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Broccoli and Carrot Industrial Solid Waste Characterization and Application in the Bread Food Matrix

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Abstract: Aiming at the necessity and possibility of reuse of the residues coming from agroindustry that still have nutritional benefits, this work aims to characterize the constituents present in beet, broccoli and carrot residue, and to evaluate the possibility of addition in bread products. the residue was dried in a vacuum oven and crushed. Chromatographic analysis was performed to determine the content of fatty acids and vitamin C, as well as chemical analysis to determine the ash content, fat, pH and acidity of the material. The residue was applied in the preparation of bread of the form type in association with carrot powder to evaluate the physicochemical characteristics of the product, through the Central Rotational Compound Design (DCCR) for the best provisions of the formulations that were studied. The specific volume, density, expansion index, acidity, pH and volume produced were evaluated, and statistically there was analysis of variance and Tukey's test. The results showed that the amount of vitamin C present in the analyzed residue is 918.57 mg / 100g. The amount of fatty acids in the sample is 2.04%. The ash and lipid content of the sample were 4.84%, and 2.13%, respectively. The pH of the residue is 4.7, while the total acidity thereof is about 10.63%. When used in combination with powdered carrots in breads, there was improvement in the parameters of specific volume and decrease in the density of the products as they were added characteristics that are desired. Therefore, the residue of beet, broccoli and carrots from the local juice industry can be used in the food industry as a source of nutrients, adding nutritional value.

Keywords: Agroindustry Waste, Chromatography, Bread Quality, Fatty Acids Profile

1. Introduction

Food loss over the entire food value chain represents a significant Loss of resources invested in food production, transport, and Storage [1]. Thus, food loss may Cause substantial environmental impact. Furthermore, economically avoidable food losses are of high importance in the efforts to combat hunger and to improve food security, not only in developing but also in developed countries. Improving the efficiency of the food value chain could help bring down the cost of food to the consumer and thus increase access to low-income households [2].

In general, composted organic wastes have high nutrient contents and exhibit high ion exchange, which can reduce the need for mineral fertilizer use through increased fertigation efficiency [3], this broadens the application potential for food formulations, aiming at nutritional enrichment.

The presence of bioactive molecules, such as fatty acids and phenolic compounds, in agro-industrial waste makes fruit and vegetable leftovers more valuable for the food industry.

Fruits and vegetables contain bioactive compounds that impart health benefits beyond basic nutrition [4].

Broccoli (Brassica oleracea L. var. Italica) contains substantial amount of health-promoting compounds such as vitamins, glucosinolates, phenolic compounds, and dietary essential minerals; thus, it benefits health beyond providing just basic nutrition, and consumption of broccoli has been increasing over the years [5], consequently, incorporating some of these broccoli health promoting compounds directly or added to pharmaceutical products (nutraceutical) or other foods (functional foods) once they have been isolated and extracted from this vegetable, is a safe and effective way to guard against many of today's most common diseases [6].

Numerous epidemiological studies indicate that Brassica vegetables in general, and broccoli in particular, protect humans against cancer, since they are rich sources of glucosinolates as well as of other phytochemicals [7].

Intensive broccoli cultivars are associated with the production of considerable waste products mainly leaves that are discarded even though they may have a similar composition to the edible parts of the plant.

These by-products could complement animal feed or be a source of nutraceuticals, which would reduce environmental impact and, at the same time, increase economic value. In the scant published scientific literature dealing with the nutritional content of broccoli leaves, as compared with the parts normally consumed, studies have been made of amino acids, fatty acids, vitamins, phenolic compounds, minerals and glucosinolates [8, 9, 10 and 11].

Carotenoids are fat soluble phytochemicals largely responsible for the red, orange and yellow color of fruits and vegetables, as for example, carrot. Interest in these compounds arises from their purported health benefits. The increased awareness of the health benefits associated with carotenoids has brought a surge of interest in identifying specific food formulations and processing conditions so as to maximize the potential of carotenoid-rich foods to confer the health benefits [12]. The cis isomers contribute 18-23% of the total carotenoid content in the carrot purees [13].

