Effect of the addition of whole grain wheat flour and of extrusion process parameters on dietary fibre content, starch transformation and mechanical properties of a ready-to-eat breakfast cereal

Ludmilla C. Oliveira[a,b], Cristina M. Rosell[b*] and Caroline J. Steel[a]

[a] University of Campinas, Department of Food Technology, School of Food Engineering, Postal Code 6121, Campinas, Brazil (millinha_oliveira@hotmail.com) (steel@fea.unicamp.br)

[b] Institute of Agrochemistry and Food Technology (IATA/CSIC), Avenida Agustin Escardino 7, Paterna, 46980 Valencia, Spain (crosell@iata.csic.es)

Running title: Whole grain wheat in the extrusion process

Abstract

This study evaluates the effect of the incorporation of whole grain wheat flour (WGWF) and of extrusion process parameters on the nutritional and technological quality of breakfast cereals. The corn flour based breakfast cereals were elaborated in a twin-screw extruder following a rotatable central composite design with varied WGWF (0-100%), feed moisture (14-24%) and zones 3 and 4 barrel temperature (76-143°C). Dietary fibre and resistant starch were significantly increased with WGWF addition. Total and digestible starch showed a decrease when WGWF increased. The RVA parameters were significantly affected by all the extrusion conditions and WGWF content. The cell structure of the extrudates was dependent of WGWF and moisture.

Key words: extrusion; whole grain; breakfast cereals; image analysis; response surface methodology.

Highlights:

- The use of the whole grain wheat flour in ready-to-eat breakfast cereals is proposed;
- Whole grain wheat flour improves the nutritional quality of extruded cereals;

- Adjustments of the extrusion process and whole grain content to overcome the technological (physical) limitations caused by the presence of fibre are suggested;
- An alternative methodology for structure analysis (not microscopy) is used with good performance for expanded extruded cereals.

1. Introduction

Currently, ready-to-eat (RTE) breakfast cereals are becoming an important part of the diet because they do not require any further preparation such as cooking. Besides this, they are affordable, convenient and nutrient-dense and may assist households in achieving recommended daily intakes of nutrients (Albertson et al., 2013). Wheat and maize are the most common raw materials for producing breakfast cereals. They are primarily used as refined flours, which have lost a great part of important nutrients like fibres, vitamins and minerals (Rosell, 2012). However, to attend the health and nutrition policies and satisfy the demands of increasingly health conscious consumers, many food processors are adding functional ingredients and fortifying with micronutrients. Special consideration is being given to the increase of dietary fibre content, mainly in grain-based products such as snacks and breakfast cereals (Peressini et al., 2015, Holguín-Acuña et al., 2008, Brennan et al., 2008). In this context, the use of whole grains in extruded products provides the answer to obtain healthier breakfast cereals. Whole grains are known by consumers as a health-promoting ingredient, because they are rich in fermentable carbohydrates such as dietary fibre, resistant starch and oligosaccharides. The intake of whole grains has been related to physiological functions like lowering the risk of cardiovascular diseases, diabetes and cancer; regulating digestion and also contributing to the immune system and to body weight management (Ye et al., 2012, Jonnalagadda et al., 2011, Marquart et al., 2007, Anderson et al., 2000, Anderson et al., 2009). The nutritional properties of dietary fibre present in whole grains make it an ideal ingredient to improve the quality of extruded products.

Extrusion cooking is a versatile, low cost and very efficient technology in food processing. It is a high-temperature and short-time process in which moistened, expansive, starchy and/or protein food materials are plasticized and cooked by a combination of moisture, pressure, temperature and mechanical shear, resulting in molecular transformations and chemical reactions. This technology is widely used by food industries in the production of ready-to-eat breakfast cereals, baby foods, flat breads, snacks, meat analogues, and modified starches (Moore, 1994, Moscicki and Zuilichem, 2011, Ryu and Ng, 2001, Castells et al., 2005, Havck and Huber, 1989). The intense mechanical shear applied to the material is able to break the covalent bonds in biopolymers, and the intense structural disruption and mixing facilitate the modification of functional properties of food ingredients and/or their texturizing (Carvalho and Mitchell, 2000, Asp and Bjorck, 1989). Nowadays, the positive and negative effects of the extrusion process on macronutrient structures, that depend on extruder conditions (temperature, feed moisture, screw speed and screw configuration) and raw-material characteristics (composition and particle size), are known (Camire et al., 1990, Jing and Chi, 2013, Sarawong et al., 2014, Slavin, 2003). The changes in chemical compounds include starch gelatinization, protein denaturation, complex formation between amylose and lipids, and degradation reactions of vitamins and pigments, depending on the type of raw material and extrusion cooking variables (Brennan et al., 2011, Singh et al., 2007, Ryu and Ng, 2001). Most of the studies carried out on the extrusion process involving wheat as a raw material have been focused on white or refined wheat flour (Ding et al., 2006, Ryu and Ng, 2001). Despite wholegrain wheat being regarded as a major ingredient to improve the nutritional properties of extruded products, only a few scientific studies have been published in this field. Specifically, Robin et al. (2012) studied the effect of whole wheat flour blended with refined wheat flour and Chassagne-Berces et al. (2011) the effect of whole grain flour and wheat or oat bran blended with refined wheat flour, corn and sugar. These authors evaluated the effect of fibre content and extrusion process parameters (temperature, feed moisture and screw speed) on physical properties of the extrudates. Ferreira et al. (2012) obtained corn-based expanded extruded snacks containing wheat bran (0-24.6%) and assessed, among others, the internal structure, expansion index and hardness of the products. The incorporation of whole grain flour or fibre rich ingredients in the formulation of expanded extruded products has been associated with a decrease in expansion, a change in the microstructure and a loss of the general technological quality. Thus, it is still a challenge to advance in the knowledge of the extrusion process to obtain both good mechanical aspects and desirable nutritional characteristics of extruded breakfast cereals.

It is known that corn flour produces the most desirable extruded products in terms of the structural properties because of the unique characteristics associated to corn starch. Therefore, the replacement of corn flour by whole grain wheat flour might be a good alternative to overcome the drawbacks derived from whole grain. That replacement requires optimizing the process variables to obtain both nutritional improvement and acceptable technological characteristics of the final product. Response surface methodology (RSM) is recommended for extrusion processing studies since it enables exploring the relationships between the responses and the experimental levels of each factor and deducing the optimum conditions (Triveni et al., 2001). The objective of this study was to study the effect of the incorporation of whole grain wheat flour (WGWF) and extrusion process variables (feed moisture and temperature) on the nutritional (dietary fibre, free sugars, digestible starch and resistant starch) and technological (pasting properties, expansion and cell structure) characteristics of ready-to-eat breakfast cereals based on corn flour, produced in a twin-screw extruder.

2. Materials and methods

2.1. Materials

Corn flour (CF) named Fecomix 425 M and whole grain wheat flour (WGWF) were obtained from Milhão Alimentos™ (Inhumas-GO, Brazil) and Anaconda™ mill (São Paulo-SP, Brazil), respectively. The CF was stored in plastic barrels and the WGWF was vacuum packed in plastic bags and stored at -21 °C until use.

2.2. Methods

2.2.1. Sample preparation and extrusion cooking

The ready-to-eat breakfast cereals were elaborated in a co-rotating ZSK 30 twin-screw extruder (Werner Pfleiderer Corporation, Ramsey, USA), following a 2^3 central composite rotatable design (CCRD), being independent variables: whole grain wheat flour (%), feed moisture (%) and barrel temperature of zones 3 and 4 (°C). Results from preliminary trials were used to select suitable extruder operating conditions and raw material levels. The outline of the experimental design and its independent variables and variation levels are presented in Table 1. Feed rate (13 kg/h), screw speed (325 rpm) and temperature of zones 1 and 2 (75 and 100 °C, respectively) were fixed.

Before extrusion, each sample set composed by corn and/or whole grain wheat flour was weighed to give a batch of 2.5 kg and mixed with distilled water, according to the experimental design, using a planetary mixer (Hypo, HB 12). The blended flour was tempered overnight at room temperature to ensure a uniform hydration level of the feeding material. The barrel diameter D was 30 mm, and barrel length L 872 mm (L/D= 29.07). A circular die was used at the end of the extruder, with diameter of 3.0 mm, and a knife with average speed of 110 rpm. The following screw configuration, composed of conveying and mixing elements, was used: 2 elements 60/30; 2 elements 42/21; 1 element 28/14; 1 element kneading block 90/5/28; 1 element 21/21; 1 element 28/14; 4 elements 20/10; 1 element kneading block; 1 element 21/21; 1 element 28/14; 5 elements 20/10; 1 element 28/14; 1 element 14/14; 1 element kneading block 45/5/14; 6 elements 20/10; 1 element kneading 45/5/20; 3 elements 20/10; 1 element 10/10; 2 elements 20/10. The extruded cereals were immediately dried in a rotary dryer at 125 °C, for 2 seconds (twice). Drying of extruded products was completed in a forced air oven at 70 °C until moisture content of 3-4 % was reached. Afterwards, products were stored in metalized bags, with light and moisture protection until further analysis.

2.2.2. Particle size distribution of flours and proximate composition of flour and extruded products

The particle size distribution of each flour was determined using a Produtest vibrator and sieves of 20, 35, 60, 80 and 100 mesh opening sizes according to AOAC method n^{o} 965.22. Sample amount of 100 g, vibration time of 20 minutes and speed 3 were set (AOAC, 2000).

Flour composition was determined following AACC (2012) Official Methods: water content (method n° 44-15.02), ash content (method n^{o} 08-01.01), fat content (method n^{o} 30-25.01), and protein content (method n^{o} 46-13.01) with a conversion factor of 5.7 for WGWF and of 6.25 for corn flour. Carbohydrate content was obtained by difference.

For the estimation of dietary fibre, samples were finely powdered to pass through a sieve of 250 µm. Total dietary fibre (TDF), insoluble dietary fibre (IDF) and soluble dietary fibre (SDF) contents were determined following AACC method n^{o} 32-07.01 (AACC, 2012). Results were expressed in percentage in dry basis. Free sugars determination was carried out following the method reported by Dura et al. (2014). Briefly, raw material and breakfast cereals samples (0.10 ± 0.01 g) were suspended in 2 mL of ethanol (80%) and incubated at 85°C in water bath for five minutes and then centrifuged (2000×*g*, 10 minutes, at room temperature). This was performed twice. Supernatants were combined to measure free sugars released. A glucose oxidase-peroxidase kit was used to quantify glucose and the absorbance was measured in an Epoch microplate reader (Biotek Epoch, Izasa, Barcelona, Spain) at 510 nm. Three replicates were assessed for each raw material or experimental point.

