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Evaluation of antioxidant and functional properties of orange pomace-based food

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ABSTRACT

Food processing wastes are promising sources of valuable compounds such as antioxidants, which may be used because of their favourable technological, nutritional and functional properties. Orange pomace (P), soybean meal (S) and wheat bran (W) were subjected to a three-component mixture design; the lower boundary was 5, 10 and 10% for pomace, soybean meal and wheat bran, while the upper boundary was 30, 80 and 70%, respectively. D-optimal design was used to obtain blends of 13 formulations analyzed for antioxidant and functional properties using standard methods. The products were high in total phenol, flavonoid, carotenoid and ferric reducing antioxidant properties (FRAP). Interactions between orange pomace and wheat bran contributed the most to the total phenol, flavonoid and FRAP, while orange pomace and soybean meal interaction contributed the most to the carotenoid content. Bulk density and water-soluble index were influenced mostly by the interaction of pomace and wheat bran while water absorption index was influenced by the interaction of orange pomace and soybean meal. The antioxidant and functional properties of the food suggested that the food could be consumed for its health- promoting benefits in addition to eliminating environmental pollution by the orange pomace.

Introduction

The increase in consumers' demand for high-quality food products has led to the usage of new technologies and ingredients. Considerable importance is given to functional foods, which, apart from their basic nutritional functions, provide physiological benefits, play an important role in disease prevention, or slow the progress of chronic diseases. Functional foods either contain (or add) a component with a positive health effect or eliminate a component with a negative one. The relationship between diet and health has focused on the role of food choices and diseases like cancer, cardiovascular disease, and allergies (Lambert 2001). Pérez-Alvarez (2008) describes that the requirements for functional food are safety, health, and taste. Many components may be added to foods to make them "functional", including ω-3 fatty acids (Hjaltason and Haraldsson, 2006), vitamins (Baro et al., 2003), probiotics (Salem et al., 2006), prebiotics (Brink et al., 2005), symbiotics (D'Antoni et al., 2004), phytochemicals (Wolfs et al., 2006), bioactive peptides (Thoma-Worringer et al., 2006), and fibre (Fernandez-Lopez et al., 2009). Scientific evidence confirming the relationship between food and health has promoted the rapid development of a new food market in recent years: the functional food market (Viuda-Martos et al., 2010). Over the past few decades, an increasing trend toward efficient utilization of natural resources has been observed around the world. The direct disposal of agro-industrial residues as waste in the environment represents an important loss of biomass, which could be bioconverted into different metabolites, with a higher commercial value. The polyphenolics from waste materials, being derived from agro-industrial production, may be used as functional food ingredients and as natural antioxidants (Zhou et al., 2009). The wastes from this processing,

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including peel, seeds and pulp, which make up nearly 50% of unprocessed fruit, are a possible source of valuable byproducts (Gowe, 2015). Other findings from Li et al. (2006) and Gorinstein et al. (2001) reported that the concentration of total phenolic compounds was higher in the peel of fruits compared to the flesh. The valuable anticarcinogenic and anti-inflammatory effects of the citrus peel against degenerative diseases as have been observed (Imran et al., 2020). Despite all these possibilities, industrial waste from orange juice remains mostly unused (Andrade et al., 2014). In providing a veritable outlet for pomace, our previous study on the development of food from pomace, found the products proximate composition to be moisture (8.50-11.03%), ash (4.00-6.00%), protein (10.84-25.40%), fat (3.50-8.00%), crude fibre (9.05-19.89%) and carbohydrate (39.18-59.34%). Also, these products were high in dietary fibre ranging from 52.97 to 66.86% (Oduntan and Arueya, 2019). Thus, this research further investigated the antioxidant and functional properties of the fibre-rich products' for their nutraceutical benefits.

Materials and methods

Sample preparations

Orange pomace was obtained after juice production at the Processing unit of the National Horticultural Research Institute, Jericho, Ibadan, Oyo State. The obtained residues were dried (Fexod AS 230 Nigeria) at 60 ± 2 °C and ground using a laboratory grinder (Fedek DS200, Nigeria) to a particle size of 500 μm and stored in air-tight high-density polyethylene till use. Wheat bran and soybean meal were purchased from SAN AGRIC Nig. Ltd, Oluyole Estate, Ring road, Ibadan, both milled and packaged and stored at 28 ± 2 °C for further use.

Experimental design and product formulation

Minitab 16 was applied for the analysis of experimental data using a commercial statistical package, Design-expert version 9.0.6 (StateaseInc, Minneapolis, USA). A mixture design (D-optimal design) was used to obtain 13 design points from three components (Figure 1). The lower limit (orange pomace – 5%; soybean meal – 10%; wheat bran – 10%) and upper bound constraints (orange pomace – 30%; soybean meal – 80%; wheat bran – 70%) for each mixture component were used to generate the design.

Extrusion cooking

Extrusion cooking was performed according to the modified Huang and Ma (2016) method. Formulated blends of wheat bran, soybean meal and orange pomace of different ratios shown in table 1 were fed manually into a single screw extruder (Model FEXOD 302) at the pre-determined moisture content (between 20 and 22 %), screw speed of 290 rpm and temperature 110 °C from experimental trials. The products were collected from the die opening of 4mm diameter, dried in a fluidized bed drier (Fexod AS 230 Nigeria) at 60 °C. Samples were allowed to cool for 10 min and packaged (Plate 1) in a 250-micron polyethylene bag for further analysis.

Determination of chemical properties of the extrudates

Total phenolic content

Total phenolic content (TPC) of extracts was determined using the Folin-Ciocalteu assay described by Chan et al. (2008).

Determination of the flavonoid content

The $AlCl_3$ method (Jagadish et al., 2009) was used for the determination of the total flavonoid content of the sample extracts.

Determination of FRAP

The reducing power of the extract was determined according to the method described by Benzie and Strain (1999).

Determination of functional properties of the extrudates

Bulk density

Bulk density (BD; g/cm^3) was calculated following Stojceska et al. (2008) expression.

Water absorption index (WAI) and water solubility index (WSI)

The WAI and WSI were determined according to the methods developed for cereals (Yagci and Gogus, 2008; Stojceska et al., 2008).

Data Analysis

The experiment was done in triplicate, and the data collected were subjected to statistical analysis using Minitab 16 of Design expert 9.0.6. Comparisons between the 13 formulations were done using analysis of variance (ANOVA) with a probability ($p < 0.05$).

