

## Stability of gluten free sweet biscuit elaborated with rice bran, broken rice and okara

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### Abstract

A challenge to the food sector has been the development of new products incorporating co-products from the food processing industry with minimal impact on their pre-determined structures and adding nutritional quality. In order to add value and develop alternatives for the use of co-products generated during the agroindustrial processing, this work aimed to study the stability of gluten-free sweet biscuits developed with soybean okara, rice bran and broken rice. The formulations were elaborated with increasing percentages of these ingredients and compared with the standard (commercial sweet biscuit) for ten months. The analyses were: weight, diameters (internal and external), thickness, specific volume, instrumental parameters of color, texture, scanning electron microscopy, water activity, proximal composition and isoflavones. The experimental sweet biscuits had characteristics of color, weight, volume and diameters (internal and external) very similar to the commercial, whereas texture, lipids and energy value decreased, and aw, moisture and protein increased during storage. The sweet biscuits showed the same stability when compared to the standard, and the

**Keywords:** new food products; food science technology; utilization of waste; bioactive compounds.

**Practical Application:** The development of new food products with high added value has become a challenge for the food industry, since the nutritional quality of the same, will depend on the raw material used. Thus, the co-products used in this experiment were considered viable alternatives for the development of new products, become a good option for the food industry, since they present themselves as good sources of bioactive compounds.

## 1 Introduction

Agroindustries are focused in the transformation and processing of raw materials from agricultural source (plant or animal) and contribute to the generation of large amounts of organic residues. These are solid or liquid materials, which are not used in the production chain, and constitute a serious problem because, apparently without viable application, they are discarded directly into the environment and, if not properly treated, can cause pollution in soil, surface water and groundwater (Mareti et al., 2010). The concern with the environment leads to the elaboration of projects aiming the sustainability of the industrial production system.

The food industry produces a number of co-products with high nutritional value and great potential for reuse. A number of studies using industrial residues from food processing have been performed with this purpose (Lacerda et al., 2009; Santos et al., 2011a; Yoshiara et al., 2012). A viable alternative, not yet exploited by the food industry, is the use of agro-industrial coproducts in the technological development of sweet biscuits, once they have nutritional support and ease of incorporation into food formulations, culminating in a final product with significant food appeal, and contributing to their total use, avoiding the irregular disposal and minimizing environmental pollution (Henningsson et al., 2004). Rice bran, broken rice, and soybean

okara still not used in food product formulations such as sweet biscuits.

Manufacture of bakery products for celiacs requires the use of preselected raw materials, which are free of proteins that could be harmful for these group of people (O'Shea et al., 2014). The number of such ingredients and their combinations is limited, and therefore the portfolio of gluten-free bread is less variable and attractive in terms of their appearance and taste in comparison with traditional bakery products (Morais et al., 2013). Gluten-free bread often reveals smaller volume, because of the lack of gluten network, which normally enables retention of carbon dioxide by the dough. Additionally it is often paler, less elastic, more crumbly and prone to staling process (Matos & Rosell, 2015).

Sweet biscuit is the product obtained by kneading and baking the dough prepared with flour, sugar, starches, fermented or not and other food substances. Its quality is related to the taste, texture, appearance and other factors. In recent years, it has emerged as a product of great commercial interest due to its practicality in the production, marketing and consumption, in addition to having long commercial life (Perez & Germani, 2007; Associação Nacional das Indústrias de Biscoitos, 2013). The broken rice also can be used as a source of carbohydrates

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and some essential amino acids, with virtually the same chemical composition of the whole rice. The okara, in turn, also has advantages such as the absence of gluten, do not alter the taste and enhance the protein content when added to processed products (Amante et al., 1999).

