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Effects of industrial by-products from orange, peach palm and soybean on the quality traits and antioxidant activity of flours: a response surface approach

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Abstract

By-products from different sources vary widely in their composition and their functional properties. The aim of this study was to produce industrial by-product flours from orange (OF), soybean (SF) and peach palm (PF), besides formulating and characterizing blends with the use of a simplex-centroid mixture design. The formulation containing 100% PF had the highest ash content (67.9 g.kg^{-1}), while the highest lipid (155.8 g.kg^{-1}) and protein (268.2 g.kg^{-1}) contents were found in the formulation that had 100% SF. Total dietary fiber content of the blends produced ranged from 577.7 to 728.5 g.kg^{-1}. The pH and the water activity varied from 4.1 to 6.7 and from 0.484 to 0.611, respectively, indicating stability and safety for consumption of the formulations. The highest swelling volume (20.8 mL.g^{-1}), water and oil absorption (7.4 and 3.6 g.g^{-1}, respectively) was found for 100% PF, which is important to the industrial applications. The simultaneous optimization showed that 100% OF is the most interesting formulation due to the best balance of insoluble to soluble fiber (2:1) and the highest total phenolic content (4.5 mg gallic acid.g^{-1}) and antioxidant activity. However, the binary and ternary mixtures of by-product flours also had interesting functional properties, and they can be used for the development of healthier foods.

Introduction

Consumption habits of populations that associate high intake of sugars, salt, saturated and trans-fatty acids with low intake of fibers, vitamins and essential minerals have led to non-transmissible chronic-degenerative diseases (Granato *et al*., 2010). To minimize the risk of such illnesses, the development of new food products that contain biologically active substances has been proposed (Granato *et al*., 2014b). Functional foods can be defined as products in which the regular ingestion leads to important physiological effects that are separate and distinct from those associated with their role as nutrients (FDA, 2004). These products can provide additional health benefits that may reduce disease risk and/or promote optimal health (ADA, 2009).

The functional food and beverage market has attracted a large number of food and drink companies (Granato *et al*., 2010). One way to develop functional products is to replace part of wheat flour by other types of flours, which are rich in nutrients (Hornstra, 2001). The use of mixed flour technology should not interfere in the final quality of the products but could provide more nutrient-rich food (Chavan and Kadam, 1993).

By-products can be processed into flours that may be used to add nutritional and financial value to productive chains (Mildner-Szkudlarz *et al*., 2015). Among them, waste that result from soybean, orange and peach palm processing stands out. Okara is a soybean by-product generated from soybean hydrosoluble extract (soymilk) and tofu production; it contains not only protein, lipid, dietary fiber and minerals, but also isoflavones (Bedani *et al*., 2014). Large quantities of okara have been produced worldwide with the increase in soybean consumption, a fact that has posed a significant disposal problem. For each kilogram of soybean processed into soybean water-soluble extract (soymilk), equal or even more weight of okara is produced (Lu *et al*., 2013). In orange juice production, only around half of the fresh orange weight is transformed into juice. Therefore, the remaining 50% is waste constituted by peel, pulp, seeds and orange leaves. The orange mesocarp (albedo) is rich in soluble fibers (Garcia-Castello, 2011). The canning process of peach palm (*Bactris gasipaes* Kunth.) only uses its central part. Thus, the waste, which consists of shell, sheath and stem, represents 84% of the rod weight. Its stem is rich

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in insoluble fibers, minerals, antioxidants and other nutrients (Bolanho *et al*., 2014). High contents of nutrients, such as dietary fibers, proteins, antioxidant phytochemicals, minerals and vitamins highlight the importance of by-products in the development of new ingredients for the food industry, since raw materials may be thoroughly used. Technology used in by-product processing should facilitate storage and application of new ingredients (Day *et al.*, 2009).

Considering the wide variation in the composition and in the functional properties of by-products from different sources, the aim of this study was to produce industrial by-product flours from orange, soybean and peach palm and formulated blends with the use of a simplex-centroid mixture design in order to obtain an ingredient with improved characteristics to be applied to food products.

Materials and Methods

Flour production from by-products

Peach palm by-product flour (PF) was obtained from the stem discarded in the processing of canned products. Orange flour (OF) was processed from the by-product of juice production (albedo) without the peel. These by-products were cut (in strips format), washed by water immersion and sanitized in sodium hypochlorite solution (50 mg/L) for 15 min, followed by rinsing in water. Soybean flour (SF) originated from the waste of soybean hydrosoluble extract (soymilk) was donated by an agro industry ground as a ground and moist mass. The by-products were oven-dried with forced air circulation (Marconi, MA 035, Piracicaba, SP, Brazil) at 60ºC for 36 h. The dried material was ground by a knife mill type Willye (Solab, SL-031, Piracicaba, SP, Brazil), passed through a set of sieves from 25 to 100 USS/ASTM and subjected to vibration for 10 min. The flour was standardized to 100 Tyler mesh sieve was then used in this study (Bolanho *et al*., 2014).

Experimental design for flour blend formulation, statistical analysis and response surface methodology

A simplex-centroid design for mixtures of three components with two centroid point replications (Table 1) was used to study the effects of single, binary and ternary mixtures of OF, SF and PF on the nutritional and functional properties of the flours under investigation. The variables studied were different percentages (0, 33.3, 50 and 100%) of the by-product flours: OF, SF and PF. The sequence of experiments was performed randomly to avoid carry=over effects. The analyses were carried out in three individual replicates and results were expressed as mean ± standard deviation. Differences among samples were highlighted by one-way ANOVA whereas the Tukey's test was used for comparing the means. Statistical analyses were performed using the Statistica 7.0 software (StatSoft, Tulsa, OK, USA) (Granato *et al*., 2014a).

The Scheffé canonical Equation 1 was used for modeling the experimental data:

$$Y = \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{23}X_2X_3 + \beta_{123}X_1X_2X_3 \quad (1)$$

where Y is the response under study, β_1, β_2, β_3, β_{12}, β_{13}, β_{23} *and* β_{123} are the regression parameters and X_1, X_2 and X_3 are the levels of flours in the blends. Positive values for binary coefficients, β_{ij}, indicate synergistic effects while negative values represent antagonism. Regression coefficients with p<0.10 were regarded as significant while goodness of fit was measured by the lack of fit test as well as by the normality of the residuals (Kolmogorov-Smirnov test). Triangular contour plots were generated from the polynomial equations for each response.