Starch hydrolysis was measured using AACC method n^{o} 32-40-01 (AACC, 2012), modified by Gularte and Rosell (2011). The pellet after free sugar extraction was incubated with porcine pancreatic α-amylase (Type VI-B, ≥10 units/mg solid; Sigma Chemical Co., St. Louis, MO, USA) in a shaking water bath at 37 ºC for 16 h. Ethanol was added to stop the enzymatic reaction and the suspension was centrifuged (2000×*g* for 5 minutes). To quantify digestible starch, the supernatant (100 µL) was diluted with 850 µL sodium acetate pH 4.5, incubated (50°C for 30 minutes) with 50 µL amyloglucosidase (3.480 U/mL) (Sigma Chemical Co., St. Louis, MO, USA) and released glucose was assessed as described above. Resistant starch after 16 h hydrolysis remained in the pellet, which was solubilized with 2 mL of 2 M KOH using a Polytron Ultra-Turrax homogenizer IKA-T18 (IKA Works, Wilmington, NC, USA) during 1 minute at speed 3. The homogenate was diluted with 8 mL 1.2 M sodium acetate pH 3.8 and incubated with 100 µL amyloglucosidase (3480 U/mL) at 50 ºC for 30 min in a shaking water bath and then centrifuged (2000×*g* for 10 minutes). The glucose content of the digestible and resistant starch was measured using a glucose oxidase-peroxidase kit as described for free sugars. Total starch was the sum of the digestible starch and the resistant starch in mg/100 mg of the sample in dry basis.

2.2.3. Pasting properties

The pasting properties of the extruded cereals were measured using a Rapid Visco Analyser 4500 (RVA 4500, Perten Instruments, Australia), adjusted with set up *Extrusion 1* no-alcohol. An amount of 5 g ground sample, previously corrected for moisture (14% basis), was dispersed in 25 mL distilled water to a total weight of 30 g. The profiles were continually recorded using the Thermocline for Windows (TCW) software, version 3. Three runs were carried out for each sample considered. For the raw materials, WGWF and CF, *Standard 1* setup was used. Pasting parameters evaluated included: peak time (minutes) – time at which peak viscosity occurred; peak viscosity (cP) – maximum viscosity after the heating portion of the test; trough viscosity (cP) – lowest viscosity after the peak viscosity just before it begins to increase again; final viscosity (cP) – viscosity at the end of the test, and setback (cP) – final viscosity minus trough viscosity (Adedokun and Itiola, 2010), which were determined from the recorded curves.

2.2.4. Cross section image analysis of extruded products

Ten spheres of each sample were selected and cut in the middle with a Stanley knife. Images of the cross section of the breakfast cereals were captured with 600 dpi in a bed scanner equipped with the software HP PrecisoScan Pro version 3.1 (HP Scanjet 4400C, Hewlett-Packard, USA), using a black background paper. The default settings for brightness (midtones 2.2) and contrast (highlights 240, midtones 2.2 and shadows 5) of the scanner software were used for acquiring the images. Images were saved in jpeg format and analyzed by the Image J software (National Institutes of Health, Bethesda, MD, USA). Measurements of scanned images were obtained in pixels and converted into mm (232 pixels along a straight line were equivalent to 1 mm) by using known length values. Data of cereal area (mm^2), perimeter (mm) and circularity (0-1) measurements were obtained after drawing the contour of the circular section of the cereal. Longitudinal and transverse diameters were marked in the figure using the straight tool to get the measures (mm) and the mean calculated. For the cell analysis, images were set to 8-bit format, the contrast was adjusted to 172, the IJ_Iso_Data algorithm was chosen, cell size range delimited as 0.10 - ∞ (analyze particles tool) and "Overlay masks" selected. The data used for each image were: particles number, mean area (mm^2), mean perimeter (mm) and mean circularity (0-1) of the particles, and the mean values were calculated for the statistical analysis. Circularity value equal to 1.0 indicates a perfect circle.

2.2.4. Statistical analysis

The experimental data were evaluated using the response surface methodology (RSM) to investigate the effect of the extrusion process (temperature and feed moisture) and the flour characteristics (whole grain wheat flour replacement) on response variables. A total number of 18 runs were defined, including four central points. The range for the independent variables considered in this study and their respective coded levels are given in Table 1. A second-order polynomial regression model was established to fit the experimental data (P<0.1) for each response variable, as shown in the following equation:

$$y_i = b_0 + \sum_{i=1}^{3} b_i x_i + \sum_{i=1}^{3} \sum_{j=1}^{3} b_{ij} x_i x_j,$$

where, y_i is the response variable; b_0, b_i and b_{ij} are the regression coefficients for constant, linear, quadratic, and interaction regression terms, respectively; x_i and x_j are the coded values of the independents variables. The results of the experimental design were analyzed using Statistica 7 software (Statsoft, Tulsa, OK, USA). The response surface plots were generated as a function of two variables, while keeping the third variable constant at the central value with basis in re-parameterized regression models (Tables 3, 4 and 5).

3. Results and discussion

Table 2 shows the proximate composition of both flours, confirming that WGWF contained higher amount of proteins, fat, ash and total dietary fibre, mainly of insoluble nature, than corn flour. In opposition, corn flour contained higher amount of resistant starch than WGWF. Regarding the particle size (Table 2), as was expected, CF showed a narrow particle size distribution, concentrated between 250 µm and 500 µm, whereas WGWF showed a bell-shaped particle size distribution.

The combination of both flours was proposed for increasing the fibre content of the breakfast cereals taking the benefit of the technological functionality provided by corn flour. An experimental design was carried out to determine the impact of the extrusion variables (feed moisture and temperature) and of the blend of flours on nutritional (dietary fibre, free sugars, digestible starch and resistant starch) and technological (pasting properties, expansion and cell structure) properties of the extruded products.

3.1. Dietary fibre (insoluble, soluble and total)

The incorporation of WGWF had a significant positive effect on the insoluble (IDF), soluble (SDF) and total dietary fibre (TDF) contents of the breakfast cereals (Table 3, Figure 1). Therefore, the fibre enriching effect of WGWF was confirmed. As expected, the effect of WGWF was more accentuated on IDF than on SDF. It is known that whole grain wheat flour contains 13.4 - 14.9% total dietary fibre, of which 11.5 - 12.7 % is insoluble and 1.1 - 2.2 % is soluble (Slavin et al., 1999, Picolli da Silva and de Lourdes Santorio Ciocca, 2005). Among the other variables studied, feed moisture had significant ($P<0.10$) negative linear and quadratic effects on IDF (Table 3, Figure 1A), indicating that IDF decreased when increasing feed moisture. The model regression coefficients (Table 3) indicated a linear significant effect of temperature on SDF and TDF (Table 3, Figure 1B). It is likely that an increase in temperature led to a redistribution of a portion of the insoluble fibre fraction to the soluble fibre fraction, attributed to a release of hemicellulose during processing in the extruded samples (Camire et al., 1990). Also, temperature might induce the breakage of molecular bonds, releasing soluble fibre that may be naturally bonded to starch or other compounds (Camire et al., 1990).

The insoluble (IDF), soluble (SDF) and total (TDF) fibre content in the defined runs varied between 1.92 and 9.38%, 1.14 and 3.03%, and 3.95 and 12.25%, respectively. The total (100%) replacement of corn flour by WGWF achieved maximum fibre content (insoluble, soluble and total). Brazilian legislation (ANVISA, 2012) establishes that a food sample must have 2.5 g dietary fibre per serving to be considered a “good” source of fibre or 5 g per serving to be “high” in fibre. Considering that the serving for breakfast cereals is 30 g (ANVISA, 2003, FDA, 2012), samples ranged from 1.19 – 3.68 g dietary fibre per serving, so samples that are “good” fibre sources can be selected from the experimental design.

3.2. Free sugar, digestible, resistant and total starch

The amount of free sugars in the extruded cereals was significantly (P<0.10) negatively affected by WGWF and positively by feed moisture. There was no free sugar content difference between corn flour and WGWF, whereby the WGWF negative effect on free sugars in the extruded cereals could only be attributed to changes induced by the extrusion process. The feed moisture had significant positive linear and quadratic effects due to its decisive role in hydrolytic reactions (Figure 2A). Digestible and total starch was significantly affected by the incorporation of WGWF (Figures 2B and 2C) and their impact was negatively linear and positively quadratic. In general, maximum and minimum digestible starch content was reached with WGWF 0% and 100%, respectively. Considering that the WGWF had lower digestible starch (Table 2), the progressive incorporation of this flour in the extruded products led to a significant decrease in digestibility. Total starch in the extruded cereals had values comprised between 57.27 and 83.76 mg/100 mg of sample, thus starch was the major compound of the extruded cereals. Temperature did not significantly affect the digestible starch content, which was plausible taking into account that the range of extrusion settings for temperature (74.6-146.4°C) always exceeded the gelatinization temperature of wheat and corn starches.

Extrusion cooking is somewhat unique because gelatinization occurs at much lower moisture levels (12–22%) than is necessary in other forms of food processes (Qu and Wang, 1994). However, no significant differences were found on the starch responses due to water supply for extrusion feeding.

Compared to raw flours (Table 2), all extruded samples had lower resistant starch content after extrusion cooking (results not shown), which indicated loss of RS under extrusion process conditions. Similar observations were reported by Faraj et al. (2004) with extrusion of pearled barley flour. According to the analysis of variance (ANOVA), the significant coefficients (P<0.10) for the resistant starch response were temperature and the interaction between moisture and temperature. Therefore, the association of heat and moisture could modulate the amount of RS in the resulting extruded cereals (Sajilata et al., 2006). Nevertheless, there is certain controversy in the scientific literature regarding the impact of extrusion on the formation of resistant starch, namely RS3 that represents the starch fraction, mainly retrograded amylose, formed during cooling of gelatinized starch (Sajilata et al., 2006). In fact, type 3 resistant starch content in waxy and regular barley flours generally decreased with extrusion cooking (Faraj et al., 2004). Conversely, a study with pastry wheat flour reported that the resistant starch content increased after extrusion compared to non-extruded flour (Kim et al., 2006). The RS content in the extrudates (1.78-4.97 g/100 g) was within the range found in processed cereal products. A database of resistant starch content in commercial cereal-based products shows values in a range of 0.5-1.5 g/100 g for whole wheat bread, 0-6.3 g/100 g for ready-to-eat breakfast cereals, being 0.7 g/100 g in bran flakes cereals and 1 g/100 g in whole wheat flakes (Murphy et al., 2008).