Considering that one of the greatest difficulties in feeding of celiac individuals is in access of products without the addition of wheat, which is one of the main ingredients of sweet biscuits and has favorable sensory characteristics and pleasing to the consumer, its substitution by broken rice flour, rice bran and okara emerge as an alternative. The preparation of sweet biscuits using such coproducts can add technological and nutrition values, besides economic and environmental benefits, in addition to increasing the protein content of the final product. Thus, the objective of this work was to study the physical and chemical stability of gluten-free sweet biscuits based on coproducts of rice and soybean, for a storage period of ten months and comparing it with the commercial sweet biscuit.

## 2 Materials and methods

### 2.1 Obtaining the raw material

The agro-industrial coproducts of rice used in the developed sweet biscuit formulations were obtained from the companies Arroz Cristal Ltda, from Aparecida de Goiânia, Goiás (Brazil). Soybeans, from which okara was obtained, was acquired in Cerealista São José, located in São Paulo, SP (Brazil). Broken grains and rice bran were obtained from the mixture of the cultivars IRGA 417 and IRGA 424, harvest 2012 (Brazil). The other ingredients that composed the formulations of experimental sweet biscuits (EC) (sodium chloride, saccharose, margarine, baking powder, dry egg, coconut essence and calcium propionate), and the commercial sweet biscuit used as standard, were purchased in local shops of Goiânia, Goiás, Brazil.

#### *Preparation of the raw material*

The broken rice were ground in a Wiley mill (Marconi, MA630, Piracicaba, Brazil) and sieve (100 mm diameter). The bran moisture content was adjusted to 21 g 100 g<sup>-1</sup>, to accelerate the enzymatic inactivation during heat treatment, according to Ramezanzadeh et al. (2000). The rice bran was roasted in microwave (CCE, M210, Manaus, Brazil) with internal volume of 21 L and a maximum power of 800 W, which corresponds to the actual power of 758 W, and batches of 150 g, by 3 min. For obtaining dehydrated soybean okara, heat treatment was carried out in the grains, in order to inactivate the antinutritional factors of legumes, following the methodology according to Devidé et al. (2012). The okara produced was dried in air circulation oven (Tecnal TE-394, Piracicaba, Brazil), generating the dehydrated okara.

### 2.2 Formulation and processing of sweet biscuits

For the preparation of sweet biscuits, a mixture experimental design (Simplex) was used in formulating the proportions of broken rice meal, dehydrated soybean okara and the toasted rice bran, according to the Patent Deposit number BR102014017133. Seven sweet biscuit formulations were developed for the

experiment, varying the concentrations of the incorporated ingredients and compared the results with a commercial sweet biscuit well accepted by the population. After weighing, the ingredients were mixed manually to obtain homogeneous dough, molded using tubes of known diameter (35 mm external and 7 mm internal) and subjected to baking. After, the sweet biscuits were allowed to stand at room temperature for cooling and subsequent chemical and physical analyzes.

### 2.3 Physical and chemical stability

The experimental sweet biscuits with most similar scores to commercial standard were chosen and stored together with the commercial sweet biscuit for a period of ten months. The parameters L\*, a\* and b\* were taken using a colorimeter (Hunterlab, ColorQuest II), in the frontal side of the sweet biscuits, wherein L\* defines lightness (L\* = 0 black and L\* = 100 white), a\* and b\* are the chromaticity coordinates (+a\* -a\* red and green, +b\* -b\* yellow and blue). Water activity was determined using an Aqualab apparatus (Aqualab CX-2) at 25 °C. To determine the rupture force of the developed sweet biscuits, a texture analyzer was used (TA.XT PLUS) and the data was expressed in Newtons (N). Scanning Electron Microscopy (SEM) was performed in a JEOL JSM - 6610 microscope, equipped with EDS, (Thermo scientific NSS Spectral Imaging). The samples were placed into aluminum stubs using double-sided tape, plated with a thin gold film (10 nm) and examined at 2.5-3.0 kV of acceleration voltage and in magnifications of 750 x and 3000 x, at 0 and 10 months. The determination of moisture, ash, protein, lipids and total carbohydrates followed the methodology proposed and the calorific value calculated by using the Atwater coefficients (carbohydrates = kcal g<sup>-1</sup>, lipids = 9.0 kcal g<sup>-1</sup>, proteins = 4.0 kcal g<sup>-1</sup>) (Association of Official Analytical Chemists, 2010). The content of isoflavones was evaluated according to the methodology according Yoshiara et al. (2012).