Carrot pulp represents another important agro-food waste. This particular waste has been shown to have high amounts of phenolic compounds and dietary fiber, which give some physical characteristics to the carrot. For example, anthocyanins and carotenoids are responsible for the color, aroma and bitterness of carrots [14]. Moreover, its phenolic acids have a strong antioxidant potential, and anthocyanins have been proven to reduce cardiovascular heart disease by decreasing the inflammation and lipid oxidation [15].

This work had as objective to develop a mixture from industrial byproducts of broccoli and carrot and its application in bread as alimentary matrix.

2. Materials and Methods

The waste of broccoli and carrot were donated by an industry from the city of Fortaleza, Ceará, Brazil and were obtained from cold extraction of juices. They were shredded and oven dried for 60 minutes at 60°C for 12 hours and had their granulometry adjusted to 20 mesh with the aid of a sieve. Then the waste was mixed to form a 50%/50% w/w blend and stored in dark glass bottles until the time of analysis.

2.1. Blend Waste Quality

2.1.1. Proximate Composition

Proximate composition of the control and optimized formulation was determined using [16]. Moisture content was determined using the oven drying method, 925.10; crude protein by Kjeldahl digestion and distillation (N x 5.66 for blend waste) was measured according to the Method 920.87. Crude fat was determined by hexane extraction using Method 945.16 and ash was determined by dry-ashing at 550°C according to the Method 923.03. Total available carbohydrates were calculated by difference, i.e. 100 − (% moisture + % protein + % fat + % ash).

2.1.2. Vitamin C

The vitamin C content of the sample broccoli and carrot waste was determined according [17] using a high performance liquid chromatograph (Perkin Elmer Corp., Series 200, Norwalk, CT, USA). The column used was a reverse C_{18} (Brownlee Validated, RP-18, Spheri-5, PerkinElmer, Waltham, Massachusetts, USA). Isocratic chromatographic separation was carried out using a mobile phase of Milli-Q water with acetic acid (0.1 mL/100 mL) and methanol in a relative proportion of 95:5 (mL: mL). The eluent flow-rate was 0.7 mL/min, and the column temperature was 30°C. Ascorbic acid was identified by comparing the retention time of the sample peak with that of the ascorbic acid standard at 254 nm. Quantification was carried out using external standardization.

To quantify vitamin C content, 10 g of sample and 5 mL of 0.045 g mL^{-1} metaphosphoric acid solution were placed into a 15 mL centrifuge tube. The tube was shaken for 30 min and centrifuged (Cientec, model 500R, Brazil) for 10 min at 5°C (3000× g). Finally, the solution was filtered using a PTFE membrane of 0.45 µm, and 40 µl was injected into the HPLC system. All the HPLC analyses were done, at least, in triplicate. For the determination of the vitamin C content, an analytical curve was constructed at concentrations of 100 (mg/L), 200 (mg/L), 500 (mg/L), 750, with areas of the chromatographic peaks of 5875721 mV, 12843355 mV, 27240878 mV, 43108820 mV and 58491152 mV, respectively corresponding to the concentrations. Thus, an equation of the type y = ax + b was obtained. The retention time of the vitamin C standard is about 5 minutes.

2.1.3. Fatty Acids Profile

After initial preparation and lyophilization of the analyzed material, lipids were extracted with a Soxhlet extractor (VELP SCIENTIFICA ser 148 Solvent Exractor). 50 mg of lipids were collected to obtain fatty acids. Fatty acid esters were obtained according to PN-EN ISO 12966-1:2015-01 and PN-EN ISO 12966-2:2011 standards and AOAC Official

Method 969.33 (1999). The ester samples were analyzed using a Varian 3800 gas chromatograph with a FID detector and a CP-Wax 52CBWCOT Fused Silica capillary column (length: 60 m, inner diameter: 0.25 mm). The initial temperature for the analysis was 120°C for 5 min and the final temperature was 210°C. The injector temperature was 260°C and the detector temperature was 260°C. The hydrogen flow rate was 30 ml/min, air flow − 300 ml/min, and helium flow − 1.4 ml/min. The volume of the injected sample was 1 µl. The results for the percentage content of fatty acids in the sample were obtained using Star GC Workstation Version 6.30.