Pasting Properties

The assessment of pasting properties in the extruded products was selected to obtain information of the impact of extrusion settings and flour blends (Table 4) on starch technological properties. The peak viscosity for the raw materials was 2659 cP and 1263 cP, for WGWF and corn flour, respectively. These results are in agreement with the results found in the literature for corn starch and whole wheat flour. Sandhu and Singh (2007) found that peak viscosities (PV) ranged from 804 cP to 1252 cP for different corn varieties (African Tall, Ageti, Early Composite, Girja, Nayjot, Parbhat, Partab, Pb Sathi and Vijay). These values are lower than the peak viscosities reported for whole wheat flour (1891 and 2683 cP) (Oro et al., 2013). Wheat and corn starches present granules with particular morphological structures and crystalline order that display particular pasting and functional properties (Singh et al., 2003). Also, the fibre present in WGWF could alter its viscosity profile. Accordingly, the extruded cereals with highest WGWF percentages and total and insoluble fibre contents showed the highest peak viscosities.

Changes in flour blends greatly affected the viscosity profiles (results not shown) of the extrudates. For instance, peak (maximum) viscosities ranged from 372 cP to 2170 cP. The analysis of variance (ANOVA) of the experimental results for pasting properties is shown in Table 4. The extruded cereals showed lower values for the pasting parameters than the native flours. Even considering that the general conditions of analysis were not exactly the same for extrudates and raw flours, as described in section *2.2.3*, the values reflect that, during the extrusion process, the heat-moisture and mechanical energy applied led to starch gelatinization, observed mainly by the lower peak time, final viscosity and set back. Native starches are more susceptible to changes in viscosity during the heating and cooling cycle than pre-gelatinized flours (Adedokun and Itiola, 2010). WGWF, moisture and temperature and the interactions among them produced significant effects on the pasting properties, as can be seen in Figure 3.

With the exception of final viscosity, WGWF showed a significant linear effect on pasting properties that was positive on peak viscosity and trough and negative on peak time and setback (Table 4, Figures 3A and 3F). Feed moisture content had a significantly positive linear effect on pasting properties, with the exception of peak viscosity (Table 4, Figure 3A). In addition, extrusion temperature significantly affected pasting properties, excluding setback; the effect was positive (linear and quadratic) for peak viscosity, but negative (linear) for the other parameters (Table 4, Figures 3A-F). Interaction effects were obtained for WGWF and temperature, showing an antagonistic effect on peak viscosity and a synergistic effect on peak time (Figures 3A and B). Temperature and feed moisture had a significant antagonistic effect on pasting parameters, excepting peak viscosity (Figures 3D, E and F). It was expected that the replacement of corn flour by WGWF reduced paste viscosity, because of the reduction in starch content available to swell (Symons and Brennan, 2004). Nevertheless, the occurrence of some interaction between fibres and starches or the viscosity provided by fibres could have led to alterations in the viscosity. The minimum viscosity reached at 95ºC (trough) was dependent on the three variables, the effects being positive for WGWF and moisture, whereas temperature affected it negatively, besides its antagonistic effect with feed moisture (Table 4). During the holding period at 95ºC, samples are subjected to constant high temperature (95°C) and mechanical shear stress (160 rpm), which, when working with native raw materials, further disrupts the starch granules (Fu et al., 2008, Rojas et al., 1999). The paste formed by ground extrudates under RVA conditions probably presented weaker bonds between polysaccharides starch derivatives and fibre components because of starch disruption resulting from its transformations in the extrusion process. This fragile structure leads to the drop in viscosity during the holding time. In addition, the opposite effect of temperature and feed moisture agrees with the higher temperature required for amylopectin melting when not sufficient water is available for starch gelatinization (Barcenas et al., 2003).

3.3. Image analysis

The incorporation of whole grain flours or fibre rich ingredients in the formulation of expanded extruded products has been associated with a decrease in the expansion of the extruded cereals (Yanniotis et al., 2007, Chanvrier et al., 2013). Up to know, changes in the structure of the extruded products have been studied by 3D image analysis carried out with X-ray tomography (Chanvrier et al., 2013, Jing and Chi, 2013, Alam et al., 2013) or by scanning electron microscopy (Dansby and Bovell-Benjamin, 2003, Singh et al., 2009, Ferreira et al., 2012). Those analyses give information about the internal cell morphology of the food at a microscopic level. Nevertheless, up to the authors' knowledge, the macroscopic structure of the cross section of extruded products has not been a point of attention, even when these measurements would really assess the impact of extrusion on product expansion. Because of this, the ImageJ® software was applied to quantify the sectional dimensions of the extruded cereals and internal structure of the scanned images.

The cross-sectional scanned images of the extruded cereals are shown in Figure 4. It was readily evident that the number of cells and their distribution was significantly different within the sample runs (Figure 4). Image analysis was carried out to determine diameter, area, perimeter and circularity of the extruded products; these parameters were selected because they reflect the expansion process after extrusion (Table 5). In general, structure was mainly influenced by WGWF and feed moisture, whereas temperature had a minor effect and only when interacted with WGWF or feed moisture (Table 5). The surface plots for diameter and perimeter showed a similar tendency (Figures 5A and 5B). WGWF and feed moisture exerted linear and quadratic effects on diameter and perimeter of the extruded products. Their effect was negative on these parameters, except for the quadratic effect of WGWF that was positive (Figure 5A and 5B). High WGWF contents resulted in more compacted products, with lower diameter, perimeter and area. The same trend was observed on the cell area, although feed moisture did not promote a quadratic effect on this parameter. WGWF, moisture and temperature had no significant effect on extrudate circularity, with the exception of the interaction of WGWF and temperature. The effect of WGWF could be primarily related to the dietary fibre content, likely due to the dilution of starch and to the rupture of the structure. The mechanistic steps of expansion include starch transformation, nucleation of bubbles, growth of bubbles and bubble collapse (Moraru and Kokini, 2003). The incorporation of wheat bran (mostly insoluble fibre) during extrusion reduces the starch content of the matrix that compromises the further expansion process, which agrees with previous findings (Brennan et al., 2008, Robin et al., 2011b, Robin et al., 2011a). Additionally to the effect of dispersed bran particles, competition for water between the fibres, present in bran, and starch may also affect the mechanical properties of the composite matrix. Decreased feed moisture leads to an increase in extrudate sectional area. During extrusion, the extent of starch gelatinization depends on the water available for starch in the extruder (Moraru and Kokini, 2003), which was modified in the presence of fibres.

Starchy extruded cereal products are usually porous and aerated due to the expansion process that occurs at the end of the die. The incorporation of WGWF also had a significant negative quadratic effect on the number of air cells within the cross section, which is related to cell density (Table 5), likely due to the collapse of the bubbles induced by fibres. The cell area was dependent on WGWF and feed moisture, being in agreement with the other analysed parameters that define the expansion process (diameter, perimeter and section area). The area of the void spaces or cells was significantly linearly reduced by WGWF and feed moisture and WGWF also induced a positive quadratic effect (Figure 5D). Also, WGWF and feed moisture showed a significant synergistic effect on the cell area, and an antagonistic effect was observed between feed moisture and temperature (Figure 5E). The results repeated the findings of other authors, which observed decreased sectional expansion, mean cell size and cell density in fibre-enriched extruded products (Chanvrier et al., 2013, Yanniotis et al., 2007, Robin et al., 2011b). Micro-computed X-ray tomography has shown that insoluble fibre induces both more compacted extruded products (with smaller product diameter) and decreased porosity structures (linked to smaller cell size). It has been proposed that the burst of the air bubbles during expansion at the interface between starch and fibres decreases the driving pressure for cell growth that limits the expansion of fibre-containing extruded products (Chanvrier et al., 2013, Chanvrier et al., 2007, Robin et al., 2011b, Yanniotis et al., 2007). The rupture of bubbles can be explained by the low chemical compatibility between the insoluble fibre particles and the continuous starch phase (Guy, 1988).

The extruded products with lower diameter, area and perimeter and lower cell area negatively influenced by fibre, corresponded to higher peak viscosity and trough in the pasting profile analysis. If the peak viscosity was higher it was probably because of fibre presence, because of the incompatibility between starch and fibre and the difficulty to form a continuous and viscoelastic phase. It is known that the expansion measurements, given by the image analysis, are governed by the physicochemical properties of the plasticized starch matrix, mainly by starch gelatinization and extrusion process severity showed in part by the pasting properties.

Even though a negative effect of fibres was observed for most of the image measurements, in the range of extrusion conditions studied, it was possible to obtain products containing WGWF with adequate technological features. Additionally, it is believed that the extrusion process led to total starch gelatinization (proved by microscopic analysis, not shown, where the images showed blocks of continuous phase with dispersed fibre particles).

4. Conclusion

The combination of wholemeal wheat flour with corn flour was a good alternative for increasing the fibre content of extruded products. Nevertheless, the incorporation of wholemeal flour in the extruded structure modified the functionality of the blend; therefore an optimization of the extrusion settings is needed. Overall, results obtained from the experimental design for obtaining extruded products with high fibre content, lower digestible starch content and intermediate expansion indicated that WGWF higher than 50% (central point), feed moisture in the central point region (17-21%) and temperature around 110°C would be advisable.

5. Acknowledgements

The authors acknowledge the financial support of São Paulo Research Foundation – FAPESP (Brazil, Project 2012/23281-2), Coordination for the Improvement of Higher Education Personnel – CAPES (Brazil, author Ludmilla Carvalho Oliveira's scholarship), Spanish National Research Council (CSIC) and the Generalitat Valenciana (Spain, Project Prometeo 2012/064). They thank the University of Campinas (UNICAMP) and the Spanish Scientific Research Council (CSIC) for allowing carrying out the study. The authors are also grateful to Milhão Alimentos^{TM} (Goiás, Brazil) for supplying the corn flour.

6. References

AACC 2012. *Approved methods of the american association of cereal chemists,* St.Paul, American Association of Cereal Chemists

ADEDOKUN, M. O. & ITIOLA, O. A. 2010. Material properties and compaction characteristics of natural and pregelatinized forms of four starches. *Carbohydrate Polymers,* 79**,** 818-824.

ALAM, S. A., JÄRVINEN, J., KIRJORANTA, S., JOUPPILA, K., POUTANEN, K. & SOZER, N. 2013. Influence of particle size reduction on structural and mechanical properties of extruded rye bran. *Food and Bioprocess Technology***,** 1-13.

ALBERTSON, A. M., FRANKO, D. L., THOMPSON, D. R., TUTTLE, C. & HOLSCHUH, N. M. 2013. Ready-to-eat cereal intake is associated with an improved nutrient intake profile among food insecure children in the United States. *Journal of Hunger & Environmental Nutrition,* 8**,** 200-220.