Multi-response optimization of the flour composition was performed with the desirability function proposed by Derringer and Suich (1980). It aimed at maximizing *in vitro* antioxidant capacity (ABTS), water absorption and total dietary fiber. For this purpose, optimization was conducted with 60 interactions at exact grid points by the software Statistica 7.0.

Composition

The proximate composition and the determination of soluble (SDF) and insoluble dietary fibers (IDF) of the flour blends were determined in agreement with AOAC (2002). Total dietary fiber content (TDF) was obtained by adding up SDF and IDF values. Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were measured in agreement with the Van Soest system (Van Soest, 1967). The estimation of the contents of cellulose and hemicellulose was based on these analyses. The results were expressed in dry basis (d.b.).

Color, physicochemical and functional properties

The instrumental color parameters of the flours were determined by measuring the CIE-Lab components, namely L^* (lightness), $+a^*$ (red) $-a^*$ (green), and $+b^*$ (yellow) $-b^*$ (blue) by the colorimeter Color Reader (Konica Minolta, CR-10, Osaka, Japan). H (hue angle) was calculated according to Equation 2 when positive results were generated for the first quadrant $(+a^*, +b^*)$ and followed Equation 3 when negative values of a^* and positive values of b^* were obtained (second quadrant) (Mclellan *et al*.,

Table 1. Proximal composition, total, insoluble and soluble dietary fiber, cellulose and hemicellulose content ($g.kg^{-1}$) in dry basis (d.b.) of by-products flours and its blends

Assay	OF (%)	SF (%)	PF (%)	Moisture ($g.kg^{-1}$)	Ash ($g.kg^{-1}$)	Lipids ($g.kg^{-1}$)	Proteins ($g.kg^{-1}$)	TDF ($g.kg^{-1}$)	IDF ($g.kg^{-1}$)	SDF ($g.kg^{-1}$)	Cellulose ($g.kg^{-1}$)	Hemicellulose ($g.kg^{-1}$)
1	100	0	0	144.0 ± 0.2^{a}	38.5 ± 1.5^{c}	24.5 ± 1.4^{e}	49.1 ± 1.4^{f}	717.2	478.9 ± 26.0^{de}	238.3 ± 19.0^{a}	207.9 ± 1.5^{c}	16.3 ± 1.0^{d}
2	0	100	0	53.0 ± 0.1^{c}	44.5 ± 1.4^{c}	155.8 ± 5.0^{a}	268.2 ± 2.5^{a}	577.7	541.7 ± 35.1^{cde}	36.0 ± 3.5^{e}	175.9 ± 6.3^{d}	$99.6\pm4.2^{a,b}$
3	0	0	100	147.0 ± 0.4^{a}	67.9 ± 2.1^{a}	37.4 ± 4.3^{d}	104.1 ± 5.0^{d}	693.8	658.7 ± 41.5^{a}	35.1 ± 1.5^{e}	263.2 ± 9.5^{a}	$99.4\pm2.6^{a,b}$
4	50	50	0	106.0 ± 1.0^{b}	41.4 ± 1.5^{c}	106.2 ± 4.4^{b}	196.8 ± 9.9^{b}	617.1	472.9 ± 32.0^{e}	144.2 ± 12.0^{b}	$197.9\pm16.4^{c,d}$	44.7 ± 2.2^{c}
5	0	50	50	99.0 ± 0.3^{b}	54.4 ± 3.3^{b}	107.7 ± 5.1^{b}	197.6 ± 8.7^{b}	599.4	566.1 ± 26.1^{bcd}	33.3 ± 2.5^{e}	$242.0\pm5.8^{a,b}$	$94.3\pm4.0^{a,b}$
6	50	0	50	143.0 ± 1.4^{a}	51.3 ± 2.2^{b}	24.5 ± 1.5^{e}	63.1 ± 5.5^{e}	612.6	507.6 ± 23.3^{cde}	105.0 ± 7.1^{c}	$242.7\pm16.0^{a,b}$	16.3 ± 1.9^{d}
7	33.3	33.3	33.3	108.0 ± 0.1^{b}	51.5 ± 2.5^{b}	79.5 ± 3.2^{c}	154.6 ± 4.5^{c}	674.2	576.8 ± 32.2^{abc}	97.4 ± 5.4^{c}	$227.4\pm14.7^{b,c}$	$98.6\pm4.5^{a,b}$
8	33.3	33.3	33.3	112.0 ± 0.3^{b}	55.2 ± 1.5^{b}	75.4 ± 1.5^{c}	159.9 ± 3.4^{c}	728.5	637.3 ± 39.5^{ab}	91.2 ± 8.3^{c}	$247.7\pm5.9^{a,b}$	102.5 ± 2.7^{a}
9	33.3	33.3	33.3	102.5 ± 0.2^{b}	55.5 ± 3.1^{b}	83.2 ± 2.0^{c}	151.0 ± 2.5^{c}	672.7	597.2 ± 25.4^{abc}	75.5 ± 6.0^{d}	$200.9\pm10.5^{c,d}$	88.8 ± 7.5^{b}

Different letters in the same column are different by Tukey test ($p<0.05$). OF – Orange by-product flour, SF – soybean by-product flour, PF – peach palm by-product flour, TDF – total dietary fiber, IDF – insoluble dietary fiber, SDF – soluble dietary fiber.

1995).

$$H\ (h^o) = \tan^{-1} (b^*/a^*) \quad (2)$$
$$H\ (h^o) = 180 + \tan^{-1} (b^*/a^*) \quad (3)$$

The pH was measured by a potentiometer (Gehaka, PG 2000, São Paulo, SP, Brazil) with 10 g sample suspended in 100 mL water. Bulk density was measured through the relation between weight and volume by a graduated cylinder. Water activity was determined at 25°C by Aqualab 3TE® (Decagon Devices Inc., Pullman, WA, USA).

