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Exploring the Suitability of Incorporating Tiger Nut Flour as Novel Ingredient in Gluten-Free Biscuit

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The effect of using tiger nut flour to improve the functional properties of gluten-free biscuit was explored. Corn flour in the biscuit formulation was replaced at three levels, 10, 20, and 30% with tiger nut flour (TNF). Biscuit containing only corn flour was used as control. Prepared biscuits were analyzed for their proximate composition, physical properties, diameter, thickness, color, texture, and were subjected to measurements using differential scanning calorimetery (DSC) and scanning electron microscopy (SEM).

Incorporation of tiger nut flour resulted in a significant increase in fibre and ash contents and in a decrease in protein content. The spread ratio of the biscuits increased significantly by increasing TNF content, which is considered a desirable quality attribute. Tiger nut-containing biscuits exhibited lower total color difference ΔE value compared to the control sample.

Thermal characteristics of TNF-containing biscuits differed significantly ($P \leq 0.05$) from the control where TNF resulted in decreased onset gelatinization temperature (*To*) and peak temperature (*Tp*). Furthermore, enthalpies of control biscuits were significantly higher than of those containing TNF; that might be due to partial gelatinization since their enthalpies were smaller than in control biscuits.

Measurement of baked biscuits texture showed that hardness and resilience values decreased when TNF content in the biscuit formulation increased. Microscopic observation revealed that TNF-containing biscuits had the most uniform and homogeneous pore distributions. These attributes probably positively influenced the quality with better surface characteristics. The results of this study revealed that incorporating TNF at the ration of 20% resulted in biscuits of superior technological quality expressed in shape, cross section structure, hardness, and surface appearance.

INTRODUCTION

Coeliac disease (CD) is a pathology affecting the upper small intestine mucosa due to an inappropriate immune response to gluten protein fractions, which are mainly present in wheat, barley and rye. It has been recognized that celiac disease is much more widespread than previously thought, and based on screening data, the prevalence of the disease may be close to 1% of the population of many countries around the world, emphasizing the importance of the market of gluten-free products [Cureton & Fasano, 2009]. Once a person is diagnosed with this disease, the total lifelong avoidance of gluten-containing cereals is required [Fasano & Catassi, 2001]. To date, a lifelong gluten-free diet is the only treatment for CD patients, despite considerable scientific advances in understanding CD and in preventing or curing its manifestations [Niewinski, 2008]. Consequently, a growing market has to be supplied with gluten-free products of acceptable quality.

Thus, research projects and development of industrial products and more precisely of gluten-free bakery products have been attracting considerable interest. Both cereal technologists and bakers are facing an inevitable challenge to formulate gluten-free bakery products. The gluten-free bakery products available on the market present poor organoleptic quality, not comparable to wheat ones. Research and development is needed to improve the structure, mouth feel, texture, acceptability and shelf-life of these products. Results from a number of recent studies have highlighted the need for an improvement in the quality of cereal based gluten-free products. Reduced volume, tough and/or dense texture, and reduced flavor development are among the problems that gluten-free bakery products are suffering from [Sumnu *et al*., 2010].

Although the tiger nut cultivation dates back to ancient Egypt, where tubers were found in sarcophagi and tombs of the earliest dynasties, it is largely unexploited [Tigernuts Traders, 2013]. Tiger nut (*Cyperus esculentus*) belongs to the family *Cyperaceae* and the order, Cyperales. Tiger nut is a tuber that is grown in the soil and is found worldwide in warm and temperate zones, occurring in Southern Europe and Africa. It has a dimension ranging from 6–10 mm and occurs in different varieties. The color is brown and has a sweet flavor when eaten. Tiger nut has been used extensively mainly for human consumption in Spain [Ukwuru *et al*., 2011; Tigernuts Traders, 2013] and eaten cold as drink. Tiger nut can be extracted into milk like liquid that is treated and bottled. The flour is used to make cakes and biscuits while oil is used for cooking [Wise, 2013].

During the last decade, considerable efforts have been directed towards improving the gluten-free product qual-

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ity from the technological point of view. Most of the studies in the literature are on the formulation of gluten-free breads. Studies on other gluten-free bakery products such as cakes, biscuits and pasta are limited. The present work aims to study the impact of incorporating tiger nut flour on the gluten-free biscuit quality. Consequently, gluten-free biscuit containing tiger nut flour at different ratios were prepared. Physicochemical properties of tiger nut flour as raw materials in gluten-free biscuit formulation and their relation to final product quality were evaluated. Proximate composition, physical properties, diameter, thickness, texture, and DSC measurements were performed. Scanning electron microscopy was also employed in order to reveal the structural differences between prepared samples.

