18.8%) of the 50% FM samples at both SS studied, but decreased them by 9.6–17.9% in the 20% FM samples. SDF was not significantly influenced by extrusion cooking. The increase of IDF content of extruded flours contributed to the increase in TDF content. Also Repo-Carrasco-Valencia, Acevedo de La Cruz, Icochea Alvarez, and Kallio (2009) reported that after extrusion cooking IDF, SDF, and TDF were decreased in kañiwa varieties. In the present study, the majority of TDF was presented mainly by the insoluble fraction, which was approximately 1.5–2.5-fold higher than the soluble fraction.

3.4. Effect of extrusion cooking on water absorption index (WAI) and water solubility index (WSI)

WAI was significantly increased (42.4–53.1%) and WSI decreased (87.9–88.4%) by increasing FM. Higher FM during extrusion cooking resulted in a lower degree of starch gelatinization and probably acts as a plasticizer caused by reduced starch degradation, also due to less shearing taking place, thus resulting in an increase in WAI and a decrease in WSI (Hagenimana, Ding, & Fang, 2006). Increasing SS caused a decrease in WAI (6.1–23.8%) and an increase in WSI of extruded samples (only at lower FM, 4.6–5.7%). Again this might be attributed to the higher amount of damaged polymer chains formed at higher shear rate, reducing the availability of hydrophilic groups to bind more water molecules, resulting in a decrease in values of WAI (Guha, Ali, & Bhattacharya, 1997). Such higher SS induced higher mechanical shear leading to more starch degradation, subsequently increasing the amount of soluble molecules and resulting in an increase WSI values. The interaction effect of FM and SS was significant for WSI. Storing of the samples had no significant effect on WAI or WSI. WAI and amylose content of starch were positively correlated; WSI and amylose content were negatively correlated.

Compared to native flour, WAI was increased after extrusion cooking at 50% FM (22.0–28.4%), but decreased after 20% FM (20.1–39.7%) at both SS studied. Generally, starch granules are susceptible to thermomechanical damage during extrusion cooking. Damaged starch has higher water retention capacity at room temperature and swelling compared to the native flour, resulting in an increase in WAI values. However, after reaching a maximum with respect to the degree of starch damage, WAI decreased under lower FM with increasing temperature. This is probably due to dextrinization or starch melting that prevails over the gelatinization phenomenon (Maskan & Altan, 2011). An inverse effect was observed on WSI. WSI of extruded flours was increased in all cases (10.1–90.3%).

Table 1
Amylose, RS contents, IDF, SDF, and TDF of extruded and native green banana flours stored at 4 °C for 0 and 24 h.

Treatment	Amylose (% w/w)	RS (% DM)	Dietary fibre (% DM)		
			IDF	SDF	TDF
Native flour	16.20 ± 0.86f	47.25 ± 2.15a	4.46 ± 0.16c	2.54 ± 0.18a	7.00 ± 0.01b
<i>Extruded flour^a</i>					
(1) 20%, 200 rpm, 0 h	17.96 ± 0.04de	1.20 ± 0.14c	3.97 ± 0.18de	2.25 ± 0.03a	6.22 ± 0.10c
(2) 20%, 400 rpm, 0 h	17.25 ± 0.65ef	0.92 ± 0.12c	3.66 ± 0.10e	2.46 ± 0.05a	6.12 ± 0.04c
(3) 50%, 200 rpm, 0 h	30.16 ± 0.12b	3.82 ± 0.14b	5.37 ± 0.16ab	2.20 ± 0.07a	7.57 ± 0.06a
(4) 50%, 400 rpm, 0 h	24.89 ± 0.81c	3.62 ± 0.11b	5.06 ± 0.14b	2.46 ± 0.06a	7.53 ± 0.05a
(5) 20%, 200 rpm, 24 h	18.92 ± 0.90d	1.34 ± 0.12c	4.03 ± 0.07d	2.34 ± 0.14a	6.37 ± 0.05c
(6) 20%, 400 rpm, 24 h	17.60 ± 0.32ef	1.11 ± 0.06c	3.68 ± 0.17e	2.50 ± 0.24a	6.19 ± 0.05c
(7) 50%, 200 rpm, 24 h	33.49 ± 0.39a	4.00 ± 0.07b	5.49 ± 0.08a	2.21 ± 0.08a	7.71 ± 0.00a
(8) 50%, 400 rpm, 24 h	26.17 ± 0.62c	3.71 ± 0.09b	5.11 ± 0.12b	2.47 ± 0.07a	7.58 ± 0.03a
Analyses of variance	<i>p</i> -Value	<i>p</i> -Value	<i>p</i> -Value	<i>p</i> -Value	<i>p</i> -Value
Feed moisture (FM)	0.000	0.000	0.000	0.315	0.000
Screw speed (SS)	0.000	0.000	0.000	0.001	0.050
FM × SS	0.000	0.818	0.831	0.443	0.629
Block	0.004	0.000	0.318	0.443	0.088
R ² (%)	98.70	99.40	98.30	67.20	98.40

