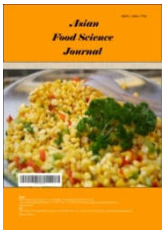

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Production of Biscuits from Mixture of Tiger Nut Flour, Milk Permeate and Soft Wheat Flour

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Authors' contributions

This work was carried out in collaboration among all authors. Author MES designed the study, wrote the protocol and correct the final draft of the manuscript. Authors AMSH and MTF managed the analyses of the study, wrote the first draft of the manuscript and managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

This study was carried out to use mixture of permeate and germinated tiger nut flour(GTNF), soft wheat flour(SWF) for production and evaluation of biscuit product. Biscuits made of 100% SWF and permeate was also prepared for comparison. Chemical composition, mixolab parameters, color attributes, baking quality, and sensory properties of biscuits were studied. GTNF was added to SWF at 10,20 and 30% level. Results revealed that GTNF characterized with its higher fiber (5.41%), fat (27.85%) and ash (3.24%).Therefore, increasing mixing level of GTNF with SWF led to increase the nutritional value of biscuits. Mixolab parameters showed that water absorption and dough stability was increased as the percentage of GTNF in SWF increase. Baking quality of biscuits showed that all biscuit samples from mixing GTNF with SWF had lower volume and specific volume than control. Results also showed that Hunter color parameters (L*, a* & b*) of biscuits were darker as mixing level of GTNF increased. This result was confirmed with the obtained sensorial results. Also, the bacteriological parameters were within the permissible bacterial limits, adopted by the Egyptian Standers, until 3-6 months of storage. Moreover, sensory evaluation of biscuits indicated that all treatments were acceptable, moreover mixture contained GTNF had superior nutritional value and could be suitable for child nutrition.

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Keywords: Soft wheat flour; permeate; germinated tiger nut flour; mixolab parameters and biscuits.

1. INTRODUCTION

Cereals and their products constitute an important part of the human diet, providing a high proportion of carbohydrates, proteins, fats, dietary fiber, B-group vitamins and minerals. More and more foods are made from whole grain [1]. Biscuits are the most popular bakery items consumed nearly by all sections of the society in Egypt. Some of the reasons for such wide popularity are low cost in compared with other processed foods (affordable cost), good nutritional quality and availability in different forms, varied taste and longer shelf-life. Bakery products are sometimes used as a vehicle for incorporation of different nutritionally rich ingredients [2,3]. Fortification with high protein legume flours could provide a good opportunity to improve the nutritional quality of protein consumed by many people. Also, fortification of wheat flour with non-wheat proteins increases protein quality by improving its amino acid profiles [4].

Tiger-nut (*Cyperus esculentus*) is an underutilized crop of the family Cyperaceae which produces rhizomes from the base and tubers that are somewhat spherical. It can be eaten raw, dried, roasted or grated and can be further subjected to further processing. Its uses in cooking and as fuel, baking flour, fish bats, milk in lieu of cow's milk and in fermentation are outlined [5]. Tiger-nut was reported to have positive effect on cholesterol level due to high content of vitamin E [6]. The nuts were found to be rich in myristic acid, oleic acid, linoleic acid with oleic being abundant [7]; rich in energy content, minerals (phosphorus and potassium) and found to be ideal for children, older persons and sportsmen [8].

Germination is a natural process that occurred during growth period of seeds in which they meet the minimum condition for growth and development [9]. During this period, reserves materials are degraded, commonly used for respiration and synthesis of new cells prior to developing embryo [10]. The process starts with the uptake of water by the quiescent dry seed and terminates with the emergence of the embryonic axis, usually the radical [11]. Several studies on the effect of germination on legumes found that germination can increase protein content, dietary fiber, reduce tannin and phytic content and increase mineral bio availability [12]. Germination also was reported to be associated with increase of vitamin concentration and bioavailability of trace elements and minerals [13]. Kaushik [14] found that germination improved calcium, copper, manganese, zinc, riboflavin, niacin and ascorbic acid contents.

Ultrafiltration (UF) of milk produces a large quantity of permeate as a by-product. It contains lactose as the major constituent in addition to soluble vitamins and salts. Therefore, permeate can be considered as a solution of nutritious significance [15]. A huge amount of UF permeate is produced annually and drained as waste. Utilization of UF permeate in the food industry will reduce environmental pollution and consider an added value.