MATERIALS AND METHODS

Raw material

Dough samples were prepared from ingredients purchased from a local market: commercial corn flour, tiger nut, sugar, vegetable margarine, milk, eggs, vanilla flavor, and leavening agent. Ingredients used for the preparation of tiger nut biscuits are listed in Table 1. All chemical reagents were of analytical grade purchased from Sigma-Aldrich (Germany).

Preparation of tiger nut flour

The method of Adeyemi [1988] was used in the preparation of tiger nut flour. Mature dried tiger nuts (*Cyperus esculentus*), were sorted to remove unwanted materials like stones, pebbles and other foreign seeds, before washing with tap water. The cleaned nuts were dried in a cabinet dryer at 60°C for 24 h. The dried nuts were milled and sieved through 600 μm pore size. The resultant flour was packed and sealed in polyethylene bags until analyzed. Corn flour in the biscuit formulation was replaced at three levels, 10, 20, and 30% with tiger nut flour (TNF).

Biscuit making

The procedure for making the biscuits according to the method of AACC [2000] is outlined in Figure 1. The dry ingredients (flour, sugar, salt and baking powder) were thoroughly mixed in a bowl by hand for 3 min, vegetable shortening was added and mixed until receiving a uniform mixture. Egg was then added and the mixture was kneaded. The batter was rolled and cut with a 5-mm diameter biscuit cutter. The biscuits were placed on baking trays, and baked at 180°C for 10 min in a baking oven. Following baking, the cookies were cooled at ambient temperature, packed in polyethylene bags and stored at 23°C prior to subsequent analysis.

TABLE 1. Formulations of tiger nut biscuits.

Ingredient (g)	Control biscuits 0% TNF	Blend (1) Biscuits 10% TNF	Blend (2) Biscuits 20% TNF	Blend (3) Biscuits 30% TNF
Corn flour	500	450	400	350
Sugar	375	375	375	375
Vegetable margarine	250	250	250	250
Whole milk	275	275	275	275
Leavening agent[a]	20	20	20	20
Egg	200.9	200.9	200.9	200.9
Vanilla flavor	1.25	1.25	1.25	1.25
Tiger nut flour	0.0	50	100	150

[a]Leavening agent is a mix formed by sodium bicarbonate, tartaric acid, corn starch, rate in mass 2:2:1

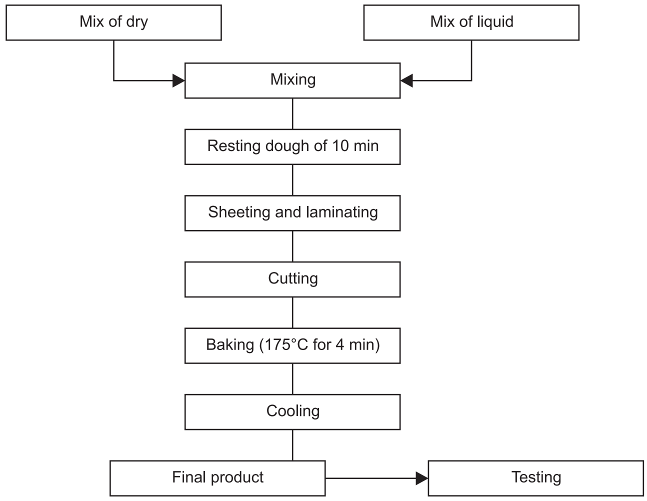

FIGURE 1. Schematic diagram of the biscuit production flow used in bench-top processing.

Proximate analysis

Proximate compositions of all baked biscuits and TNF used in the biscuit preparation were evaluated using standard methods. Moisture, protein, fat, crude fiber and ash contents were estimated by AACC approved methods [2000] while carbohydrate was calculated by difference.