Different letters (a–f) within the same column as compared to the native flour differ significantly ($p < 0.05$). Values are means of triplicate ± standard deviation.

^a Feed moisture (%), screw speed (rpm), stored at 4 °C for 0 or 24 h.

3.5. Effect of extrusion cooking on pasting properties

The pasting properties of the extruded flours were all lower compared to native flour, except for breakdown and setback values in the extruded flours at higher FM, demonstrating that extrusion cooking at all setting studied partly/fully gelatinized the starch of the flours (Table 2). However the varied FM and SS had significant influences on the pasting properties of the flour, and they interacted significantly. Within the same SS, lower FM resulted in lower peak viscosity, hot paste viscosity, cold paste viscosity, breakdown and setback (by 86.9–89.7%, 94.8–95.8%, 92.7–93.6%, 82.9–86.8%, and 89.9–92.7%, respectively). This can be attributed to the fact that a higher degree of gelatinization and starch degradation occurred during extrusion cooking under lower FM, it is a likely significant factor associated with low viscosity profiles. The cold paste viscosity is a direct measure of the viscosity of the gel formed after retrogradation. The cold paste viscosity of all extruded flours increased upon cooling, due to the aggregation of the starch molecules. The cold paste viscosities of the 20% FM samples, however, were still lower than that of the higher 50% FM samples. Breakdown is a measure for the degree of starch granule disintegration influenced by mechanical shear during the holding phase (Adedokun & Itiola, 2010). The 20% FM samples exhibited a lower breakdown, implying that in these samples a lower mechanical shear-thinning occurred during the holding period at 95 °C. Additionally, the lower setback value indicated a reduced starch retrogradation after cooling. These results were in agreement with other studies (Kim et al., 2006; Sompong, Siebenhandl-Ehn, Berghofer, & Schoenlechner, 2011). Regarding the effect of SS, it was found that all pasting properties of extruded flours decreased by 19.2–25.4%, 20.5–25.6%, 14.6–19.2%, 18.5–25.3%, and 8.8–12.9%, respectively when increasing SS. Obviously, higher starch degradation occurred due to higher shear forces at increased SS. Cool storage of the extruded flours significantly increased the pasting properties, especially peak viscosity.

Extrusion cooking, especially under lower FM causes greater friction and energy dissipation to the flour, resulting in dextrinization or degradation of starch, and an increased formation of water-soluble molecules (Hagenimana et al., 2006; Repo-Carrasco-Valencia et al., 2009). Extruded flours with lower WAI and higher WSI showed lower viscosity and stickiness of end-products, which is

of interest for children's foods and beverages. Correlation analyses showed that pasting properties were influenced by the amylose content of the starch. It is known, that the amylose content provides an indication of the gelling ability of starch (Adedokun & Itiola, 2010). Amylose leaching is commonly associated with breakdown viscosity (Menegassi et al., 2011). The highest breakdown value was found in the higher FM sample with the highest amylose content. The sample extruded under higher FM and lower SS caused less starch degradation and thus corresponded with the highest amylose content, exhibited the highest setback values, indicating an increase in retrogradation tendency, and leading to higher RS formation.