In this study, it developed a biscuit composed of germinated tiger nut and milk-permeate as a nutritive/healthy stable functional food. Study of the physical, chemical, bacteriological and sensory properties of the produced biscuit, during a storage time of 12 months was another goal.

2. MATERIALS AND METHODS

2.1 Materials

Tiger-Nut (*Cyperus esculentus*) was purchased from Al Azhar market, Cairo, Egypt. Strong wheat flour, Soft wheat flour, Sugar, Milk powder, eggs, shortening, vanilla and baking powder were obtained from the local market, Cairo, Egypt (Dokki, Egypt).Permeate was obtained from Dairy unit, Animal Production Research Institute, Ministry of Agriculture, Dokki, Giza, Egypt.

2.2 Methods

2.2.1 Preparation of germinated tigernut flour (GTNF)

Tigernut seeds were separately sorted, cleaned and washed in cold tap water. The seeds were soaked in cold tap water for 12 hrs at room temperature (27ºC). After soaking, the seeds were drained and spread on a clean jute bag and also covered with a damp cotton cloth and left for 72 hrs to germinate. Water was sprinkled at 12 hrs interval to facilitate the germination process. At the end of germination, root hairs were removed from the germinated seeds. The seeds

were dried at 60ºC in an air-draft oven. The dried nuts were milled and sieved through 600 µm pore size. The resultant flour was packed and sealed in polyethylene bags until analyzed [16].

2.2.2 Preparation of mixture

Wheat Flour was well blended with germinated tigernut flour (GTNF)to produce individual mixtures containing 0, 10, 20and 30% GTNF. All samples were stored in airtight containers and kept at 5-7ºC till use.

2.2.3 Rheological properties

Rheological properties of dough's were evaluated using Mixolabetest according to AACC [17].

2.2.4 Preparation and evaluation of baking quality and sensory properties of biscuits

The biscuits were prepared by mixing 200 g wheat flour and their blends containing 10, 20and 30% GTNF. Biscuits were prepared according to the method of AOAC [18] with some modifications in the recipe. The dry ingredients (flour, sugar, salt, and baking powder) were thoroughly mixed in a bowl by hand for 3 min. 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 200°C for 25 min in a baking oven. Following baking, the biscuits were cooled at ambient temperature, packed in polyethylene bags and stored at near ambient temperature (28±2°C) prior to subsequent analysis and sensory evaluation. Weight, volume, specific volume, diameter, thickness (height) and spread ratio of biscuits were recorded, every parameter was measured in triplicate and the mean was calculated. Organoleptic characteristics of biscuits were evaluated according to Hussein [19] where each formula was subjected to sensory analysis by 20 panelists. Each panelist was asked to assign scores 0-20 for color, odor, taste, texture, appearance and overall acceptability.

2.2.5 Color determinations

Objective evaluation of color for raw materials and products (biscuits) were measured. Hunter a*, b* and L* parameters were measured with a color difference meter using a Hunter color meter (Tristimulus Colour Machine) with the CIE lab color scale (Hunter, Lab Scan XE - Reston VA, USA) in the reflection mode. The instrument was standardized each time with white tile of Hunter Lab Colour Standard (LX No.16379): X= 72.26, Y= 81.94 and Z= 88.14 (L*= 92.46; a*= -0.86; b*= -0.16) [20].

2.3 Analytical Methods

2.3.1 Chemical composition

Moisture, ash, crude protein, fat and crude fiber contents were determined in raw materials and products (biscuits) according to the methods outlined in AOAC [18]. Carbohydrates were calculated by difference as mentioned as follows: Carbohydrates = 100 – (% protein + % fat + % ash + % crude fiber).

Table 1. Formulation of biscuit

Ingredients	Samples				
	Control	Control permeate	10%	20%	30%
Wheat flour(g)	200	200	180	160	140
Germinated tiger nut flour (g)	--	--	20	40	60
Sugar (g)	60.0	60.0	60.0	60.0	60.0
Milk powder (g)	30.0	30.0	30.0	30.0	30.0
Fresh egg (g)	30.0	30.0	30.0	30.0	30.0
Shortening (g)	80.0	80.0	80.0	80.0	80.0
Vanilla powder (g)	4.00	4.00	4.00	4.00	4.00
Baking powder (g)	1.50	1.50	1.50	1.50	1.50
Salt (g)	1.50	1.50	1.50	1.50	1.50
Water (ml)	15.0	--	--	--	--
Permeate (ml)	--	15	15	15	15

2.4 Bacteriological Analysis

2.4.1 Samples preparation

25 gram of each sample was mixed and homogenized in sterile mixer, and diluted with buffered peptone water to make the sufficient dilutions for the microbiological analysis. Ten-folds dilutions of homogenates samples were prepared and inoculated onto plates of selective media. Microbiological analyses were followed according to Egyptian Standards [21].

