

Nutritional and functional properties of extruded cassava-soy composite with grape pomace

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Abbreviations:

RDS Rapidly digestible starch

SDS Slowly digestible starch

IVSD *In-vitro* starch digestibility

EGI Estimated glyceic index

IDF Insoluble dietary fibre

SDF Soluble dietary fibre

FTIR-ATR Fourier transform infrared-attenuated total reflectance

Keywords: Dietary fibre, enzymatic inhibition, extrusion cooking, phenolic compounds, starch digestibility

Abstract

Cassava-soy composite is extruded with grape pomace at 0, 10 and 20% addition levels. Some nutritional, functional, rheological properties, total phenolic contents and antioxidant activity of the extruded products are analyzed. Kinetics of starch digestibility show that extrusion cooking leads to significant ($p < 0.05$) increase in the rate of starch digested but the addition of grape pomace lowers the rate of starch digestibility and estimated glycaemic index. The water absorption capacity of composites decreases while the solubility index increases with increasing addition of grape pomace. FTIR spectra show an increase in β -sheets formation as the addition of grape pomace increases. The composite with 20% grape pomace has the lowest viscosity and all composites exhibit a shear thinning behavior. Extrusion cooking probably leads to depolymerization of dietary fibre and redistribution of insoluble dietary fibre to soluble dietary fibre ratio. The total phenolic content and antioxidant activity of cassava-soy composite are significantly ($p < 0.05$) increased with the addition of grape pomace. The results indicate that grape pomace can be added to starch-rich foods with extrusion cooking to produce instant products with potential health promoting properties.

1 Introduction

Grape pomace is a by-product of wine and grape juice production. It is estimated that more than half of grapes cultivated worldwide are used in the wine industry [1] leaving behind a vast proportion of waste [2]. Grape pomace is mostly used in animal feed [4] and as fertilizer [5]. It is characterized by having a high dietary fibre content (Kumar *et al.* 2010) and phenolic compounds [6]. The red grape pomace contains between 51.09 – 56.31% total dietary fibre [2]. The phenolic compounds are mainly condensed tannins, anthocyanins and resveratrol, and they are known to possess antioxidant and radical scavenging properties [7]. The dietary fibre components of grape pomace are pectin, cellulose and hemicelluloses [8].

Dietary fibre and phenolic compounds have been proven to be of immense health benefits [9]. The benefits include their ability to influence carbohydrate metabolism [9]. Dietary fibre is classified into soluble and insoluble dietary fibre. The soluble dietary fibre is mostly associated with the ability to influence post-prandial glucose response due to its viscous nature by slowing down gastric emptying [10], lower rate of starch hydrolysis [11], slow down diffusion of starch hydrolysis products to the intestinal gastric mucosa and reduction in glucose absorption [12]. Insoluble dietary fibre adds bulk to diet and reduces faecal transit time [13].

Several authors have reported that phenolic compounds can influence carbohydrate hydrolysis [14, 15]. A variety of phenolic compounds have been shown to inhibit the activities of α -amylase and/or α -glucosidase. The inhibitory phenolic compounds include flavonoids (flavanones, anthocyanins, flavanols, isoflavones, and flavones), phenolic acids and tannins (proanthocyanidins and ellagitannins). Phenolic compounds are postulated to bind to active or

secondary sites of digestive enzymes [16] and/or bind to substrate thus reducing starch hydrolysis [17].

Extrusion cooking is used to better utilize food by-products [18] and produce dietary fibre enriched foods [19]. Extrusion cooking is an efficient and versatile processing technique which combines high temperature and pressure within a short time to cook food materials [20]. Extrusion cooking is advantageous because of its ability to blend varieties of ingredients to produce a wide range of food products with improved nutritional qualities, many of which cannot be produced easily by any other process [21]. Food ingredients and their components undergo several transformations during extrusion cooking giving rise to extrudates with improved functionalities. The changes in the functional properties are related to the nutritional properties of extrudates [22].

There have been studies carried out on improving the nutritional properties of extrudates by addition of fruit by-products. Fruits blend waste (orange peel, grape seed and tomato pomace) was extruded with rice grits by Yağcı and Göğüş [23]. The authors reported that starch digestibility decreased with increasing addition of the fruit blend waste. This was attributed to the presence of non-starch polysaccharides in the fruit blend waste, that limited contact between digestive enzyme and starch. In another study, Dehghan- Shoar *et al.* [24] extruded tomato waste (tomato skin, seed and paste) and corn grits. The authors reported a decrease in starch digestibility of extrudates with the highest ratio of tomato waste. It was suggested that this might be due to complex formation between starch and pectin, and the starch-pectin complex might have limited accessibility of α -amylase to starch for digestion.

This study is a continuity of work done by Muoki *et al.* [25] on extruded cassava-soy composite porridge. This product has been introduced in some communities in northern Mozambique, but we are unaware if it is commercially available. The effect of added functional ingredients on nutritional and quality parameters of extrudates is important in order to create a platform for developing new food products from food by-products. The objective of this study was therefore to investigate the effects of extrusion cooking and grape pomace addition at 0, 10 and 20% level on some of the nutritional, functional, and potential health promoting properties of cassava-soy composite.

2 Materials and methods

2.1 Raw materials

High quality cassava flour produced according to FAO method [26] (fresh cassava roots were peeled, washed, grated, pressed, disintegrated, dried, milled, sifted, and the flour was bagged) with particle size of $\leq 250 \mu\text{m}$ was purchased from Thai Farm International (Ogun State, Nigeria). The flour contained 84.4% starch, 9.8% moisture, pH of 5.7 and 0.5% crude fibre. Toasted defatted soy flour with particle size of $\leq 212 \mu\text{m}$ was purchased from Petrow foods (Johannesburg, South Africa). Grape pomace was kindly donated by Brenn-O-Kem (Pty) Ltd (Wolseley, South Africa).

2.2 Extrusion cooking

Cassava and defatted toasted soy were uniformly mixed in a ratio of 65 to 35% (w/w db) respectively. Grape pomace was added at 0, 10 and 20% (w/w) levels and mixed thoroughly with the cassava and soy composite. A co-rotating twin screw extruder (TX 32, CFAM, Potchefstroom, South Africa) with five heating zones set at 60/80/100/140/140 °C respectively

was used. Moisture dosing rate of 3 l/h and feed rate of 25 kg/h was used with a die opening of 3 mm. The screw speed was maintained at 200 rpm. Extrudates were immediately dried in an oven at 90 °C for 5 min and ground with an analytical mill (A11, IKA, Staufen, Germany). The ground extruded products were kept in plastic sample bottles and refrigerated at 4 °C in preparation for analysis.

2.3 Physico-chemical characteristics

Twenty extrudates from each treatment were measured for expansion ratio. This was determined as the diameter of extrudates divided by the diameter of the die exit (3 mm) [27]. The Bulk density (g/cm^3) of the extrudate was expressed as the weight of the extrudate divided by its volume [28].

Proximate composition (ash and crude fat) were determined according to the AOAC methods 942.05 and 920.39A [29] respectively. Dumas combustion according to the AACC International crude protein combustion method 46 – 30 [30] was used to determine protein ($\text{N} \times 6.25$). The method described by McCleary, Solah and Gibson [31] was used to determine total starch. Thermostable α -amylase (CAS 9000-90-2) and amyloglucosidase (CAS 9032-08-0) from Megazyme (K-TSTA 07/11) (Megazyme International, Bray, Ireland) were used to hydrolyse starch to glucose. Glucose was then quantified colorimetrically by glucose oxidase-peroxidase reaction and the absorbance was read at 510 nm. Total starch was expressed as starch proportion (%) of total sample weight and correction was made for moisture to determine starch on a dry weight basis.