3.6. Effect of extrusion cooking on free, bound, and total phenolic contents

Varied FM and SS and their interaction had significant influences on the free, bound and total phenolic content (Table 4). Increased FM within the same SS studied resulted in a significant decrease of total phenolics by 12.4–29.2% due to a significant decrease in free phenolics (17.5–42.8%) and bound phenolics (7.9–19.7%). Sharma, Gujral, and Singh (2012) reported a significant decrease in total phenolic content (8–29%) for eight extruded barley cultivars when increasing FM (from 15% to 20%) and barrel temperature (from 150 °C to 180 °C) and also Sompong et al. (2011) found similar results. Increasing SS, within the same FM content resulted in a slight increase in free, bound, and total phenolic contents by 8.1–14.1%, 10.6–10.7%, and 9.33–11.9%, respectively at lower 20% FM samples, whereas increasing SS at higher 50% FM samples led to a slight decrease in free, bound, and total phenolic contents by 13.4–15.6%, 0.9–2.9%, and 5.8–7.5%, respectively (the effect of SS on free phenolics was not significant). In general, an increase in SS leads to a decrease of torque development due to a reduced filled length and residence time, while an increase in mechanical energy input to the system with increasing shear rate can be observed (Altan, McCarthy, & Maskan, 2009; Guha et al., 1997; Iwe, Zuilichem, & Ngoddy, 2001). In this study, it was found that the decreased torque development associated with shorter residence time at increased SS and lower FM affected more dominantly the phenolic content than the effect of the increased shearing. Increasing SS at higher 50% FM samples, however, led to destruction of

Table 2

WAI (g/g), WSI (%) and pasting properties of extruded and native green banana flours stored at 4 °C for 0 and 24 h by the Brabender Viscograph E (unit: BU).

Treatment	WAI	WSI	Peak viscosity	Hot paste viscosity	Cold paste viscosity	Breakdown	Setback
Native flour	2.44 ± 0.01b	7.60 ± 0.06e	1292.5 ± 6.4a	749.5 ± 7.8a	1064.0 ± 0.0a	543.0 ± 1.4d	314.5 ± 7.8d
<i>Extruded flour^a</i>							
(1) 20%, 200 rpm, 0 h	1.93 ± 0.24c	73.04 ± 0.68c	120.0 ± 12.7e	15.5 ± 2.1e	53.0 ± 8.5e	104.5 ± 10.6e	37.5 ± 6.4e
(2) 20%, 400 rpm, 0 h	1.47 ± 0.04d	77.45 ± 0.76a	114.0 ± 11.3e	13.0 ± 1.4e	39.0 ± 7.1e	101.0 ± 9.9e	26.0 ± 5.7e
(3) 50%, 200 rpm, 0 h	3.35 ± 0.08a	8.45 ± 0.15de	1162.0 ± 21.2b	371.0 ± 2.6b	751.0 ± 15.6b	791.0 ± 3.8a	380.0 ± 7.1a
(4) 50%, 400 rpm, 0 h	3.13 ± 0.05a	9.25 ± 0.20d	867.0 ± 7.1d	276.0 ± 2.7d	607.0 ± 14.1d	591.0 ± 5.7c	331.0 ± 1.4c
(5) 20%, 200 rpm, 24 h	1.95 ± 0.38c	74.77 ± 0.90b	132.5 ± 12.0e	17.0 ± 2.8e	55.5 ± 7.8e	115.5 ± 9.2e	38.5 ± 4.9e
(6) 20%, 400 rpm, 24 h	1.50 ± 0.04d	78.41 ± 1.59a	116.0 ± 8.5e	16.0 ± 2.8e	41.5 ± 2.1e	100.0 ± 5.7e	25.5 ± 4.9e
(7) 50%, 200 rpm, 24 h	3.41 ± 0.12a	8.84 ± 0.21d	1179.5 ± 20.5b	383.5 ± 10.6b	765.5 ± 21.9b	796.0 ± 1.1a	382.0 ± 1.3a
(8) 50%, 400 rpm, 24 h	3.20 ± 0.19a	9.48 ± 0.68d	953.5 ± 16.3c	305.0 ± 2.8c	653.5 ± 13.4c	648.5 ± 13.4b	348.5 ± 10.6b
Analyses of variance	<i>p</i> -Value	<i>p</i> -Value	<i>p</i> -Value	<i>p</i> -Value	<i>p</i> -Value	<i>p</i> -Value	<i>p</i> -Value
Feed moisture (FM)	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Screw speed (SS)	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FM × SS	0.060	0.000	0.000	0.000	0.000	0.000	0.003
Block	0.456	0.008	0.029	0.058	0.054	0.135	0.212
R ² (%)	96.20	99.90	99.80	99.70	99.80	99.60	99.90

Different letters (a–d) within the same column as compared to the native flour differ significantly ($p < 0.05$).