Determination of physical properties of biscuits

The diameter (D) and thickness (T) of the biscuit were measured to calculate the spread ratio (SR) according to AACC method 10–50D AACC [2000]. The diameter of the biscuit was measured by placing six edge-to-edges horizontally and rotating at 90° angle for a duplicate reading. The thickness of biscuit was measured by placing six cookies on the top of each other, followed by a duplicate reading recorded by shuffling biscuits. All the measurements were done in two replicates of six biscuits each one and all the readings were divided by six to get the values per biscuit. The spread ratio (SR) was calculated according to the following formula: SR= (D/T) X 10.

Color

Measurement of the upper surface (crust) color of the biscuits was carried out with Hunter color meter (Hunter Associates Laboratory, Inc., Reston, VA, USA). Four replicates of each blend were measured. The results were expressed in accordance with the CIELAB system where:

L (L = 0 [black], L = 100[white]), a (-a = greenness, +a = redness), b* (-b = blueness, +b = yellowness).

The total color difference (ΔE) between the control biscuit and the TNF-containing biscuits was calculated as follows:

$$\Delta E = [(L_c - L_s) + (a_c - a_s) + (b_c - b_s)]^{½}$$

where: subscript c = control and subscript s = samples containing TNF.

Differential scanning calorimetry (DSC)

DSC measurements were performed with Shimadzu DSC-50 in the four blends and in the corresponding biscuits of the control and the TNF-containing biscuits. The samples were heated to 200°C at a 10°C/min heating rate. The onset temperature (To), peak temperature (Tp), end set temperature (Te) and (ΔH) of the enthalpy was calculated from the DSC thermo grams. The enthalpy was expressed in J/g of dried blends and baked biscuits.

Texture profile analysis of biscuit

Hardness and resilience analysis of the baked biscuit samples was conducted using The TVT Texture Analyzer (Perten instruments) according to TVT Method 10.0. The analyzer was set to perform single cycle measurements which are used for the determination of the first bite force of a product. The measurement speed of 2 mm/s and a distance of 5 mm were applied. A force–time diagram was taken for each test. The force-time plots were analyzed for peak breaking force (g) and time (s) to reach the peak. Textural attributes were measured in three independent samples and the presented values are mean values. Data were subject to analysis of variance to assess the effect of incorporation of tiger nut flour on textural attributes.

Scanning electron microscopy(SEM)

Baked biscuit samples were scanned using JSM-6400 scanning electron microscope (JEOL, Tokyo, Japan). Prior to examination, samples were sputter coated with gold-palladium to render thermoelectrically conductive by using HUMMLE-VII Sputter Coating Device (Anatech Electronics, Garfield, N.J., USA). The micrographs were taken at magnification of 200x for the surface and cross section parts of the biscuits.

Statistical analysis

The mean and standard deviation of parameters from proximate analysis, physical properties, DSC, and texture analyses were calculated and differences between the formulations were evaluated by analysis of variance (ANOVA), using the SPSS 17.0 statistical software programme (SPSS Inc., Chicago, IL, USA).

RESULTS

Proximate composition

Proximate composition of the flour samples is presented in Table 2. The protein content (%) of the composite flour decreased from 8.8 to 4.1 as tiger nut ratio increased. This may be attributed to the low protein content of tiger nut [Bamishaiye & Bamishaiye, 2011]. Although there was a reduction in the protein content of substituted samples with tiger nut flour, a notable enhancement of fiber content in the range of 3.5 to 5.6 was achieved. Tiger nut addition also resulted in 109.1 to 124.2% increase in the ash content of the composite flour.

The fat content increased from 105.2 to 112.5% proportionately as tiger nut flour increased. This might be due to high fat content (32.8%) of the tiger nut flour [Bamishaiye & Bamishaiye, 2011]. The tubers contain up to 30% of non--drying oil which is used for cooking and for making soap [Nwaoguikpe, 2010]. Hence, defatting the nut before utilization may yield a better result. The carbohydrate content (%) of the composite flour decreased from 68.7 to 54.7% indicating low carbohydrate content of the tiger nut flour. Inclusion of tiger nut flour into corn flour at levels of 10 to 30% resulted in a notable increase in fiber and ash contents and decreased content of protein.

Physical quality

Changes in the physical properties of biscuits are shown in Table 3. The spread of the biscuits increased significantly

TABLE 3. Effect of fat reduction on physical quality of biscuits.