### 2.4 Statistical analysis

The statistical analyses were carried out using the Sisvar Software (Ferreira, 2003). From analysis of variance, polynomial regression models were selected based on the significance of each model and also by the coefficient of determination.

## 3 Results and discussion

Three from the seven initially developed formulations (Formulations 1, 3 and 6) showed very similar values to the sweet biscuits currently found in the market for coloration (L\* and b\* parameters), internal and external diameters, weight, specific volume and rupture force.

The significant differences observed among the developed formulations, might have occurred by different percentages of the supplemental ingredients in each formulation (Table 1), leading to changes in weight and providing greater water retention, due to the fiber content of the sweet biscuits. Santos et al. (2011a), also reported higher values compared to standard, when researching cookies prepared with buriti flour, with and without addition of oatmeal in the formulation.

**Table 1.** Means of color ( $L^*$ ,  $a^*$  and  $b^*$ )  $A_w$ , internal and external diameter (mm), thickness (mm), weight (g), specific volume ( $\text{mL g}^{-1}$ ) and hardness (N) of sweet biscuit formulations based on coproducts of rice (rice bran and broken rice) and soybean (okara).

	Luminosity	Chroma $a^*$	Chroma $b^*$	$A_w$	Internal Diameter	External Diameter	Thickness	Weight	Specific Volume	Hardness
1	33.34c	26.05b	41.22b	0.12	9.44a	39.33a	6.95b	5.79b	1.31a	3810.51a
2	31.23d	28.89a	38.38c	0.13	8.94a	38.28a	7.14b	6.05b	1.31a	3310.58b
3	30.61d	29.69a	37.35c	0.11	8.98a	38.55a	7.90ab	6.60 <sup>a</sup>	1.30a	2262.98c
4	28.72e	26.70b	34.16d	0.12	9.36a	38.25a	6.46b	5.55b	1.24a	2066.23d
5	34.94c	26.00b	42.81b	0.13	9.45a	38.92a	8.33ab	7.04 <sup>a</sup>	1.16a	3912.13a
6	40.54b	24.38b	19.79e	0.1	9.40a	39.09a	7.89ab	5.90b	1.57a	3423.56b
7	45.56a	25.60b	50.94a	0.14	9.55a	39.17a	7.71ab	6.49b	1.26a	2119.65cd
<b>Commercial sweet biscuit</b>	<b>34.35</b>	<b>29.51</b>	<b>43.45</b>	<b>0.21</b>	<b>11.1</b>	<b>44.19</b>	<b>8.04</b>	<b>5.47</b>	<b>1.99</b>	<b>3624.29</b>

\*Means followed by the same letter in the column do not differ at 95% confidence (Tukey 0.05).

The addition of toasted rice bran, broken rice flour and soybean okara in the formulation of sweet biscuits, when compared to standard commercial, resulted in a product with lighter coloration, besides having lower water activity ( $A_w$ ) and smaller specific volume, internal and external diameters. Perez & Germani (2007) reported similar values to those detected in this experiment for the variables weight, specific volume and internal and external diameters in cookies with substitution of 10, 15 and 20g  $100\text{g}^{-1}$  of eggplant flour.

According to the data obtained for moisture, ash, lipids and energy value, the experimental and the commercial sweet biscuits were significantly influenced by the interactions of the factors type and storage time ( $p < 0.05$ ), whereas the protein and carbohydrate were only affected by the isolated factor storage time ( $p < 0.05$ ).

The moisture and protein contents increased during storage (Figure 1), however, until the fifth month, commercial sweet biscuit ( $6.18\text{ g }100\text{ g}^{-1}$ ) had greater moisture than experimental sweet biscuit ( $5.52\text{ g }100\text{ g}^{-1}$ ).