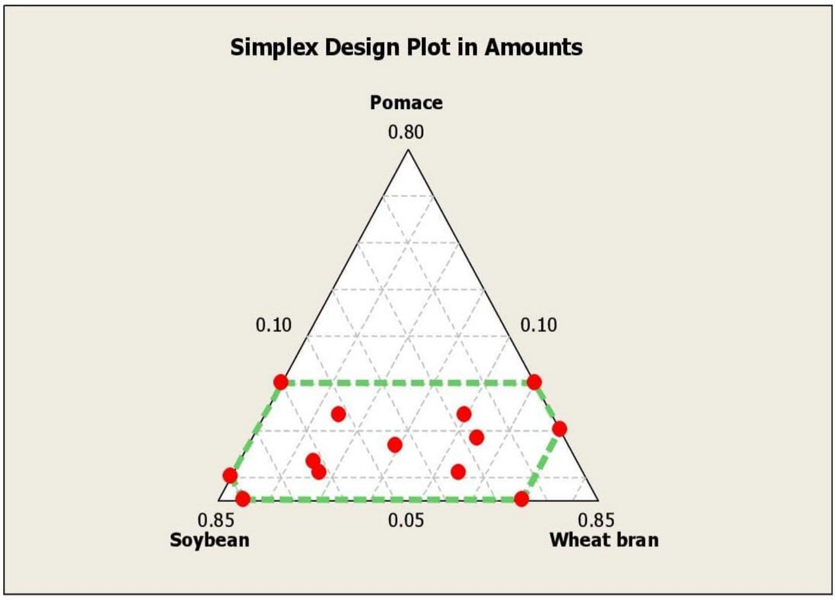

Figure 1. Constrain region of D optimal design plot

Table 1. Design matrix for ingredient formulations of orange pomace-based food

Run Order	PtType	Blocks	Pomace (%)	Soybean meal (%)	Wheat bran (%)
1	-1	1	13	62	25
2	0	1	17	44	39
3	1	1	5	80	15
4	1	1	10	80	10
5	-1	1	23	52	25
6	1	1	20	10	70
7	1	1	5	25	70
8	-1	1	23	27	50
9	-1	1	11	62	27
10	-1	1	11	35	55
11	-1	1	18	27	55
12	1	1	30	60	10
13	1	1	30	10	60

Plate 1. Extruded blends of pomace, soybean meal and wheat (1-13 = trial runs)

Results and discussion

Total phenolic content

Total phenolic content (TPC) of the extrudates ranged from 0.47 to 0.76 mg/g (Figure 2); maximum content was observed in blend 2 (17% pomace, 44% soybean meal,39% wheat bran), while blend 8 (23% pomace, 27% soybean meal, 50%wheat bran) had the minimal phenolic content. There were significant variations ($p < 0.05$) among 13 blends. The values were lower than the values reported for biscuits enriched with mango peel powder by Ajila et al. (2008). The existence of phenol in small quantities in food is believed to have nutraceutical properties, to inhibit the proliferation of cancer cells and to protect neurons against oxidative stress (Silva et al., 2008). The antioxidant property of phenolic moieties is primarily a result of their redox properties, which could play a vital function in the absorption and removal of free radicals, the decomposition of singlet oxygen and triplet oxygen or peroxide singlet oxygen and triplet oxygen or peroxide decomposition (Oduntan et al., 2014). These natural antioxidants remove harmful free radicals associated with advanced cancer and other deteriorating diseases, including poor brain function (Dillard et al., 2000).

A normal plot of residuals of TPC showed a good linear relationship (Figure 3) and confirmed the adequacy of the model. Phenolics are present in plants, and when phytochemicals are ingested in plant foods such as these, phytochemicals add to the absorption of normal antioxidants in the diet. Numerous findings revealed that antioxidants inhibit the incidence of deteriorating diseases like certain cancers, neurodegenerative and cardiovascular diseases, cataracts, ageing and oxidative stress dysfunctions (Hollman, 2001).

The predicted TPC trace graph shows that the increase in total phenolic compounds occurred with an increase in pomace to the optimum point, after which a further increase led to its decrease, while an increase in soybean meal and wheat bran reduced the total phenolic content, as shown in Figure 4.

Flavonoid content

Flavonoid in the extrudates ranged from 2.13 to 8.21 mg/g (Figure 5). Blend 2 (17%pomace, 44% soybean meal, 39% wheat bran) had the highest value for flavonoid while blend 8 (23% pomace, 27% soybean meal, 50% wheat bran) had the lowest value. Significant variations ($p < 0.05$) were observed among the blends while R2 was 0.4485. Flavonoids were shown to have many beneficial properties, including antimicrobial, anti-inflammatory enzymatic inhibition, estrogenic, antiallergic, antioxidant, and antitumor effects (Harbone and Williams, 2000). The existence of a substantial quantity of bioactive complexes like flavonoids in these pomace-based food assures their substantial nutritive value (Saura-Calixto and Goni, 2005). A higher concentration of flavonoid was observed with a higher soybean meal quantity and a small quantity of orange pomace.

The predicted trace plot of flavonoid shows an increase in pomace and wheat bran resulting in a quadratic reduction in the flavonoid quantity and an increase in the soybean meal increased the value of flavonoid until it reached the optimal point after which further increase led to a decrease in the flavonoid content (Figure 6).

Carotenoid content of the extruded blends

The content of carotenoids in extrudates ranged from 0.02 to 0.14 mg / g (Figure 7). This was higher than the value of biscuits enriched with mango peel powder stated by Ajila et al. (2013), but smaller than the values stated for crispbreads with carrot and pumpkin by-products. The highest value (0.14mg/g) was observed in a blend of 5% pomace, 25% soybean meal and 70% wheat bran while the lowest (0.02mg/g) was observed in three blends with a higher amount of pomace and soybean meal. Carotenoids are photosynthetic auxiliary pigments that cannot be produced by the human body. These colourants that are converted by the human body into vitamin A must be supplemented by ingestion (Van den Berg et al., 2000). An increase in carotenoid concentration was observed with a high quantity of wheat bran and a small quantity of pomace. This is consistent with the results of Luthria et al. (2015), who confirm that the highest concentrations of carotenoids were observed in the germ fraction, followed by the bran and endosperm fraction. It was further observed that the concentration of total carotenoids in wheat ranges from 0.7 g/g in durum wheat from Spain to as high as 13.6 g/g in Einkorn accessions from Italy.

