World Journal of Dairy & Food Sciences 9 (2): 127-134, 2014
ISSN 1817-308X
© IDOSI Publications, 2014
DOI: 10.5829/idosi.wjdfs.2014.9.2.1136

Nutritional Value and Sensory Profile of Gluten-Free Tiger Nut Enriched Biscuit

[1]Zahra S. Ahmed, [1]Safaa S. Abozed and [2]M.S. Negm

[1]Department of Food Science, Food Science and Nutrition Division,
National Research Centre, Dokki, Giza, Egypt
[2]Food Technology Research Institute, Agricultural Research Center, Giza, Egypt

Abstract: The objective of this study was to evaluate the nutritional value, the antioxidant activity and sensory quality of biscuits containing four different concentrations of tiger nut flour (TNF) (0, 10, 20 and 30%) as partial replacement for corn flour. Prepared biscuits were subjected to chemical analysis, mineral content, phytochemical content, antioxidants activities and sensory evaluation. Supplementation with TNF resulted in significant increase ($p<0.05$) in fiber, Fe, Zn, total polyphenols and antioxidative activity. The principal component analysis (PCA) was performed on sensory attributes to test variation among biscuits samples. Panelists rated samples prepared with TNF as having higher moistness, softer and airier texture than that of control. TNF enriched biscuits imparts positive characteristics associated with biscuits such as granular structure and nutty flavor. The use of 20% of TNF in the preparation of biscuits have well-developed potential to appeal consumers and to impart health benefits.

Key words: Antioxidants · Biscuits · Gluten-free · PCA sensory analysis

INTRODUCTION

Tiger nut (*Cyperus esculentus* L.) is an underutilized crop which belongs to family Cyperaceae and was found to be a cosmopolitan perennial crop of the same genus as the papyrus plant. Tiger nut has been demonstrated to be rich in dietary fiber content, quality oil and contains moderate amount of gluten free protein [1]. In addition, tiger nut protein contains higher essential amino acids than those proposed in the protein standard by the FAO/WHO/UNU [2]. The tiger nut is non-allergenic and recommended for those who are suffering from Celiac disease. Considering the nutritive and health benefits of the underutilized tiger nuts, it should be encouraged so as to solve the problem of celiac disease i.e. malabsorption of certain proteins in the diet, mainly gluten. Celiac Disease severely impairs intestinal absorption that can lead to severe malnutrition and is caused by a severe sensitivity to gluten [3]. These individuals are intolerant to cereals such as wheat, oats, rye and barley. The only way to ensure a life free of complications is to adhere strictly to a 100% gluten-free diet [4]. The growing interest in the market by patients of Celiac Disease keeps the demand for gluten-free products. Tiger nut can be used in many food applications, e.g. it can be used in snack processing to increase crispiness of chips and crackers [5]. It also reduces cracker hardness, improve the texture of the product and reduce breakage [6]. The use of TNF in making biscuits is explored in our lab and results proved that biscuits of superior quality can be prepared [1]. Biscuits belong to the group of food products that are very popular in daily diet of almost all profiles of consumers [7], having not only the nutritive purpose but influencing also on emotional status of consumers with the effects even on the positive mood enhancement [8]. Biscuits are characterized with quite long shelf life, which results in their availability almost everywhere at any time [6, 9]. Therefore, the alteration of composition of biscuits directed to enhancement of their nutritive and/or functional properties or to enabling of their consumption to the groups of consumers with special needs and demands has been the subject of interest of many researchers. The objective of this study was to evaluate nutritional and functionality characteristics of tiger nut flour enriched biscuit.

Corresponding Author: Zahra Saleh Ahmed, Department of Food Science,
Food Science and Nutrition Division, National Research Centre, Dokki, Giza, Egypt.

MATERIALS AND METHODS

Raw Materials and Chemicals: Ingredients used for the preparation of tiger nut biscuits were purchased from local market: commercial corn flour, tiger nut, sugar, vegetable margarine, milk, eggs, vanilla flavor and leavening agent. Mature cleaned tiger nut (*Cyperus esculentus* L.) were dried in a cabinet dryer at 60°C for 24h then milled and sieved through 600 µm aperture size. The resultant flour was packed and sealed in polythene bags until analyzed. All chemicals were procured from Sigma-Aldrich and Fluka.