2.4 Water absorption capacity and Water solubility index

Water absorption and solubility index were determined according to the method by Gujral and Singh [32]. Raw and extruded flours (2.5 g) were dispersed in 30 mL of distilled water at 30 °C for 30 min in a shaking water bath, and the mixture was vortexed every 5 min. The sample solution was then centrifuged at 3169 x g for 15 min (Hermle Z366K, Wehingen, Germany) and the supernatant was decanted into an aluminium pan of known weight. Water absorption capacity was recorded as weight of pellet (g) obtained per gram of dry ground sample. The amount of dry solids recovered after evaporating the supernatant in an oven at 100 °C overnight was expressed as percentage dry solid in the 2.5 g sample and defined as water solubility index.

2.5 Nitrogen solubility index

Nitrogen solubility index was determined according to the AACC Method 46-23, [30] with modification. About 1g flour samples were dispersed in 20 mL of 0.1M NaCl solution at pH 7 and stirred continuously for 1 h at 30 °C. The suspension was centrifuged (9154.3 x g, 15min, 4°C) and the supernatant filtered through a Whatman No. 1 filter paper. The residue was re-washed twice in 10mL of 0.1M NaCl solution at pH 7. The filtrate was frozen (-18 °C) overnight and freeze-dried with a freeze dryer (13KL, Instruvac Lyophilizer, Midrand, South Africa) for 4 days. The nitrogen content of the freeze-dried sample was determined using a Dumatherm (DT, Gerhardt Königswinter, Germany). Nitrogen solubility index was expressed as a percentage of the total nitrogen content of freeze-dried sample divided by total nitrogen content in flour sample on a dry basis

2.6 In-vitro protein digestibility

A multi-enzyme method according to Vilakati *et al.* [33] was used to determine the *in-vitro* protein digestibility of raw and extruded samples. Five (5) mL of multi-enzyme solution containing 1.6 mg trypsin (14,600 U/mg), 3.1 mg α -chymotrypsin (48 U/mg) and 1.3 mg peptidase (102 U/g) per mL kept in ice was added to sample suspension containing 6.25 mg protein/mL with pH adjusted to pH 8.0 with 0.1M NaOH and incubated at 37 °C. The solution was maintained at this temperature and stirred continuously while the pH drop in the suspension was recorded over 10 min at 1 min interval. The percentage *in-vitro* protein digestibility was calculated using the linear regression equation below;

$$Y = 210.46 - 18.10X \quad (i)$$

Where Y is the percentage *in-vitro* protein digestibility and X is the pH of sample suspension after 10 min hydrolysis [32].

2.7 FTIR-ATR Spectroscopy

Protein secondary structure of samples (raw and extruded) was determined by FTIR spectroscopy as described by Anyango *et al.* [34]. Samples were stored in a desiccator containing silica gel for 72 h at ambient temperature to ensure minimal moisture content before spectroscopic analysis. Absorbance spectra were recorded using a Perkin Elmer Spectrum 100 with a universal attenuated total reflectance accessory (Perkin Elmer, Connecticut, USA). Samples were scanned over a frequency range of 4000 cm^{-1} to 600 cm^{-1} and averaged from a total of 32 scans with a resolution of 8 cm^{-1} . Spectra measurements were corrected for background. The FTIR-ATR spectra were deconvoluted using Lorentzian filter with a resolution enhancement factor of 2 and a bandwidth of 8 cm^{-1} . A rubber band correction of 64 baseline

points was used for correcting absorbance spectra. The relative proportions of α -helical conformations and the β -conformation were determined using the equation below. The α/β ratio were calculated:

$$\% \alpha\text{-helical conformation} = \frac{\text{Abs } \alpha\text{-helix peak}}{\text{Abs } \alpha\text{-helix} + \beta\text{-sheet peak}} \times 100\% \quad (\text{ii})$$

$$\% \beta\text{-sheet conformation} = \frac{\text{Abs } \beta\text{-sheet peak}}{\text{Abs } \alpha\text{-helical peak} + \text{Abs } \beta\text{-sheet peak}} \times 100\% \quad (\text{iii})$$

$$\alpha:\beta \text{ ratio} = \frac{\% \alpha\text{-helical conformation}}{\% \beta\text{-sheet conformation}} \quad (\text{iv})$$

Where:

Abs α -helix peak = absorbance at $\approx 1647 \text{ cm}^{-1}$ after baseline correction [34].

Abs β -sheet peak = Absorbance at $\approx 1620 \text{ cm}^{-1}$ after baseline correction [34].

2.8 In-vitro kinetics of starch digestibility (IVSD)

The method according to Goñi *et al.* [35] was used with slight modification. A sample (raw and extruded) containing 50 mg starch was used per assay and 1 mL of boiling water was added to each sample for easy dispersion before 10 mL of HCl-KCl buffer (pH 1.5, 0.05 M) and 0.2 mL of solution containing 1 mg of pepsin from porcine gastric mucosa (Sigma Aldrich P7000-100G) were added followed by incubation at 40 °C for 60 min with constant agitation. Ten mL of tris-maleate (0.05 M) at pH 6.9 was added and pH was adjusted with 1 M NaOH to 6.9. The volume was made up to 25 mL with tris-maleate buffer and the 0 min aliquot of 0.1 mL was taken before the addition of 5 mL tris-maleate buffer (pH 6.9) containing 2.6IU of pancreatic α -amylase with activity of 19.6 units/mg (Sigma-Aldrich A-3176) followed by incubation at 37 °C with constant

shaking. Aliquots of 0.1 mL was taken at 5 min and then at intervals of 30 min until 3 h. The tubes containing the aliquots taken were placed in boiling water for 15 min to inactivate α -amylase. Then, 1 mL of 0.4 M sodium-acetate buffer (pH 4.75) and 90 μ L of amylogucosidase (*Aspergillus niger*) with an activity of 64.7 U/mg (EC 3.2.1.3, CAS 9032-08-0) was added and incubated at 6 0°C for 45 min. Glucose concentration was measured using glucose oxidase-peroxidase kit and the rate of in-vitro starch digestion was expressed as the percentage of the total starch digested at time intervals (0, 5, 30, 60, 90, 120 and 180 min).

2.9 Estimated glycemic index

The first order equation proposed by Goñi *et al.* [35] was used to describe the kinetics of starch hydrolysis:

$$C = C_{\infty}(1 - e^{-kt}) \quad (\text{v})$$

Where C is concentration of starch hydrolysed at time t, C_{∞} is the percentage (%) of starch hydrolyzed after 180 min, k is digestibility rate constant (min^{-1}) and t is time (min). The parameters K and C_{∞} were estimated for each treatment based on the data obtained from the *in-vitro* hydrolysis procedure. The equation by Jaisut *et al.* [36] was used to calculate the area under curve (AUC):

$$AUC = C_{\infty} (t_f - t_0) - \left(\frac{C_{\infty}}{K}\right) \left(1 - \exp\left(-k(t_f - t_0)\right)\right) \quad (\text{vi})$$

Where t_f is the final time (180 min), t_0 is the initial time (time 0). The hydrolysis index (HI) was defined as the area under the hydrolysis curve of sample divided by the corresponding area of white bread. Glycaemic index was then estimated using the equation according to Goñi *et al.* [35]:

$$EGI = 39.71 + 0.549HI \quad (\text{vii})$$

Estimated glyceic load = estimated glyceic index/100 x Available carbohydrate

2.10 Nutritionally important starch fractions

The enzymatic hydrolysis method of Goñi *et al.* [35] was used to obtain the rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) fractions. The RDS was defined as the percentage of starch digested at 30 min, the SDS as the percentage of starch digested at 120 min and the RS was defined as the sum of RDS and SDS subtracted from the total starch.