To determine the swelling volume of the particles (SV), 1 g sample was mixed with excess of distilled water and stirred for 2 h. After complete decantation, the volume occupied by the sample in the beaker was named swelling volume. The water absorption index (WAI) was measured by weighing 2 g sample in centrifuge tubes, adding 20 mL water and stirring continuously in a shaker. The mixture was centrifuged at 3000 x g for 10 min. The residue was weighed and the WAI was obtained by the weight ratio between the wet sediment and the dry matter. The oil absorption index (OAI) was determined the same way as the water absorption one, by using commercial soybean oil (Seibel and Beléia, 2009).

Total phenolic content and in vitro *antioxidant activity*

The extraction of antioxidants from flours was carried out by using 1 g sample in 10 mL of a 80% ethyl alcohol solution, with shaker agitation for 4 h (Hung *et al*., 2009). This procedure was carried out twice. The ethanolic extract was used for the determination of total phenolic content (TPC) by the Folin-Ciocalteau method (Swain and Hillis, 1959). TPC was determined by a standard curve prepared with gallic acid (Sigma, New Orleans, LA, USA). Results were expressed in milligrams of gallic acid equivalent per gram of sample.

The *in vitro* antioxidant activity (AA) of the ethanolic extracts was measured by different methods. The free radical scavenging toward ABTS (2,2-azino-bis-3-ethylbezothiazoline-6-sulfonic acid, Sigma-Aldrich Chemicals) was carried out with sample aliquots of 30 µL added to 3 mL ABTS solution with absorbance of 0.70±0.05 measured at 734 nm by a UV/VIS spectrophotometer (Femto 700 Plus, São Paulo, SP, Brazil). After a 6-minute reaction, the final absorbance was read at 734 nm (Re *et al*., 1999). The radical scavenging activity toward DPPH radical (2,2-diphenyl-1-picrylhydrazyl, Sigma-Aldrich Chemicals) was determined by using 1 mL acetate buffer 100 mmol/L pH 5.5, 1 mL absolute ethyl alcohol, 0.5 mL DPPH 250 µmol/L solution and 50 µL sample. After 30 min, absorbance was read at 517 nm by the spectrophotometer (Brand-Williams *et al*., 1995). The ferric reducing antioxidant power (FRAP) of the extracts was carried out with 2.7 mL FRAP reagent, 90 µL sample and 270 µL distilled water. After 30 min, absorbance was read at 595 nm by a spectrophotometer (Benzie and Strain, 1996). Results of these methodologies are expressed as µmol of Trolox equivalent per g of flour.

Results and Discussion

Composition

The composition of the by-product blends (Table 1) showed that the 100% PF had the highest ash content (67.9 $g.kg^{-1}$ d.b.) among the formulations. Assay 2 (100% SF) had the highest lipid (155.8 $g.kg^{-1}$ d.b.) and protein (268.2 $g.kg^{-1}$ d.b.) contents

Table 2. Color, functional and physicochemical properties of by-products flours and its blends

Assay	OF (%)	SF (%)	PF (%)	SV ($mL.g^{-1}$)	WA (g water.g^{-1})	OA (g oil.g^{-1})	Density ($g.mL^{-1}$)	pH	Aw	Instrumental color L*	a*	b*
1	100	0	0	10.3 ± 0.6^{cde}	5.6 ± 0.0^{c}	2.3 ± 0.0^{b}	0.5 ± 0.0^{b}	4.1 ± 0.3^{e}	0.522 ± 0.3^{b}	79.1 ± 0.6^{d}	0.3 ± 0.0^{c}	32.5 ± 0.4^{a}
2	0	100	0	12.3 ± 0.5^{bc}	3.7 ± 0.1^{f}	2.0 ± 0.0^{c}	0.6 ± 0.0^{a}	6.7 ± 0.2^{a}	0.583 ± 0.9^{a}	80.9 ± 0.3^{c}	2.3 ± 0.2^{a}	22.6 ± 0.6^{d}
3	0	0	100	20.8 ± 0.9^{a}	7.4 ± 0.3^{a}	3.6 ± 0.1^{a}	0.4 ± 0.0^{c}	6.1 ± 0.2^{b}	0.494 ± 0.4^{b}	86.2 ± 0.5^{a}	-0.3 ± 0.0^{de}	21.1 ± 0.2^{e}
4	50	50	0	9.0 ± 0.8^{f}	4.7 ± 0.1^{e}	2.1 ± 0.0^{bc}	0.5 ± 0.0^{b}	5.3 ± 0.5^{c}	0.611 ± 0.1^{a}	82.0 ± 0.2^{c}	-0.1 ± 0.0^{d}	29.3 ± 0.5^{b}
5	0	50	50	10.0 ± 0.0^{de}	5.2 ± 0.1^{d}	2.3 ± 0.1^{b}	0.4 ± 0.0^{c}	6.0 ± 0.1^{b}	0.495 ± 0.4^{b}	81.8 ± 0.6^{c}	1.4 ± 0.1^{b}	24.1 ± 0.6^{d}
6	50	0	50	12.5 ± 0.8^{b}	5.9 ± 0.1^{b}	2.2 ± 0.0^{bc}	0.4 ± 0.0^{c}	4.9 ± 0.3^{d}	0.484 ± 0.3^{c}	83.8 ± 0.4^{b}	-0.6 ± 0.0^{de}	26.7 ± 0.6^{c}
7	33.3	33.3	33.3	11.5 ± 0.9^{bcd}	5.3 ± 0.1^{c}	2.2 ± 0.0^{bc}	0.5 ± 0.0^{b}	5.5 ± 0.1^{c}	0.490 ± 0.2^{c}	84.0 ± 0.4^{b}	-0.4 ± 0.0^{de}	26.3 ± 0.8^{c}
8	33.3	33.3	33.3	11.1 ± 0.2^{bcd}	5.5 ± 0.0^{c}	2.8 ± 0.0^{bc}	0.5 ± 0.0^{b}	5.4 ± 0.2^{c}	0.497 ± 0.1^{b}	84.5 ± 0.4^{b}	-0.4 ± 0.0^{de}	25.7 ± 0.8^{c}
9	33.3	33.3	33.3	11.8 ± 0.5^{bcd}	5.6 ± 0.1^{c}	2.2 ± 0.1^{bc}	0.4 ± 0.0^{c}	5.5 ± 0.0^{c}	0.494 ± 0.3^{b}	83.7 ± 0.4^{b}	-0.5 ± 0.0^{de}	25.9 ± 0.8^{c}