ANDERSON, J. W., BAIRD, P., DAVIS JR, R. H., FERRERI, S., KNUDTSON, M., KORAYM, A., WATERS, V. & WILLIAMS, C. L. 2009. Health benefits of dietary fiber. *Nutrition Reviews,* 67**,** 188-205.

ANDERSON, J. W., HANNA, T. J., PENG, X. & KRYSCIO, R. J. 2000. Whole grain foods and heart disease risk. *Journal of the American College of Nutrition,* 19**,** 291S-299S.

ANVISA 2003. Resolução da Diretoria Colegiada nº 359, de 23 de dez. de 2003.
. *In:* AGÊNCIA NACIONAL DE VIGILÂNCIA SANITÁRIA DO MINISTÉRIO DA SAÚDE (ANVISA, B. (ed.).

ANVISA 2012. Resolução da Diretoria Colegiada, nº 54, de 12 de nov. de 2012. *In:* BRAZIL), A. N. D. V. S. D. M. D. S. (ed.).

AOAC 2000. *Approved Methods of the American Association of Official Analytical Chemists,* Gaithersburg, USA., American Association of Official Analytical Chemists.

ASP, N.-G. & BJORCK, I. 1989. Nutritional properties of extruded foods. *In:* C. MERCIER, P. L., U.N. HARPER (ed.) *Extrusion Cooking.* St Paul, MN, USA American Association of Cereal Chemists (AACC).

BRENNAN, C., BRENNAN, M., DERBYSHIRE, E. & TIWARI, B. K. 2011. Effects of extrusion on the polyphenols, vitamins and antioxidant activity of foods. *Trends in Food Science & Technology,* 22**,** 570-575.

BRENNAN, M. A., MONRO, J. A. & BRENNAN, C. S. 2008. Effect of inclusion of soluble and insoluble fibres into extruded breakfast cereal products made with reverse screw configuration. *International Journal of Food Science & Technology,* 43**,** 2278-2288.

CAMIRE, M. E., CAMIRE, A. & KRUMHAR, K. 1990. Chemical and nutritional changes in foods during extrusion. *Critical Reviews in Food Science & Nutrition,* 29**,** 35-57.

CARVALHO, C. W. P. & MITCHELL, J. R. 2000. Effect of sugar on the extrusion of maize grits and wheat flour. *International Journal of Food Science & Technology,* 35**,** 569-576.

CASTELLS, M., MARIN, S., SANCHIS, V. & RAMOS, A. 2005. Fate of mycotoxins in cereals during extrusion cooking: a review. *Food Additives and Contaminants,* 22**,** 150-157.

CHANVRIER, H., APPELQVIST, I. A. M., BIRD, A. R., GILBERT, E., HTOON, A., LI, Z., LILLFORD, P. J., LOPEZ-RUBIO, A., MORELL, M. K. & TOPPING, D. L. 2007. Processing of novel elevated amylose wheats: functional properties and starch digestibility of extruded products. *Journal of agricultural and food chemistry,* 55**,** 10248-10257.

CHANVRIER, H., DESBOIS, F., PEROTTI, F., SALZMANN, C., CHASSAGNE, S., GUMY, J.-C. & BLANK, I. 2013. Starch-based extruded cereals enriched in fibers: a behavior of composite solid foams. *Carbohydrate Polymers,* 98**,** 842-853.

CHASSAGNE-BERCES, S., LEITNER, M., MELADO, A., BARREIRO, P., CORREA, E. C., BLANK, I., GUMY, J.-C. & CHANVRIER, H. 2011. Effect of fibers and whole grain content on quality attributes of extruded cereals. *Procedia Food Science,* 1**,** 17-23.

DANSBY, M. & BOVELL-BENJAMIN, A. 2003. Physical Properties and Sixth Graders' Acceptance of an Extruded Ready-to-Eat Sweetpotato Breakfast Cereal. *Journal of Food Science,* 68**,** 2607-2612.

DING, Q.-B., AINSWORTH, P., PLUNKETT, A., TUCKER, G. & MARSON, H. 2006. The effect of extrusion conditions on the functional and physical properties of wheat-based expanded snacks. *Journal of Food Engineering,* 73**,** 142-148.

DURA, A., BŁASZCZAK, W. & ROSELL, C. M. 2014. Functionality of porous starch obtained by amylase or amyloglucosidase treatments. *Carbohydrate Polymers,* 101**,** 837-845.

FARAJ, A., VASANTHAN, T. & HOOVER, R. 2004. The effect of extrusion cooking on resistant starch formation in waxy and regular barley flours. *Food Research International,* 37**,** 517-525.

FDA 2012. CFR-Code of Federal Regulations Title 21. *In: Food and drugs [USA]: Food and drug administration (FDA). Departament of Health & Human Service. Food for human consumption. Food labeling. General provisions. Section 101.12. Reference amounts customarily consumed per eating occasion. Available in http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=101.12.*

FERREIRA, R. E., CHANG, Y. K. & STEEL, C. J. 2012. Influence of wheat bran addition and of thermoplastic extrusion process parameters on physical properties of corn-based expanded extruded snacks. *Alimentos e Nutrição Araraquara,* 22**,** 507-520.

FU, L., TIAN, J.-C., SUN, C.-L. & LI, C. 2008. RVA and farinograph properties study on blends of resistant starch and wheat flour. *Agricultural Sciences in China,* 7**,** 812-822.

GULARTE, M. A. & ROSELL, C. M. 2011. Physicochemical properties and enzymatic hydrolysis of different starches in the presence of hydrocolloids. *Carbohydrate Polymers,* 85**,** 237-244.

GUY, R. 1988. Extrusion and co-extrusion of cereals. *Food structure: its creation and evaluation/[edited by] JMV Blanshard, JR Mitchell.*

HAVCK, B. W. & HUBER, G. R. 1989. Single screw vs twin screw extrusion. *The American Association of Cereal Chemists,* 34**,** 930-939.

HOLGUÍN-ACUÑA, A. L., CARVAJAL-MILLÁN, E., SANTANA-RODRÍGUEZ, V., RASCÓN-CHU, A., MÁRQUEZ-ESCALANTE, J. A., PONCE DE LEÓN-RENOVA, N. E. & GASTELUM-FRANCO, G. 2008. Maize bran/oat flour extruded breakfast cereal: A novel source of complex polysaccharides and an antioxidant. *Food Chemistry,* 111**,** 654-657.

JING, Y. & CHI, Y.-J. 2013. Effects of twin-screw extrusion on soluble dietary fibre and physicochemical properties of soybean residue. *Food Chemistry,* 138**,** 884-889.

JONNALAGADDA, S. S., HARNACK, L., LIU, R. H., MCKEOWN, N., SEAL, C., LIU, S. & FAHEY, G. C. 2011. Putting the whole grain puzzle together: Health benefits associated with whole grains—summary of American Society for Nutrition 2010 Satellite Symposium. *The Journal of Nutrition,* 141**,** 1011S-1022S.

KIM, J. H., TANHEHCO, E. J. & NG, P. K. W. 2006. Effect of extrusion conditions on resistant starch formation from pastry wheat flour. *Food Chemistry,* 99**,** 718-723.

MARQUART, L., JONES, J. M., COHEN, E. A. & POUTANEN, K. 2007. The Future of Whole Grains. *Whole Grains and Health.* Blackwell Publishing Professional.

MOORE, G. 1994. Snack food extrusion. *In:* FRAME, N. D. (ed.) *The technology of extrusion cooking.* St Paul, MN: American Association of Cereal Chemists.

MORARU, C. & KOKINI, J. 2003. Nucleation and expansion during extrusion and microwave heating of cereal foods. *Comprehensive Reviews in Food Science and Food Safety,* 2**,** 147-165.

MOSCICKI, L. & ZUILICHEM, D. J. V. 2011. Extrusion-Cooking and Related Technique. *In:* MOSCICKI, L. (ed.) *Extrusion-Cooking Techniques: Applications, Theory and Sustainability.* Weinheim, Germany: Wiley-VCH.

MURPHY, M. M., DOUGLASS, J. S. & BIRKETT, A. 2008. Resistant starch intakes in the United States. *Journal of the American Dietetic Association,* 108**,** 67-78.

ORO, T., LIMBERGER, V. M., DE MIRANDAI, M. Z., DOS SANTOS RICHARDS, N. S. P., GUTKOSKI, L. C. & DE FRANCISCO, A. 2013. Propriedades de pasta de mesclas de farinha integral com farinha refinada usadas na produção de pães. *Ciência Rural,* 43.

PERESSINI, D., FOSCHIA, M., TUBARO, F. & SENSIDONI, A. 2015. Impact of soluble dietary fibre on the characteristics of extruded snacks. *Food Hydrocolloids,* 43**,** 73-81.

PICOLLI DA SILVA, L. & DE LOURDES SANTORIO CIOCCA, M. 2005. Total, insoluble and soluble dietary fiber values measured by enzymatic–gravimetric method in cereal grains. *Journal of Food Composition and Analysis,* 18**,** 113-120.

QU, D. & WANG, S. 1994. Kinetics of the formations of gelatinized and melted starch at extrusion cooking conditions. *Starch‐Stärke,* 46**,** 225-229.

ROBIN, F., DUBOIS, C., CURTI, D., SCHUCHMANN, H. P. & PALZER, S. 2011a. Effect of wheat bran on the mechanical properties of extruded starchy foams. *Food Research International,* 44**,** 2880-2888.

ROBIN, F., DUBOIS, C., PINEAU, N., LABAT, E., THÉODULOZ, C. & CURTI, D. 2012. Process, structure and texture of extruded whole wheat. *Journal of Cereal Science,* 56**,** 358-366.

ROBIN, F., DUBOIS, C., PINEAU, N., SCHUCHMANN, H. P. & PALZER, S. 2011b. Expansion mechanism of extruded foams supplemented with wheat bran. *Journal of Food Engineering,* 107**,** 80-89.

ROJAS, J. A., ROSELL, C. M. & BENEDITO DE BARBER, C. 1999. Pasting properties of different wheat flour-hydrocolloid systems. *Food Hydrocolloids,* 13**,** 27--33.

ROSELL, C. 2012. The nutritional enhancement of wheat flour. *In:* CAUVAIN, S. (ed.) *Breadmaking: Improving quality.* . Second ed. UK: Woodhead Publishing, .

RYU, G. H. & NG, P. K. W. 2001. Effects of selected process parameters on expansion and mechanical properties of wheat flour and whole cornmeal extrudates. *Starch - Stärke,* 53**,** 147-154.