2.11 Soluble and insoluble dietary fibre determination

This was determined according to AOAC 991.43 [37] method using the total dietary fibre megazyme kit (K-TDFR). A sample of 1 g was dissolved in 40 mL of mes-tris (pH 8.2, 0.05 M) buffer solution and thermostable α -amylase (*Bacillus licheniformis*) with an activity of 3,000 U/ml (EC 3.2.1.1, CAS 9000-90-2) was added to hydrolyze starch to dextrans at 100 °C. Protease (Subtilisin A from *Bacillus licheniformis*) with an activity of 350 tyrosine U/ml (EC 3.4.21.62, CAS 9014-01-1) was used to solubilize protein. Amylogucosidase (*Aspergillus niger*) with an activity of 3,300 U/ml (EC 3.2.1.3, CAS 9032-08-0) was used to hydrolyze starch fragments to glucose. The sample and enzyme mixture were filtered, and the residue was washed with ethanol and acetone to obtain the insoluble dietary fibre (IDF) portion. Four volumes of ethanol heated to 60 °C was added to the filtrate to precipitate the SDF and was left to stand for 1 h after which it was filtered. The soluble dietary fibre (SDF) residues were washed with 78% and 95% (v/v) of ethanol followed by washing with acetone. The IDF and SDF residues were dried overnight at

100 °C. The SDF and IDF residues were corrected for protein and ash for the final calculation of SDF and IDF values.

2.12 Dynamic Viscosity

The method according to D'Silva, Taylor and Emmambux, [38] was used to determine flow properties with slight modification. Flow properties were measured with a Physica MCR 101 Rheometer with Rheoplus software[®], (Anton Paar, Ostfildern, Germany). Flour slurry containing 10% solid (w/v) was prepared and held at 50 °C for 5 min to equilibrate and the slurry was transferred into the rheometer cup and maintained at 50 °C. The sample was stirred with a vane over a shear rate range of 0.01 to 1000 s⁻¹ and the shear stress was determined. To describe the time independent flow behavior, the experimental data (shear stress- shear rate) were fitted by Power law model;

$$\tau = K\dot{\gamma}^n \quad \text{(viii)}$$

Where, τ is shear stress (Pa), $\dot{\gamma}$ is the shear rate (s⁻¹), K is the consistency co-efficient (Pa.sⁿ) and n is the flow behavior index.

2.13 Particle size determination by wet sieving

Ten % (w/v) sample suspension was prepared with distilled water and passed through a stack of sieves with aperture sizes (500, 212, 180, 75, 38 μm). The residue retained on each sieve was dried at 60 °C to constant weight in an oven. The dried residue was expressed as percentage mass retained and the smallest fraction was calculated as difference between initial sample weight and sum of residues retained in sieves.

2.14 Preparation of extracts for total phenolic content and antioxidant capacity assays

The method described by Kayitesi [39] was used to prepare extracts from raw and extruded cassava-soy composite substituted with 0, 10 and 20% grape pomace using acidified methanol (1% conc. HCl in methanol) as solvent. Each sample (3 g) was extracted with 30 ml of solvent in three steps; 10 ml of solvent was initially added to 3 g of sample in a conical flask, stirred for 3 h to allow diffusion of phenolics from cellular matrix after which they were centrifuged at 1917 $\times g$ (Hermle Z366K, Wehingen, Germany) for 10 min at ambient temperature (25 °C). The supernatant was decanted and the residue was rinsed with 10 ml of solvent, stirred for 20 min and centrifuged using conditions described earlier. The supernatant was decanted and the rinsing was repeated. The supernatants recovered were stored in an air tight glass bottle covered with aluminum foil and stored in the cold room at -20 °C within 1 week of analysis.

2.15 Total phenolics determination

The total phenolics content of extracts of cassava-soy composite substituted with and without grape pomace was determined spectrophotometrically using the Folin Ciocalteu procedure modified for 96-well microplate described by Apea-Bah *et al.* [40]. Extracts or catechin standard were dissolved in 1% conc. HCl in methanol. About 18.2 μL was added to the microplate, after which 36.4 μL of 10% (v/v) Folin-Ciocalteu reagent in water was added. Thereafter, 145.4 μL of 700 mM sodium carbonate was added and the plate was incubated for 2 h in the dark at 25 °C. A standard catechin calibration curve was obtained by using concentrations of 0.1 to 0.5 mg/mL in acidified methanol. The absorbance of the extracts and catechin standards were read at 750 nm using a microplate reader (Multiskan FC, Thermo Fisher Scientific, Shanghai, China) and the results were expressed as mg catechin equivalents (CE)/g sample on dry weight basis.

2.16 Determination of antioxidant capacity

The antioxidant capacity (radical scavenging capacity) of extracts was determined using the 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS⁺) assay. The ABTS⁺ radical scavenging activity of the extracts was determined according to the modified method of Appea-Bah et al. [40]. A working solution was prepared from the addition of 58 mL phosphate buffer at pH 7.4 to 2 mL of ABTS mother solution (prepared by adding equal volumes of 3 mM potassium persulphate and 8 mM ABTS salt and left in the dark for 16 h). Working solution (190 μ L) was added to 10 μ L of the extract in a 96-well microplate and the mixture was incubated in the dark for 30 min at ambient temperature (25 °C). Absorbance was read at 750 nm. Trolox was used as the standard and the results were expressed as micromole Trolox equivalent per gram (μ mol TE/g) on dry weight basis.

2.17 Statistical Analysis

The main effects of treatment (extrusion cooking and grape pomace addition level 0, 10 and 20%) on dependent variables were calculated by two-way analysis of variance (ANOVA) using SPSS software version 20 (SPSS Inc., Armonk, NY). Means were compared using Fisher's least significant test (LSD) at 5% level of significance. The results of analysis were reported as mean obtained from triplicate experiments \pm standard deviation.

3 Results and discussion

The effects of grape pomace addition and extrusion cooking on physico-chemical and functional properties of cassava-soy composite are shown in Table 1. The addition of grape pomace to cassava-soy composite led to a decrease in starch and crude protein content. This is expected as

Table 1: Effects of extrusion cooking and grape pomace addition on some chemical composition, physical and functional properties of cassava-soy composite