Different letters in the same column are different by Tukey test ($p<0.05$). OF – Orange by-product flour, SF – soybean by-product flour, PF – peach palm by-product flour, SV – swelling volume, WA – water absorption, OA – oil absorption, A_w – water activity.

whereas the blends containing 50% of SF (assays 4 and 5) had the highest levels of these nutrients by comparison with the other blends. Flour blends containing SF (assays 4, 5, 7, 8, 9) had between 2-fold to 3-fold protein content (154.6 - 197.6 $g.kg^{-1}$ d.b.) in comparison with assay 6 which did not have this flour (63.1 $g.kg^{-1}$ d.b.).

Total dietary fiber content ranged from 577.7 to 728.5 $g.kg^{-1}$ d.b. (Table 1). Assay 1 (100% OF) had the highest content of soluble dietary fiber (238.3 $g.kg^{-1}$ d.b.). The IDF values (472.9 – 658.7 $g.kg^{-1}$ d.b.) were 2-fold to 19-fold higher than SDF values. In terms of health benefits, both types of fiber complement each other and it is considered a well balanced proportion if the content of IDF and SDF varied from 50 to 70% and 30 to 50%, respectively. OF has 67% of IDF and 33% of SDF; thus, it showed the most balanced DF composition among the formulations under analysis.

The analysis of acid (170.2 – 268.1 $g.kg^{-1}$ d.b.) and neutral detergent fiber (196.7 –351.4 $g.kg^{-1}$ d.b.) - data not shown - led to the determination of two fibrous components. The cellulose content was higher than the hemicellulose one, ranging from 175.9 to 263.2 $g.kg^{-1}$ d.b. and from 16.3 to 102.5 $g.kg^{-1}$ d.b., respectively (Table 1). Cellulose forms about one third of dietary fiber in vegetables and its insolubility in water helps to increase the fecal volume, promoting regular bowel movements (Mudgil and Barak, 2013). Hemicellulose, which can be both soluble and insoluble in water, is important for intestinal regulation, since it helps to increase the number of beneficial bacteria in the gut and directly binds cholesterol, preventing its absorption in the intestine (Mudgil *et al*., 2012).

Color, physicochemical and functional properties

Color is one of the most important quality parameters of foods. When some flour is added to a food product, its color may imply modification in the sensorial properties and limit its potential application. Among the produced flours, assay 3 (100% PF) showed the highest lightness and the lowest b^* value, while the other blends were darker and more yellow - L^* reduction and b^* increase (Table 2). The addition of PF to formulations 5, 6, 7 caused lighter color than assay 4, due to its color characteristics. The hue angle of flours and blends is closer to 90° (data not shown), which is the yellowness quadrant. To obtain lighter color in the flours under study, drying temperature is important; therefore, the chosen temperature was 60° C. Previous studies showed that higher temperatures cause browning of the flours, due to reactions that occur among their components, such as sugars and proteins (Bolanho *et al*., 2013). It is important that the fiber-rich ingredient has no negative effect on the color, texture, flavor and taste of the product (Kunzek *et al*., 2002).

The density of the blends ranged from 0.4 to 0.6 $g.mL^{-1}$. Akubor and Ukwuru (2003) found similar values of density in soy flour (0.6 $g.mL^{-1}$). Giami *et al.* (2000) stated that high density would facilitate closer packing and improve handling of large quantities of flour.

Assay 1 (100% OF) had the lowest mean pH value (4.1). The other blends had pH above 4.8; they are in the range of low acid foods (p>4.5), which are more subject to microbial growth. On the other hand, aw values (Table 5) ranged from 0.484 to 0.611. Thus, the formulations can be considered safe for storage and incorporation into food products, since these parameters are inadequate for microbial growth

Table 3. Total phenolic content and in vitro antioxidant activity evaluated by FRAP, DPPH and ABTS methods

Assay	OF (%)	SF (%)	PF (%)	TPC (mg gallic acid.g^{-1})	FRAP (μmol Trolox.g^{-1})	DPPH (μmol Trolox.g^{-1})	ABTS (μmol Trolox.g^{-1})
1	100	0	0	4.5±0.3^{a}	49.7±0.5^{a}	67.6±0.5^{a}	4.3±0.2^{a}
2	0	100	0	0.1±0.0^{e}	7.6±0.6^{e}	26.8±0.4^{e}	0.6±0.0^{e}
3	0	0	100	1.3±0.1^{d}	17.2±0.3^{d}	29.6±0.6^{d}	2.3±0.1^{c}
4	50	50	0	2.7±0.2^{c}	29.9±1.3^{c}	55.2±1.1^{b}	2.9±0.2^{b}
5	0	50	50	1.1±0.1^{d}	16.3±0.2^{d}	28.0±0.3^{de}	1.6±0.0^{d}
6	50	0	50	3.3±0.0^{b}	37.5±0.7^{b}	56.5±0.3^{b}	2.9±0.1^{b}
7	33.3	33.3	33.3	2.7±0.0^{c}	30.9±2.8^{c}	47.8±0.9^{c}	2.5±0.1^{c}
8	33.3	33.3	33.3	2.5±0.0^{c}	28.5±1.8^{c}	50.5±0.7^{c}	2.7±0.2^{c}
9	33.3	33.3	33.3	2.6±0.0^{c}	33.7±2.0^{c}	52.9±0.5^{c}	2.4±0.1^{c}

– peach palm by-product flour, TPC – total phenolic content.

(Khouryieh and Aramouni, 2012).