Values are means of triplicate ± standard deviation.

^a Feed moisture (%), screw speed (rpm), stored at 4 °C for 0 or 24 h.**Table 3**

Correlation coefficients amongst the content of amylose, RS, IDF, SDF, TDF, WAI, WSI and pasting properties values.

	Amylose	RS	IDF	SDF	TDF	WAI	WSI	Peak viscosity	Hot paste viscosity	Cold paste viscosity	Breakdown	Setback
Amylose	1											
RS	0.939**	1										
IDF	0.954**	0.983**	1									
SDF	-0.421	-0.236	-0.385	1								
TDF	0.927**	0.994**	0.982**	-0.205	1							
WAI	0.929**	0.972**	0.966**	-0.352	0.953**	1						
WSI	-0.912**	-0.995**	-0.971**	0.207	-0.987**	-0.966**	1					
Peak viscosity	0.969**	0.987**	0.979**	-0.308	0.976**	0.960**	-0.980	1				
Hot paste viscosity	0.966**	0.988**	0.981**	-0.294	0.980**	0.957**	-0.980**	0.998**	1			
Cold paste viscosity	0.955**	0.994**	0.981**	-0.273	0.985**	0.964**	-0.990**	0.997**	0.998**	1		
Breakdown	0.969**	0.985**	0.978**	-0.315	0.973**	0.960**	-0.978**	1.000**	0.996**	0.996**	1	
Setback	0.941**	0.996**	0.979**	-0.251	0.987**	0.967**	-0.996**	0.993**	0.993**	0.998**	0.992**	1

** Significant at $p < 0.01$.

these phenolic contents mainly due to the effect of increased shearing. Storing at 4 °C for 24 h significantly increased the content of free, bound and total phenolics by 2.4–14.8%, 3.1–5.0%, and 3.3–8.6%, respectively.

In general, and as expected extrusion cooking caused a significant decrease in total phenolic content (32.3–55.0%), mostly due to a reduction in their free phenolics (60.0–79.1%), while bound phenolics significantly increased (6.8–28.2%). A decrease of total and free phenolics was also found in previous studies (Altan et al., 2009; Mora-Rochin et al., 2010; Sharma et al., 2012). High temperature causes either decomposition or alteration of the molecular structure of phenolic compounds leading to a reduced chemical reactivity or extractability due to a certain degree of polymerisation (Altan et al., 2009; Brennan et al., 2011; Nayak, Berrios, Powers, & Tang, 2011; Sharma et al., 2012). However, the observed increase in bound phenolics could be due to the disruption of cell walls by all extrusion conditions studied, resulting in an increased release of these phenolics.

The average contribution of free to total phenolics in all extruded samples ranged from 33.4–41.9% (for the stored samples) and 35.9–42.6% (for the non-stored samples), respectively, whereas bound phenolics contributed 58.1–66.6% and 57.4–64.1%, respectively. In native flour, on the other hand, the average

contribution of free to total phenolics was 72.0%. These observations for native flour are in agreement with previous data reported by Subba Rao and Muralikrishna (2002) for millet and by Hung and Morita (2008) for buckwheat flours, but in contrast with the results from Adom and Liu (2002) for oats, corn, wheat, and rice flours, and by Adom et al. (2003) for wheat varieties. Therefore, the ratio of free to total phenolics in native banana flour seems to be more similar to millet and buckwheat flours, than to other cereals.

3.7. Effect of extrusion cooking on antioxidant capacities of free, bound, and total phenolics

The antioxidant capacity of extruded and native flours were determined using FRAP, DPPH and ABTS.⁺ assays. The results are presented in Table 5. FM and SS had a significant interaction effect on antioxidant capacities of free, bound and total phenolics of extruded samples.

Within the same SS increasing FM caused a decrease of the antioxidant activity of total phenolics (FRAP, ABTS.⁺ and DPPH) due to a significant decrease of antioxidant activity mainly in free phenolics. Decrease of antioxidant activity in bound phenolics was lower. Within the same FM, increasing SS, especially at lower FM, led to an increase in the antioxidant activity of total phenolics due to a

Table 4

Free, bound, and total phenolics of extruded and native samples from green banana flour stored at 4 °C for 0 and 24 h.