| Treatment | Grape pomace addition (%) | Moisture (%) | Crude fat (%) | Protein (%) | Crude fibre (%) | Ash (%) | Starch (%) | Bulk density | Expansion ratio | WAI ² (g/g) | WSI ³ (%) |
|------------------|---------------------------|------------------------|------------------------|-------------------------|-------------------------|------------------------|-------------------------|--------------------------|------------------------|--------------------------|-------------------------|
| Raw | 0 | 8.3 ^d ± 0.1 | 1.0 ^d ± 0.0 | 18.4 ^c ± 0.2 | 4.0 ^a ± 0.1 | 2.1 ^a ± 0.0 | 53.9 ^b ± 0.5 | ND ¹ | ND | 1.77 ^a ± 0.04 | 7.9 ^a ± 0.1 |
| | 10 | 8.6 ^e ± 0.1 | 1.8 ^e ± 0.0 | 17.4 ^a ± 0.2 | 8.8 ^b ± 0.1 | 3.2 ^c ± 0.1 | 47.2 ^c ± 0.4 | ND | ND | 1.78 ^a ± 0.02 | 9.7 ^b ± 0.3 |
| | 20 | 8.1 ^c ± 0.1 | 2.1 ^f ± 0.0 | 17.0 ^a ± 0.1 | 11.6 ^c ± 0.0 | 4.1 ^c ± 0.1 | 43.0 ^a ± 0.2 | ND | ND | 1.80 ^a ± 0.02 | 10.6 ^b ± 0.1 |
| Extrusion cooked | 0 | 5.8 ^b ± 0.0 | 0.3 ^a ± 0.0 | 19.4 ^d ± 0.1 | 4.0 ^a ± 0.0 | 2.4 ^b ± 0.0 | 54.7 ^b ± 0.3 | 0.26 ^a ± 0.01 | 3.7 ^c ± 0.2 | 2.44 ^d ± 0.01 | 45.7 ^c ± 0.8 |
| | 10 | 4.2 ^a ± 0.1 | 0.4 ± 0.0 | 17.9 ^b ± 0.1 | 8.8 ^b ± 0.1 | 3.4 ^d ± 0.1 | 47.9 ^c ± 0.4 | 0.31 ^b ± 0.00 | 2.8 ^b ± 0.0 | 2.21 ^c ± 0.01 | 47.6 ^d ± 0.3 |
| | 20 | 4.1 ^a ± 0.0 | 0.6 ^c ± 0.0 | 17.6 ^b ± 0.1 | 11.4 ^c ± 0.1 | 4.2 ^e ± 0.0 | 43.8 ^a ± 0.6 | 0.34 ^c ± 0.01 | 2.5 ^a ± 0.1 | 2.05 ^b ± 0.02 | 48.1 ^d ± 0.1 |

Values are means ± standard deviations of 3 independent experiments. Values within the same column followed by different letters are significantly different (p<0.05).

¹ Not determined in raw composites

²Water absorption index.

³Water solubility index

starch and protein content in grape pomace is low. Crude fat and ash content of cassava-soy composite increased with increase in level of grape pomace addition. Red grape pomace has been reported to contain higher ash and fat content [41], hence the increase in fat and ash content as level of addition of grape pomace to cassava-soy composite increased. After extrusion cooking, crude fat was decreased, and ash content was significantly ($p<0.05$) increased. There was no significant interaction between grape pomace addition level and treatment on starch ($p<0.80$), protein ($p<0.06$) and crude fibre content ($p<0.84$) while a significant interaction effect was observed for crude fat ($p<0.00$) and ash ($p<0.01$). This indicates that the effect of grape pomace addition on crude fat and ash was different for raw and extruded composites.

The variations in addition level of grape pomace had a significant ($p<0.05$) effect on bulk density and expansion ratio of the cassava-soy composite. It was observed that as the level of addition of grape pomace increased from 0 to 20%, the bulk density of extrudates increased and the expansion ratio decreased. Altan *et al.* [6] also reported an increase in bulk density and decrease in expansion ratio when grape pomace addition level to barley increased during extrusion cooking. This was associated with increasing fibre content in the food ingredient mix and sugar present in grape pomace which may have lowered melt temperature and thus reduce the vapor pressure of water. The dilution of starch as grape pomace level increased could influence the macro-structure of extrudates by causing a reduction in the amount of extensible polymers in the bubble cell walls [42].

Bulk density is an important parameter in the production of expanded and formed foods and it is closely associated with expansion ratio [43]. The presence of inert materials such as dietary fibre are reported to disrupt the stretching and setting of bubble films [44]. Fibre particles could hinder

the growth of air cells by puncturing them before they reach their maximum expansion limits [45] and this leads to formation of extrudates with a dense structure and smaller air cells [46].

There was a significant main effect of grape pomace addition on water absorption (WAI) and solubility index (WSI). Extrusion cooking significantly ($p < 0.01$) increased the WAI and WSI of extrudates (Table 1). The increase was probably due to starch gelatinization and depolymerization which led to an increase in availability of hydrophilic groups in the food system [47]. WAI decreased significantly ($p < 0.001$) as the addition of grape pomace in cassava-soy composite increased in extrudates (Table 1).

The WSI values of extruded composites were between 45.7 to 48.1% and the mean values showed that WSI increased as grape pomace addition level increased from 0 to 20% (Table 1). A similar trend in results was reported by Altan *et al.* [42] in the study on incorporation of tomato or grape pomace to barley flour. The decrease in WAI as by-products (tomato and grape pomace) increased was attributed to the induced stress during extrusion cooking which may have opened the structure of fibre thus allowing more water to enter into contact with hydrophilic groups [42]. Depolymerization and dextrinization of starch which occurs during extrusion cooking may also have contributed to the increase in WAI after extrusion cooking [48].

The increase observed in the WSI as grape pomace addition increased could be related to fibre modification during extrusion cooking as a result of high shear which led to the release of low molecular weight compounds [44]. Similarly, Larrea *et al.* [49] reported an increase in WSI of extruded orange pulp and wheat, and this increase was largely attributed to degradation of components of fibre during extrusion cooking. The highest value of WSI was found at the highest level (20%) of grape pomace addition to cassava-soy composite (48.1%). This result may

be related to availability of more sugar in the system due to sugar content of grape pomace in addition to the fibre content. Onyango *et al.* [50] suggested that an increase in WSI with increasing sugar concentration was a sign of increased solubilization of starch.

The addition of grape pomace to cassava-soy composite lowered the NSI and IVPD of cassava-soy composite (Table 2). Extrusion cooking also slightly decreased IVPD of extruded composites with grape pomace but there was no significant ($p>0.05$) difference in IVPD of composite without grape pomace before and after extrusion cooking. The NSI of composites was significantly ($p<0.05$) lowered after extrusion cooking. There was significant interaction between grape pomace addition level and treatment on IVPD and nitrogen solubility ($p<0.02$, $p<0.00$ respectively). This means that the effect of addition of grape pomace at different level on IVPD and NSI was different for raw and extruded cassava-soy composites. Nitrogen solubility can be related to protein digestibility as an increase in soluble nitrogen is often accompanied by an increase in protein digestibility. The high temperature used during extrusion cooking (140 °C) may promote unfolding of protein structure. The unfolded proteins can interact to form cross-links through formation of covalent inter-molecular disulphide bonds [51]. Cross-linking of proteins may prevent enzyme access to peptide bonds by blocking sites of enzyme attack [52].

The polyphenols present in grape pomace may also have contributed to the reduction observed in IVPD and NSI as polyphenols are known to interact with proteins to form indigestible complexes which are less susceptible to proteolytic digestion [53]. Arimboor and Arumugan, [54] similarly reported that interactions of sea buckthorn polyphenols with proteins led to decrease in the *in-vitro* protein digestibility of sea buckthorn. Also, Labuckas *et al.* [55] found that the presence of polyphenols decreases the protein solubility of walnut flour.

Hydrogen bonding and hydrophobic interaction are reported to be the primary attractive forces between protein molecule and phenolic group [56]. The aromatic nuclei and hydroxyl group of the aromatic ring of the phenolic compounds provide the principal binding sites for protein-phenolic complexation [57]. It has been suggested that hydrogen bonding between the phenolic hydroxyl group and the NH- and CO- groups of proteins are involved in protein-phenolics interaction [58]. These interactions result in formation of insoluble complexes which ultimately reduces the digestibility of proteins. Phenolic compounds may also directly bind to proteolytic enzymes. Covalent attachment between phenolics and the reactive nucleophilic sites in the enzyme have been reported to result in the inhibition of actions of proteolytic enzymes [59] and this results in decrease in *in-vitro* protein digestibility.