Regarding the functional properties (Table 2), assay 3 (100% PF) had the highest swelling volume (21.2 mL.g^{-1}) and water absorption (7.3 g of water.g^{-1}), while, in the other formulations, these values ranged from 9.0 to 12.5 mL.g^{-1} and 3.7 to 5.9 g of water.g^{-1}, respectively. Fibers contribute markedly to the hydration properties; it is important because they can influence consistency, thickness, viscosity and adhesion, besides affecting the texture quality of food products such as soups, sauces and baked goods (Kunzek *et al*., 2002). The highest oil absorption was also found in assay 3 (3.6 g of oil.g^{-1}) whereas, in the other formulations, values varied from 2.0 to 2.8 g of oil.g^{-1}. This property is attributed to the combination of fat to non-polar groups of proteins or others lipophilic groups. It is an interesting fact because the higher the OA, the higher the flavor and aroma retention (Jitngarmkusol *et al*., 2008).

Total phenolic content and in vitro *antioxidant activity*

Formulation 1 (100% OF) had the highest total phenolic content (4.5 mg gallic acid.g^{-1}) and antioxidant activity analyzed by FRAP, DPPH and ABTS assays - 49.7, 67.6, 4.3 μmol Trolox.g^{-1} respectively - (Table 3). According to Fernández-López *et al.* (2009), the major groups of phenolic compounds occurring in orange by-products were flavanones (the most important ones), flavones and hydroxycinnamic acids. Among the other blends, formulation 6 (50% OF and 50% PF) had the highest content of TPC (3.3 mg gallic acid.g^{-1}) and AA evaluated by the FRAP methodology (37.5 μmol Trolox.g^{-1}). This formulation had similar scavenging capacity DPPH and ABTS free radicals as compared to assay 4 (50% OF and 50% SF). Simas *et al*. (2009) found 1.3 mg gallic acid.g^{-1} in flour produced from king palm by-product. Phenolic compounds are important molecules that react with free radicals *in vivo*, decreasing the risk of development of a wide variety of illnesses (Rajendran *et al.,* 2014). Kunzek *et al*. (2002) highlighted that flours made of by-products should contain a balanced ratio of soluble and insoluble fractions and adequate amounts of bioactive components (phenolic compounds and/or carotenoids).

Response surface modeling (RSM)

The experimental design followed by RSM analysis was used for choosing the best formulation, from both nutritional and functional points of view. The triangular contour plots are shown in Figure 1 and the generated models with all statistical data are presented in Table 4. To obtain blends with high protein content, the addition of higher levels of SF and the interaction between SF and OF, as well as SF and PF, is worthy (Figure 1A). The model was highly significant ($p<0.001$) and able to explain 99.95% of data variability. No lack of fit was observed ($p=0.891$). With regard to total dietary fiber, the effect of each component (SF, PF, OF) and the ternary mixture increased ($p<0.001$) the content of fibers in the blends considerably. The RSM model showed that the model presented $R^2=0.8445$ with no lack of fit ($p=0.576$) and that the residuals followed a normal distribution (KS=0.218, $p=0.248$); thus, the data fit the quadratic model satisfactorily. Likewise, the RSM models generated for insoluble ($R^2=0.8666$, $R^2_{adj}=0.7865$, $p=0.013$) and soluble dietary fibers ($R^2=0.9894$, $R^2_{adj}=0.9789$, $p<0.001$) had high determination coefficients and residuals were normally distributed (KS=0.188, $p=0.476$; KS=0.155, $p=0.771$). These data clearly indicate the suitability of the generated models to explain the experimental results. The triangular contour plots showed that either the PF