This suggested an increase in the nutraceutical properties of the products in these formulations. Significant variation ($p < 0.05$) was perceived among the 13 blends. Carotene, in addition to being a safe source of vitamin A, is also a healthy food colourant. β-Carotene has been shown to be a highly active singlet oxygen scavenger that may be useful in preventing free radicals (Obradović et al., 2015). It was believed that food rich in beta-carotene may protect against the threat of developing certain cancers, may prevent skin diseases and vision problems (Konrade et al., 2018).

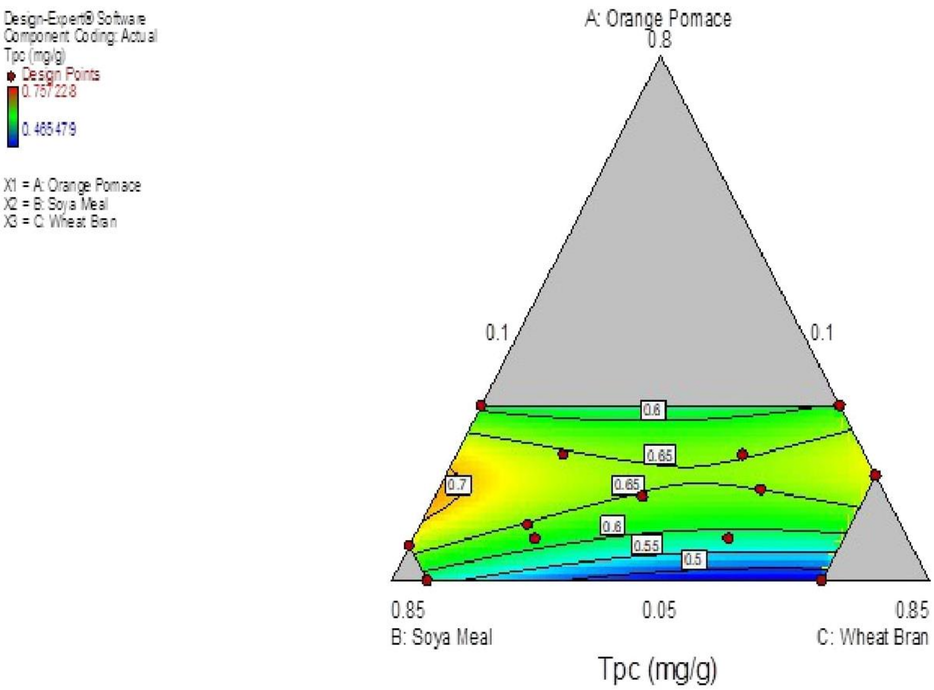

Figure 2. Mixture contour plot of total phenolic content of the products

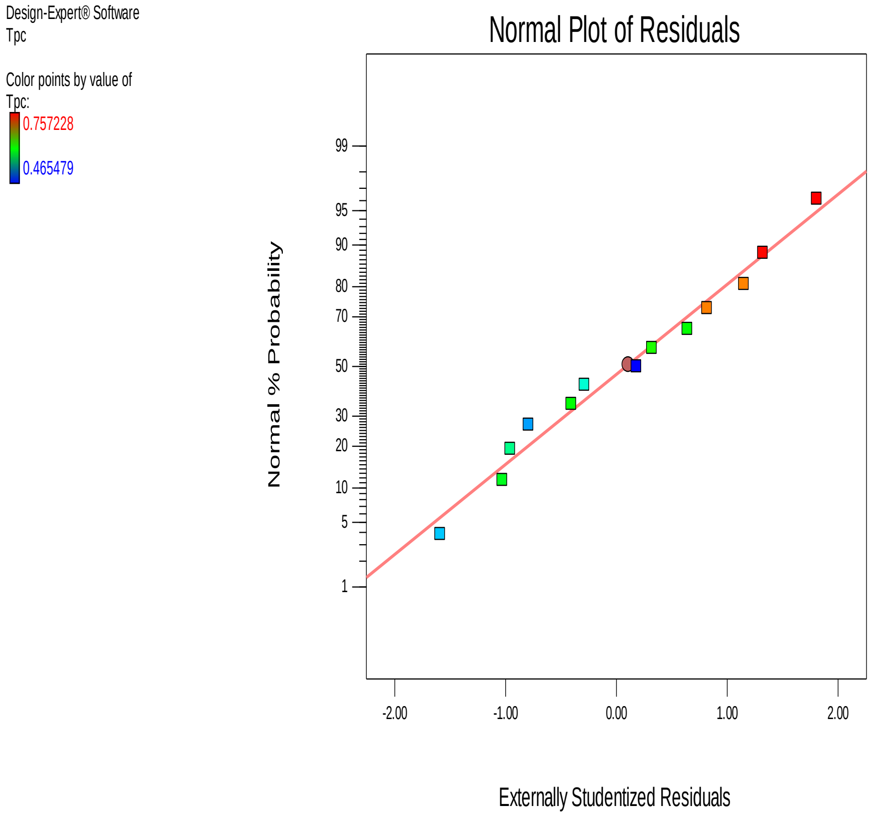

Figure 3. Normal plot of residuals of total phenolic content

The total daily consumption of carotenoids in the western diet is about 6 mg (Leitzmann, 2016). The predicted carotenoid trace plot of the components is shown in figure 8; an increase in the pomace content after the reference mix led to a sharp decrease in carotenoid content, an increase in soybean meal also led to a reduction in the amount of carotenoidwhile the increase in wheat bran resulted in an increase in carotenoid content.