The ATR-FTIR deconvoluted spectra of raw and extruded composites of cassava-soy composite with 0, 10 and 20% grape pomace were determined to examine the effect of grape pomace addition and extrusion cooking on the protein secondary structure of cassava-soy composites (Figure 1). The amide I band was observed at 1627 – 1637 for the raw composites and at 1620 to 1647 cm^{-1} for the extruded composites. The amide I band which appears between 1680 and 1600 cm^{-1} primarily results from CN stretching, CCN deformation, and in-plane NH bending modes of groups in the polypeptide chain [60, 61]. The amide II vibrations are reported to be an out of phase combination of mainly the NH bending vibrations and CN stretch [62] and they were observed between 1550 and 1500 cm^{-1} [61].

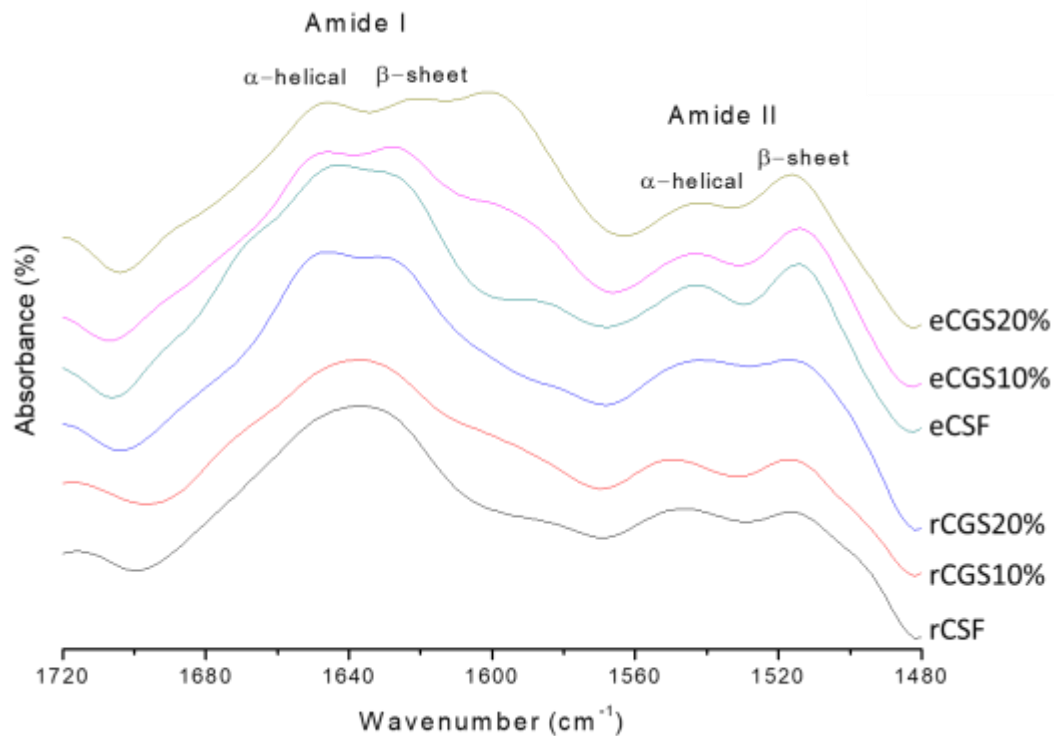


Figure 1: FTIR spectra of raw and extruded cassava-soy composite with and without grape pomace. rCSF is raw cassava-soy flour, rCGS10% is raw cassava-soy flour with 10% grape pomace, rCGS20% is raw cassava-soy flour with 20% grape pomace, eCSF is extruded cassava-soy flour, eCGS10% is extruded cassava-soy flour with 10% grape pomace, eCGSS20% is extruded cassava-soy flour with 20% grape pomace.

There were no clear peaks of α -helical conformation in the raw composite without grape pomace and that which contained 10% grape pomace. Only the raw composite which contained 20% grape pomace showed a visible peak at 1647 cm^{-1} and this is associated with the α -helix of the protein secondary structure. Due to the absence of clear peaks showing the α -helical conformation in the amide I region of the raw composites, the ratio of α/β conformation could not be determined in these composites. The extruded composites on the other hand all showed visible peaks of α -helical and β -sheet conformation both in the amide I and amide II regions (Table 2). There was a significant decrease in α -helix to β -sheet ratio from 1.04 to 0.95 in the amide I region and from 0.84 to 0.77 in the amide II region of the extruded composites as the

Table 2. Effects of extrusion cooking and grape pomace addition on the *in-vitro* protein digestibility, nitrogen solubility, protein secondary structure, soluble and insoluble dietary fibre content of cassava-soy composite.

| Treatment | Grape pomace addition (%) | IVPD (%) | NSI (%) | Amide I | | Amide II | | Amide I Ratio ($\alpha:\beta$) | Amide II Ratio ($\alpha:\beta$) | IDF (%) | SDF (%) |
|------------------|---------------------------|-------------------------|--------------------------|---|--|---|--|----------------------------------|-----------------------------------|-------------------------|-------------------------|
| | | | | α -helix wave number (cm ⁻¹) | β -sheet wave number (cm ⁻¹) | α -helix wave number (cm ⁻¹) | β -sheet wave number (cm ⁻¹) | | | | |
| Raw | 0 | 91.4 ^d ± 0.1 | 50.0 ^e ± 0.5 | ND | 1637 | 1547 | 1516 | ND | 1.11 ^e ± 0.01 | 8.4 ^b ± 0.1 | 3.2 ^a ± 0.3 |
| | 10 | 88.3 ^c ± 0.7 | 16.8 ^{cd} ± 0.1 | ND | 1638 | 1551 | 1516 | ND | 1.01 ^d ± 0.01 | 13.1 ^d ± 0.2 | 4.4 ^b ± 0.1 |
| | 20 | 85.0 ^b ± 0.5 | 16.2 ^c ± 0.2 | ND | 1627 | 1544 | 1515 | ND | 0.95 ^c ± 0.02 | 18.7 ^f ± 0.2 | 6.1 ^c ± 0.2 |
| Extrusion cooked | 0 | 90.8 ^d ± 0.3 | 17.2 ^d ± 0.1 | 1643 | 1625 | 1542 | 1514 | 1.04 ^c ± 0.01 | 0.84 ^b ± 0.01 | 7.2 ^a ± 0.2 | 4.3 ^b ± 0.2 |
| | 10 | 86.2 ^b ± 0.2 | 11.5 ^b ± 0.1 | 1647 | 1627 | 1543 | 1514 | 0.98 ^b ± 0.01 | 0.80 ^a ± 0.01 | 9.7 ^c ± 0.4 | 6.8 ^d ± 0.4 |
| | 20 | 82.8 ^a ± 0.7 | 9.9 ^a ± 0.2 | 1647 | 1620 | 1543 | 1512 | 0.95 ^a ± 0.01 | 0.77 ^a ± 0.02 | 14.4 ^e ± 0.3 | 10.8 ^e ± 0.4 |

Values are means ± standard deviations of 3 independent experiments. Values within the same column followed by different letters are significantly different (p<0.001)

Protein secondary structure was determined by ATR-FTIR spectra of amide I and amide II region

IVPD = *in-vitro* protein digestibility

NSI = nitrogen solubility index

ND = not detected in the composites

IDF = insoluble dietary fibre

SDF = soluble dietary fibre

ND = not detected in the samples

level of addition of grape pomace increased from 0 to 20% (Table 2). This indicates that grape pomace addition promoted an increase in β -sheet formation with a consequent decrease in α -helical conformation. Mehanna *et al.* [63] reported shifts in the ratio of α/β conformation in the amide I and II regions of whey isolate after addition of tannic acid and this was attributed to binding between tannic acid and whey protein.