2.2. Bread Processing

Bread samples were prepared with different broccoli and carrot at different proportions as presented in Table I. Second order design matrix used for the evaluation of the effects of process variables on some physical properties of dough and bread.

All ingredients were mixed in a semi-industrial mixer, following a preliminary mixing of dry ingredients for 1 min at low speed. Mixing was done for 3 minutes at medium speed and for 6 minutes at high speed. A quantity of water was added in the mixer at the beginning of the medium speed period.

Dough temperature at the end of mixing was T = 26.0°C ±1.0°C. After the mixture the dough was left to rest for 5 min and then divided in pieces of 100.0 g and hand-molded in an ellipses form. Dough fermentation was performed in fermentation chamber (Perfecta, Brazil) at 28.0°C±2.0°C and 70.0% relative humidity for two hours in fermentation process. The doughs were baked without stream at 220.0°C for 20 minutes and cooled at room temperature (28.0°C±2°C).

Table 1. *Second order design matrix used for the evaluation of the effects of process variables on some physical properties of dough and bread.*

Run	Broccoli waste (X_1)	Carrot waste (X_2)	Broccoli waste (%)	Carrot waste (%)
1	-1	-1	3.75	3.75
2	+1	-1	6.25	3.75
3	-1	+1	3.75	6.25
4	+1	+1	6.25	6.25
5	-1,41	0	2.5	5.0
6	+1,41	0	7.5	5.0
7	0	-1,41	5.0	2.5
8	0	+1,41	5.0	7.5
9	0	0	5.0	5.0
10	0	0	5.0	5.0
11	0	0	5.0	5.0

2.3. Bread Quality

2.3.1. pH and Total Titratable Acidity

Total titratable acidity (TTA) was determined after homogenization of 10 g of dough with 90 ml of distilled water, and expressed as the amount (ml) of 0.1 M NaOH required to neutralize the solution, using phenolphthalein as indicator.

The pH value of bread was determined by a pHmeter (Model 507, Crison, Milan, Italy).

2.3.2 Maximum Expansion Factor

The dough pieces have been considered as having the geometry of a truncated ellipse. The volume expansion of the dough piece was evaluated from the vertical and horizontal expansion. It was assumed that the ratio between all the dimensions, of the truncated ellipse observed at the beginning of the expansion, was kept constant during the expansion and/or contraction of the dough, according to methodology proposed by [18].

The dough volume for each measurement time was calculated based on the formula of the ellipse and its rotation around the y axis, as shown in equation 1.

$$V\ (cm^3) = \pi a^2\ (\frac{2b}{3} + b' - \frac{b\prime^3}{3b^2}) \quad (1)$$

Where: a = ellipse width (cm); B = ellipse height - from the center to the upper ellipse (cm); B '= height of the ellipse - from the center to the bottom (cm).

The volume expansion factor (cm³) - FEV was calculated using equation 2.

$$VEF = \frac{V - V_0}{V_0} \quad (2)$$

2.3.3. Volume Produced During Fermentation Process

The volume produced was calculated from dough portions of approximately 15 g, which were placed in previously sterilized beakers of 50 mL for measuring the volumes of the dough during the fermentation process. For the calculation of the volume produced (ΔV) the subtraction between the final volume produced and the initial dough volume in the beakers was used, the results expressed in mL.

2.3.4. Specific Volume

The breads were weighed after cooling and their volume (mL) was determined by rapeseed displacement method. The specific volume (mL/g) was calculated as loaf volume/bread weight.

2.4. Statistical Analysis

Response surface methodology was used for the optimization of variables in the present study. A central composite design (CCRD) was used to study the effect of two independent variables at five levels on response pattern and to determine the optimum combination of variables. Eleven experiments were conducted to study the effect of processing parameters like broccoli and carrot wastes levels on physical properties of bread. The independent variables optimized were X_1 (Broccoli waste), X_2 (Carrot waste) for dependent response Y_1 (Maxium Expansion Factor), Y_2 (Volume Produced), Y_3 (Specific Volume), Y_4 (Total titratable acidity) and Y_5 (pH). The second degree polynomial model proposed for responses was:

$$Y = \beta_o + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X^2 + \beta_{22} X^2 + \beta_{12} X_1 X_2 \quad (3)$$

The coefficients of the polynomial were represented by b_0 (constant term), b_1 and b2 (linear effects), b_{11} and b_{22}

quadratic effects) and b_{12} (interaction effects).