Ferric reducing antioxidant properties of the extrudates (FRAP)

The values of FRAP of the extrudates were between 2.13 and 24.33 mg/g (Figure 9). These values were higher than 5.2 – 12.8 μmol TE/g reported for snacks enriched with powdered tomato by Wojtowicz et al. (2017). The ferric reducing antioxidant power (FRAP) is a widely acknowledged indicator of phenolic activity similar to the reducing power (Dong et al., 2014). Higher FRAP was observed at a higher quantity of wheat bran and a lower quantity of orange pomace. The predicted FRAP trace of the components showed that an increase in pomace brought about a sharp reduction in FRAP, also a rise in soybean meal decreased FRAP, while an increase in wheat bran led to the increase of FRAP (Figure 10). This shows the importance of wheat bran in the formulations and is confirmed in Equation (1). The components and their interactions show that pomace had the least impact on FRAP; an increase in pomace may lead to a decrease in the ferric reducing antioxidant power of the blends. Pomace wheat bran (AC) interaction with the highest coefficient (192.80) had the greatest impact on FRAP (equation 1).

$$FRAP = -140.08A + 9.69B + 22.82C + 183.97AB + 192.80\,AC - 30.24BC \quad (1)$$

Functional properties of the extrudates

Bulk density

The extrudate bulk density (BD) ranged from 0.42 to 0.61 g/cm3 (Table 2). The values were higher than those reported for guava pomace and rice flour extrudates by Srikanth et al. (2012), but comparable to sweet potato and tomato pomace extrudates (Dhungana et al., 2014). Bulk density is usually a measure of the physical properties and characteristics of extrudates (Escalante-Aburto et al., 2013). It describes the expansion of products in all directions. Bulk density was higher with high soybean meal concentration and low orange pomace concentration. This corroborated the previous studies showingwhen materials high in fibre and protein were added to starch products, the density of the product increased (Veronica et al., 2006). The predicted bulk density trace diagram of the components (Figure 11) shows that an increase in pomace and wheat bran led to a decrease in bulk density, while an increase in soybean meal led to an increase in bulk density.

The more the soybean meal, the higher the bulk density of the extrudates, which may be the result of a high content of lipids and protein of soybean meal which influenced the rheological characteristics of the blend as supported by Yagci and Gogus (2008).

Equation (2) shows that pomace had the contributed to the bulk density the least with a coefficient of -0.45 while pomace wheat bran (1.60) interaction contributed to the bulk density the most.

$$\text{Bulk density} = -0.45A + 0.50B + 0.28C + 1.40AB + 1.60AC + 0.48BC \quad (2)$$

Water solubility index

The values for the water solubility index (WSI) for the extrudates ranged from 4.67 to 14.67 % (Table 2). The values were higher than the values reported for sweet potato and tomato pomace (Dhungana et al., 2014) and red lentil-carrot pomace snack (Alam and Kumar, 2014). Water solubility and absorption parameters were used to describe the extruded products and are useful to predict the behaviour of the extruded material during future processing. Solubility in water also specifies the number of small molecules dissolved in water (Ajita et al., 2017).

Low WSI is associated with the occurrence of water-insoluble compounds formed by the macromolecules of amylose, proteins and lipid compounds of the raw materials (Wojtowicz et al., 2018). It is an indicator of the extent of starch degradation. This means that at lower WSI, the starch degrades only slightly and such a condition results in a lower solubility of particles in the formulation (Hernandez-Diaz et al., 2007). Higher WSI values were observed in an increased soybean meal content and low pomace concentration, also at high concentrations of wheat bran and low concentration of pomace. This is due to the low carbohydrate content of the blends. The predicted WSI trace plot of components indicates that an increase in the content of soybean meal and wheat bran led to an increase in water solubility index until it reached its maximum while a further increase led to a decrease in WSI (Figure 12).

WSI serves as a sign of the breakdown of molecular compounds such as starch, fibre and protein (Suksomboon et al., 2011, Seth and Rajamanickam, 2012).

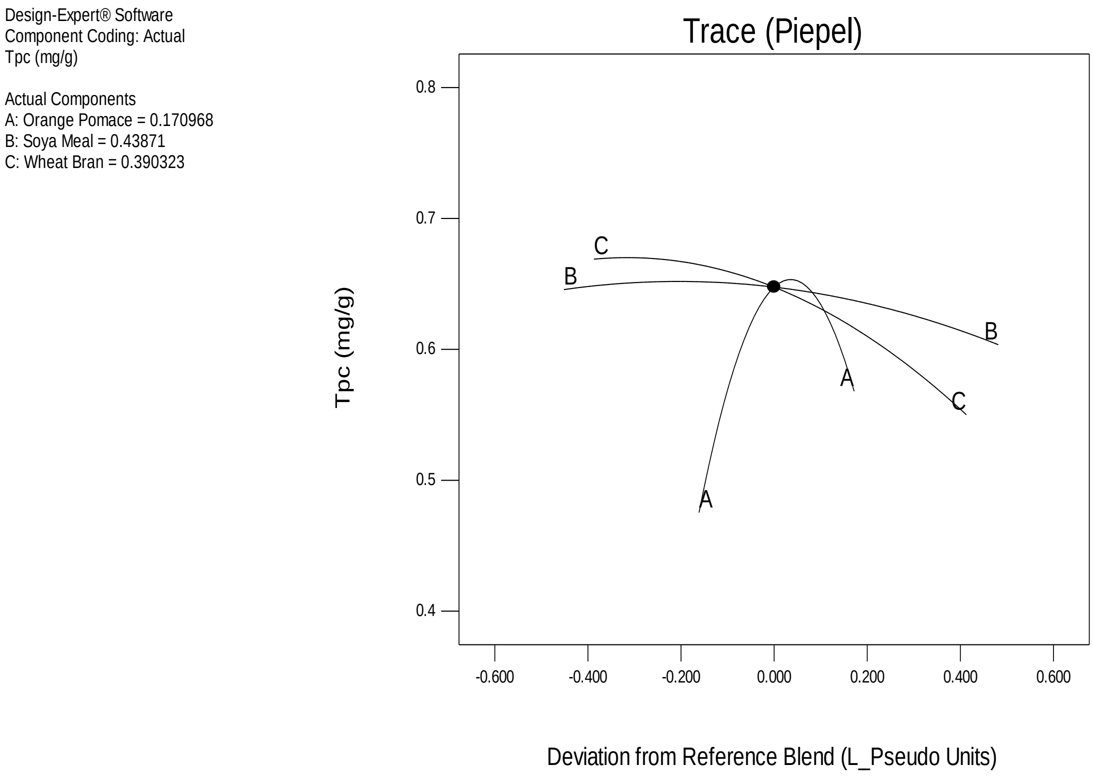

Figure 4. Component interaction effects on the total phenolic content of the products