The aerobic bacterial count was carried out using plate agar count after 24-72± 2hrs incubation at 35± 1ºC, colony forming units were counted and calculated per gram of sample, according to ES [22]. Coliform group was determined using solid medium method onto plates of violet red bile agar medium; plates were incubated for 24 hrs at 35ºC. Coliform group to be counted will produce purple colonies surrounded by purple halos(ES) [23]. Ten ml mixture was transferred to selenite cystein broth and incubated at 35ºC for 72 hrs. Plates of *Salmonella* and *Shigella* ager were streaked and incubated at 35ºC for 24 hrs. Growth of *Salmonella typhimurium* is appears as colourless colonies with black centres. (ES) [24]. Enumeration of *E. coli* used MacConkey broth medium was carried out 1ml dilution onto the 9ml medium, tubes were incubated for 24 hrs at 44.5ºC. Enumeration of *S. aureus* in samples was carried out by spreading 0.l ml of each of sufficient (expected) dilution onto the surface agar medium. Baird Parker media supplemented with egg yolk and potassium tellurite solution. Plates were incubated at 37ºC for 48 hrs (ES) [25]. *Bacillus cereus* was determined by the surface plating technique onto the *Bacillus cereus* agar medium, supplemented with polymyxin B and egg yolk (ES) [26]. Enumeration of yeasts and moulds were carried out using the potato dextrose agar medium. Plates were incubated at 25°C for 3-7 days, colonies of yeasts and moulds were counted and calculated per gram of sample(ES) [27].

2.5 Statistical Analysis

The obtained results were evaluated statistically using analysis of variance as reported by McClave& Benson [28].

3. RESULTS AND DISCUSSION

3.1 Gross Chemical Composition of Raw Materials

Table 2 showed the chemical composition of raw materials such as Soft wheat flour (SWF), Germinated Tiger nut Flour(GTNF) and Permeate. The moisture content of Permeate indicated the highest content 94.16%, while the SWF and GTNF13.11 and 8.26 %, respectively. The protein content of raw materials ranged between 0.31 and 9.70%, where protein content maximized in SWF (9.70%) and minimized in Permeate (0.30%). While GTNF maximized in lipid, ash and fiber (27.85, 3.24 and 5.41%), respectively. SWF had the maximum value for total carbohydrates (75.31%). Similar results have been reported by Hussein, Hussein, Ahmed & Hussein and Okoye & Ene [19,29,30,31].

3.2 Mixolab Parameters

Mixolab parameters were used to identify correlations between thermo-mechanical behavior of mixed SWF (72% extraction) with GTNF at different mixing rate (10%, 20% and 30%). Table (3) indicated that water absorption of 72% SWF recorded lowered value (54.2%) compared to SWF mixed with at different mixing rate which recorded the highest value of water absorption (59.8%). This result agreed with Ahmed & Hussein [30]. They stated that water absorption of gelatinized corn flour mixed with GTNF was increased compared with control. On the other hand, dough stability were increased gradually with increasing the levels of GTNF where the highest value of dough stability reached to 9.6 min in case of mixing 30% GTNF with 72% wheat flour, while it was 6.07 min in 72% wheat flour.