Preparation of Biscuit: Table 1 shows the control and different biscuit blends with 10, 20 and 30% corn replacement with TNF. Biscuits were made according to the method of AACC, 10-31.03 [10]. 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 uniform. Egg was then added and the mixture kneaded. The batter was rolled and cut with a 5mm diameter biscuit cutter. The biscuit was placed on baking trays and baked at 180°C for 10 min in baking oven. Following baking, the biscuits was cooled at ambient temperature, packed in polyethylene bags and stored at 23°C prior to subsequent analysis.

Nutritional Evaluation of Biscuits:

Marconutritive Composition Analysis: Protein, fat and ash contents were estimated using the standard methods of analysis AOAC [11]. All the measurements were done in three replicates. Available carbohydrate content was obtained by difference, subtracting 100g minus the sum of grams of water, protein, lipids, ash and crude fibre. The method has been chosen due to its simplicity, since it has been proven to be as accurate as other commonly used methods for estimation of available carbohydrates in starchy foods [12].

Total Mineral Content: The content of potassium, phosphorous, calcium, magnesium, sodium, iron, manganese, zinc and copper was determined as described by AACC [10] method using Atomic Absorption Spectrophotometer Atomic1100B Perkin-Elmer; while phosphorus was determined using Perkin-Elmer UV/VIS Spectrometer Lambda 2. All samples were determined in triplicates.

Determination of Total Phenolic Content (TPC): Total phenolic content of biscuit samples were determined using Folin-Ciocalteau colourimetric method as described previously [13-15]. Briefly, ground biscuit samples (200 mg) were extracted with acidified methanol (HCl/methanol/water, 1:80:10, v/v) (4 ml) at room temperature for 2h. The extracts obtained were oxidized with Folin-Ciocalteu reagent and the reaction mixture was neutralized with sodium carbonate. The mixture was incubated at room temperature for 90 min and its absorbance was measured at 725 nm. Acidified methanol was used as the blank. Gallic acid was used as the standard and total phenolic content expressed as mg gallic acid equivalents/g sample dry weight. All analyses were performed in triplicate.

Total Falvonoid Content (TFC): Flavonoid contents of biscuit samples were determined using the aluminum chloride colorimetric method of Chang *et al.* [16] based on the method of Woisky and Salatino [17]. The appropriate dilution of extracts (0.5 ml) were mixed with 1.5 ml of 95% ethanol, followed by 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm with Shimadzu UV-160A spectrophotometer. The flavonoid content was calculated using a standard calibration of rutin solution and expressed as micrograms of rutin equivalent (RE) per gram of sample. All the measurements were done in three replicates.

Table 1: Formulations of tiger nut biscuits.

	(TNF) Tiger Nut Flour (%)			
Ingredient, g	Control0 % TNF	Blend (1)10 % TNF	Blend (2)20 % TNF	Blend (3)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 agenta	20	20	20	20
Egg	200.9	200.9	200.9	200.9
Vanilla flavour	1.25	1.25	1.25	1.25
Tigre 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

DPPH Radical Scavenging Activity Assay: Di(phenyl)-(2,4,6-trinitrophenyl) iminoazanium (DPPH) radical scavenging capacities of biscuit samples were determined by the reduction of the reaction color between DPPH solution and sample extracts as previously described by Huang *et al.*[18]. A final concentration of DPPH solution used was 0.15 mM. DPPH solution (3.9 ml) was mixed with sample solution (0.1 ml). The mixture was kept in the dark at ambient temperature. The absorbance of the mixtures was recorded at 515 nm for exactly 30 min. Blank was made from 3.9 ml of DPPH and 0.1 ml methanol and measured absorbance at t = 0. All the measurements were done in three replicates.

The scavenging of DPPH was calculated according to the following equation [19]:

% DPPH scavenging = {(Abs (t=0) - Abs (t=30) / Abs (t=0)} x 100

Where Abs (t=0) = absorbance of DPPH radical + methanol at t = 0 min;

Abs (t=30) = absorbance of DPPH radical + phenolic extracts at t = 30 min.