Samples	Width (W) (mm)	Thickness (T) (mm)	Spread ratio (W/T)
Control biscuits 0% TNF	50.5±0.70[cd]	9.7±0.33[a]	5.2
Biscuits 10% TNF	51.6±0.41[c]	9.3±0.92[ab]	5.5
Biscuits 20% TNF	53.6±0.54[b]	8.9±0.74[c]	6.0
Biscuits 30% TNF	55.5±1.13[a]	8.6±0.34[cd]	6.5

Means followed by different superscript within a column are significantly different (p<0.05).

TABLE 2. Proximate composition of the composite flour samples (%).

Samples	Moisture	Protein	Fat	Fiber	Ash	Total carbohydrate
Corn Flour	9.10±0.11[a]	10.62±0.13[a]	4.00±0.06[f]	3.60±0.11[e]	2.50±0.00[b]	79.28±0.72[a]
Tiger Nut Flour (TNF)	3.95±0.10[f]	4.89±0.03[e]	31.15±0.25[e]	5.85±0.18[a]	3.75±0.00[a]	54.36±0.44[b]
Control biscuits 0% TNF	7.17±0.03[b]	8.87±0.06[b]	31.12±0.13[d]	3.50±0.05[f]	1.65±0.00[f]	54.86±0.39[c]
Biscuits 10% TNF	6.39±0.05[c]	6.61±0.05[c]	32.75±0.22[c]	3.81±0.07[b]	1.80±0.01[e]	55.03±0.46[b]
Biscuits 20% TNF	5.79±0.01[d]	5.79±0.03[d]	33.65±0.17[b]	4.22±0.09[c]	1.92±0.01[d]	54.42±0.52[d]
Biscuits 30% TNF	5.60±0.08[e]	4.11±0.01[f]	35.01±0.12[a]	5.65±0.05[b]	2.05±0.01[c]	51.13±0.65[f]
LSD at 0.05	0.074	0.019	0.002	0.085	0.074	0.056

Means followed by different superscript within a column are significantly different (p<0.05).

when TNF was increased in the formulation. Biscuits containing 30% TNF had a spread of 55.5 mm, which reduced to 50.5 mm for control biscuit. Similarly, there was also a significant decrease in the thickness of these biscuits from 8.6 mm to 9.7 mm. This elastic nature of the dough would be responsible for such an effect on the spread and thickness of the respective biscuits.

The diameter of the cookies was negatively correlated to their protein content. These results are in agreement with the earlier studies reported by Gaines [2004] and Singh *et al.* [1993], who also reported a decrease in spread factor with increased protein content in the cookies. It was observed that biscuits prepared from high protein flour had greater thickness and lower spread factor as compared to weak flour biscuits which had more width [Maache-Rezzoug *et al*., 1998]. It may be due to the fact that protein has higher binding power and thus restricts the spread of biscuits. Biscuits having higher spread ratios are considered most desirable [Eissa *et al*., 2007].

Color

The color parameter values (L, a, and b) of the biscuits' upper surface (crust) are shown in Table 4. The surface of the control biscuit was significantly lighter (L= 74.12) and slightly yellower than in biscuits made with TNF. The addition of the TNF communicated darkness to the biscuits' surface. The development of color, *i.e.* browning, in biscuits is the result of two simultaneously occurring processes: the Maillard reaction where sugars interact with amino acids, and caramelization which is a direct degradation of sugars [Zanoni *et al*., 1995]. The samples with TNF contained a lower level of proteins and had lower moisture content, which contributed to a reduction in the Maillard reaction. Gallagher *et al.* [2005] reported that as the levels of protein decreased from 8.8 to 4.1% in control and 30% TNF biscuit formulation, L values decreased from 70.1 to 52.2, respectively. Ozturk *et al*. [2009] also found that bread crust color values decreased as protein content decreased as well.

In terms of the total color difference (ΔE) between the control biscuit and the biscuits containing the TNF, all samples exhibited ΔE value lower than the control sample. This means that all of them were darker than the control biscuits, having lower values of L, and higher values of b and a. Consonantly, there was a reduction in the typical golden or very light brown color caused by Maillard reactions in the external crust; although Maillard reactions take place throughout the biscuit dough, browning takes place more intensely on the external surface, promoted by high temperatures and a low moisture content. According to Hadiyanto *et al*. [2007], baking of most products is finished before caramelisation (and carbonisation) reactions at temperatures above 150°C start: even though oven temperatures above 150°C are being used, the temperature of the surface seldom exceeds this value.