Table 2. Chemical composition of raw materials

Samples	Approximate chemical composition					
	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Fiber (%)	CHO (%)
SWF	13.11±0.56	0.50±0.021	9.70**±**0.021	0.92**±**0.042	0.55±0.041	75.31**±**0.44
GTNF	8.26±0.44	3.24±0.007	8.37**±0.31**	27.85**±**1.42	5.41±0.11	46.30**±**0.50
P	94.16±1.16	0.44±0.0	0.31**±0.014**	0.025**±0.007**	ND	5.07**±**0.021

Where: SWF= Soft wheat flour; GTNF= Germinated Tiger Nut Flour; P= Permeate; Cho= carbohydrate

Table 3. Mixolab rheological parameters

Samples	Water absorption (%)	Dough stability (min)	DDT (min)	C1 (Nm)	C2 (Nm)	C3 (Nm)	C4 (Nm)	C5 (Nm)
SWF 72% Extraction	54.2	6.07		3.08	16.88	25.68	32.95	45.02
SWF +10%GTNF	55.0	6.30		3.02	16.72	23.72	34.23	45.00
SWF +20%GTNF	59.8	8.88		1.53	16.68	24.37	29.42	45.02
SWF +30%GTNF	58.0	9.60		7.28	16.68	24.77	31.45	45.02

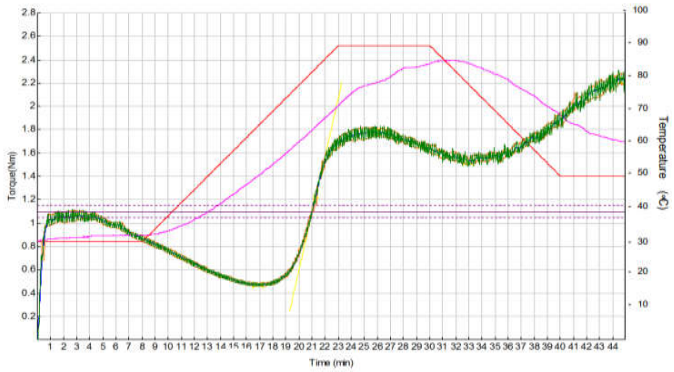
Where: SWF=Soft wheat flour; GTNF = Germinated Tiger Nut Flour

Moreover, protein weakness values (C2) were affected by mixing levels of GTNF where C2 values were increased lightly with increasing the level of GTNF. In spite of protein weakness, Izydorczyk [32] found that the presence of β-glucan in barley seems to override the negative effects associated with the dilution of wheat gluten upon mixing with fiber and starch, and leads to a strengthening of the dough. The increase in dough strength, due to β-glucan addition, also depends on the quality of the wheat flour that is used, with a greater effect for poor bread-making flour than for good bread-making flour. Also, Dhaka [33] indicated that the lower values of C2 led to produce dough's were less tolerant to mixing as compared to the higher values.

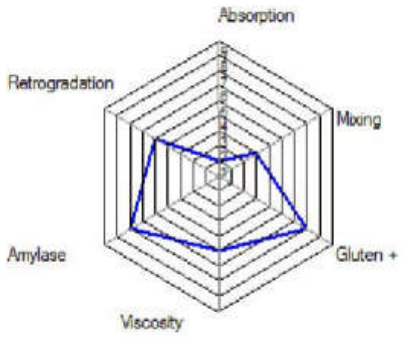
On the other hand, starch gelatinization values (C3) were affected by mixing levels of TNF, where the lowest C3 (23.72 Nm) was recorded by wheat flour mixed with 10%, followed by 20% TNF (24.37 Nm) and 30% GTNF (24.77NM), so increasing mixing level of GTNF to SWF increased gradually the values of C3.Concerning the stability of the hot gel (C4 values), the lowest value (29.42 Nm) was obtained by mixed SWF with 20% GTNF, in contrast to the highest value (34.23 Nm) was found by SWF +10% GTNF. Likewise, the values of starch retro gradation during cooling period (C5) were progressively decreased when the mixing levels of barley flour was increased where, the mixolab characteristic of C5-C4, represent the shelf life of the end product as reported by Capouchová [34]. However, low value of mixolab characteristic C5 represents the rate of retro gradation, verifies the worse quality of the starch part of the wheat grain [35].

3.3 Baking Quality of Biscuit

Results presented in Table 4 showed the weight (g), volume (cm^3), specific volume (v/w), diameter (cm), thickness (cm) and spread ratio (%) of biscuits. Biscuit diameter is significantly increased with increasing mixing level of GTNF, while the volume showed a significant rise upon addition of GTNF generally. This effect may be due to the higher fiber content in GTNF as well as the emulsifying properties reported by Ballesteros and El-Shebini [36,37]. Meanwhile, diameter and spread ratio were decreased significantly compared to the control as affected with GTNF.