Sensory Evaluation: The survey panel evaluated four randomly presented biscuit samples for upper and lower surface appearance (US/LS), fracture (FR), structure (ST), chewiness (CH), flavor (FL) and overall acceptability (OA) using an 10-cm unstructured line scale, with anchors was used for each descriptor on the sensory ballot and the panelists placed a vertical mark on the scales according to their perception of each sensory attribute ranging from zero (0) denoting not (e.g. not fractured) to (5) denoting extreme (e.g. extremely fractured). The descriptive analysis of the biscuits was replicated three times with a fresh batch each time. Products tested included four experimental biscuits with replacement TNF at 0 (control), 10, 20 and 30%. A panel of 26 (12 female and 14 male assessor in the age range of (20-50 years) who had been selected and trained according to guidelines in ISO/5496 [20] by exposure to the four experimental biscuits in three training sessions of 2h each. A set of 3-digit randomly-coded biscuits were used separately in a booth for evaluation. In between samples, panelists were served drinking water (25±1°C) to cleanse their palates. Ethical approval to conduct the test was granted by the National Research Centre Ethics Committee. Before engaging in the sensory test the panelists signed consent form informing them of the nature of the biscuit samples they would evaluate.

Statistical Analysis: The mean and standard deviation of parameters from proximate analysis, mineral content and antioxidant activities 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). Principal component analysis (PCA), which is a multivariate approach designed for multi-correlated data, was done using XLSTAT 2010.2.03 on the whole sensory data set. Average sensory response values over replicates were used in data analysis.

RESULTS

Macro-Nutritive Composition of Biscuits: Macronutritive evaluation of tested biscuits included the estimation of total proteins, fat, fibre, ash and carbohydrate are presented in Fig. 1. Total protein content of the investigated biscuits ranged from 8.87 in control biscuit to 4.11 g/100 g in 30% TNF containing samples (g/100 g dry matter). Total fibre content ranged from 3.50 in the control biscuit up to 5.65 in 30% TNF containing biscuits (g/100 g dry matter). The content of total fat of biscuit samples was high, ranging from 31.12% in control to 35.01 % in sample enriched with TNF. The content of carbohydrates was 54.86 (g/100 g dry matter) in the control sample and it was significantly reduced in all modified samples by substituting a share of corn flour with TNF. Obtained values ranged from 55.03 g/100 g dry matter in the sample with 10% TNF to 51.13 g/100 g dry matter in 30% TNF enriched sample. The contents of mentioned components were converted to food energy using an Atwater general factor system according to FAO recommendations [21]. Conversion factors used were 4.0 kcal g^{-1} for proteins and carbohydrates; 9.0 kcal g^{-1} for fats; 2.0 kcal g^{-1} for dietary fibre Table 2. According to the acceptable macronutrient distribution ranges for energy given by dietary reference intakes (DRIs) consumption of examined biscuit provides an unbalanced intake of energy since the energy contribution of proteins is lower and contribution of fats higher than recommended values FNB Food [22].

Minerals Content: Supplementation of biscuits with TNF significantly ($p < 0.05$) increased the levels of trace metals iron and zinc from 81.2 (mg/g) for control to 99.0 for 30% TNF biscuits; and from 3.5 for control to 6.3 for 30% TNF biscuits respectively Table 3. The content of potassium, phosphorus, calcium, manganese, sodium, magnesium and cupper concentration was reduced in biscuit as TNF content increase. The higher iron and