No significant differences in the crumb (cross section) color parameter values were found between the TNF-containing formulations (data not shown). However, the color of the biscuit surface was darker than the crumb in all cases, as expected. It is interesting that the addition of TNF did not alter the crumb color of the biscuits to any measurable extent.

DSC measurements

In order to evaluate the degree of starch gelatinization after baking and to understand the contribution of TNF to the structural characteristics of the biscuits, DSC measurements were performed. Onset temperature (To), peak temperature (Tp), and end set temperature, (Te) were recorded from the thermo curve. Gelatinization energy (enthalpy, ΔH) was the area that was calculated by drawing a straight line between onset temperature and end set temperature and was determined in Joules per gram (J/g) on a dry weight basis of blends and baked biscuits made of them.

All biscuit samples showed an endothermic peak at 62.25, 59.73, 52.11, and 52.29°C for control, 10, 20,and 30% TNF, respectively, which corresponds to the melting of starch crystals (Table 5). Increasing the rate of TNF substitution led to a decrease in both *To* and *Tp* for blends. In comparison to three blends, onset and peak gelatinization temperatures of samples were statistically higher. Moreover, a significant decrease in enthalpies by increasing the TNF for blends and biscuits made of them is due to the complete pregelatinization so that they required less energy when they went through DSC heating. Wang & Sastry [1997] found that lower enthalpies by DSC mean enhanced starch gelatinization taking place before and so less energy was needed for fully gelatinized starch than it did for raw samples.

The enthalpies expressed per weight of dried blends were decreased from 10.5 to 7.9 J/g for the control biscuits and these containing 30% TNF, respectively, which reflects the lower starch content available for gelatinization. The same trend was reported for biscuits containing TNF (8.3 to 6.1 J/g for the control and 30% TNF, respectively) however with

TABLE 4. Biscuit color parameter values for control and tiger nut-containing formulations.

Samples	L*	a	b	ΔE**
Corn flour CF	83.01± 1.32^{a}	1.08± 0.04^{f}	17.77± 0.03^{f}	84.89± 1.55^{a}
Tiger nut flour TNF	59.29±1.23^{e}	9.66±0.23^{c}	31.64±0.03^{c}	67.89±1.94^{e}
Control biscuits 0% NF	74.12±1.12^{b}	2.74±0.11^{e}	25.51±0.01^{e}	76.75±1.63^{b}
Biscuits 10% TNF	70.51±1.11^{c}	5.65±0.23^{d}	29.31±0.05^{d}	74.88±1.24^{c}
Biscuits 20% TNF	60.68±1.03^{d}	10.63±0.65^{b}	36.79±0.11^{b}	70.02 ±1.71^{d}
Biscuits 30% TNF	52.28±1.34^{f}	10.65±0.33^{a}	39.54±1.03^{a}	66.41±1.80^{f}
LSD at 0.05	0.064	0.018	1.26	0.085

Values are means (N = 4) ± standard deviations. Means in the same column followed by different superscript are significantly different ($p<0.05$). L*: Lightness; a*: redness; b*: yellowness. ** ΔE : Total color difference.

TABLE 5. Temperature and enthalpy of starch gelatinization (ΔH) of dry blends and biscuits correspondent to the control and tiger nut flour-containing formulations.

Samples	DSC parameters				
	To (°C)	*Tp* (°C)	*Te* (°C)	ΔT (°C)	Δ*H* gel J/g
Corn Flour (CF)	70.12	73.85	78.20	8.08	10.52
Blend (1) 90% Corn Flour + 10% TNF	65.10	69.40	74.10	9.00	9.22
Blend (2) 80% Corn Flour + 20% TNF	57.15	61.73	66.18	9.03	8.34
Blend (3) 70% Corn Flour + 30% TNF	55.95	59.51	65.13	9.28	7.95
Biscuits					
Control biscuits 0% TNF	62.25	68.17	68.50	6.25	8.30
Biscuits 10% TNF	59.73	62.13	67.85	8.12	7.66
Biscuits 20% TNF	52.11	59.66	64.02	11.91	7.15
Biscuits 30% TNF	52.29	56.19	59.35	7.16	6.12

To = onset temperature, *Tp* = peak temperature, *Te* = end set temperature, Δ*T*= the temperature range (*Te-To*), ΔH gel= Enthalpy of gelatinization.

lesser magnitude. The control blends showed higher enthalpy per weight of dried sample than the TNF-containing samples, which is associated to the lower starch content present in the TNF samples (corn flour was replaced by TNF).