Table 4. Regression coefficients and statistical parameters of the RSM models

Parameters	Regression coefficients	Standard error	t-value	p-value	-95% confidence	95% confidence
Protein content						
(A)OF	4.235	0.249	17.005	<0.001	3.442	5.027
(B)SF	25.295	0.249	101.575	<0.001	24.502	26.087
(C)PF	8.915	0.249	35.797	<0.001	8.122	9.707
AB	11.427	1.089	10.491	0.002	7.961	14.894
AC	-4.612	1.089	-4.237	0.024	-8.077	-1.148
BC	2.868	1.089	2.634	0.078	-0.597	6.332
R^2	0.9995					
R^2_{adj}	0.9986					
P-value	<0.001					
Total dietary fiber						
(A)OF	60.603	2.112	28.698	<0.001	54.740	66.466
(B)SF	52.786	1.961	26.922	<0.001	47.342	58.229
(C)PF	58.483	2.112	27.694	<0.001	52.620	64.346
AC	-28.571	11.091	-2.576	0.062	-59.366	2.223
ABC	208.425	48.941	4.259	0.013	72.543	344.308
R^2	0.8445					
R^2_{adj}	0.6891					
P-value	<0.001					
Insoluble dietary fiber						
(A)OF	38.627	2.302	16.777	<0.001	32.708	44.546
(B)SF	49.647	2.302	21.563	<0.001	43.728	55.566
(C)PF	54.311	2.302	23.588	<0.001	48.392	60.230
ABC	174.552	52.540	3.322	0.021	39.494	309.611
R^2	0.8666					
R^2_{adj}	0.7865					
P-value	0.013					
Soluble dietary fiber						
(A)OF	20.719	0.738	28.075	<0.001	18.670	22.768
(B)SF	3.719	0.738	5.039	0.007	1.670	5.768
(C)PF	3.022	0.805	3.753	0.020	0.786	5.258
AC	-35.150	3.520	-9.986	0.001	-44.924	-25.377
BC	22.850	3.520	6.491	0.003	13.076	32.623
R^2	0.9894					
R^2_{adj}	0.9789					
P-value	<0.001					
Ash content						
(A)OF	3.270	0.131	25.051	<0.001	2.934	3.606
(B)SF	4.202	0.131	32.191	<0.001	3.866	4.538
(C)PF	5.738	0.131	43.958	<0.001	5.402	6.074
ABC	12.384	2.979	4.158	0.009	4.727	20.041
R^2	0.9725					
R^2_{adj}	0.956					
P-value	<0.001					
Lipid content						
(A)OF	1.868	0.408	4.577	0.006	0.819	2.918
(B)SF	14.984	0.408	36.705	<0.001	13.935	16.034
(C)PF	3.244	0.361	8.992	<0.001	2.316	4.171
AB	4.156	1.946	2.136	0.086	-0.847	9.158
R^2	0.9925					
R^2_{adj}	0.9881					
P-value	<0.001					
Cellulose content						
(A)OF	18.337	0.671	27.313	<0.001	16.611	20.062
(B)SF	16.782	0.759	22.099	<0.001	14.830	18.734
(C)PF	22.743	0.759	29.950	<0.001	20.791	24.695
BC	9.947	3.618	2.749	0.040	0.647	19.246
R^2	0.9026					
R^2_{adj}	0.8441					
P-value	0.001					
Hemicellulose content						
(A)OF	0.946	0.627	1.508	0.206	-0.796	2.687
(B)SF	8.871	0.582	15.235	<0.001	7.255	10.488
(C)PF	8.426	0.627	13.435	<0.001	6.684	10.167
AC	-13.143	3.294	-3.990	0.016	-22.289	-3.997
ABC	107.419	14.535	7.390	0.002	67.064	147.775
R^2	0.9785					
R^2_{adj}	0.9571					
P-value	0.001					
Water absorption						
(A)OF	5.504	0.189	29.188	<0.001	5.042	5.965
(B)SF	3.708	0.189	19.663	<0.001	3.246	4.169
(C)PF	7.144	0.188	37.902	<0.001	6.683	7.605
R^2	0.9571					
R^2_{adj}	0.9428					
P-value	<0.001					
Oil absorption						
(A)OF	2.112	0.281	7.527	<0.001	1.425	2.798
(B)SF	1.912	0.281	6.814	<0.001	1.225	2.598
(C)PF	3.259	0.280	11.624	<0.001	2.573	3.946
R^2	0.6436					
R^2_{adj}	0.5248					
P-value	0.045					
Swelling volume						
(A)OF	10.300	0.366	28.113	0.001	8.724	11.876
(B)SF	12.300	0.366	33.572	0.001	10.724	13.876
(C)PF	20.820	0.366	56.826	<0.001	19.244	22.396
AB	-9.200	1.795	-5.126	0.036	-16.923	-1.477
AC	-12.200	1.795	-6.797	0.021	-19.923	-4.477
BC	-26.240	1.795	-14.619	0.005	-33.963	-18.517
ABC	62.619	9.707	6.451	0.023	20.851	104.386
R^2	0.9972					
R^2_{adj}	0.9887					
P-value	0.001					
Total phenolic content						
(A)OF	460.857	19.375	23.787	<0.001	407.065	514.649
(B)SF	73.509	20.867	3.523	0.024	15.572	131.445
(C)PF	147.949	20.867	7.090	0.002	90.012	205.885
BC	-395.914	109.599	-3.612	0.023	-700.20	-91.619
ABC	1834.631	483.609	3.794	0.019	491.917	3177.345
R^2	0.9852					
R^2_{adj}	0.9704					
P-value	<0.001					
Antioxidant activity - FRAP						
(A)OF	52.455	2.844	18.441	<0.001	45.495	59.415
(B)SF	10.295	2.844	3.619	0.011	3.335	17.255
(C)PF	21.023	2.843	7.394	<0.001	14.066	27.980
R^2	0.941					
R^2_{adj}	0.9213					
P-value	<0.001					
Antioxidant activity - DPPH						
(A)OF	67.302	2.132	31.560	<0.001	61.381	73.222
(B)SF	26.932	1.954	13.781	<0.001	21.506	32.358
(C)PF	29.733	1.954	15.215	<0.001	24.307	35.159
AB	37.091	9.327	3.977	0.016	11.194	62.988
AC	36.704	9.322	3.937	0.017	10.822	62.585
R^2	0.9891					
R^2_{adj}	0.9782					
P-value	<0.001					
Antioxidant activity - ABTS						
(A)OF	4.285	0.136	31.543	<0.001	3.908	4.662
(B)SF	0.660	0.124	5.300	0.006	0.314	1.006
(C)PF	2.360	0.124	18.956	<0.001	2.014	2.706
AB	1.946	0.594	3.275	0.031	0.296	3.596
AC	-1.454	0.594	-2.448	0.071	-3.102	0.195
R^2	0.9907					
R^2_{adj}	0.9814					
P-value	<0.001					

OF – Orange by-product flour, SF – soybean by-product flour, PF – peach palm by-product flour.

or the combination between PF and SF increased the insoluble fiber content (Figure 1C) whereas OF was the main contributor to the soluble fiber content (Figure 1D). The ash content was significantly ($p<0.001$) increased not only by the PF but also by the ternary mixture of the three flours (Figure 1E). The mathematical equation was able to explain up to 97% of the data. Residuals did not present trends (plack of fit=0.910) and followed a normal distribution (KS=0.176, p=0.578). Experimental results for lipid content were fitted suitably (R^2=0.9925, R^2_{adj}=0.9881, $p<0.001$) and indicated that the SF was the major contributor to increase the total lipid content of the flours (Figure 1F). The cellulose content was positively affected by all three flours, especially by PF, followed by OF (Figure 1G). The RSM equation satisfactorily adjusted the experimental data (R^2=0.9026, R^2adj=0.8441, $p<0.001$) with no lack of fit (p=0.845) and normally distributed residuals (KS=0.236, p=0.159). The same trend was observed for hemicellulose, that is, the multiple linear regression model was adequate to describe the results (KS=0.167, p=0.668, plack of fit=0.295) in which PF and SF were the main components which increased the hemicellulose content (Figure 1H) while OF did not contribute to increase this variable (p=0.206).