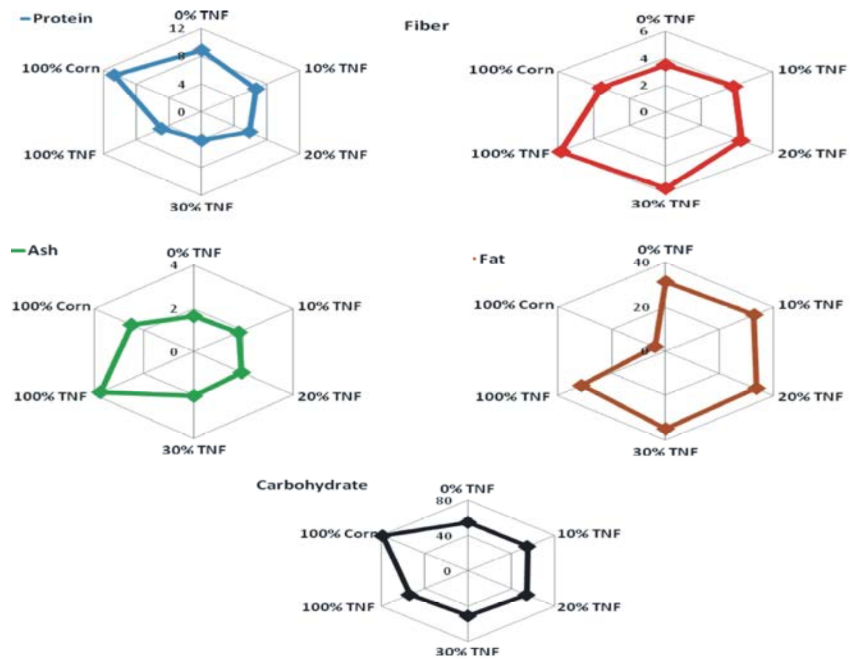

Fig 1: Marconutritive composition of enriched tiger nut biscuits

Table 2: Macronutritive composition g/100g, energetic value and contribution of macro-nutritive compounds to the total energetic value of investigated biscuits.

	% of TNF*			
Items	0	10	20	30
Protein	8.87±0.06^{b}	6.61±0.05^{c}	5.79±0.03^{d}	4.11±0.01^{f}
**% Energy	6.50	4.8	4.2	3.0
Fat	31.12±0.13^{d}	32.75±0.13^{c}	33.65±0.17^{b}	35.01±0.12^{a}
% Energy	51.7	53.7	54.8	56.7
Fiber	3.50±0.05^{f}	3.81±0.07^{b}	4.22±0.09^{c}	5.65±0.05^{b}
% Energy	1.3	1.4	1.6	2.0
Ash	1.65±0.00^{f}	1.80±0.01^{e}	1.92±0.01^{d}	2.05±0.01^{c}
% Energy	0.0	0.0	0.0	0.0
Carbohydrate	54.86±0.39^{c}	55.03±0.46^{b}	54.42±0.52^{d}	53.18±0.65^{f}
% Energy	40.5	40.1	39.4	38.3
kcal/100 g(S)	542	548.93	552.13	555.55

*Tiger Nut Flour

% contribution of energy to the total energetic value of investigated biscuits

Different letters in the same row indicate significant differences (significance level 95%)

Table 3: Minerals composition (mg/kg) of tiger nut flour and biscuits with various levels of substitutions .

	% of TNF				
Minerals	0	10	20	30	100
K	169.5 ± 0.0^{c}	187.3 ± 3.4^{b}	183.8± 4.2^{b}	185.3±0.5^{b}	446.4±2.1^{a}
P	13.4±0.1^{b}	13.1±0.1^{c}	9.8±0.0^{d}	4.4±0.1^{e}	16.1±0.1^{a}
Ca	24.7±0.1^{b}	18.8±0.1^{c}	15.7±0.1^{d}	15.3±0.1^{d}	162.5±0.7^{a}
Mg	27.4±0.1^{d}	27.8±0.2b^{c}	28.0±0.1^{b}	27.6±0.0^{cd}	42.1±0.0^{a}
Na	270.6±0.5^{a}	262.5±0.7^{c}	264.8±0.9^{b}	215.9±0.2^{d}	141.7±0.3^{e}
Fe	11.0±0.161^{e}	18.2±0.1^{d}	90.1±0.0^{c}	99.0±0.2^{b}	117.6±0.0^{a}
Mn	2.0±0.0^{b}	1.2±0.0^{d}	1.2±0.0^{d}	1.4±0.0^{c}	4.6±0.1^{a}
Zn	3.5±0.1^{d}	6.3±0.1^{c}	6.7±0.2b^{c}	6.9±0.4^{b}	89.0±0.1^{a}
Cu	1.4±0.1^{c}	2.2±0.0^{e}	2.7±0.1^{b}	3.6±0.1^{a}	0.5±0.0^{d}

Different letters in the same row indicate significant differences (significance level 95%)

lower sodium contents demonstrated the health benefit of the TNF supplemented biscuits. Calcium, magnesium and iron are minerals that are deficient in gluten-free products and in the gluten-free diet [23-26].