SAJILATA, M., SINGHAL, R. S. & KULKARNI, P. R. 2006. Resistant starch–a review. *Comprehensive Reviews in Food Science and Food Safety,* 5**,** 1-17.

SANDHU, K. S. & SINGH, N. 2007. Some properties of corn starches II: Physicochemical, gelatinization, retrogradation, pasting and gel textural properties. *Food Chemistry,* 101**,** 1499-1507.

SARAWONG, C., SCHOENLECHNER, R., SEKIGUCHI, K., BERGHOFER, E. & NG, P. K. 2014. Effect of extrusion cooking on the physicochemical properties, resistant starch, phenolic content and antioxidant capacities of green banana flour. *Food Chemistry,* 143**,** 33-39.

SINGH, J., KAUR, L., MCCARTHY, O. J., MOUGHAN, P. J. & SINGH, H. 2009. Development and characterization of extruded snacks from New Zealand *Taewa* (Maori potato) flours. *Food Research International,* 42**,** 666-673.

SINGH, N., SINGH, J., KAUR, L., SINGH SODHI, N. & SINGH GILL, B. 2003. Morphological, thermal and rheological properties of starches from different botanical sources. *Food Chemistry,* 81**,** 219-231.

SINGH, S., GAMLATH, S. & WAKELING, L. 2007. Nutritional aspects of food extrusion: a review. *International Journal of Food Science & Technology,* 42**,** 916-929.

SLAVIN, J. 2003. Why whole grains are protective: biological mechanisms. *Proceedings of the Nutrition Society,* 62**,** 129-134.

SLAVIN, J. L., MARTINI, M. C., JACOBS, D. R. & MARQUART, L. 1999. Plausible mechanisms for the protectiveness of whole grains. *The American journal of clinical nutrition,* 70**,** 459s-463s.

SYMONS, L. & BRENNAN, C. 2004. The Influence of (1→ 3)(1→ 4)-β-D-Glucan-rich Fractions from Barley on the Physicochemical Properties and In Vitro Reducing Sugar Release of White Wheat Breads. *Journal of Food Science,* 69**,** C463-C467.

YANNIOTIS, S., PETRAKI, A. & SOUMPASI, E. 2007. Effect of pectin and wheat fibers on quality attributes of extruded cornstarch. *Journal of Food Engineering,* 80**,** 594-599.

YE, E. Q., CHACKO, S. A., CHOU, E. L., KUGIZAKI, M. & LIU, S. 2012. Greater whole-grain intake is associated with lower risk of type 2 diabetes, cardiovascular disease, and weight gain. *The Journal of Nutrition,* 142**,** 1304-1313.

FIGURE CAPTIONS

Figure 1. Response surface plots for insoluble fibre (A); soluble fibre (B) and total dietary fibre (C) as a function of WGWF, feed moisture and temperature.

Figure 2. Response surface plots for free sugars (A), digestible starch (B), resistant starch (C) and total starch (D) as a function of WGWF, feed moisture and temperature.

Figure 3. Response surface plots for pasting properties of expanded extruded cereals as a function of WGWF, feed moisture and temperature.

Figure 4. Cross-sectional scanned images of extruded cereals. The numbers 1-18 correspond to the runs of the Central Composite Rotatable Design (CCRD).

Figure 5. Response surface plots for image analysis parameters as a function of WGWF, feed moisture and temperature.

Table 1. Ranges of the independent variables and their corresponding real and coded levels.

Independent variables	Code	Levels				
		- α	-1	0	1	+ α
Whole grain wheat flour (%)	x_1	0	20.24	50.00	79.76	100.00
Feed moisture (%)	x_2	13.96	16.00	19.00	22.00	24.04
Temperature (°C)	x_3	76.40	90.00	110.00	130.00	143.60

$\alpha=2n^{1/4}$; n= number of independents variables; α=1.68.

Table 2. Characteristics of the flours used as raw materials.

		Whole grain wheat flour	Corn flour
Proximate composition (%)			
	Moisture	10.82	11.4
	Protein	12.7	5.6
	Fat	1.72	1.22
	Ash	1.6	0.28
Carbohydrates (mg/100 mg)			
	Free sugars	0.06	0.07
	Digestible starch	23.41	54.05
	Resistant starch	6.82	10.59
	Total starch	30.23	64.64
Dietary fibres (%)			
	Soluble fibre	2.36	0.80
	Insoluble fibre	10.46	2.09
	Total dietary fibre	12.82	2.89
Particle size distribution (%)	>840µm	0.60	0.13
	840µm-500µm	6.28	3.32
	500µm-250µm	43.12	72.54
	250µm-177µm	26.58	19.68
	177µm-149µm	16.94	1.78
	<149µm	6.13	2.28

Table 3. Estimated regression coefficients of second-order polynomial models for dietary fibre and starch fractions of extruded breakfast cereals.

Parameter	Insoluble fibre (%)	Soluble fibre (%)	Total fibre (%)	Free sugars (mg/100mg)	Digestible starch (mg/100mg)	Resistant starch (mg/100mg)	Total starch (mg/100mg)
Constant	5.70	1.99	7.61	0.04	57.66	2.17	59.83
WGWF (x_1)	2.22*	0.44*	2.67*	-0.02*	-4.52*	-0.32	-4.84*
WGWF (x_1x_1)	-	0.08	-	-	4.28*	0.21	4.48*
Moisture (x_2)	-0.13	0.11	-	0.04*	-1.15	0.26	-0.89
Moisture (x_2x_2)	-0.33*	0.24	-	0.02*	1.95	-0.21	1.74
Temperature (x_3)	-	0.31*	0.23*	-	-0.74	0.58*	-0.16
Temperature (x_3x_3)		-0.10	-	-	1.16	0.54*	1.70
WGWF x Moisture (x_1x_2)	-	-0.11	-	-	-0.77	-0.18	-0.94
WGWF x Temperature (x_1x_3)	-	0.02	-	-	2.02	-0.15	1.87
Moisture x Temperature (x_2x_3)	-	0.13	-	-	-0.72	0.49*	-0.23
R² (%)	98.38	71.24	97.97	93.89	82.98	80.48	84.18

* Statistically significant at *P*<0.10.

Table 4. Estimated regression coefficients of the second-order polynomial models for pasting properties of extruded breakfast cereals.

Parameter	Peak viscosity (cP)	Peak time (min)	Trough (cP)	Final Viscosity (cP)	Setback (cP)
Constant	860.373	3.62	308.31	775.63	467.32
WGWF (x_1)	114.35*	-0.41*	75.38*	-	-43.41*
WGWF (x_1x_1)	-	-	-	-	-
Moisture (x_2)	-	0.40*	41.06*	144.52*	103.51*
Moisture (x_2x_2)	-	-	-	-	-
Temperature (x_3)	473.30*	-0.70*	-45.29*	-78.63*	-
Temperature (x_3x_3)	179.406*	-	-	-	-
WGWF x Moisture (x_1x_2)	-	-	-	-	-
WGWF x Temperature (x_1x_3)	-200.15*	0.26*	-	-	-
Moisture x Temperature (x_2x_3)	-	-0.21*	-50.23*	-139.33*	-89.10*
R² (%)	94.29	97.45	74.62	76.51	74.69

* Statistically significant at *P*<0.10.

Table 5. Estimated regression coefficients of second-order polynomial models for cell structure image analysis data of extruded breakfast cereals.

Parameter	Diameter (mm)	Area (mm^2)	Perimeter (mm)	Extrudate circularity	Cell number	Cell area (mm^2)	Cell circularity
Constant	8.87	57.15	30.89	0.81	12.21	1.38	0.31
WGWF (x_1)	-0.62*	-10.64*	-2.26*	0.01	0.61	-0.40*	0.01
WGWF (x_1x_1)	0.41*	7.91*	1.39*	0.01	-0.72*	0.24*	-0.01
Moisture (x_2)	-0.84*	-11.46*	-2.85*	-0.01	-0.56	-0.18*	-0.01
Moisture (x_2x_2)	-0.44*	-	-1.41*	-0.01	0.40	-	0.02
Temperature (x_3)	-	-	-	0.01	0.57	-	-0.02
Temperature (x_3x_3)	-	-	-	0.00	-0.28	-	0.01
WGWF x Moisture (x_1x_2)	-	-	-	-0.01	-0.02	0.23*	0.01
WGWF x Temperature (x_1x_3)	-	-	-	0.02*	0.09	-	-0.02
Moisture x Temperature (x_2x_3)	-	-	-	-0.01	0.57	-0.23*	0.01
R² (%)	81.84	71.64	81.17	72.99	70.03	78.57	50.44

* Statistically significant at *P*<0.10.

Figure 1.