Thereafter, they equaled statistically, reaching an average of  $5.40\text{ g }100\text{ g}^{-1}$ . The difference in behavior during the storage might be due to the preparation of the sweet biscuits, in which different processes were used for commercial sweet biscuit and experimental sweet biscuit. Another major factor in the results of both types of sweet biscuits could be related to the particle size of the raw material incorporated into formulations, once they directly influences the absorption of water by increasing its proportion during storage (Robertson & Eastwood, 1981; Mongeau & Brassard, 1982; Anderson & Eastwood, 1997; Cho et al., 1997). According to Ordorica-Falomir & Paredes-López (1991), the flour or any other ingredient, that absorbs water during the dough mixing can reduce its expansion factor, which results in products with small size or very high weight.

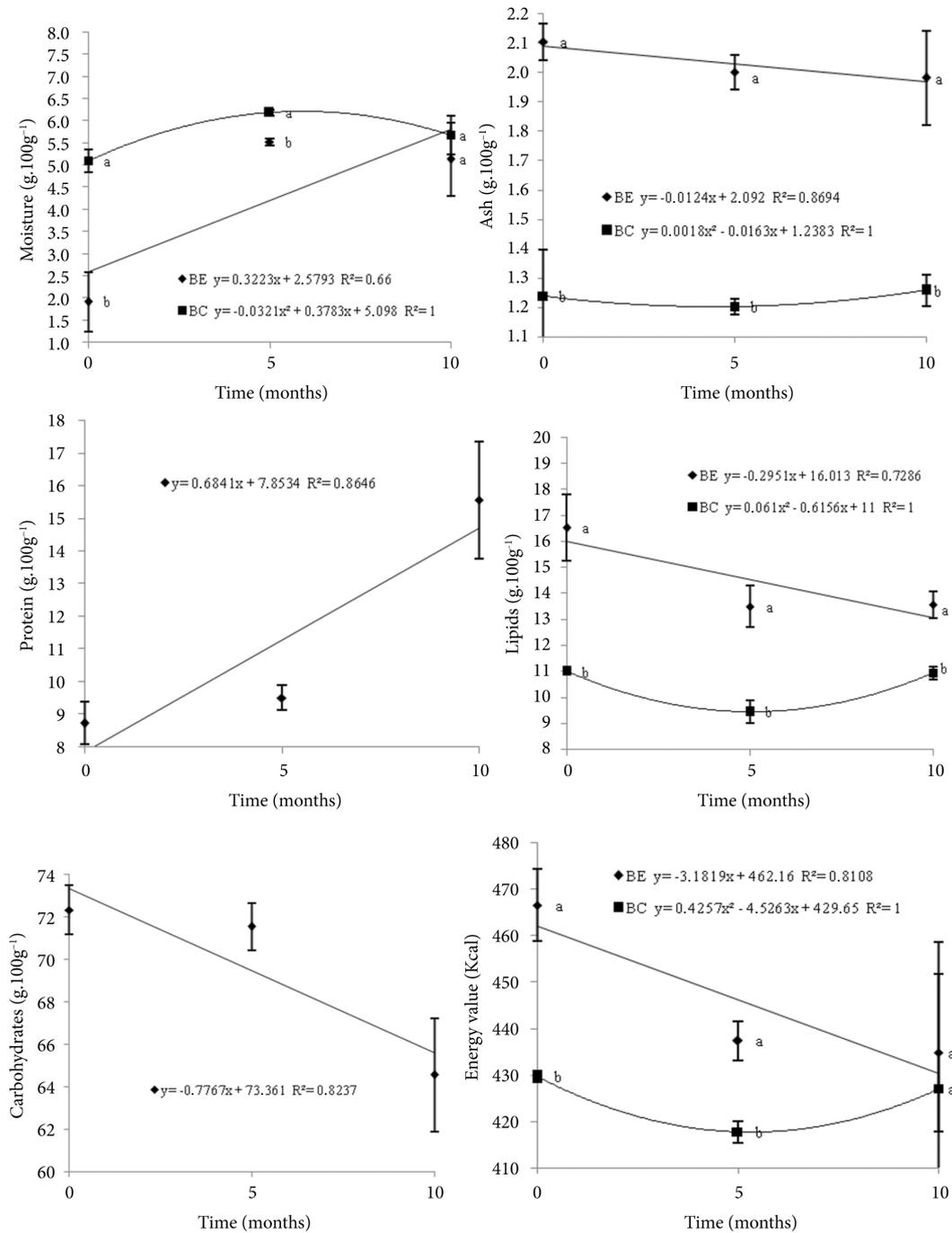
According to Moretto & Feett (1999), the packaging for cookies must have low permeability to water vapor. Despite the variation of moisture content, the two analyzed sweet biscuits were within the standards established by the Brazilian legislation, which stipulates maximum moisture limit for sweet biscuits and wafers of up to  $14.0\text{ g }100\text{ g}^{-1}$  w/w (Brasil, 1978). Lower values