The comparison of the enthalpies per weight of the backed biscuits, or per weight of the four blends, reflected a decrease in the transition energy in the biscuits in comparison to the corresponding dry blends, which reflects an increase in the extent of starch gelatinization in the biscuits in comparison to the corresponding blend. Moreover, changes in lipid and protein content would modulate the thermal and mechanical properties of starch gelatinization [Biliaderis & Juliano, 1993; Marshall *et al.*, 1990]. A decrease in gelatinization temperature from lipid and protein removed starch [Crowther, 2012] and a decrease in peak temperature from amaranth starches treated with low alkaline protease [Radosavljevic *et al*., 1998] has been observed.

Texture profile analysis (TPA)

The texture profile (force *vs.* time) curve for the treatments was used for the estimation of hardness (HRD), and resilience (RES), (Figure 2). Hardness (HRD) is the peak force measured during the first compression cycle (*i.e.,* first bite). Resilience (RES) is how well a product "fights to regain its original position". Resilience is measured as the ratio of area, covered under maximum force to base line and area, covered under initial point to the maximum force.

Measurement of baked biscuit texture in the texture analyzer showed that the hardness value decreased when tiger nut flour content in the biscuit formulation was increased. Resilience was also significantly reduced. According to Annor *et al*. [2006], the harder texture of the cookies is attributed to the increased protein content and its interaction during dough development and baking.

Variation in TPA profile due to various levels of tiger nut flour incorporation might be due to lipid content as well as protein and starch quality. Sudha *et al*. [2007] reported that fat coats the surface of the flour particles inhibiting the development of the gluten proteins. The free fat therefore disrupts the gluten network resulting in softer doughs [Menjivar & Faridi, 1994].

Scanning electron microscopy (SEM)

Microscopic observations revealed that the replacement of corn flour with TNF in different percentages affected the microstructure of the produced biscuits. Structural differences between control and TNF-containing biscuits were evident as it can be seen in Figures 3 and 4. The images obtained at 200x magnification correspond to the cross section (crumb) and surface (crust), respectively. According to

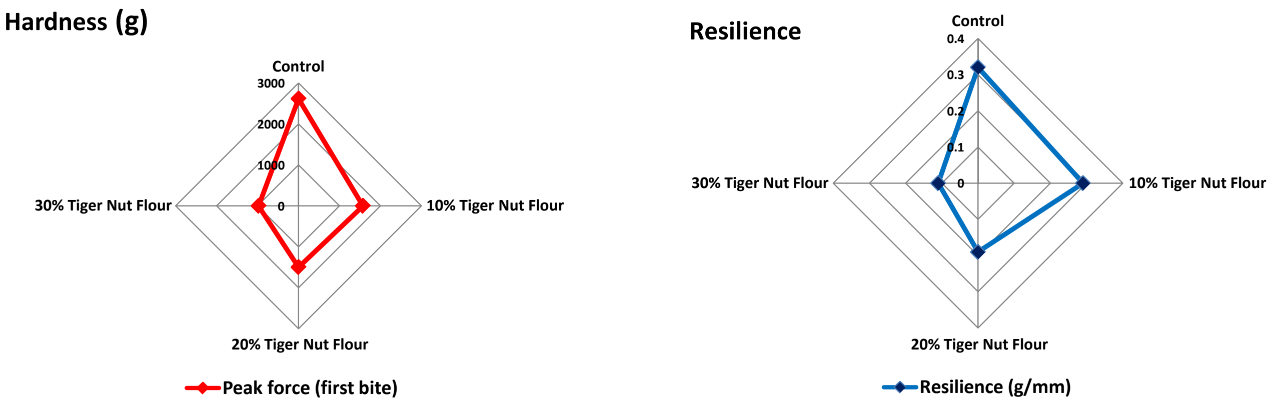

FIGURE 2. Hardness and resilience parameters of corn biscuit incorporated with different amounts of tiger nut flour.