Antioxidants Activity: Profiling of antioxidant potential of tiger nut biscuits was assessed by applying three different antioxidant assays namely total phenol content, total falvonoid content and DPPH radical scavenging activity Table 4. The total phenolic content of the four biscuit samples was examined, since the phenolics might be the major contributor of the antioxidant activities. Significant amounts of total phenolics were detected in all biscuit samples and ranged from 2.11 in control to 1.98 (mg/g) in 30% TNF biscuits. No significant difference ($p<0.05$) between TNF biscuit compared to control. The DPPH radical scavenging activity ranged from 6.51 in control to 7.58±0.1 in 30% TNF biscuits. The ability to scavenge DPPH by the extracts of TNF supplemented biscuits was significantly higher ($p<0.05$) than the ability of an extract obtained using the control biscuit. The total flavoniod content in the control and enriched biscuits showed that incorporation of TNF increased the content of TFC in the enriched biscuits from 4.56 to 6.57 mg/100ml Table 4. With increasing the level of TNF incorporation, the total falvoniod increased. TN enriched biscuits were characterized with higher antioxidant potential in comparison to control biscuits due to the incorporation of phenolic compounds, which had been shown to possess antioxidant activity [27, 28]. Although total phenol and total falvoniod are good in antioxidant activity and scavenging ability on DPPH radicals, they are additives and used or present in milligram levels in foods. However, biscuits could be consumed in gram levels as food. Therefore, TNF biscuit could be developed as a functional food with more effective antioxidant properties.

Sensor Evaluation: Quality characteristics of biscuits included the estimation of several parameters: shape, appearance of upper surface, appearance of lower surface, fracture, structure, chewiness and flavour. The scores for each parameter ranged from 1 to 5. Total score was obtained by summing the scores for each parameter. None of the sensory attributes had mean sensory scores significantly lower ($P<0.05$) than the grand mean scores for the control samples. Panelists rated samples prepared from TNF as having higher moistness, softer and airier texture than that of control as seen in Fig. 2. The highest sensory scores were given to the biscuits supplemented with 20%TNF and statistical analysis of sensory scores confirmed that it was significantly different from other biscuits and thus regarded as most accepted sample. Other TNF biscuits also scored better in all other sensory attributes of appearance, flavor and texture where the score for overall acceptability for biscuits 10% and 30% TNF was 14.12 and 14.41 respectively compared to 14.11

Table 4: Antioxidant properties of biscuit enriched with tiger nut flour.

Sample	TPCmg/g	DPPH%	TFCmg/100ml
0 % TNF biscuit	2.11±0.1a	6.51±0.4b	4.56±0.0bc
10% TNF biscuit	2.01±0.0a	7.03±0.2b	4.78±0.2bc
20% TNF biscuit	2.06±0.1a	7.29±1.0b	5.89±1.3ab
30% TNF biscuit	1.98±0.0a	7.58±0.1b	6.57±0.2a
100% TNF	2.16±0.1a	14.61±0.7a	4.01±0.1c

Different letters in the same row indicate significant differences (significance level 95%).

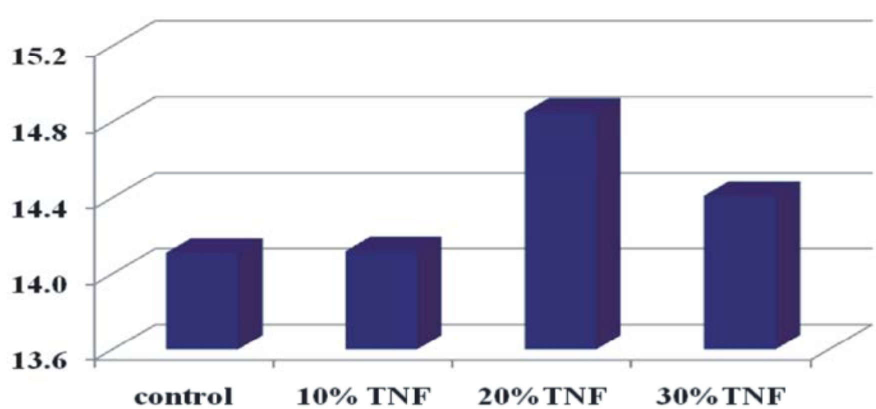

Fig 2: Mean intensity ratings for the sensory attributes of enriched tiger nut biscuits.