were reported by Madrona & Almeida (2008), in cookies produced with different percentages of okara ( $3.2\text{ g }100\text{ g}^{-1}$ ).

Regarding the ash content, it was observed that the sweet biscuits differed throughout the storage, possibly due to the raw materials used for the sweet biscuit preparation. The elaborated sweet biscuit decreased whereas commercial had a linear trend. This behavior in the ash content might be related to the increase detected in moisture content. According to Brasil (1978), the sweet biscuits must have a maximum of  $3.0\text{ g }100\text{ g}^{-1}$  w/w of ash. This parameter is important for classification of the wheat flour (main ingredient of marketed sweet biscuit, since the greater the ash content, the more impure is the flour, once our samples had pericarp and bran in the formulations. Considering the maximum level required by the Brazilian law, both sweet biscuits were found to be adequate. The ash content present in commercial sweet biscuit is close to the value reported by Ascheri et al. (2006), in cookies with addition of  $10\text{ g }100\text{ g}^{-1}$  of flour from jabuticaba bagasse, and those of the study of (Zoulias et al., 2000) in cookie using flaxseed flour in different proportions ( $1.6$  and  $1.9\text{ g }100\text{ g}^{-1}$ ). The ash content, although crudely represents the mineral content of the sample, from the data obtained for experimental sweet biscuit ( $2.03\text{ g }100\text{ g}^{-1}$ ) and commercial sweet biscuit ( $1.23\text{ g }100\text{ g}^{-1}$ ), the elaborated sweet biscuit can be considered nutritionally superior.

A decrease was observed for lipid, carbohydrate levels and energetic value, which possibly can be explained by the increase in the moisture content of the samples during storage. Higher values for lipids, carbohydrates and energy were found by Perez & Germani (2007); Santos et al. (2011b), in cookie formulations developed with flours from eggplant and buriti, respectively. Regarding the water activity and rupture force, both sweet biscuits were significantly influenced by the interactions of the factors sweet biscuit type and storage time ( $p < 0.05$ ), as shown in Figure 2.