The data obtained from experimental runs was analyzed for analysis of variance (ANOVA) and regression models using STATISTICA software (StatSoft, Tulsa, OK). A second-order polynomial model was fitted to the data to obtain regression equations. The model terms were examined for statistical significance. The significance of the models was analyzed using model analysis, lack of fit test and coefficient of determination (R^2). The effect of variables at linear, quadratic and interactive level on the response was analyzed.

3. Results & Discussion

Figure 1 shows the waste blend (50%/50% w/w) of broccoli and carrot chromatogram.

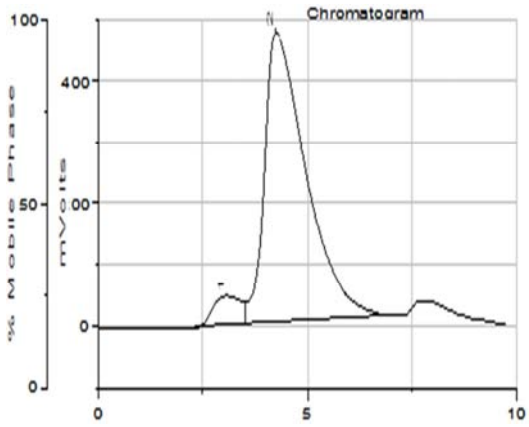

Figure 1. *The waste blend of broccoli and carrot chromatogram.*

The area generated by the curve is inserted in the equation obtained to calculate the concentration of vitamin C in the sample in mg/L. Based on the analytical curve generated, an area equivalent to 55305248 mV, a concentration of 918.57 mg of vitamin C present in the sample analyzed every 100 g can be observed, characterizing the waste blend of broccoli and carrots as rich in vitamin C and with a good potential for use in food matrices.

The Figure 2 presents the Vitamin C analytical curve.

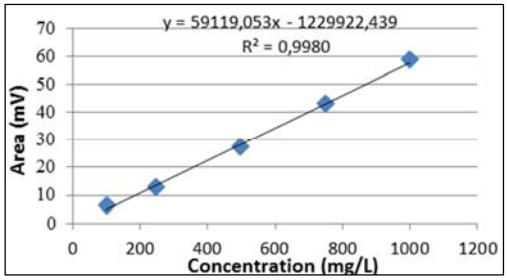

Figure 2. *Vitamin C Analytical Curve.*

According the [19] is recommended a daily intake of 90 mg of vitamin C per day for a diet of 2.000 kcal. In this way, the inclusion of 10% of the waste blend is sufficient to supply the nutritional demand of this component per day. Because vitamin C is water soluble, its risk of severe toxicity from overeating is low, since all excess is easily excreted in the urine. A very large daily intake, usually above 2000 mg per day, is needed to make it toxic.

[20] found broccoli vitamin C content values ranging from 35 35 mg 100 g^{-1} to 75 mg 100 g^{-1}. [21], the vitamin c present in broccoli ranges from 89.30 mg 100 g^{-1} to 106.40 mg 100 g^{-1}. The vitamin C content in flower buds of freshly harvested broccoli was 96.52 mg 100 g^{-1} on average [22] therefore; the broccoli waste contributes strongly to the result obtained for vitamin C.

The vitamin C content for carrots is 35.57 mg 100 g^{-1} [23] and 33.07 to 37.98 mg 100 g^{-1} [24] According [25], the majority of broccoli stem cell reflected by large amounts of insoluble dietary fiber (32.62±0.63 g/100 g dry weight).

The Figure 3 presents the wastes blend fatty acids profile obtained by chromatography.