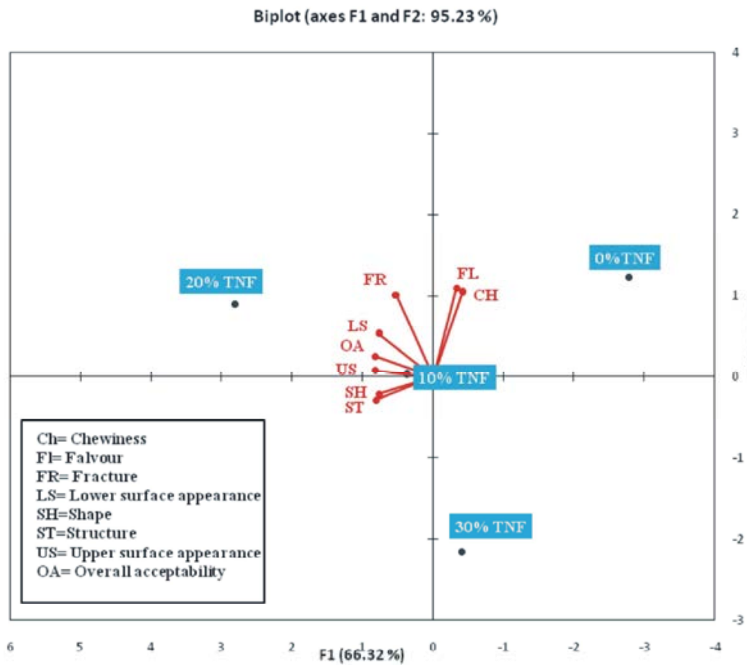

Fig 3: Principal components analysis (PCA) biplot showing the biscuits (• % refers to percentage substitution of corn flour weight by tiger nut flour) in relation to the descriptive attributes based on the descriptive sensory data (n=26 judges × 3 replicates).

score for control. Sample's mean values rated by each panel were then submitted to Principal Component Analysis (PCA) to study sensory attributes sample relationships among tested biscuits. PCA is a well known method for the extraction of the relevant information in multivariate data sets by means of a small number of orthogonal variables called principal components (PCs) [29]. The bi-plot (product-attribute) space using principal components are shown in Fig. 3. It is evident that the attributes discriminating the biscuits containing tiger nut flour from the formulation containing only corn flour are appearance of lower and upper surface, chewiness and overall acceptability. In general, this group of biscuits differentiated in shape, appearance and structure. The supplemented biscuits were airier than the control and therefore rated higher for structure where, 20% enriched TNF biscuits was more porous with mainly granular structure whereas the control sample was compact. In general, panelist scored sensory attributes for TNF containing biscuits as having a more intense stimulus than the control. Texture and flavor were identified as important attributes required for further formulation improvement of a TNF containing product. Supplementation of corn biscuits with TNF imparts positive characteristics associated with biscuits such as moistness, softer, as well as airier texture and nutty flavor.

CONCLUSION

Tiger nut enhanced corn biscuit contained more functional components and more effective antioxidant properties. The sensory analysis indicated that the product with 20% TNF was the best within TNF supplemented ones. Consumption of 100 g of tiger nut enriched biscuits could significantly contribute to an improved total dietary antioxidant intake. The inclusion of tiger nut flour in the formulations of convenient food is beneficial for consumers as it may contribute to more variant and balanced diet.

ACKNOWLEDGEMENT

We would like to extend appreciation to Dr. Mohamed Fathy for statistical analysis assistance.

REFERENCES

1. Ahmed, S.Z. and M.S. Hussein, 2014. Exploring the suitability of incorporating tiger nut flour as novel ingredients in gluten free biscuit. Polish Journal of Food and Nutrition Sciences, 64(2): 27-33.
2. FAO/WHO/UNU, Expert Consultation. Energy and Protein Requirements. World Health Organization Geneva. 1985.