Treatment	Phenolic contents (mg GAE/ 100 g db)		
	Free	Bound	Total
Native flour	158.83 ± 1.88a	61.47 ± 2.48f	220.30 ± 0.59a
<i>Extruded flour^a</i>			
(1) 20%, 200 rpm, 0 h	53.22 ± 2.73d	73.72 ± 0.93cd	126.95 ± 3.66d
(2) 20%, rpm, 0 h	57.91 ± 2.84c	82.11 ± 3.60ab	140.02 ± 0.76c
(3) 50%, 200 rpm, 0 h	39.27 ± 1.68f	67.89 ± 4.69de	107.16 ± 3.01f
(4) 50%, 400 rpm, 0 h	33.15 ± 0.31g	65.93 ± 1.99ef	99.09 ± 2.30g
(5) 20%, 200 rpm, 24 h	54.53 ± 0.66cd	76.80 ± 0.61bc	131.33 ± 0.06d
(6) 20%, 400 rpm, 24 h	63.48 ± 1.84b	85.67 ± 3.22a	149.15 ± 1.38b
(7) 50%, 200 rpm, 24 h	44.98 ± 1.19e	70.08 ± 0.09de	115.06 ± 1.10e
(8) 50%, 400 rpm, 24 h	38.93 ± 0.61f	69.43 ± 1.22de	108.36 ± 1.82f
Analyses of variance	<i>p</i> -Value	<i>p</i> -Value	<i>p</i> -Value
Feed moisture (FM)	0.000	0.000	0.000
Screw speed (SS)	0.702	0.007	0.003
FM × SS	0.000	0.001	0.000
Block	0.000	0.017	0.000
R ² (%)	97.60	92.90	98.80

Different letters (a–g) within the same column as compared to the native flour differ significantly (*p* < 0.05).

Values are means of triplicate ± standard deviation.

^a Feed moisture (%), screw speed (rpm), stored at 4 °C for 0 or 24 h.**Table 5**Antioxidant activities (FRAP, ABTS,⁺ and DPPH) of free, bound and total phenolics of extruded and native samples from green banana flour stored at 4 °C for 0 and 24 h.

Treatment	FRAP (mmol Fe (II)/100 g db)			ABTS, ⁺ (mmol TEAC/100 g db)			DPPH (mmol TEAC/100 g db)		
	Free	Bound	Total	Free	Bound	Total	Free	Bound	Total
Native flour	5.44 ± 0.17a	1.12 ± 0.21d	6.56 ± 0.03a	2.99 ± 1.00a	0.42 ± 0.09a	3.41 ± 1.09a	2.16 ± 0.08a	0.32 ± 0.03a	2.48 ± 0.03a
<i>Extruded flour^a</i>									
(1) 20%, 200 rpm, 0 h	1.29 ± 0.27cd	1.36 ± 0.01abc	2.65 ± 0.18de	0.63 ± 0.06b	0.52 ± 0.18a	1.15 ± 0.23b	0.94 ± 0.13c	0.34 ± 0.02a	1.28 ± 0.07c
(2) 20%, 400 rpm, 0 h	1.91 ± 0.67bc	1.49 ± 0.06ab	3.40 ± 0.43bc	0.89 ± 0.29b	0.55 ± 0.06a	1.44 ± 0.36b	1.27 ± 0.17b	0.34 ± 0.00a	1.62 ± 0.11b
(3) 50%, 200 rpm, 0 h	0.63 ± 0.02de	1.34 ± 0.06bc	1.97 ± 0.03ef	0.25 ± 0.01b	0.47 ± 0.01a	0.71 ± 0.01b	0.55 ± 0.00d	0.33 ± 0.01a	0.89 ± 0.01d
(4) 50%, 400 rpm, 0 h	0.52 ± 0.02e	1.28 ± 0.03cd	1.80 ± 0.01f	0.18 ± 0.01b	0.44 ± 0.00a	0.62 ± 0.01b	0.55 ± 0.00d	0.33 ± 0.00a	0.88 ± 0.00d
(5) 20%, 200 rpm, 24 h	1.32 ± 0.20cd	1.44 ± 0.01abc	2.75 ± 0.13cd	0.63 ± 0.09b	0.60 ± 0.01a	1.23 ± 0.07b	0.95 ± 0.11c	0.35 ± 0.01a	1.29 ± 0.17c
(6) 20%, 400 rpm, 24 h	2.05 ± 0.47b	1.54 ± 0.03a	3.59 ± 0.31b	1.00 ± 0.31b	0.55 ± 0.03a	1.55 ± 0.28b	1.33 ± 0.24b	0.36 ± 0.00a	1.69 ± 0.17b
(7) 50%, 200 rpm, 24 h	0.69 ± 0.05de	1.41 ± 0.03abc	2.10 ± 0.01def	0.29 ± 0.05b	0.54 ± 0.03a	0.83 ± 0.02b	0.60 ± 0.04d	0.34 ± 0.00a	0.94 ± 0.03d
(8) 50%, 400 rpm, 24 h	0.64 ± 0.04de	1.36 ± 0.01abc	1.99 ± 0.02ef	0.22 ± 0.15b	0.53 ± 0.09a	0.74 ± 0.24b	0.58 ± 0.04d	0.34 ± 0.01a	0.92 ± 0.02d
Analyses of variance	<i>p</i> -Value	<i>p</i> -Value	<i>p</i> -Value	<i>p</i> -Value	<i>p</i> -Value	<i>p</i> -Value	<i>p</i> -Value	<i>p</i> -Value	<i>p</i> -Value
Feed moisture (FM)	0.000	0.000	0.000	0.000	0.090	0.000	0.000	0.087	0.000
Screw speed (SS)	0.048	0.073	0.038	0.116	0.613	0.250	0.007	0.648	0.008
FM × SS	0.017	0.000	0.007	0.021	0.942	0.046	0.004	0.368	0.004
Block	0.532	0.001	0.289	0.522	0.102	0.240	0.512	0.087	0.424
R ² (%)	86.70	90.50	88.30	86.40	38.60	84.20	92.60	42.50	92.20