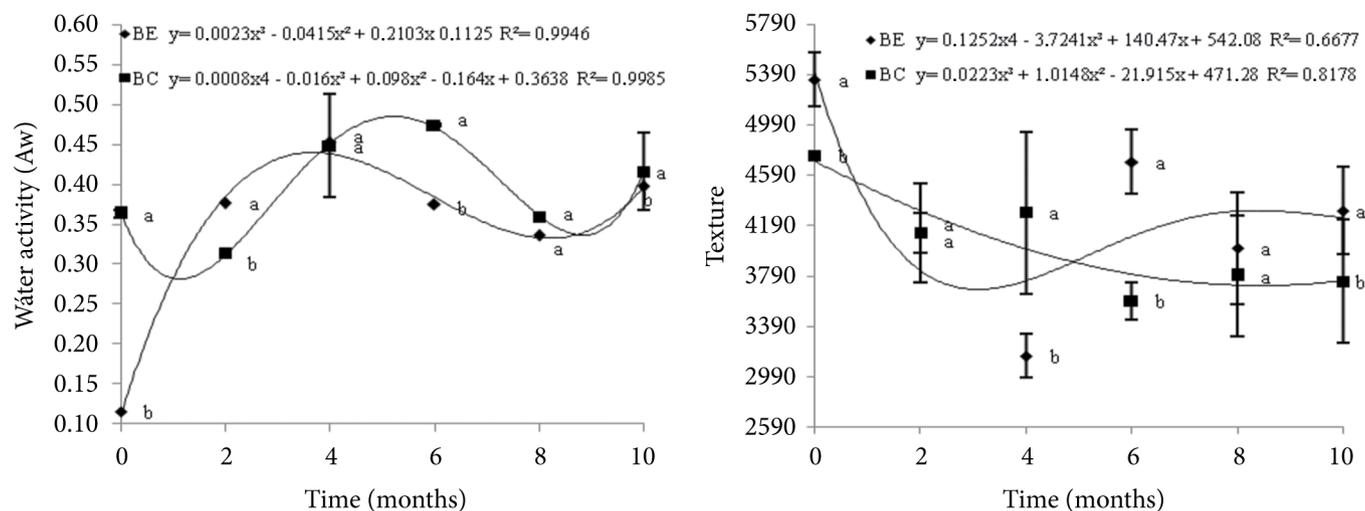
During the storage, the water activity increased, and the rupture force decreased in both sweet biscuits (Figure 2). It was observed that the increase in water activity and in the moisture interfered on the product texture, causing softening by incorporation of water in their chemical structures. The water activity of EC and CC varied throughout the storage period. The experimental



**Figure 1.** Regression equations and coefficients of determination of the moisture, ash, protein, lipids, carbohydrates and energy value of sweet biscuits produced with co-products from rice (rice bran and grits) and soybean (okara) based agroindustries - experimental (EC) - and commercial (CC), stored for 10 months. Means followed by the same letter in each time represent statistical similarities between the sweet biscuits, a 5% probability, using the T test.

sweet biscuit showed the greatest variation probably due to the incorporation of the ingredients used in the formulations, or it may have been provided by the structural difference of the packages of the sweet biscuits. The incorporation of rice bran, broken rice and okara in EC probably increased the content of fibers in the sweet biscuit, which can directly influence the texture of the product, thereby determining higher rupture levels.

According to Collar et al. (2007), the fiber content can modify the consistency, texture, rheological properties and sensory characteristics of food products. According to Scott (1957), the lower water activity levels can ensure microbiological stability of cookies. Thus, the sweet biscuits tend to be microbiologically safer with  $a_w$  below 0.6. Maache-Rezzoug et al. (1998), studied the influence of water, lipids and sucrose in dough for sweet



**Figure 2.** Mean values observed, regression equations and coefficients of determination of water activity and rupture force of sweet biscuits produced with co-products of rice (rice bran and broken rice) and soybean (okara) agroindustries - experimental (EC) - and commercial (CC), stored for 10 months. Means followed by the same letter in each time represent statistical similarities between sweet biscuits, at 5% probability, by the T test.

biscuits and concluded that greater amounts of sucrose produced excessively soft dough, due to competition with the water of the system. In contrast, sucrose tends to crystallize in the cold cookie, making it more crispy, but with a tendency to flaking, by dispersion of starch molecules and proteins, preventing the formation of solid dough, which improves the texture of the product. The texture is a purely sensory concept in foods, in general, one of the most important attributes that affect preference and acceptance by consumers. Therefore, CC was able to maintain their crunchy texture, regarding the rupture force analysis during storage. Even Okara being rich in isoflavones and used in the developed formulations, these compounds were not detected in the compositions supplemented with this raw material (Table 2).