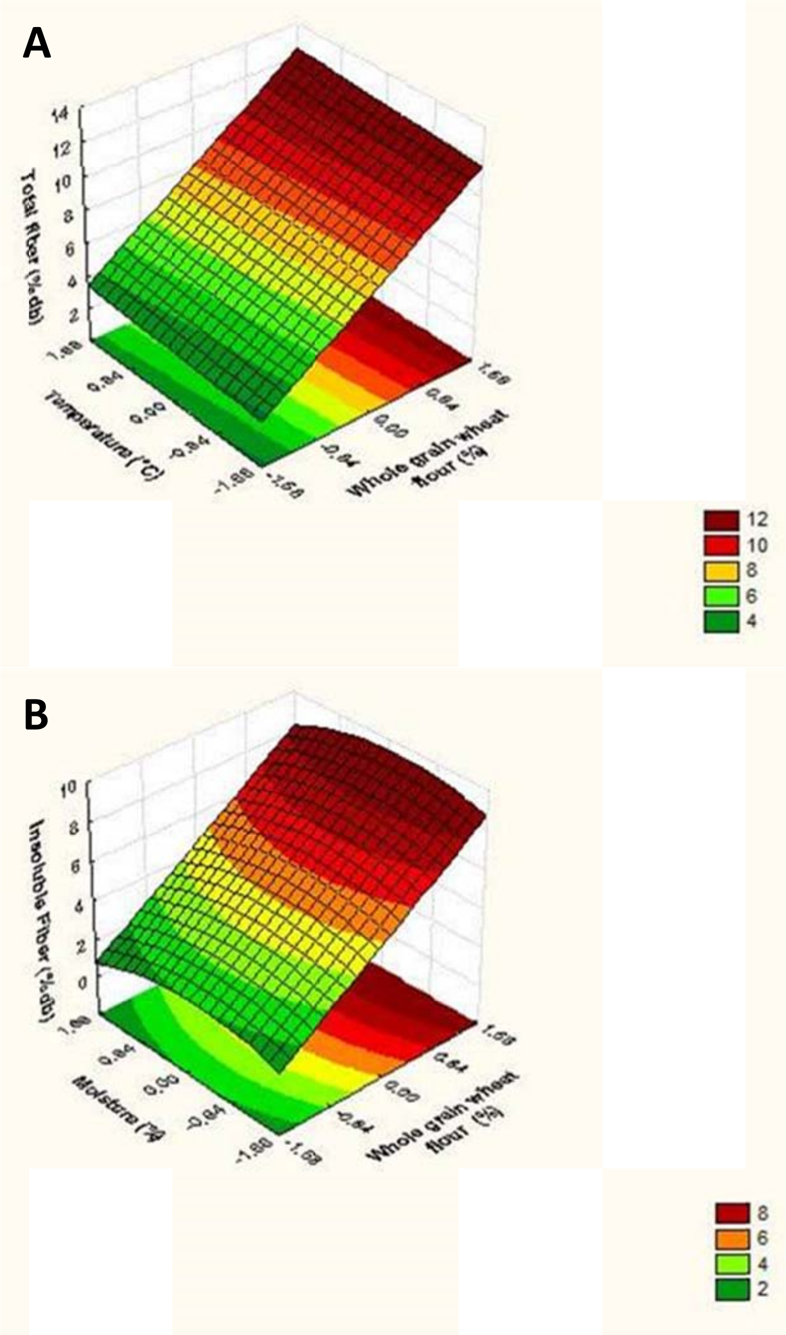
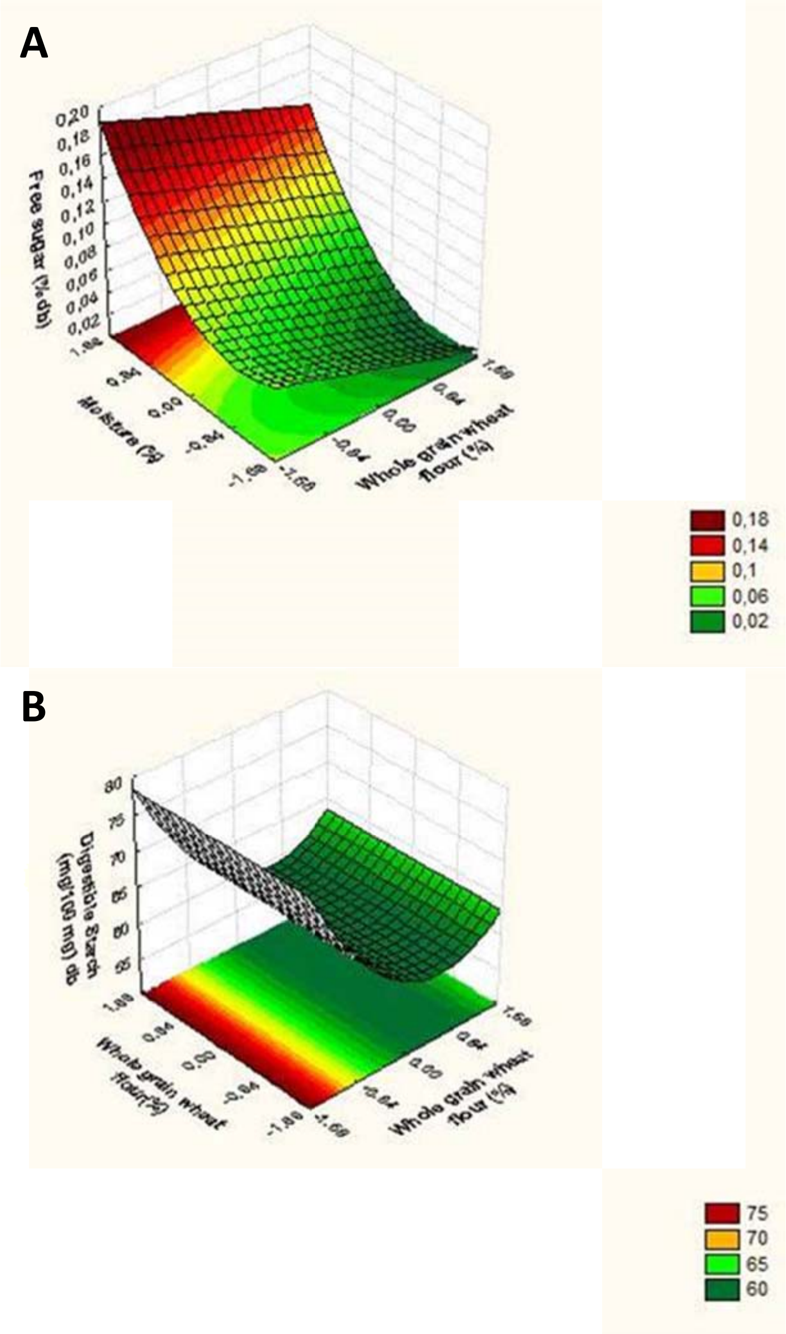

A
Total fiber (% db)
14
12
10
8
6
4
2
1.68
0.84
0.00
-0.84
-1.68
Temperature (°C)
-1.68
-0.84
0.00
0.84
1.68
Whole grain wheat flour (%)
12
10
8
6
4
B
Insoluble Fiber (% db)
10
8
6
4
2
0
1.68
0.84
0.00
-0.84
-1.68
Moisture (%)
-1.68
-0.84
0.00
0.84
1.68
Whole grain wheat flour (%)
8
6
4
2

Figure 2.


A
Free sugar (% db)
0,20
0,18
0,16
0,14
0,12
0,10
0,08
0,06
0,04
0,02
Moisture (%)
1,68
0,84
0,00
-0,84
-1,68
Whole grain wheat flour (%)
0,18
0,14
0,1
0,06
0,02
B
Digestible Starch (mg/100 mg) db
80
75
70
65
60
55
Whole grain wheat flour (%)
Whole grain wheat flour (%)
1,68
0,84
0,00
-0,84
-1,68
75
70
65
60