Different (a–f) within the same column as compared to the native flour differ significantly (*p* < 0.05).

Values are means of triplicate ± standard deviation.

^a Feed moisture (%), screw speed (rpm), stored at 4 °C for 0 or 24 h.**Table 6**Correlation coefficients amongst TPC and their antioxidant activities (FRAP, ABTS,⁺, DPPH) in free, bound and total phenolics.

	TPC	FRAP			ABTS, ⁺			DPPH					
		Free	Bound	Total	Free	Bound	Total	Free	Bound	Total			
TPC	Free	1	0.898**	0.983**	0.921**	0.872**	0.933**	0.927**	0.552*	0.938**	0.931**	0.610*	0.934**
	Bound		1	0.963**	0.827**	0.830**	0.844**	0.827**	0.448	0.827**	0.873**	0.619*	0.876**
	Total			1	0.905**	0.877**	0.920**	0.909**	0.523*	0.916**	0.931**	0.629**	0.934**

* Significant at *p* < 0.05.** Significant at *p* < 0.01.

significant increase in the antioxidant activity of free phenolics compared to a small increase in the bound phenolics. At 50% FM there was no significant influence of SS. The increase in antioxidant activity with increased SS was probably due to the shorter residence times at higher SS.

Compared to native flours, extrusion cooking led to a significant decrease in total antioxidant capacities, mainly due to a significant decrease in the antioxidant activities of the free phenolics, whereas the antioxidant activities of the bound phenolics was not or only slightly increased. The antioxidant capacities in free, bound, and

total phenolics showed a significant positive correlation with the content of free, bound, and total phenolics measuring by FRAP, ABTS,⁺ and DPPH (Table 6).

Although all extruded samples have lower amounts of free phenolics than bound phenolics, these free phenolics showed higher antioxidant capacities, which is similar to buckwheat flours extracts (Hung & Morita, 2008).

4. Conclusions

The interaction between FM and SS significantly affected the amylose content, WSI, pasting properties, TPC and their antioxidant activities (FRAP, ABTS,⁺, DPPH) in free and bound phenolics in the extruded banana flours. Storage condition was also an important factor to improve the final extruded flours properties, amylose content was significantly increased, as well as RS, and content of free, bound and total phenolics. Regarding the samples extruded at low FM, although they were low in RS, WAI, pasting properties, the high TDF, WSI, TPC and their antioxidant activities (FRAP, ABTS,⁺, DPPH) in free and bound phenolics make them of interest in utilising as functional ingredients in a variety of cereal foods in the future.

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