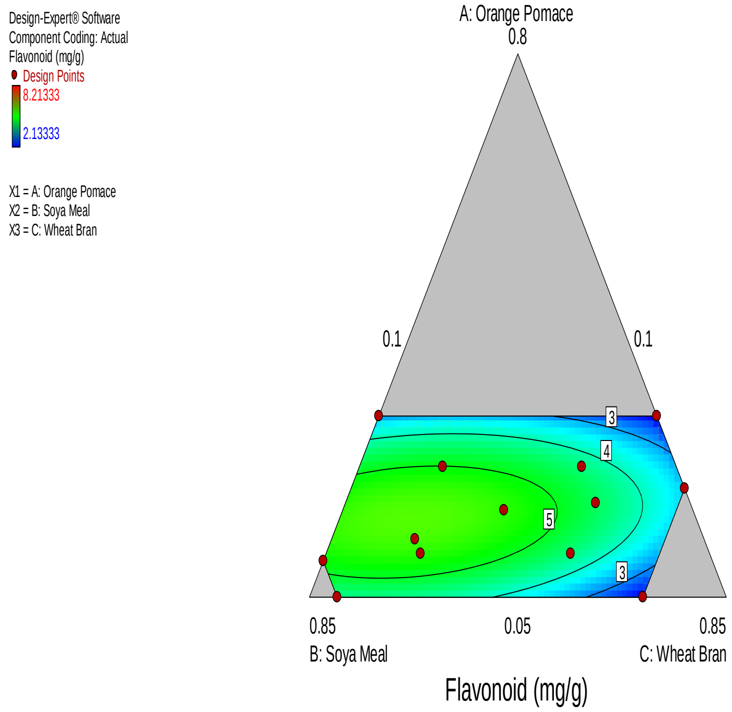

Figure 5. Mixture contour plot of flavonoid of the products

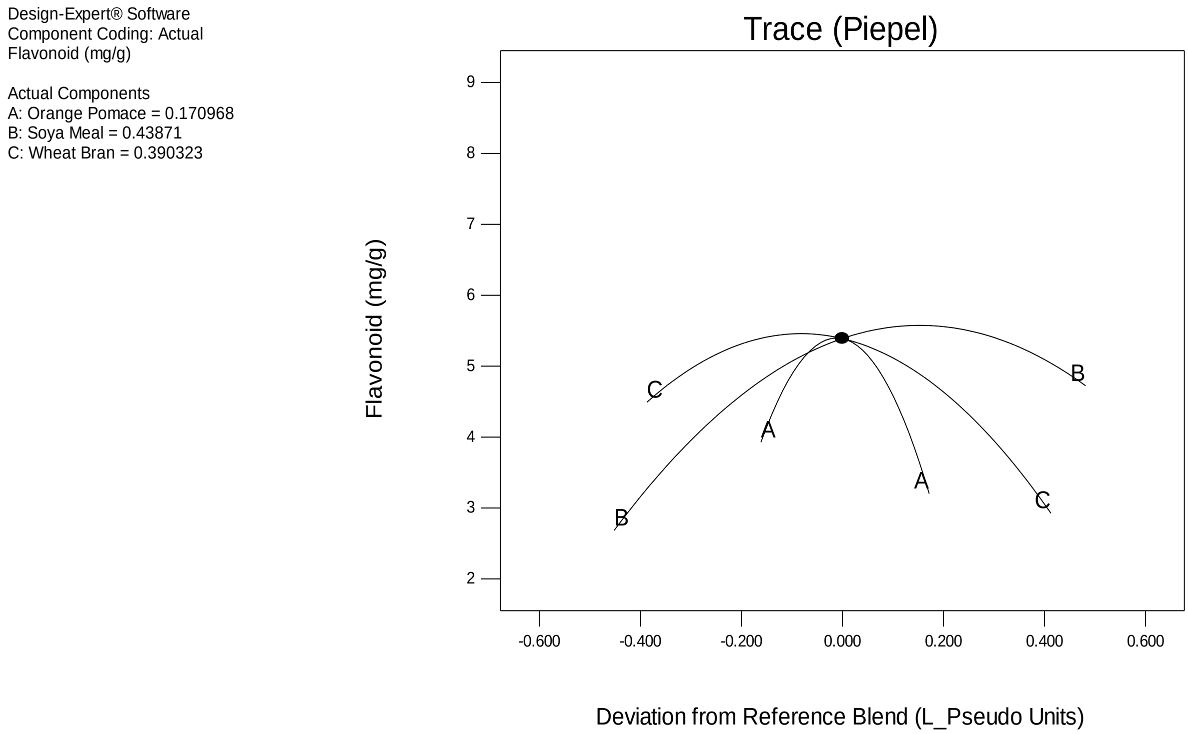

Figure 6. Component interaction effects on the flavonoid of the products

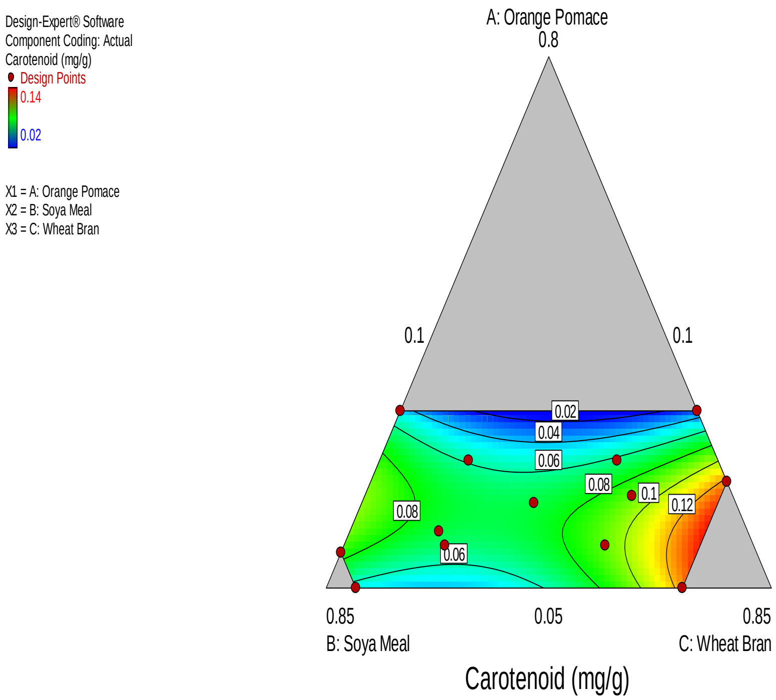

Figure 7. Mixture contour plot of carotenoid

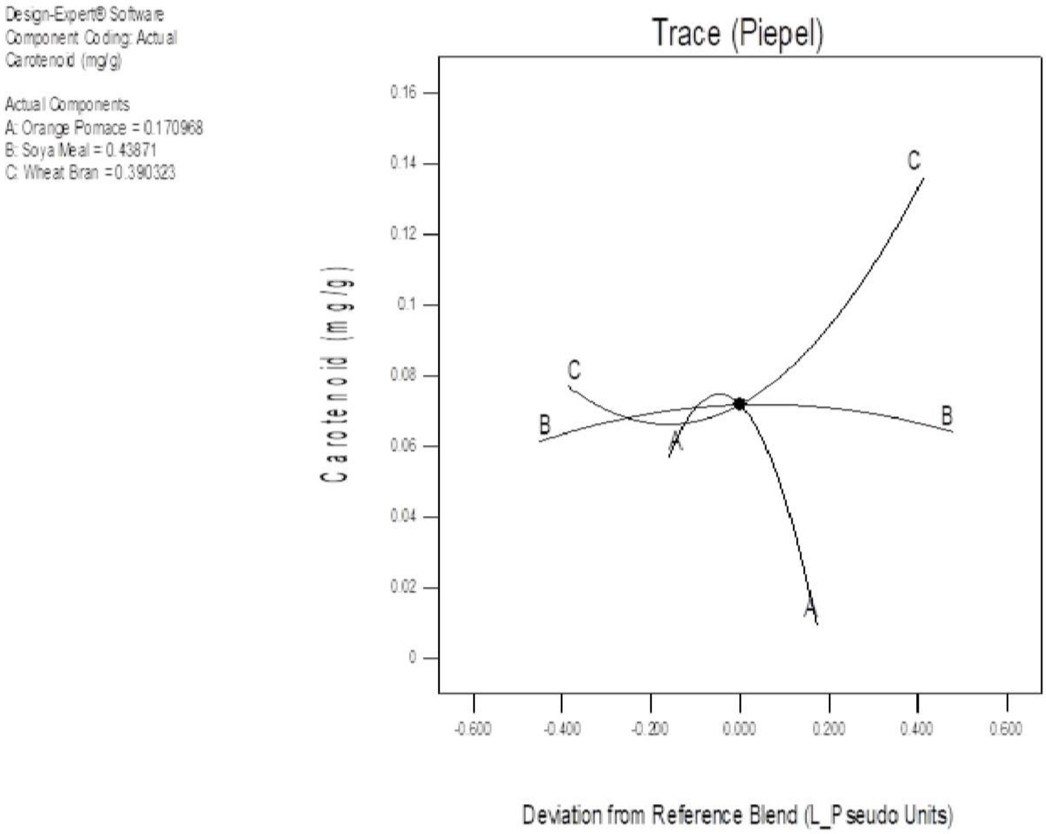

Figure 8. Component interaction effects on the carotenoid

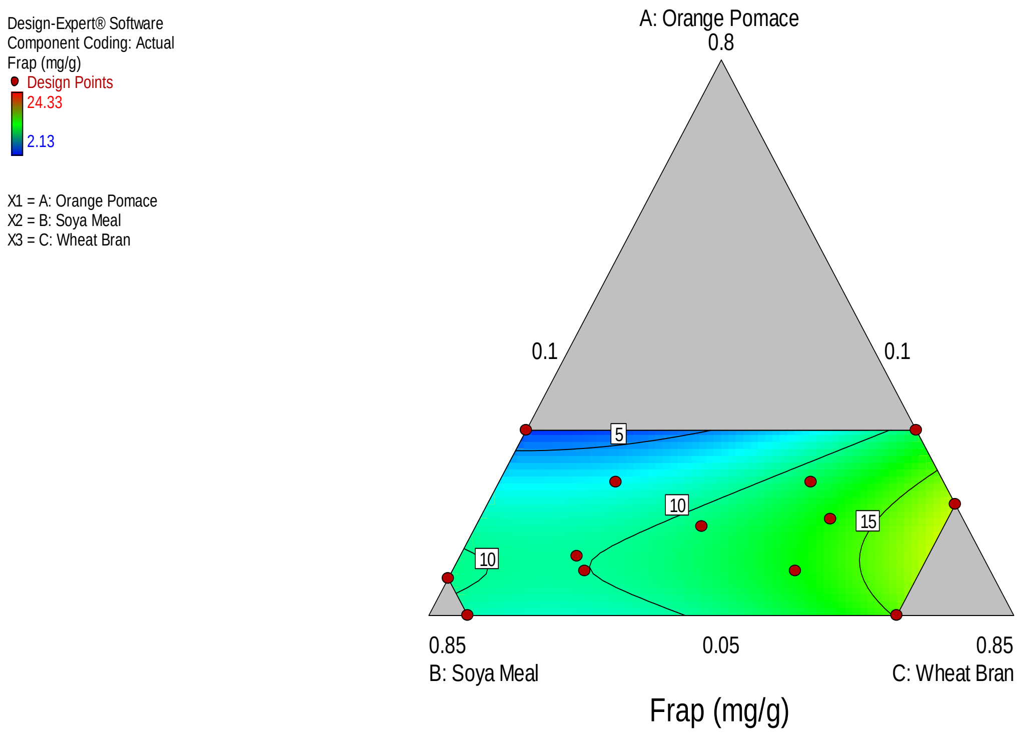

Figure 9. Mixture contour plot of ferric reducing antioxidant properties

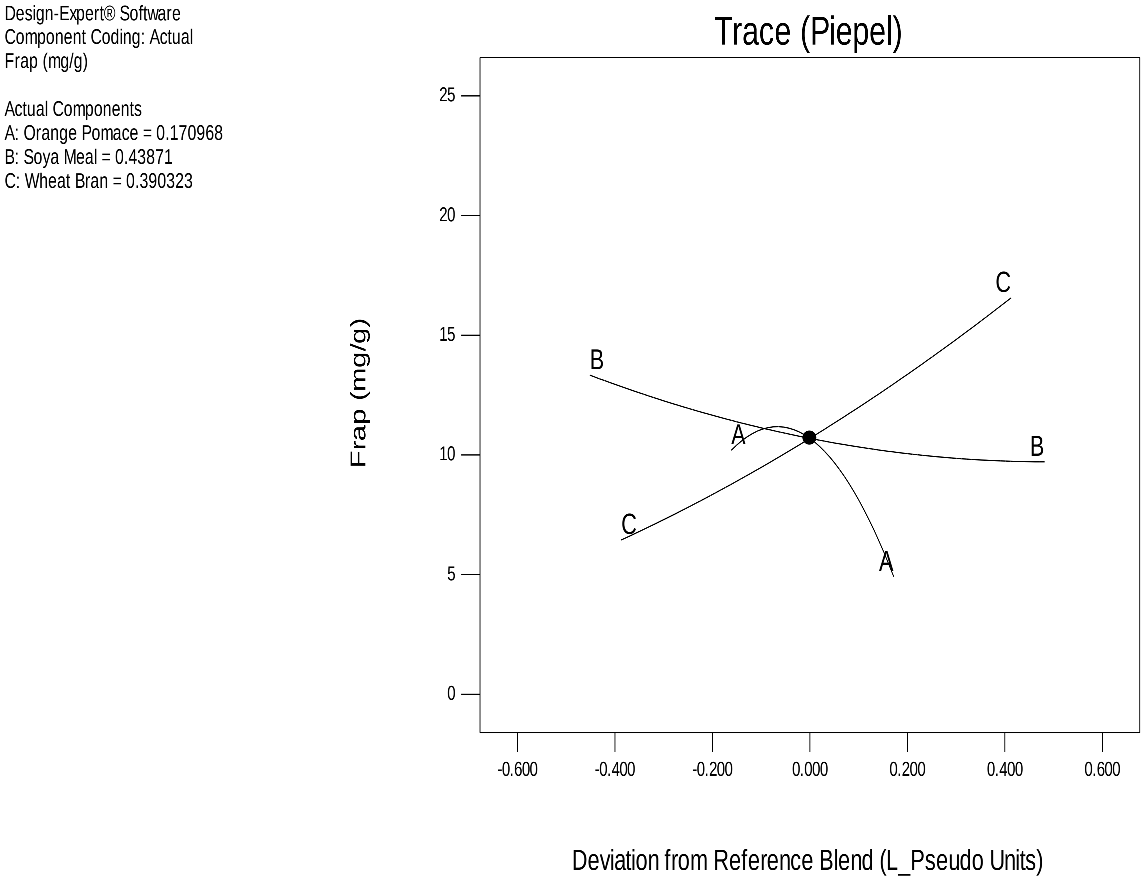