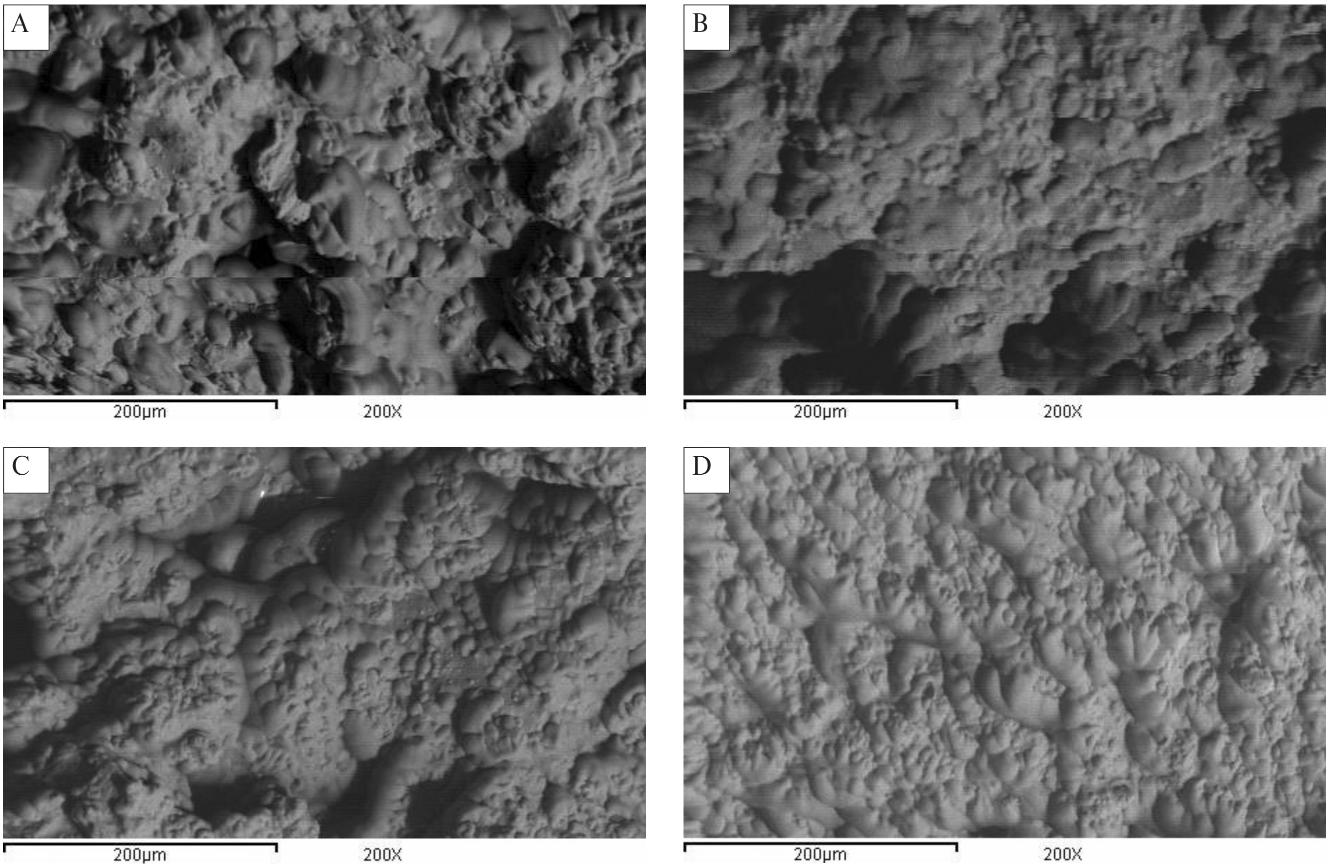

FIGURE 3. SEM micrographs (200x) for surface structure (crust) of biscuits (A. control, B. 10% TNF, C. 20% TNF, D. 30% TNF).