Water absorption was positively increased by the PF followed by OF and SF and no binary/ternary mixture was significant (Figure 1I). The regression model was able to explain almost 96% and the linear model fitted the data well (KS=0.238, p=0.148, plack

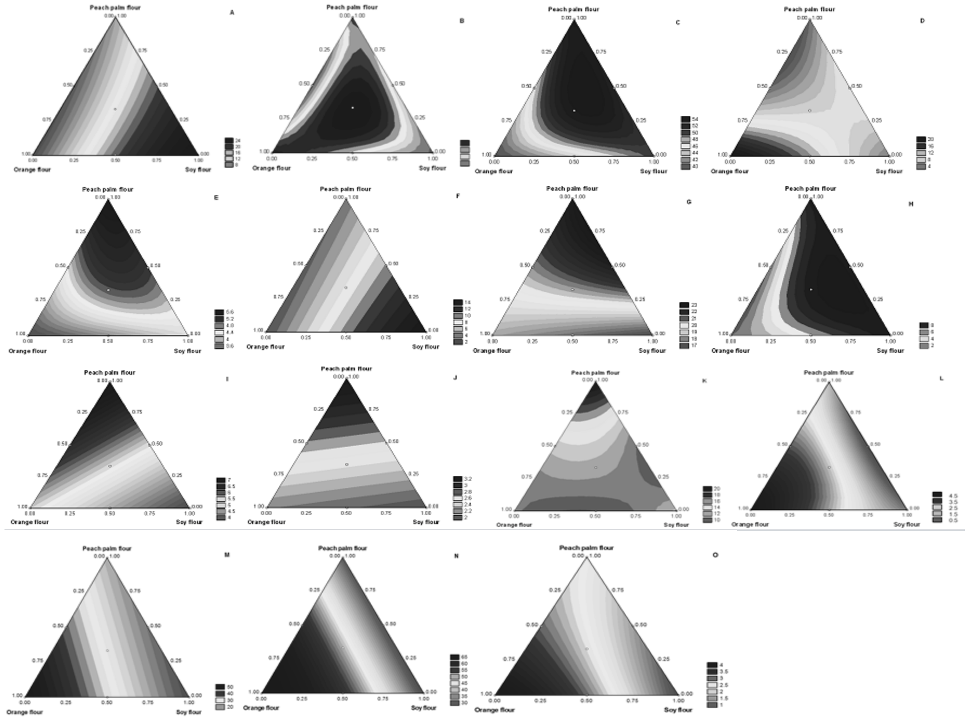

Figure 1. Triangular plots to explain the experimental data obtained for total protein content (A), total dietary fiber (B), insoluble fiber (C), soluble fiber (D), ash content (E), lipid content (F), cellulose content (G), hemicellulose content (H), water absorption (I), oil absorption (J), swelling volume (K), total phenolic content (L), FRAP (M), DPPH (N), and ABTS (O).

of fit=0.300). Oil absorption seemed to be correlated to the content of PF while the other flours did not seem to increase this variable (Figure 1J) although a statistical significance was observed in Table 4. The regression model for oil absorption had a low determination coefficient ($R^2<0.700$); thus, this equation cannot be used for prediction purposes. Among the functional properties, the swelling volume was positively correlated ($p<0.05$) with water absorption (r=0.70) and oil absorption (r=0.89). It increased when PF was added to the flour blends (Figure 1K).

The addition of OF to the blends led to higher content of total phenolic compounds (Figure 1L) and antioxidant activity, since the TPC was positively correlated ($p<0.05$) with the antioxidant activity evaluated by the ABTS (r=0.94), DPPH (r=0.98) and FRAP (0.99) methods. The RSM model generated for TPC was highly significant ($p<0.001$): it had $R^2=0.9852$, $R^2_{adj}=0.9704$ and fitted the experimental results well (plack of fit=0.498, KS=0.117, p=0.975). Regarding the antioxidant activity assays, the RSM was also applied to try to explain the experimental results mathematically. For FRAP, the linear model seems to adjust the data well (plack of fit=0.327, KS=0.180, p=0.548); it explains about 94% of data variability and was highly influenced by the content of OF, followed by PF (Figure 1M). Likewise, DPPH was also more influenced by OF (Figure 1N). The interaction between OF and SF, as well as the combination of OF and PF, had a positive effect on the radical scavenging activity toward the DPPH radical. The quadratic model explained almost 99% of data variability. Additionally, no lack of fit was observed (p=0.704) and residuals had a normal distribution (KS=0.201, p=0.366). The scavenging activity of the ABTS radical was highly influenced by OF ($p<0.001$), followed by PF and, to a lesser extent, by SF and the combination of OF and SF. This quadratic model explained up to 99% of data variability. Figure 1O shows the triangular plot in which the experimental results can be better observed.

The simultaneous optimization was conducted to increase the content of total fibers, antioxidant activity and water absorption of the blended flours. It may be observed that the flour containing only orange by-product met this condition. The d-value of this optimization model was 0.99867, meaning that this formulation was the best solution that satisfies the initial requirement regarding the maximization of the selected responses.

Conclusion

The produced by-product flours were important to the nutritional, functional and physicochemical properties of the blends under study. The RSM was helpful to assess the effects of three different flours on the quality traits of blends that can be used by food companies as potentially functional ingredients. From the experimental data, orange flour had the highest antioxidant activity, total phenolic content and soluble dietary fiber; the optimization procedure confirmed this initial finding. The binary and ternary mixtures of orange, peach palm and soybean by-product flour had interesting functional properties that can be better explored in the formulation of healthier products.

References

ADA - American Dietetic Association. 2009. Position of the American Dietetic Association: functional foods. Journal of the American Dietetic Association 109 (4): 735-746.

Akubor, P. I. and Ukwuru, M. U. 2003. Functional properties and biscuit making potential of soybean and cassava flour blends. Plant Foods for Human Nutrition 58 (3): 1-12.

AOAC - Association of Official Analytical Chemists. 2002. Official methods of analysis of AOAC International. 17th ed. Gaithersburg: AOAC International.

Bedani, R., Rossi, E. A., Cavallini, D. C. U., Pinto, R. A., Vendramini, R. C., Augusto, E. M., Abdalla, D. S. P. and Saad, S. M. I. 2014. Influence of daily consumption of symbiotic soy-based product supplemented with okara soybean by-product on risk factors for cardiovascular diseases. Food Research International 73 (10): 142-148.

Benzie, I. F. F. and Strain, J. J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. Analytical Biochemistry 239 (1): 70-76.

Bolanho, B. C., Danesi, E. D. G. and Beléia, A. P. 2013. Peach palm (*Bactris gasipaes* Kunth) characterization and the potential of by-products flour processing. Food Science and Technology Research 19 (6): 1061-1069.

Bolanho, B. C., Danesi, E. D. G. and Beléia, A. P. 2014. Characterization of flours made from peach palm (*Bactris gasipaes* Kunth) by-products as a new food ingredient. Journal of Food and Nutrition Research 53 (1): 51-9.