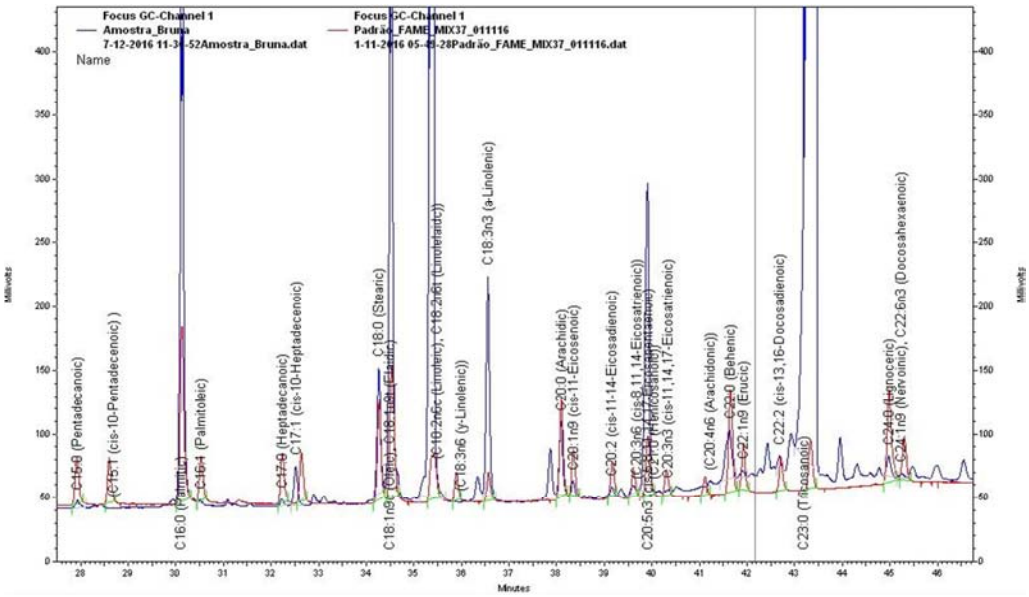

Figure 3. *Wastes blend fatty acids profile.*

The fatty acids present in the sample are confirmed by the figure, the chromatogram illustrates, in millivolts per minute, the comparison of the peaks of the sample, represented by the blue color lines, with the standards (FAME MIX37-011116) established, represented by the lines in red.

The Table 2 presents the wastes blend (Broccoli/Carrot 50/50 w/w) fatty acids profile detailed.

Table 2. *Wastes blend fatty acids profile.*

Fatty Acids	**% in the sample**
C4:0 (Butyric acid)	0,0007
C6:0 (Caproic acid)	Not detected
C8:0 (Caprylic acid)	Not detected
C10:0 (Capric acid)	Not detected
C11:0 (Undecanoic acid)	Not detected
C12:0 (Lauric acid)	0,0028
C13:0 (Tridecanoic acid)	Not detected
C14:0 (Myristic acid)	0,0021
C14:1 (Myristoylic acid)	Not detected
C15:0 (Pentadecanoic acid)	0,0021
C15:1 (Pentadecenoic acid)	Not detected
C16:0 (Palmitic acid)	0,1819
C16:1 (Palmitoleic acid)	0,0004
C17:0 (Heptadecanoic acid)	0,0015
C17:1 (Heptadecenoic acid)	0,0104
C18:0 (Stearic acid)	0,0281
C18:1n9c (Oleic acid) and C18:1n9 (Elaidic acid)	0,2413
C18:2n6c (Linoleic acid) and C18:2n6 (Linolelaic acid)	0,5132
C18:3n6 (Linolenic acid)	Not detected
C18:3n3 (Trans-Linolenic acid)	0,0901
C20:0 (Arachidonic acid)	0,0168
C20:1n9 (Gadoic acid)	0,0035
C20:2 (Eicosadienoic acid)	0,0027
C20:3n6 (Eicosatrienoic acid)	0,0042
C21:0 (Heneicosanoic acid) and C20:3n3 (Eicosatrienoic acid)	0,0546
C20:4n6 (Arachidonic acid)	0,0009
C20:5n3 (Eicosapentaenoic acid)	Not detected
C22:0 (Behenic acid)	0,0017
C22:1n9 (Erucic acid)	0,0017
C22:2 (Docosahexaenoic acid)	0,0039
C23:0 (Tricosanoic acid)	0,8631
C24:0 (Lignoceric acid)	0,0046
C24:1n9 (Nervonic acid) and C22:6n3 (Docosahexaenoic acid)	0,0025
Fatty Acids Classes	% in the sample
Saturated fatty acids	1.1607
Unsaturated fatty acids	0.9301
Monounsaturated fatty acids	0.2576
Polyunsaturated fatty acids	0.6699
Omega-3 (ω-3) fatty acids	0.1448
Omega-6 (ω-6) fatty acids	0.5184
Omega-9 (ω-9) fatty acids	0.2492