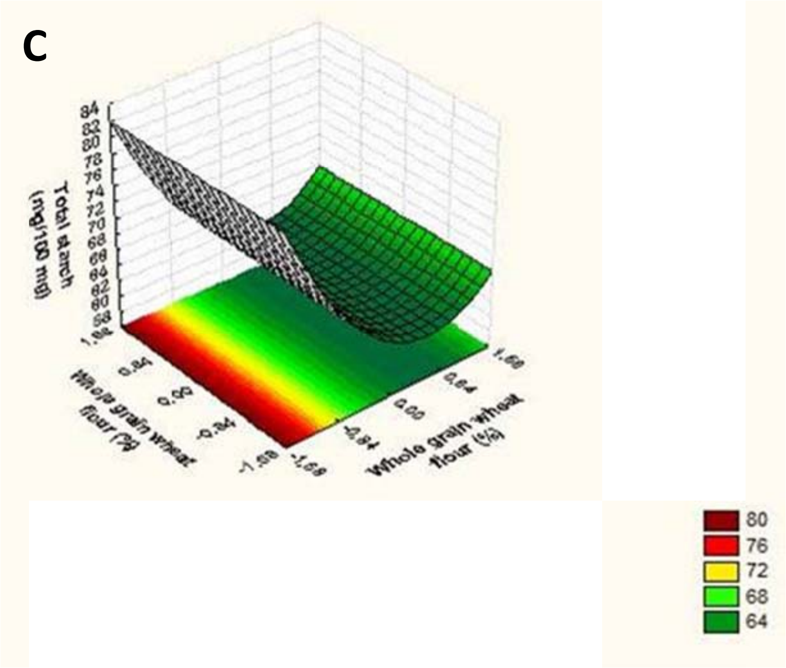
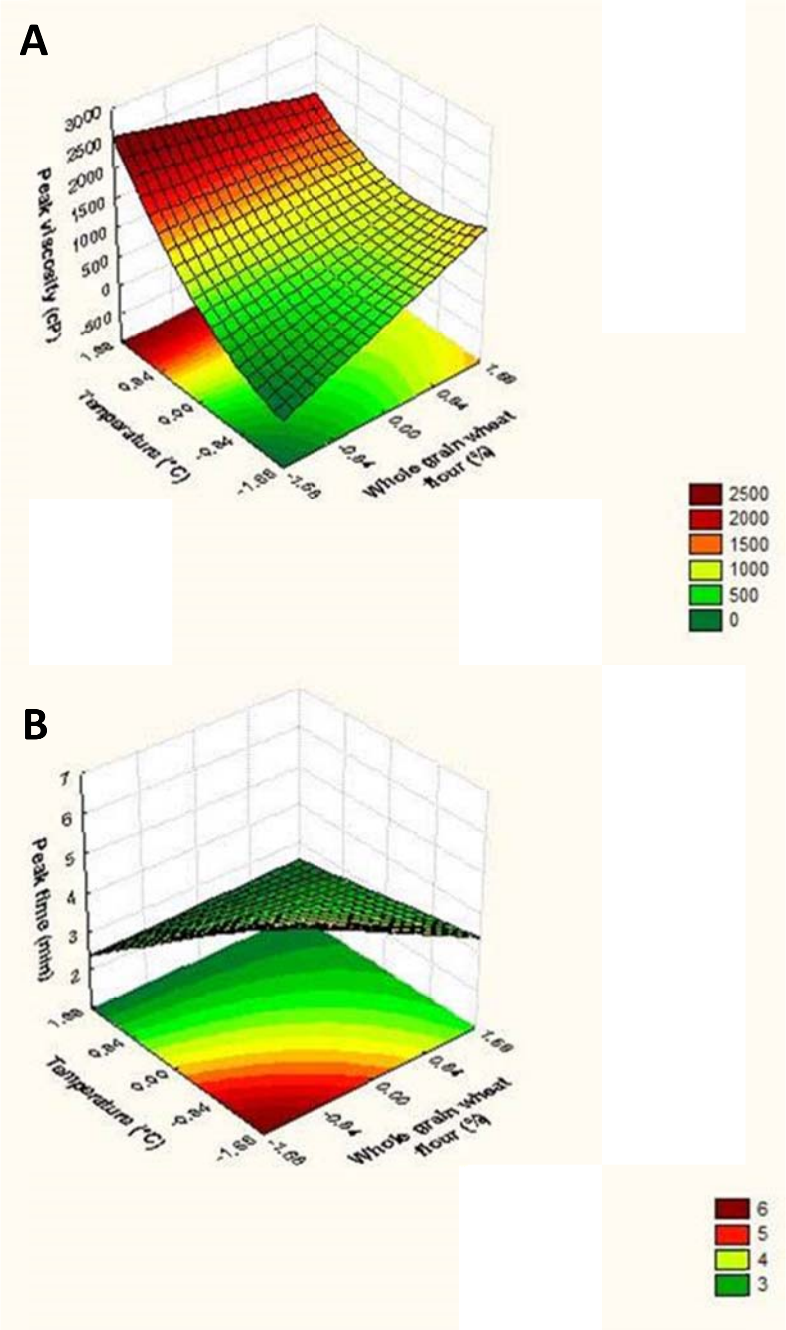
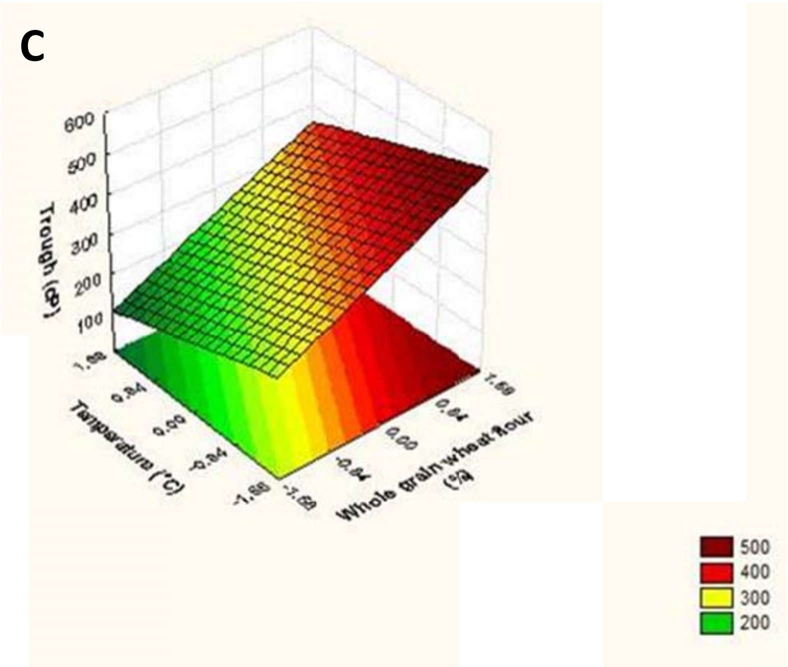
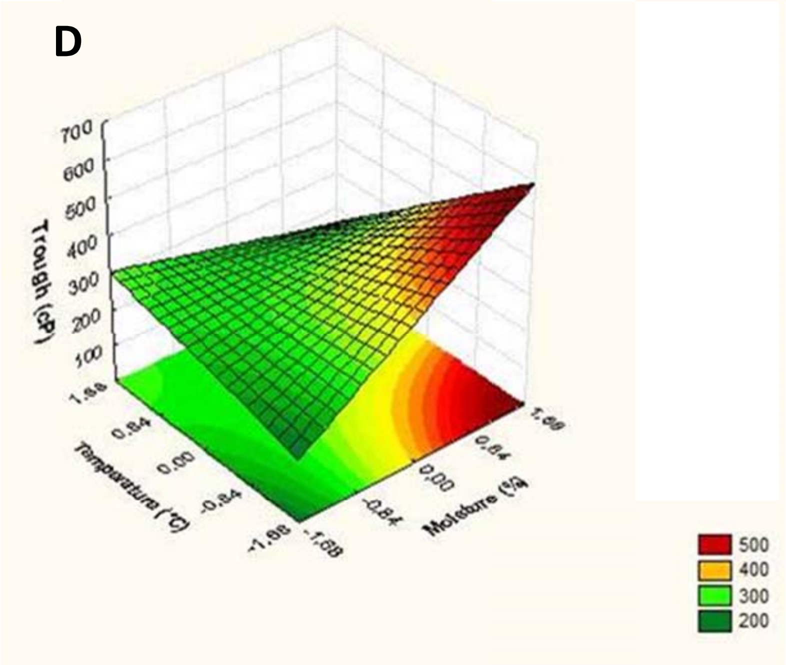
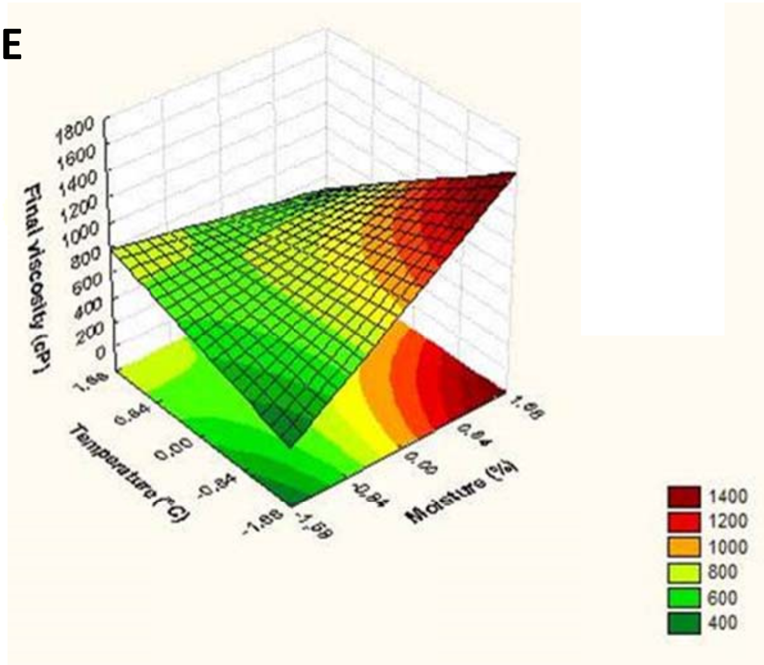

C
Total starch (mg/100 mg)
84
82
80
78
76
74
72
70
68
66
64
62
60
58
Whole grain wheat flour (%)
1.68
0.84
0.00
-0.84
-1.68
Whole grain wheat flour (%)
1.68
0.84
0.00
-0.84
-1.68
80
76
72
68
64

Figure 3.


A
Peak viscosity (cP)
3000
2500
2000
1500
1000
500
0
-500
Temperature (°C)
1.68
0.84
0.00
-0.84
-1.68
Whole grain wheat flour (%)
-1.68
-0.84
0.00
0.84
1.68
2500
2000
1500
1000
500
0
B
Peak time (min)
7
6
5
4
3
2
Temperature (°C)
1.68
0.84
0.00
-0.84
-1.68
Whole grain wheat flour (%)
-1.68
-0.84
0.00
0.84
1.68
6
5
4
3


C
600
500
400
300
200
100
Trough (cP)
1.68
0.84
0.00
-0.84
-1.68
Temperature (°C)
-1.68
-0.84
0.00
0.84
1.68
Whole grain wheat flour (%)
500
400
300
200


D
700
600
500
400
300
200
100
Trough (cP)
1.68
0.84
0.00
-0.84
-1.68
Temperature (°C)
-1.68
-0.84
0.00
0.84
1.68
Moisture (%)
500
400
300
200


E
Final viscosity (cP)
1800
1600
1400
1200
1000
800
600
400
200
0
Temperature (°C)
1.68
0.84
0.00
-0.84
-1.68
Moisture (%)
1.68
0.84
0.00
-0.84
-1.68
1400
1200
1000
800
600
400

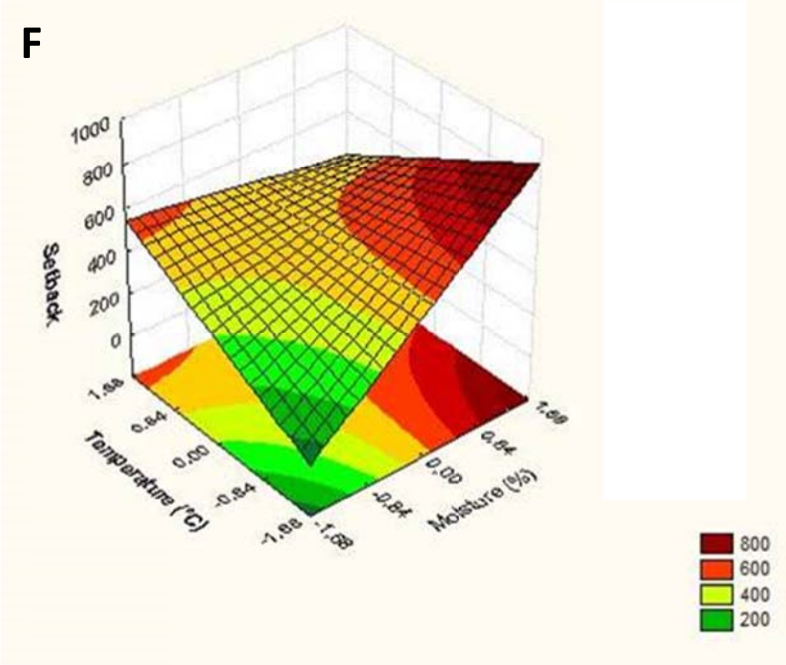

F
Setback
1000
800
600
400
200
0
Temperature (°C)
1.68
0.84
0.00
-0.84
-1.68
Moisture (%)
1.68
0.84
0.00
-0.84
-1.68
800
600
400
200

Figure 4.