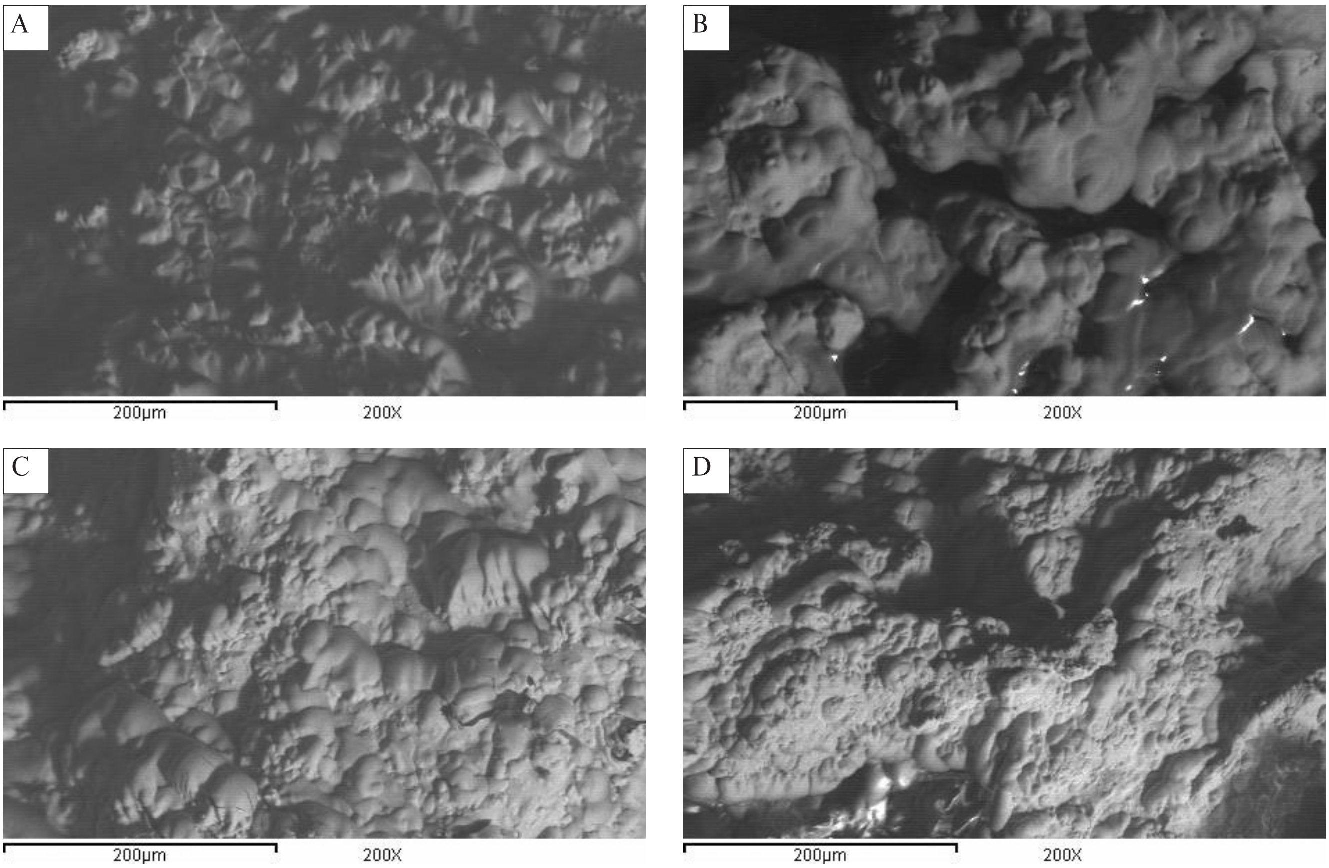

FIGURE 4. SEM micrographs (200x) for inner structure (crumb) of biscuits (A. control, B. 10% TNF, C. 20% TNF, D. 30% TNF).

the obtained micrographs the structure consisted presumably of small densely packed starch granules incorporated in interrupted protein matrix. Some starch granules were intact in spite of the thermal treatment due to an insufficient amount of added water in the entire used biscuit formulations.

More uniform structures were obtained when TNF was added into the biscuit formulations. Control biscuit had both small and large pores and the distribution was not homogeneous. The crust appeared as a continuous sheet of proteins with embedded starch granules, which were not gelatinized because of the rapid dehydration of the surface at the oven temperature (Figure 3a-d). The inner structure (crumb) of the biscuit showed both intact and gelatinized granules (Figure 4a-d).

CONCLUSIONS

Measurements performed in this study revealed that mixtures of corn and tiger nut flour can be successfully incorporated into gluten-free cereal-based products, resulting in biscuits of acceptable technological quality expressed in shape, cross section structure, hardness, and appearance of top and bottom surfaces.

Physicochemical analyses performed on formulations containing TNF resulted in cohesive structure of cookies which was revealed by scanning electron microscopy. The prepared biscuits will be further subjected to sensorial as well as nutritional evaluation.

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