Isoflavones are substances having in common the structure derived from 3-phenyl-benzopyran-4-one and found in relatively high concentrations in legumes (Accame, 2001). As expected, significant amounts of different forms of isoflavones (mg/100 g) were found in the raw material used to prepare the sweet biscuit that used the soy by-product (okara). Esteves & Monteiro (2001) and Nahás et al. (2003) state that the main isoflavones found in soybeans and their derivatives are daidzein, genistein and glycitein, which are present with various forms of glycosidic conjugates, depending on the extension of processing or fermentation. However, in the experimental sweet biscuit, no isoflavone was detected. Wang & Murphy (1994), explain that during the processing steps of soy derived products, significant losses of some isoflavones can occur and also changes in their profile. These authors reported that the main isoflavones present in the unprocessed soy grain are malonylgenistin, genistein, daidzein and malonyldaidzin, and these forms are present in okara, used in this work. Soybeans and defatted soy flour contain significant levels of malonyl-glycosyl isoflavones, and lesser amounts of b-glycosylated forms and only traces of the conjugates acetyl-glycosyl (Barnes, 1998). Among these,

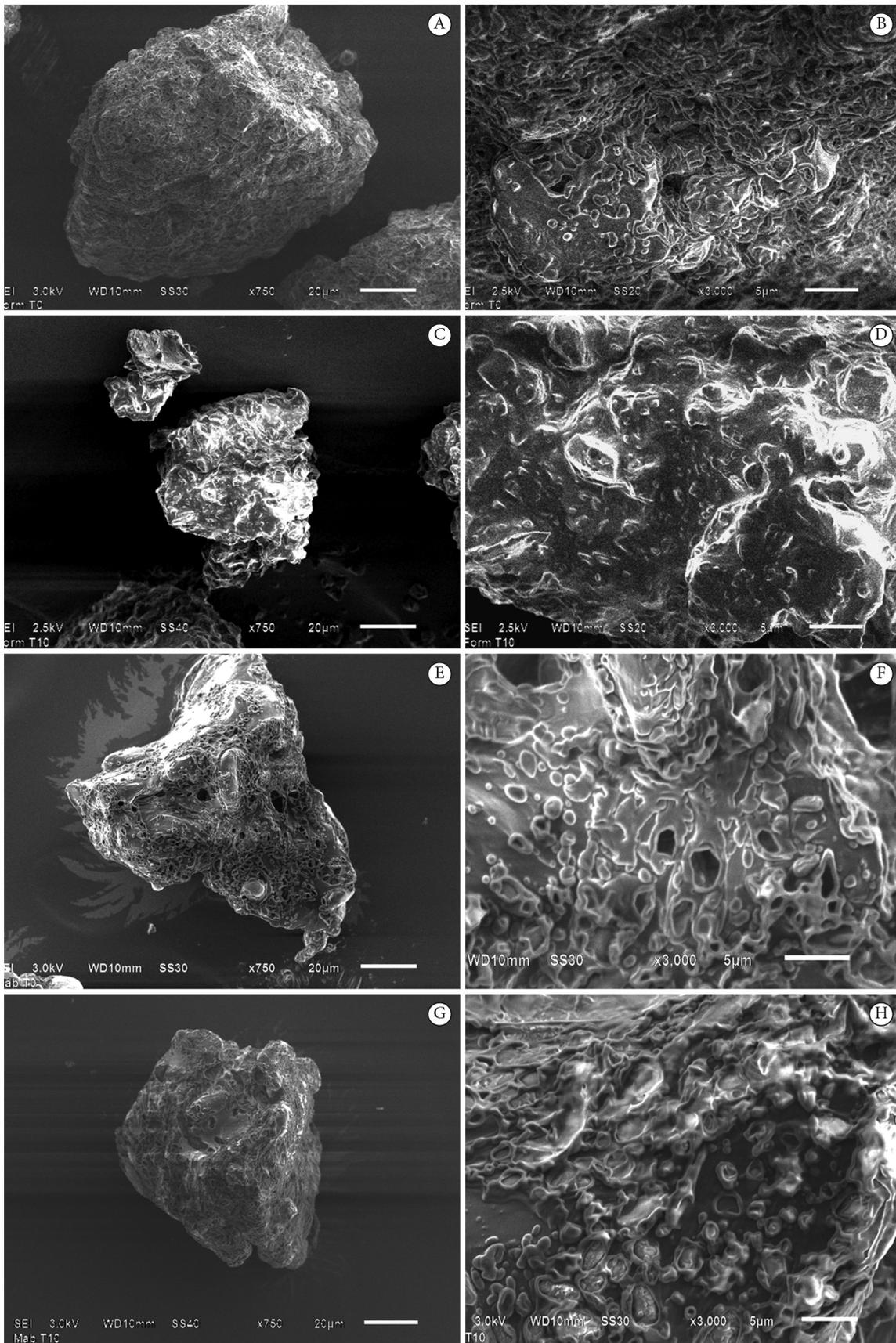
**Table 2.** Content of different forms of isoflavones found in okara and in the sweet biscuits produced with coproducts of rice (rice bran and broken rice) and soybean (okara) agroindustries.