The formation of β -sheet is reported to be indicative of protein aggregation [61]. The increase in proportion of β -sheets has been linked to decrease in *in-vitro* protein digestibility due to the highly hydrophobic nature of β -sheets [64]. Wang *et al.* [65] reported increase in surface hydrophobicity of soy protein with increasing β -sheets conformation. High hydrophobicity has been suggested to reduce solubility of legume proteins through facilitating protein-protein interaction and aggregate formation which thereby limits enzyme access to susceptible sites [66].

Starch digestion was generally higher in extrusion cooked porridges compared to the starting raw materials (Figure 2). Total starch digestion (TSD) of cassava-soy composites was significantly reduced ($p < 0.001$) by addition of grape pomace. The porridge with 20% grape pomace exhibited the lowest TSD (%). Starch digestibility kinetic parameters (C_{∞} , K, HI, EGI and EGL) were significantly ($p < 0.01$) increased by extrusion cooking (Table 3) but the addition of grape pomace lowered the values of these parameters. The lower K value (0.020) in composite with 20% grape pomace compared to the composite without grape pomace (0.031) indicates that there was a decrease in the rate of starch digestibility with addition of grape pomace. The EGL of extrusion cooked porridges were 44.7, 35.0 and 24.3 for cassava-soy porridge, porridges with 10 and 20% grape pomace respectively.

Table 3: Effects of extrusion cooking and grape pomace addition on in-vitro starch digestibility kinetic parameters, starch fractions and pH of cassava-soy composite and reference sample.

| Treatment | Grape pomace addition (%) | C_{∞} (%) | K (min^{-1}) | HI (%) | EGI | EGL | RDS (%) | SDS (%) | RS (%) | pH |
|------------------|---------------------------|----------------------------|------------------------------|--------------------------|-------------------------|-------------------------|-------------------------|--------------------------|-------------------------|--------------------------|
| Raw | 0 | 55.8 ^b ± 2.4 | 0.020 ^a ± 0.00 | 53.8 ^c ± 1.4 | 69.3 ^c ± 1.2 | 30.3 ^d ± 0.4 | 13.5 ^a ± 0.5 | 45.0 ^{cd} ± 0.7 | 41.6 ^c ± 0.2 | 6.30 ^d ± 0.01 |
| | 10 | 48.6 ^a ± 0.5 | 0.017 ^a ± 0.00 | 49.4 ^b ± 1.5 | 66.9 ^b ± 2.1 | 22.5 ^b ± 0.5 | 11.1 ^a ± 1.1 | 46.0 ^{cd} ± 0.4 | 43.0 ^c ± 0.9 | 5.28 ^b ± 0.00 |
| | 20 | 46.4 ^a ± 1.1 | 0.015 ^a ± 0.01 | 47.2 ^a ± 0.4 | 65.6 ^a ± 0.8 | 16.3 ^a ± 0.7 | 8.5 ^a ± 0.7 | 48.6 ^d ± 0.5 | 43.9 ^c ± 1.2 | 4.53 ^a ± 0.01 |
| Extrusion cooked | 0 | 98.6 ^e ± 0.7 | 0.031 ^c ± 0.01 | 98.2 ^f ± 0.7 | 94.1 ^f ± 1.3 | 44.1 ^f ± 1.1 | 70.1 ^d ± 0.9 | 28.9 ^a ± 1.0 | 1.3 ^a ± 0.1 | 6.15 ^c ± 0.00 |
| | 10 | 85.2 ^d ± 2.9 | 0.027 ^{bc} ± 0.00 | 86.4 ^e ± 0.5 | 87.2 ^e ± 0.6 | 35.6 ^e ± 0.4 | 62.0 ^c ± 1.3 | 35.2 ^b ± 2.6 | 2.8 ^b ± 0.2 | 5.28 ^b ± 0.01 |
| | 20 | 80.2 ^c ± 1.5 | 0.020 ^a ± 0.00 | 79.6 ^d ± 0.7 | 83.4 ^d ± 0.3 | 26.5 ^c ± 0.6 | 53.4 ^b ± 1.9 | 43.4 ^c ± 1.3 | 3.2 ^b ± 0.2 | 4.55 ^a ± 0.00 |
| | Bread | 99.1 ^e ± 0.4 | 0.029 ^{bc} ± 0.01 | 100.0 ^g ± 0.1 | 94.6 ^g ± 0.6 | 64.8 ^g ± 0.1 | 73.1 ^d ± 1.1 | 25.7 ^a ± 0.7 | 1.1 ^a ± 0.2 | nd |

Values are means ± standard deviations of 3 independent experiments. Values within the same column followed by different letters are significantly different (p<0.01)

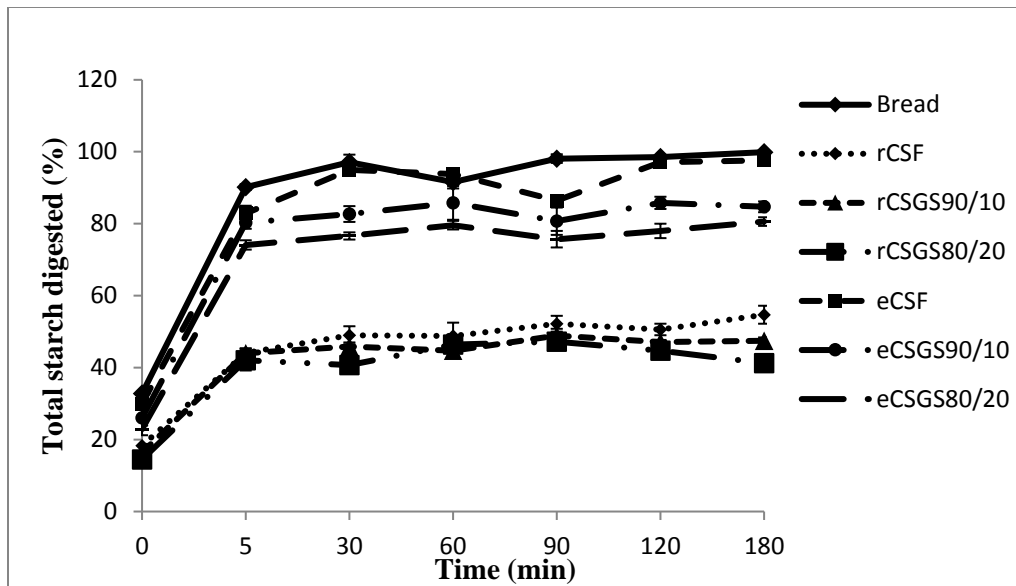
C_{∞} = % starch digested after 180 min

HI, k and GI were calculated from the equation: $AUC = \frac{C_{\infty}}{k} (1 - \exp(-k(t - t_{f_0})))$ proposed by Goni *et al.* 1997

EGL per g solids was estimated as $EGI * S / 100$ where S is starch content (g/100g solids)

White bread was used as the reference for calculating EGI

nd = not determined in bread because it was not needed



rCSF is raw cassava-soy flour
 rCSGS90/10 is raw cassava-soy flour with 10% grape pomace
 rCSGS80/20 is raw cassava-soy flour with 20% grape pomace
 eCSF is extruded cassava-soy flour
 eCSGS90/10 is extruded cassava-soy flour with 10% grape pomace
 eCSGS80/20 is extruded cassava-soy flour with 20% grape pomace
 White Bread was used as reference

Figure 2: Effects of grape pomace addition and extrusion cooking on the kinetics of starch digestion of cassava-soy composite

The estimated rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) fractions of the raw and extrusion cooked composites are shown in Table 3. These starch fractions (RDS, SDS and RS) determined from *in-vitro* starch digestion can be used as a prediction tool of *in-vivo* glucose response. The raw composites had lower RDS while SDS and RS were high. The addition of grape pomace significantly ($p < 0.001$) increased the SDS of cassava-soy porridge. The reference (white bread) had the highest RDS and least SDS while the extruded composite which had 20% grape pomace composite had the highest SDS and least RDS amongst the extruded samples. The estimated resistant starch (RS) of the reference was significantly lower to that of extruded composites with and without grape pomace.