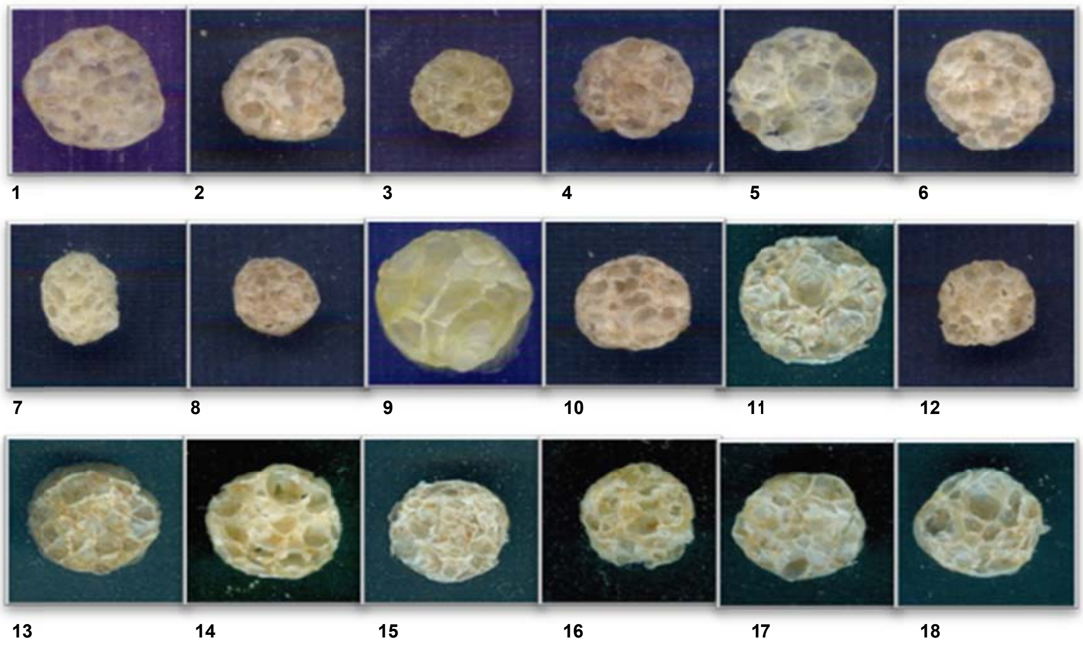
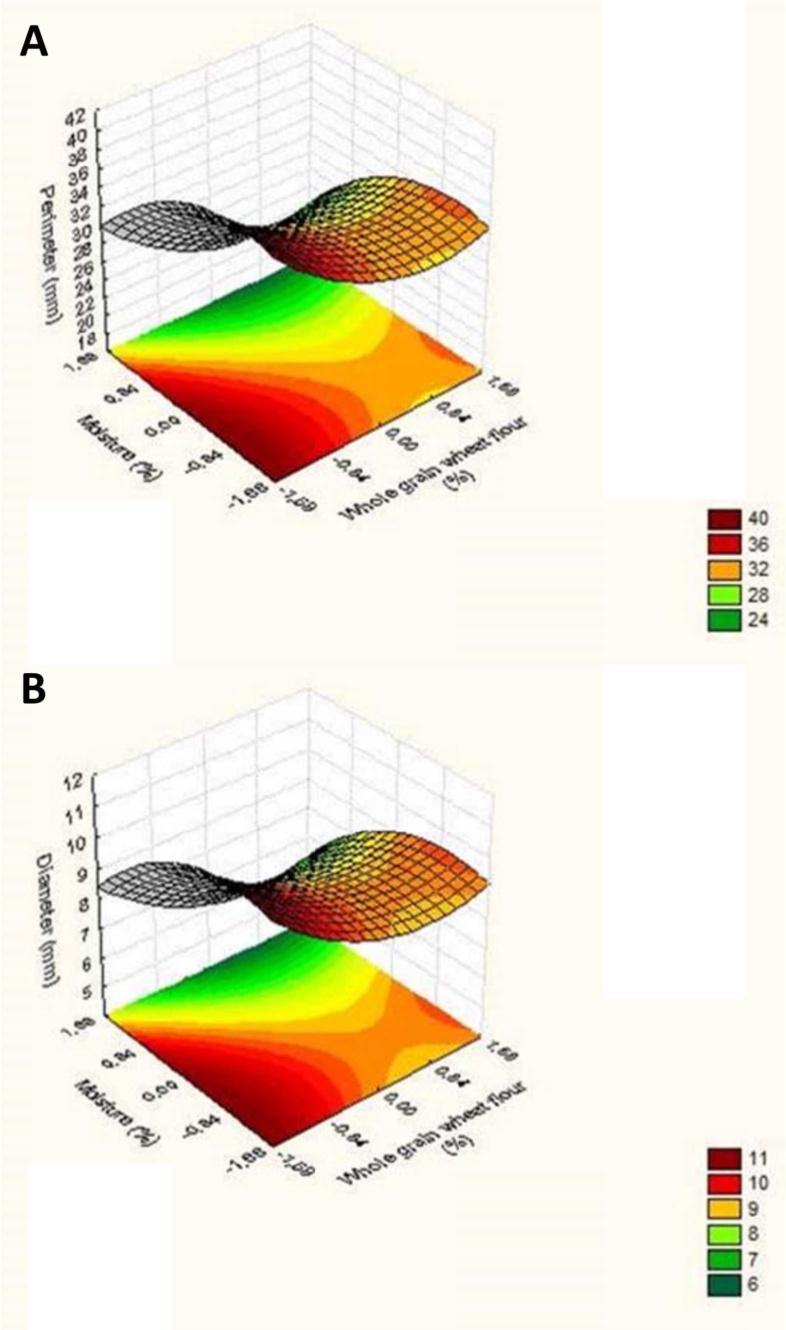
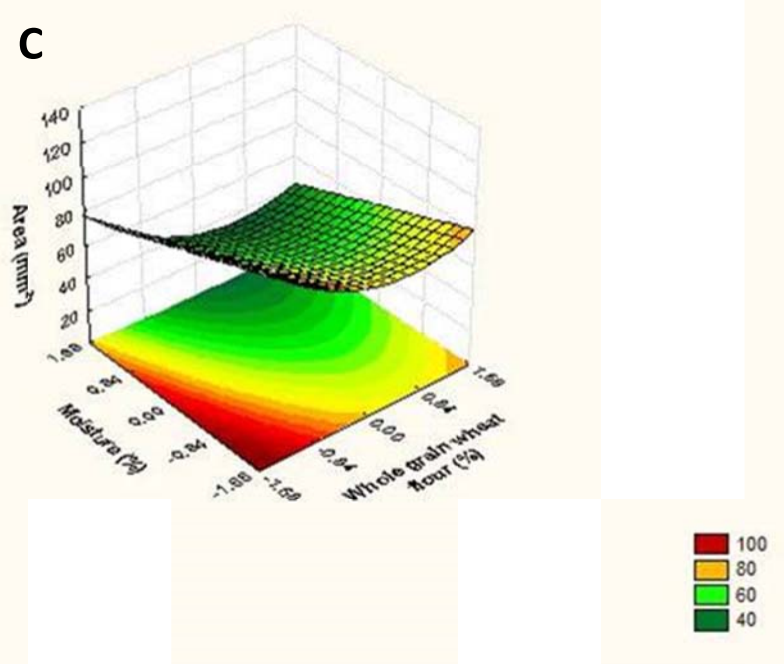
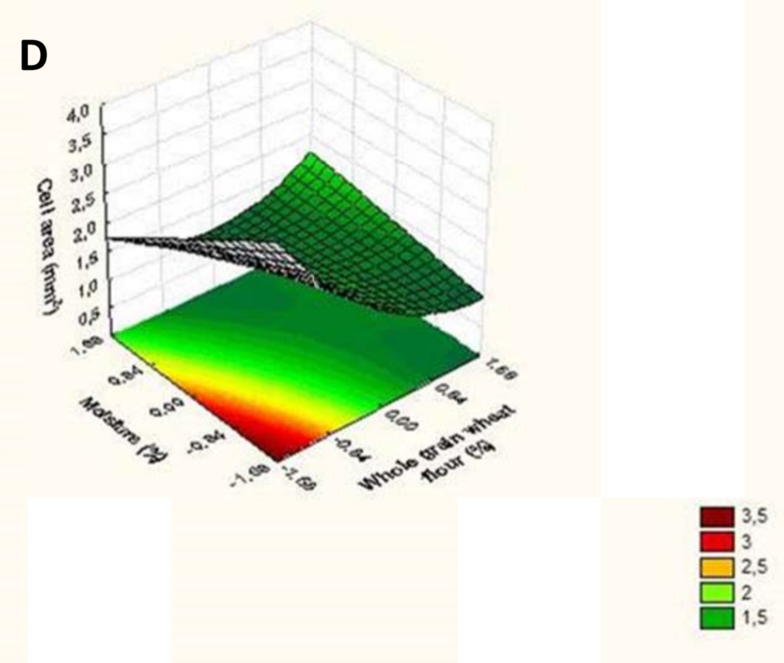
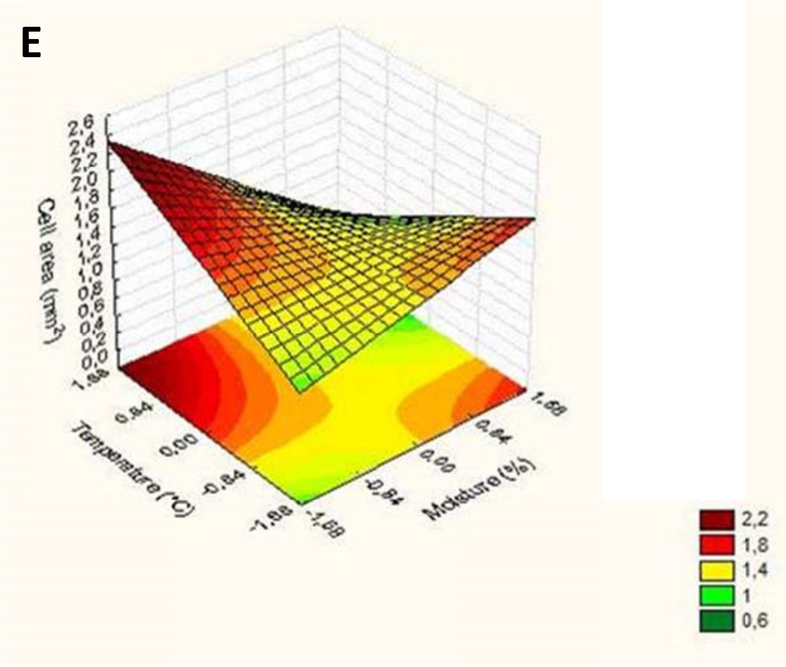


Figure 5.


A
Perimeter (mm)
42
40
38
36
34
32
30
28
26
24
22
20
18
Moisture (%)
1.68
0.84
0.00
-0.84
-1.68
Whole grain wheat flour (%)
-1.68
-0.84
0.00
0.84
1.68
40
36
32
28
24
B
Diameter (mm)
12
11
10
9
8
7
6
5
Moisture (%)
1.68
0.84
0.00
-0.84
-1.68
Whole grain wheat flour (%)
-1.68
-0.84
0.00
0.84
1.68
11
10
9
8
7
6


C
Area (mm²)
140
120
100
80
60
40
20
Moisture (%)
1.68
0.84
0.00
-0.84
-1.68
Whole grain wheat flour (%)
1.68
0.84
0.00
-0.84
-1.68
100
80
60
40


D
Cell area (mm²)
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
Moisture (%)
1.68
0.84
0.00
-0.84
-1.68
Whole grain wheat flour (%)
1.68
0.84
0.00
-0.84
-1.68
3,5
3
2,5
2
1,5


E
Cell area (mm²)
2,6
2,4
2,2
2,0
1,8
1,6
1,4
1,2
1,0
0,8
0,6
0,4
0,2
0,0
Temperature (°C)
1,68
0,84
0,00
-0,84
-1,68
Moisture (%)
-1,68
-0,84
0,00
0,84
1,68
2,2
1,8
1,4
1
0,6