3. Sanchez, H.D., C.A. Osella and M.A. De La Torre, 2002. Optimization of Gluten-Free Bread Prepared from Cornstarch, Rice Flour and Cassava Starch. Journal of Food Science, 67(1): 416-419.
4. Gallagher, E., T.R. Gormley and E.K. Arendt, 2003. Crust and crumb characteristics of gluten free breads. Journal of Food Engineering, 56: 153-161.
5. Gallagher, E., S. Kenny and E.K. Arendt, 2005. Impact of dairy protein powders on biscuit quality. European Food Research and Technology, 221: 237-243.
6. Pareyt, B. and J.A. Delcour, 2008. The role of wheat flour constituents, sugars and fat in low moisture cereal based products: A review on sugar-snap cookies, Critical Review of Food Science and Nutrition, 48: 824-839.
7. Sharif, M.K., M.S. Butt, F.M. Anjum, M. Nasir, R. Minhas and M.N. Qayyum, 2003. Extension of cookies shelf life by using rice bran oil. International Journal of Agricultural Biology, 5: 455-457.
8. Turner, S.A., A. Luszczynska, L. Warner and R. Schwarzer, 2010. Emotional and uncontrolled eating styles and chocolate chip cookie consumption, A controlled trial of the effects of positive mood enhancement. Appetite, 54: 143-149.
9. Rukshan, A., 2001. Consideration of issue of special flour production for confectionery products made with flour. Wheat-Bread, 3: 53-58.
10. AACC, 2000. Approved methods of American Association of Cereal Chemists. St. Paul, MN: Am. Assoc. Cereal Chem. Inc.
11. A.O.A.C., 1995. Official Methods of Analysis. (16^{th} Ed.), Arlington, VA, USA: Association of Official Analytical Chemists.
12. Menzes, E.W, A.T. de Melo, G.H. Lima and F.M. Lajolo, 2004. Measurement of carbohydrate components and their impact on energy value of foods. Journal of Food Composition Analysis, 17: 331-338.
13. Inglett, G.E., D. Chen, M. Berhow and S. Lee, 2011. Antioxidant activity of commercial buckwheat flours and their free and bound phenolic compositions. Food Chemistry, 125: 923-929.
14. Waterhouse, A.L., 2001. Determination of Total Phenolics. In: Wrolstad R.E. Editor. Current protocols in Food Analytical Chemistry (Il.1.1-Il.1.8). New York: John Wiley & Sons Inc.
15. Yu, L. and K. Zhou, 2004. Antioxidant properties of oat bran extracts from Platte wheat grown at different locations. Food Chemistry, 90: 303-307.
16. Chang, C.C., M.H. Yang, H.M. Wen and J.C. Chern, 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal Food and Drug Analysis, 10: 178-182.
17. Woisky, R. and A. Salatino, 1998. Analysis of propolis: Some parameters and procedures for chemical quality control. Journal of Apicultural Research, 37: 99-105.
18. Huang, D.J., B.X. Ou and R.L. Prior, 2005. The chemistry behind antioxidant capacity assays. Journal of Agriculture and Food Chemistry, 53(6): 1841-1856.
19. Liyana-Pathirana, C.M. and F. Shahidi, 2007. Antioxidant and free radical scavenging activities of whole wheat and milling fractions. Food Chemistry, 101: 1151-1157.
20. ISO 5496, 1992. Sensory Analysis -Methodology - Initiation and Training of Assessors in the Detection and Recognition of Odors.
21. FAO Food and Nutrition Paper 77 Food energy-methods of analysis and conversion factors, Food and Agriculture Organization of the United Nation, Rome. 2003.
22. FNB Food and Nutrition Board. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids (Macronutrients), 2005.
23. Hopman, E.G.D., S. Le Cessie, M.E. Von Blomberg and M.L. Mearin, 2006. Nutritional management of the gluten-free diet in young people with celiac disease in the Netherlands. Journal of Pediatric Gastroenterology and Nutrition, 43: 102-108.
24. Kupper, C., 2005. Dietary guidelines and implementation for celiac disease. Gastroenterology, 128(4): S121-S127.
25. Thompson, T., M. Dennis, L.A. Higgins, A.R. Lee and M.K. Sharrett, 2005. Gluten-free diet survey: are Americans with coeliac disease consuming recommended amounts of fibre, iron calcium and grain foods? Journal of Human Nutrition Dietetics, 18: 163-169.
26. Thompson, T., 2000. Folate, iron and dietary fiber contents of the gluten-free diet. Journal American Dietetic Association, 100: 1389-1395.

27. Halosava, M., V. Fiedlerova, H. Smrcinova, M. Orsak, L. Lachman and S. Vavreinova, 2002. Buckwheat-the source of antioxidant activity in functional foods. Food Research International, 35: 207-211.
28. Sun, T. and C.T. Ho, 2005. Antioxidant activities of buckwheat extracts. Food Chemistry, 90: 743-749.
29. Wold, S., K. Esbensen and P. Geladi, 1987. Principal Component Analysis. Chemometrics and Intellectual Lab System, 2: 37-52.