The type of starch, physical state of starch, rheological characteristics of food, its texture, the presence of other non-starch components and the interactions that occur between starch and these non-starch components are all factors that could influence enzymatic digestibility [67]. Native starches are reported to have a high resistance to enzymatic hydrolysis due to their highly organized and intact crystalline structure [68] hence the lower digestibility observed in the starting raw materials.

Extrusion cooking may promote physical disruption of the organized granular structure of native starches and this leads to gelatinization and disintegration of starch [69]. Starch gelatinization and disintegration facilitates amyolytic hydrolysis by making starch more accessible to digestive enzyme thus increasing starch digestibility [70] and this may be responsible for significant increases in digestibility observed in extrudates after extrusion cooking.

The reduction observed in EGI and EGL when grape pomace was added to cassava-soy may be attributed to the inhibitory effects polyphenols present in grape pomace have on starch digestive enzymes. The ability of polyphenols to inhibit activities of alpha amylase and alpha glucosidase is well reported in literature [14, 71]. Phenolic compounds bind to the active and secondary sites of digestive enzymes therefore making them inactive [72]. The activities of alpha amylase are reported to be inhibited by tannins while smaller phenolics such as phenolic acids inhibit the activities of alpha-glucosidase [73].

The inhibitory effects of tannins on alpha-amylase is reported to be concentration dependent. Polymers have higher inhibitory activity against alpha-amylase than oligomers while reverse is the case for alpha-glucosidase [74]. Miao *et al.* [75] in a study on grape pomace extract inhibition of alpha-amylase *in-vitro* reported a non-competitive inhibition of pancreatic alpha-

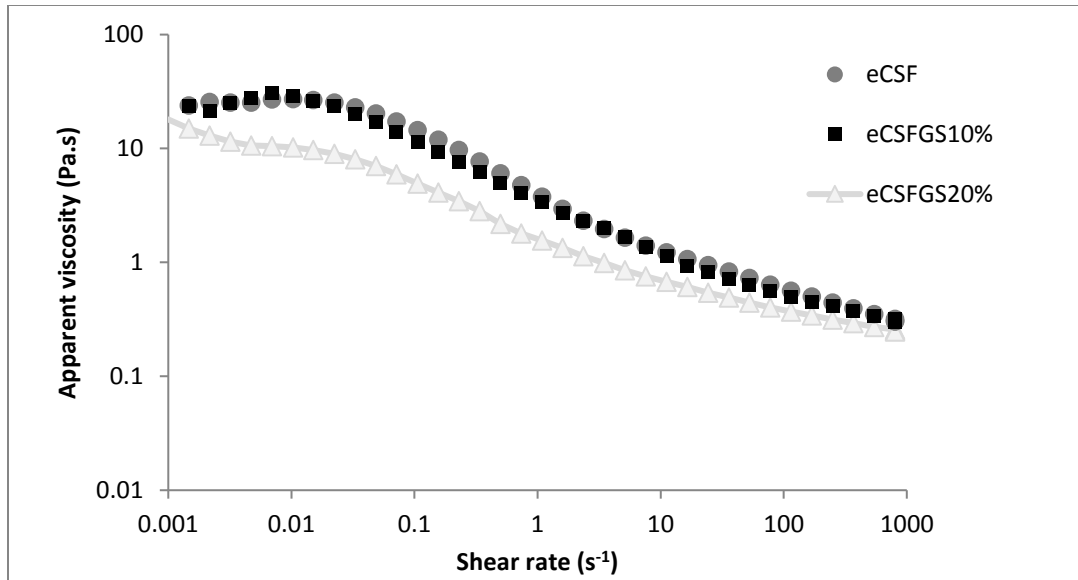
amylase by grape pomace extract. The authors reported that grape extract polyphenol occupied one of the binding sites of alpha-amylase, interacted with side chains of alpha-amylase and formed hydrogen bonds and Van der Waal forces with residues of the catalytic site [75].

In a study on effects of antioxidant of grape pomace extract on post-prandial hyperglycemia in diabetic mice, Hogan *et al.* [76] reported that grape pomace extract inhibited alpha glucosidase activity and promoted decrease in post prandial glucose response. Also, Barrett *et al.* [16] suggested that tannins in grapes had a high inhibitory effect on alpha amylase and alpha glucosidase. It is important to note that pH could not have had an effect on starch digestibility because buffers were used to ensure that all the treatments were at the same pH throughout the assay.

Grape pomace addition significantly ($p < 0.01$) increased the insoluble dietary fibre (IDF) and soluble dietary fibre (SDF) of cassava-soy composite (Table 2). Soluble dietary fibre (SDF) contents of cassava-soy composite with and without grape pomace increased with a decrease in insoluble dietary fibre (IDF) after extrusion cooking (Table 2). Significant interaction between treatment and grape pomace addition level was observed in IDF and SDF ($p < 0.00$, $p < 0.02$ respectively). There was an about 54.5% increase in SDF content of composite with 10% grape pomace and the highest increase was observed in cassava-soy composite with 20% grape pomace addition level with about 77% increase. Redistribution in IDF to SDF ratio was also reported in extrusion cooking of lemon fibre by Méndez-García *et al.* [77]. Martínez-Boustos *et al.* [78] also reported that extruded blends of sugar bagasse fibre, whey protein concentrate, and corn starch had higher SDF and decreased IDF content compared to the unextruded blends. The authors suggested that solubilization and fragmentation of dietary fibre during extrusion cooking led to the increase in soluble dietary fibre.

Mechanical stress during extrusion cooking has been associated with the breakdown of polysaccharide glycosidic bonds giving rise to the release of oligosaccharides hence the increase in SDF contents [79]. According to Ralet *et al.* [80], extrusion cooking leads to solubilization of IDF and it also promotes the reduction in molecular weight of non-cellulosic polysaccharides and pectin. Grape pomace dietary fibre primarily consists of cellulose, hemicellulose and pectin [3]. Several authors have shown that extrusion cooking solubilizes insoluble pectic polysaccharide [81, 82]. Pectin consists of α -(1,4)- linked homogalacturonan and rhamnogalacturonan which are highly branched with various neutral sugars [83]. The high shear during extrusion cooking may result in breakage of α -(1,4)-glycosidic linkages in the galacturonan backbone of pectic polysaccharides and this would lead to increase in solubility [84].

The cassava-soy composite with 20% grape pomace had the lowest apparent viscosity (Figure 3). The zero-shear viscosity, K and n -value of the cassava-soy (the control) and the composite with 10% grape pomace level statistically ($p < 0.001$) differed from the composite with 20% grape pomace (Table 4). All samples exhibited a shear thinning behavior ($n < 1$). The addition of grape pomace significantly ($P < 0.001$) lowered the hysteresis area of cassava-soy composite (Table 4).



eCSF is extrusion cooked cassava-soy flour
 eCSB90/10 is extrusion cooked cassava-soy flour with 10% grape pomace
 eCSB80/20 is extrusion cooked cassava-soy flour with 20% grape pomace

Figure 3: Effect of grape pomace addition on apparent viscosity of extruded cassava-soy composite at 50 °C as a function of shear.