Table 2 presents more clearly the fatty acids evaluated and those detected, with their respective percentages of presence in the sample. It is also worth mentioning the presence in a significant quantity of unsaturated fatty acids (0.9301%), polyunsaturated fatty acids (0.6699%), with emphasis to Omega-6 fatty acids (0.5184%).

The fatty acids in wastes blend were concentrated in 16 to 24 carbon atoms in chain length. Similar results were found by [26] when studying the fatty acid profile of carrot seeds through supercritical extraction. [27] proved that the fatty acid composition of the broccoli leaves were 18:3n3 (α-linolenic), 18:2n6 (linoleic) and 16:0 (palmitic acid). In this way, the fatty acid profile found for the wastes blend presents mixed characteristics of broccoli and carrot, being considered a supplier of fatty acids, mainly, of the class omega.

The prescription Omega-3 fatty acid products are indicate as an adjunct to diet to reduce triglyceride levels in patients with severe (≥500 mg/dL) hypertriglyceridemia at a dose of 4 g/day [28]. The lipid content in the wastes blend was 2.13%±0.12%, below that obtained by [29] to broccoli flours, therefore, [30] reported values ranged between 2.5-4.2%.

The low lipid and moisture content (6.83%±0.09%) of the wastes blend corroborates to the increase of its useful life, since the damages through lipid oxidation are not intense, according to a similar argument used by [31] to cauliflower soup powder.

Acidity is an important parameter in the evaluation of the state of conservation of a food product. The acidity values found in wastes blend powder ranged from 10.63% to 10.65%. The pH values found ranged from 4.72 to 4.80. In this way, because it is an acid product, low humidity and lipid content, its useful life is probably high.

Thus, the wastes blend has the potential to be applied in a food matrix, in this case, bread. Therefore, different amounts of wastes broccoli and carrot powder were added in bread formulations and the effect of this on the physical properties of quality was verified.

Figure 4 presents the response surfaces for the maximum expansion factor, produced and specific volume.

It can be estimated that the volume produced and the maximum dough expansion factor is high (> 3.00 cm^3) when the addition of up to 4% of broccoli waste powder and carrot waste powder occurs together. It has been found that carrot powder contributes to the increase in the maximum expansion factor when broccoli is maintained at up to 3.5%, behavior that can be verified through the regression model presented in Table 3. A similar result was found for the volume produced, where the optimization zone comprises volumes between 20 mL and 24 mL.

The specific volume was significantly ($p \leq 0.05$) influenced by the addition of broccoli and carrot wastes. The proposed mathematical model designed a minimum region, obtaining values of 2.43 mL/g, when 5.33% of broccoli waste powder and 4.64% of carrot waste powder were added. Therefore, the addition of these wastes by up to 3% promotes breads with specific volumes greater than 4.00 mL/g.