Figure 10. Component interaction effects on the ferric reducing antioxidant properties of the products

Table 2. Functional variables of extruded blends

Blend S/N	Orange pomace (%)	Soybean meal (%)	Wheat bran (%)	Bulk density (g/cm³)	Water solubility index (%)	Water absorption index (g/g)
1	13	62	25	0.58 ± 0.00^{b}	4.67 ± 2.31^{e}	2.93 ± 0.16^{e}
2	17	44	39	0.61 ± 0.01^{a}	9.33 ± 1.15^{bcd}	2.91 ± 0.01^{e}
3	5	80	15	0.59 ± 0.01^{b}	10.00 ± 0.00^{bcd}	3.19 ± 0.14^{de}
4	10	80	10	0.53 ± 0.01^{c}	12.67 ± 1.15^{ab}	3.18 ± 0.07^{de}
5	23	52	25	0.53 ± 0.01^{c}	8.67 ± 2.31^{bcde}	3.61 ± 0.06^{bcd}
6	20	10	70	0.44 ± 0.00^{g}	8.00 ± 0.00^{cde}	3.86 ± 0.05^{b}
7	5	25	70	0.46 ± 0.01^{f}	8.00 ± 2.00^{cde}	3.52 ± 0.20^{bcd}
8	23	27	50	0.46 ± 0.01^{f}	8.67 ± 1.15^{bcde}	3.69 ± 0.20^{b}
9	11	62	27	0.48 ± 0.01^{e}	12.67 ± 3.05^{ab}	3.67 ± 0.11^{b}
10	11	35	55	0.48 ± 0.01^{e}	11.33 ± 3.05^{abc}	3.49 ± 0.14^{bcd}
11	18	27	55	0.51 ± 0.01^{d}	14.67 ± 4.16^{a}	3.62 ± 0.18^{bc}
12	30	60	10	0.53 ± 0.01^{c}	6.00 ± 2.00^{de}	3.24 ± 0.06^{cde}
13	30	10	60	0.42 ± 0.01^{h}	8.00 ± 2.00^{cde}	4.29 ± 0.24^{a}

Averages of three replicates followed by different letters in the same column are significantly different ± Standard deviation