Table 4: Effect of grape pomace addition on power law (k and n -values) parameters, zero-shear viscosity, viscosity at shear rate 50 s^{-1} and hysteresis area of extruded cassava-soy composite

| Treatment | K -value (Pa.s) ⁿ | Power law index (n -value) | $\eta_{a, 50}$ (mPa.s) | η_0 ($\times 10^3 \text{ mPa.s}$) | Hysteresis area Pa/ (S· ml) |
|---|-----------------------------------|-------------------------------------|---------------------------|---|-----------------------------------|
| Extrusion cooked cassava-defatted toasted soy flour | $3.78^b \pm 0.49$ | $0.56^a \pm 0.1$ | $728^b \pm 15$ | $24^b \pm 2$ | $17357^c \pm 10$ |
| With 10% grape pomace | $3.14^b \pm 0.70$ | $0.56^a \pm 0.0$ | $629^b \pm 28$ | $23^b \pm 2$ | $14682^b \pm 33$ |
| With 20% grape pomace | $2.15^a \pm 0.06$ | $0.61^b \pm 0.1$ | $473^a \pm 1$ | $14^a \pm 3$ | $13365^a \pm 18$ |

Values are means \pm standard deviations of 3 independent experiments.
 Values within the same column followed by different letters are significantly different ($p \leq 0.05$)

Apparent viscosity of non-starch polysaccharides depends on ionically charged groups, molecular weight, concentration of dietary fibre, surrounding structures [85] and pH [86]. The

low viscosity recorded for the composites containing grape pomace despite the increase in soluble dietary fibre after extrusion cooking could be as a result of breakdown of high molecular weight polysaccharides to low molecular weight in the soluble fractions [87] which are less viscous. Another contributing factor to low viscosity as suggested by Nyman and Haska, [88] is degradation of attached side chains of dietary fibre during storage by endogenous pectinases. In this case, the grape pomace might have been stored for a long period after crushing of grape fruits to expel juice, hence the lower viscosity observed in the composites which had grape pomace.

Pectin is estimated to be about 34% in grape pomace and it is one of the major components of grape pomace dietary fibre [89]. Pectin is reported to be a process-sensitive non-starch polysaccharide which can undergo depolymerization at relatively intermediate heating temperature (≥ 50 °C) [90]. Heating is found to change the conformational properties of pectin in solution and this directly impacts on its functionality [91]. pH also significantly influences viscosity. The pH values recorded for the extruded composites with and without grape pomace are shown in Table 3. The cassava-soy composite with 20% grape pomace had the lowest pH value of 4.55 while that with 10% grape pomace was 5.28 and the control without grape pomace had a pH value of 6.15. Pectin depolymerization is reported to be dominated by acid hydrolysis at pH 4.5 [92, 90] and this may also have contributed to the low viscosity recorded for the composite with 20% grape pomace.

Svanberg *et al.* [87] reported a reduction in apparent viscosity of carrots after boiling compared to microwave heating. The authors suggested that the reduction may be due to intensity of treatment associated with boiling that caused more glycosidic linkages breakage and loss of inter-molecular association of water-soluble polysaccharides. Also, the viscosity of Brussels

sprouts was reported to be lower to that of green beans after heat processing and the authors attributed this to depolymerization of high molecular weight polysaccharides with a consequent increase in low molecular weight polysaccharides [93].

Extrusion cooking makes use of high shear during processing and this facilitates extensive degradation of dietary fibre polysaccharides and cleavage of some glycosidic linkages that results in increased solubility and possibly also in reduced viscosity [94]. It can thus be suggested that despite the higher concentration of SDF in the composite with 20% grape pomace, the molecular weight of these SDF portion was very low and as such could not promote viscosity hence why the apparent viscosity was the lowest.

The effects of extrusion cooking and grape pomace addition on particle size distribution of cassava-soy composites are shown in Table S1. The addition of grape pomace increased the mass retained in the higher mesh sieves while the smallest portion retained in the sieve size below 38 μm was highest in the control (cassava-soy composite). Extrusion cooking significantly ($p < 0.001$) decreased the particle size of extrudates as percentage mass of extrudates retained in sieves 500 and 212 μm were significantly ($p < 0.01$) lower after extrusion cooking compared to the starting raw materials particularly in the composites which contained grape pomace.

The addition of grape pomace resulted in an increase in the total phenolics and antioxidant activity of cassava-soy composite (Table 5). As the level of addition of grape pomace increased, the total phenolic content and antioxidant activity also increased. Extrusion cooking significantly ($p < 0.05$) lowered the total phenolic content and antioxidant activity of cassava-soy composite. Similar findings were reported by Wani and Kumar [95] in extrusion cooking of a nutritious snack made from a combination of fenugreek seed flour, fenugreek leave flour, oat, dried green

pea and corn flour and the reduction in total phenolic content was attributed to a decrease in amount of free phenolics. In another study, Caltinogu *et al.* [96] ascribed the reduction in total phenolic content and antioxidant activity to increasing temperature from 100 to 120 °C.

Table 5: Effects of extrusion cooking and grape pomace addition on the total phenolics contents (TPC) and antioxidant activity (AA) of cassava-soy composite

| Treatment | Grape pomace addition (%) | Total phenolics contents (mg CE/g) | Antioxidant activity (μmol TE/g) ABTS assay |
|------------------|----------------------------------|---|--|
| raw | 0 | 2.1 ^b ± 0.2 | 7.7 ^b ± 0.2 |
| | 10 | 5.5 ^d ± 0.2 | 10.6 ^c ± 0.3 |
| | 20 | 7.7 ^f ± 0.1 | 13.5 ^d ± 0.3 |
| Extruded | 0 | 0.9 ^a ± 0.0 | 5.5 ^a ± 0.6 |
| | 10 | 3.5 ^c ± 0.2 | 8.5 ^b ± 0.1 |
| | 20 | 5.9 ^e ± 0.1 | 9.8 ^c ± 0.3 |

Values are means ± standard deviations of 3 independent experiments.

Values within the same column followed by different letters are significantly different (p≤0.001)

The high temperature in the extruder barrel facilitates degradation of bioactive phenolic compounds with consequent loss of their antioxidant capacity [97]. Also, high temperature could alter the molecular structure of phenolic compounds thereby leading to either a decline in chemical reactivity or reduction in extractability of these phenolic compounds and loss of antioxidant activity because of a certain degree of polymerization [98]. Dietary phenolics have proven antioxidant activity [99]. They help in the prevention of some processes involved in the development of degenerative diseases such as cancer by protecting the DNA from oxidative damage [100], CVDs by inhibiting oxidation of LDL cholesterol [101] and type-2 diabetes through their hypoglycemic effect [102].

4 Conclusions

The reduction observed in the starch digestibility of cassava-soy composite as grape pomace addition increased may be related to the dietary fibre and phenolic compounds present in grape pomace. These phenolics can inhibit the activities of starch hydrolyzing enzymes thereby delaying the digestion of starch and absorption of glucose. Although extrusion cooking reduced the total phenolics and antioxidant activity of extrudates however, significant amounts were still retained. This study indicates that grape pomace can be added to starch-rich foods as a functional ingredient with the use of extrusion cooking to produce fibre-rich extrudates which has the potential to lower glycemic index.

Acknowledgements:

This work is supported by National Research Foundation (NRF) of South Africa and the DST/NRF-Centre of excellence in Food Security.

The authors have declared no conflict of interest

Novelty: This research elucidates on the observed reduced estimated glycaemic index of composites of extruded cassava-soy containing grape pomace and this was attributed to the phenolic compounds present in grape pomace

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