Isoflavone forms (mg 100 g <sup>-1</sup> )*	Soybean Okara	Experimental Sweet biscuit
Daidzin	27.64	0.00
Genistin	42.93	0.00
Glycitin	0.00	0.00
Malonyl-daidzin	15.74	0.00
Malonyl-genistin	39.48	0.00
Malonyl-glycitin	0.00	0.00
Acetyl-daidzin	0.00	0.00
Acetyl-genistin	0.00	0.00
Acetyl-glycitin	0.00	0.00
Daidzein	7.93	0.00
Genistein	11.53	0.00
Glycitein	0.00	0.00

\*1.4 µL aliquots of the filtrate, in triplicate, injected into the liquid chromatograph.

genistein and daidzein as glicosiloflavonas show less biological activity than their aglycone forms, genistein and daidzein, and are the main forms found in soybean, constituting 50 to 90% of flavonoids in soybean flour (Fukutake et al., 1996; Park et al., 2002). Park et al. (2002), studied the conversion of malonyl beta-glycosyl isoflavones, an isoflavone glycoside present in some Brazilian soybean cultivars, and observed changes in the composition of isoflavones extracted at 100 °C or above, once they are thermolabile. The fact that isoflavones undergo changes that lead to loss of this compound can be the explanation for their absence in the elaborated sweet biscuits of this work.

The scanning electron microscopy, evaluated before and after storage of biscuits, revealed a greater uniformity and compaction of the product for both sweet biscuits at time zero. After ten months, a greater porosity was observed in



**Figure 3.** Scanning Electron Microscopy Images of experimental sweet biscuit at time 0 (A), and after 10 months (B), and commercial sweet biscuit at time 0 (C) and after 10 months (D). Magnifications of 750 x and 3000 x. The word A and B are the 1st of left and right, respectively, and G and H are the latest of left and right, respectively.

both formulations, which might be associated with increase in moisture content (Figure 1), water activity (Figure 2), leading to a decrease in rupture force (Figure 2) and a non-uniformity and decompression of sweet biscuits. In the microscopy of the experimental sweet biscuits, fragments irregular in shape and size were observed, possibly due to the incorporation of the various ingredients in the formulation. Furthermore, there is the appearance of holes in the surface of BC in observed in the 3000 x magnification (Figure 3). According to Chiang & Yeh (2002), these voids (holes) can be related to the interaction of proteins with other ingredients used in the preparation of the final product.

#### 4 Conclusion

The sweet biscuits of broken rice, rice bran and soybean okara showed the same stability when compared to commercial. This coproduct is good alternative for incorporation into sweet biscuit recipes.

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