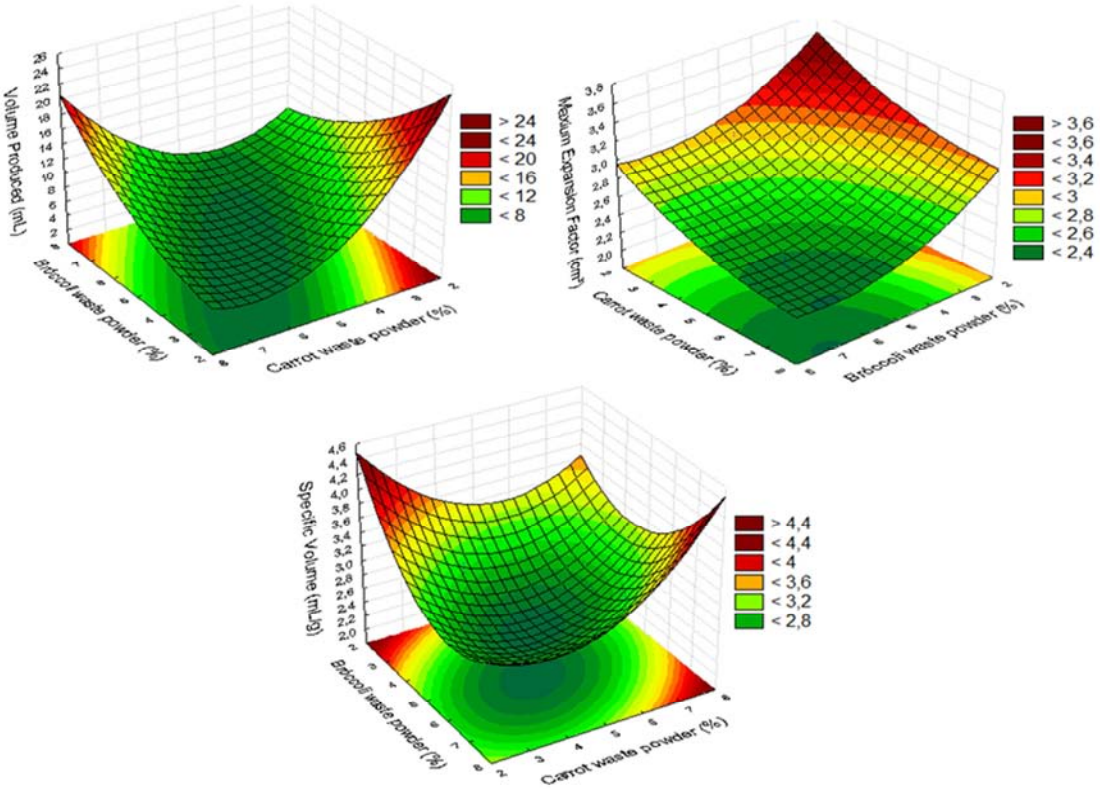

Figure 4. *Surface response of volume produced, maximum expansion factor and specific volume of breads with different proportions of broccoli and carrot waste.*

The Table 3 presents the Mathematical model by regression analysis for the bread physical properties.

Table 3. *Mathematical model by regression analysis ($p \leq 0.05$).*

Physical Properties	Model	R^2
Specific Volume (mL/g)	$Y = 2.44 + 0.19X_1^2 - 0.06X_2 + 0.12X_2^2 + 0.11X_1X_2$	0.8492
Produced Volume (mL)	$Y = 5.40 + 0.69X_1^2 - 0.59X_2 + 1.26X_2^2 + 1.50X_1X_2$	0.9244
Maxium Expansion Factor (cm^3)	$Y = 2.55 - 0.14X_1 + 0.06X_1^2 - 0.12X_2 + 0.02X_2^2$	0.9328

The reduction of dough fermentative potential and, consequently, the specific volume of the breads can be explained by some hypotheses. Due to its high ash content (6.55% for broccoli waste powder and 8.17% for carrot waste powder), They are potentially damaging to gluten network according [32]. The incorporation of fibers or minerals allows a poor formation or weakening of the gluten network, allowing this carbon dioxide to escape, reducing the bread specific volume. Therefore, this incorporation can promote the escape of the carbon dioxide produced during the fermentation process, which can reduce the bread specific volume as observed in this study.

4. Conclusions

It was found that the broccoli and carrot waste from a local juice industry still contained significant amounts of vitamin C and fatty acids, which could be increased in the human diet in the form of powder, for example. If it is reused, the waste can add commercial and nutritional values to new processed products. In breads, as the flour of the residue was added in association with the powdered carrot, it was observed the increase of the specific volume and decrease of the density of the products, characteristics that are well accepted by the great part of the consumers.

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