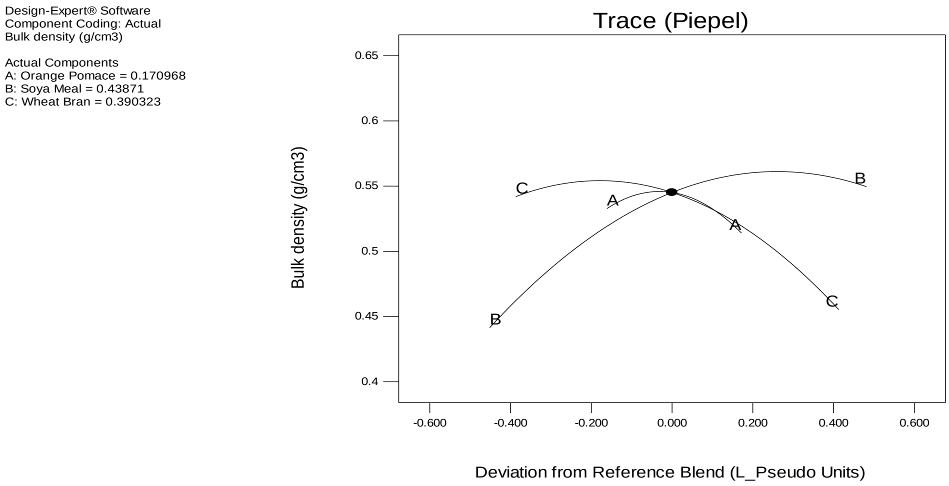

Figure 11. Component interaction effects on the bulk density of the products

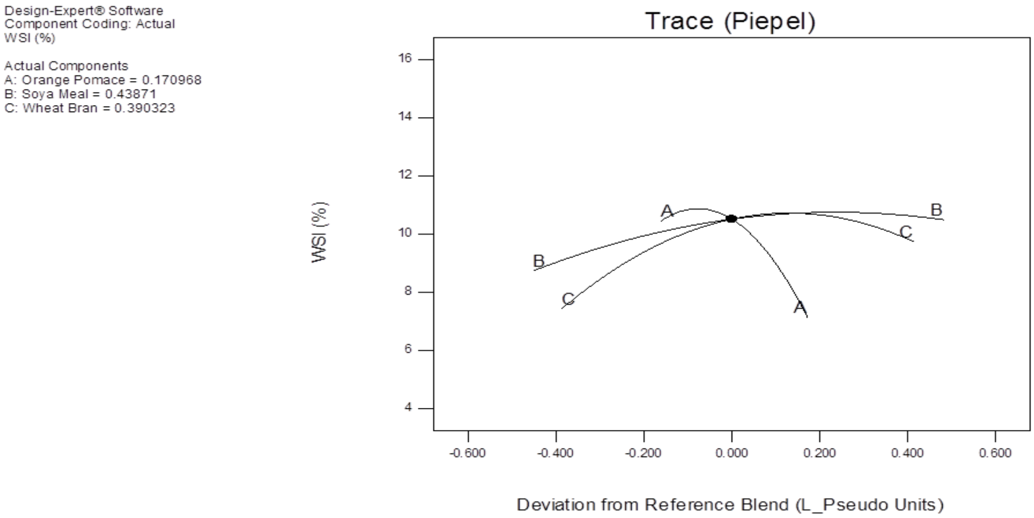

Figure 12. Component interaction effects on the water solubility index

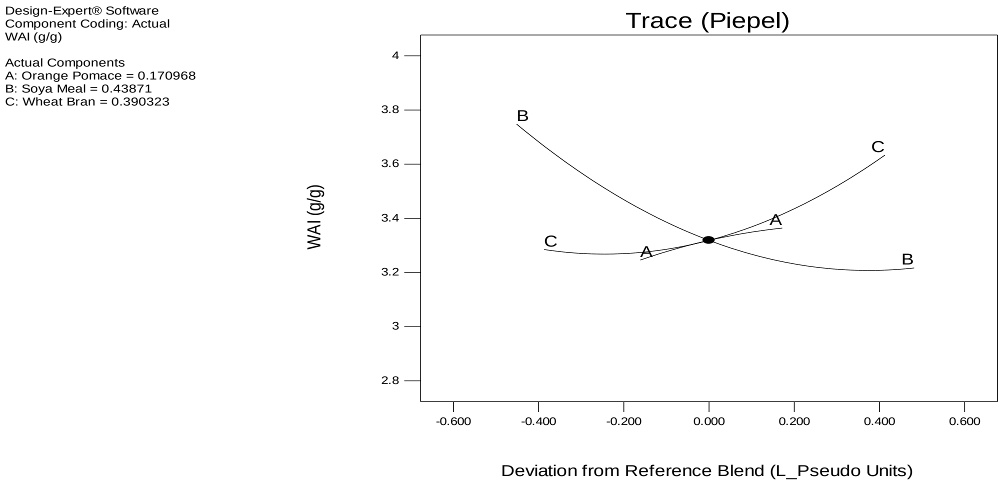

Figure 13. Component interaction effects on the water absorption index of the products

The increase in soybean meal content may be related to the solubilisation of proteins through extrusion caused by mechanical cutting (Ghumman et al., 2016).

Water absorption index

The water absorption index (WAI) of the extrudate was between 2.91 and 3.86 (Table 2). This value is lower than the 3.2 – 5.9 reported for snacks supplemented with tomato powder by Wojtowicz et al. (2018).
The water absorption index showed the amount of water restrained by the extrudate. WAI is ascribed to the distribution of starch in excess water, and the concentration is increased by the extent of starch destruction due to gelatinization and extrusion-induced breakup, which corresponds to a molecular weight decrease of amylose and amylopectin molecules (Yagci and Gogus, 2008). A decrease in the water absorption index with an increasing amount of soy flour can be linked to the lubricating effect of its lipids (Navarro-Cortez et al., 2016), which makes the oil in soybean to interfere with the water uptake. The predicted WAI trace plot shows an increase in wheat bran and pomace, which led to an increase in WAI while an increase in soybean meal led to the reduction in WAI (Figure 13). The high value of the water absorption index in products refers to their dietary fibre content and their ability to retain water in their matrix. This property may increase the faecal weight and possibly reduce the absorption of nutrients from the gastrointestine after ingestion (Gallaher and Schneeman, 2001).
Soybean meal wheat bran (BC) interaction affected WAI the leastwhile wheat bran contributed the most to the water absorption index of the products (Equation 3).

$$WAI = 3.02A + 3.31B + 4.19C + 0.51AB + 0.47AC - 2.07BC \quad (3)$$

Conclusions

The extrudates were high in TPC, flavonoid, carotenoid and ferric reducing antioxidant properties. Interactions between orange pomace and wheat bran contributed the most to the TPC, flavonoid and FRAP while pomace soybean meal interaction contributed the most to the carotenoid content.
Bulk density (BD) and water solubility index (WSI) were influenced mostly by the interaction of pomace and wheat bran while WAI was influenced by the interaction of orange pomace and soybean meal. Transformation of this waste makes it possible for citrus processing companies to reduce their cost of treatment, generate additional profits from what was previously considered waste and thus improve their competitiveness as well as reduce their environmental pollution.
The functional and antioxidant properties of the orange pomace-based food suggested that the food could be consumed for its health promoting benefits.

Author Contributions: Dr Oduntan, A. Oluwakemi conceptualised, designed, and carried out the experiment. She also analysed, interpreted the results and wrote the manuscript. Dr Arueya, Gibson supervised the experiment, proof read, corrected, and also contributed to the manuscript.

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*Conflicts of Interest: "*The authors declare no conflict of interest."

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