Brand-Williams, W., Cuvelier, M. E. and Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity. Lebensmittel-Wissenschaft Technologie - Food Science and Technology 28 (1): 25-30.

Chavan, J. K. and Kadam, S. S. 1993. Nutritional enrichment of bakery products by supplementation with nonwheat flours. Critical Reviews in Food Science and Nutrition 33 (3): 189-226.

Day L, Seymour R. B., Pitts K. F., Konczak I. K. and Lundin L. 2009. Incorporation of functional ingredients into foods. Trends in Food Science Technology 20 (9): 388-395.

Derringer, G. and Suich, R. 1980. Simultaneous optimization of several response variables. Journal of Quality Technology 12 (4): 214–219.

Food and Drug Administration (FDA). (October 2004). Bureau Circular No. 16. Probiotics. Retrieved on April 28, 2007 from FDA Website: *http://www.fda.gov.ph/attachments/article/17107/bc%2016%20s%202004.pdf.*

Fernández-López, J., Sendra-Nadal, E., Navarro, C., Sayas, E., Viuda-Martos, M. and Alvarez, J. A. P. 2009. Storage stability of a high dietary fibre powder from orange by-products. International Journal of Food Science and Technology 44 (4): 748-756.

Garcia-Castello, E. M., Mayor, L., Chorques, S., Argüelles, A., Vidal-Brotóns, D. and Gras, M. L. 2011. Reverse osmosis concentration of press liquid from orange juice solid wastes: Flux decline mechanisms. Journal of Food Engineering 106 (3):199-205.

Giami, S. Y., Adindu, M. N., Akusu M. O. and Emelike, J. N. T. 2000. Compositional, functional and storage properties from raw and heat processed African breadfruit (*Treculia africana* Decne) seeds. Plant Foods Human Nutritional 55 (4): 357-368.

Granato, D., Grevink, R., Zielinski, A. A. F., Nunes, D. S. and Van Ruth, S. M. 2014. Analytical strategy coupled with response surface methodology to maximize the extraction of antioxidants from ternary mixtures of green, yellow, and red teas (*Camellia sinensis* var sinensis). Journal of Agricultural and Food Chemistry 62 (42): 10283-10296.

Granato, D., Calado, V. M. A. and Jarvis, B. 2014. Observations on the use of statistical methods in food science and technology. Food Research International 55:137-149.

Granato, D., Branco, G. F., Nazzaro, F., Cruz, A. G. and Faria, J. A. F. 2010. Functional foods and nondairy probiotic food development: trends, concepts, and products. Comprehensive Reviews in Food Science and Food Safety 9 (3): 292-302.

Hornstra, G. 2001. Importance of polyunsaturated fatty acids of the n-6 and n-3 families for early human development. European Journal of Lipid Science Technology 103 (6): 379-389.

Hung, P. V., Maeda, T., Miyatake, K. and Morita, N. 2009. Total phenolic compounds and antioxidant capacity of wheat graded flours by polishing method. Food Research International 42 (1): 185-190.

Jitngarmkusol, S.; Hongsuwankul, J. and Tananuwong, K. 2008. Chemical compositions, functional properties, and microstructure of defatted macadamia flours. Food Chemistry 110 (1): 23-30.

Khouryieh H. and Aramouni F. 2012. Physical and sensory characteristics of cookies prepared with flaxseed flour. Journal Science Food Agricultural 92(11):2366-2372.

Kunzek H., Müller S., Vetter S. and Godeck R. 2002. The significance of physic chemical properties of plant cell wall materials for the development of innovative food products. European Food Research and Technology 214(5):361-376.

Lu, F., Liu, Y. and Li, B. 2013. Okara dietary fiber and hypoglycemic effect of okara foods. Bioactive Carbohydrates and Dietary Fiber 2 (2): 126-32.

Mclellan, M. R., Lind, L. R. and Kime, R. W. 1995. Hue angle determinations and statistical analysis for multiquadrant Hunter L,a,b data. Journal of Food Quality and Safety 18 (3): 235-240.

Mildner-Szkudlarz, S., Siger, A., Szwengiel, A. and Bajerska, J. 2015. Natural compounds from grape by-products enhance nutritive value and reduce formation of CML in model muffins. Food Chemistry 172: 78-85.

Mudgil, D. and Barak, S. 2013. Composition, properties and health benefits of indigestible carbohydrate polymers as dietary fiber: A review. International Journal of Biological Macromolecules 61 (6): 1-6.

Mudgil, D., Barak, S. and Khatkar, B. S. 2012. X-ray diffraction, IR spectroscopy and thermal characterization of partially hydrolyzed guar gum. International Journal of Biological Macromolecules 50 (4): 1035-1039.

Rajendran, P., Nandakumar, N., Rengarajan, T., Palaniswami, R, Gnanadhas, E. N., Lakshminarasaiah, U., Gopas, J. and Nishigaki, I. 2014. Antioxidants and human diseases. Clinica Chimica Acta 436: 332-347.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. and Rice-Evans, C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine 26 (9): 1231-1237.

Seibel, N. F. and Beléia, A. P. 2009. Características químicas e funcionalidade tecnológica de ingredientes de soja [*Glycine Max* (L.) Merrill]: carboidratos e proteínas. Brazilian Journal of Food Technology 12 (2): 113-122.

Simas, K. N., Vieira, L. N., Podestá, R., Müller, C. M. O., Vieira, M.A., Beber, R. C., Reis, M. S., Barreto, P. L. M., Amante, E. R. and Castanho-Amboni, R. D. M. 2009. Effect of king palm *(Archontophoenix alexandrae)* flour incorporation on physicochemical and textural characteristics of gluten-free cookies. International Journal of Food Science and Technology 44 (3): 531-538.

Swain, T. and Hillis, W. E. 1959. The phenolic constituents of Prunus domestica - the quantitative analysis of phenolic constituents. Journal of Science Food Agriculture 10 (1): 63-68.

Van Soest, P. J. 1967. Development of comprehensive system of feed analyses and its application to forages. Journal of Animal Science 26 (1): 119-128.