Control (SWF)



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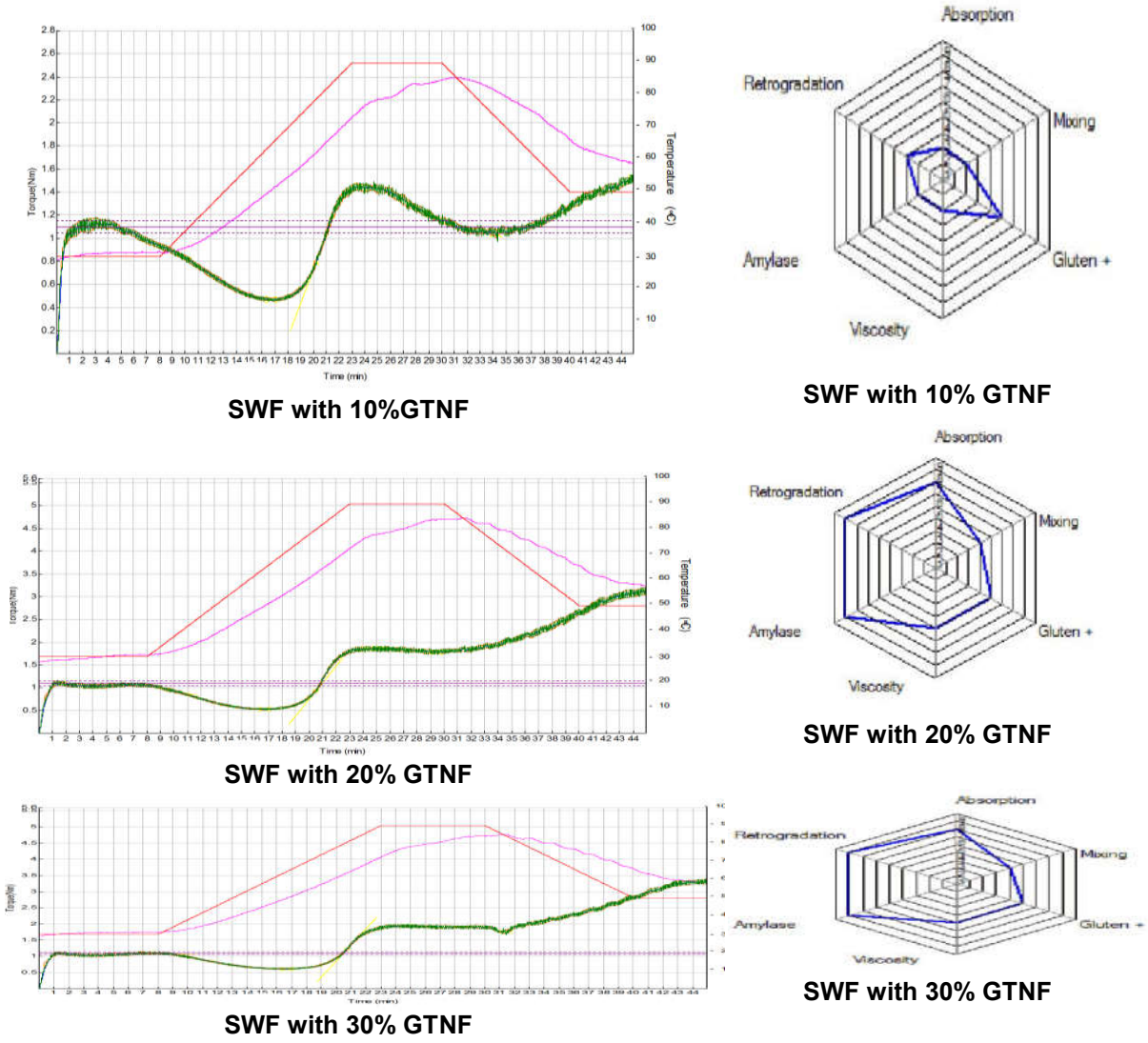

Fig. 1. Mixograms of SWF72% incorporated with GTNFat different levels

Where: SWF= Soft wheat flour; GTNF= Germinated Tiger Nut Flour

Table 4. Physical properties of biscuit

Samples	Physical parameters					
	Weight (g)	Volume (cm^3)	Specific volume (cm^3/g)	Diameters (cm)	Thickness (cm)	Spread ratio (%)
Control	8.4^{a}±0.27	13.7^{ba}±0.47	1.64^{a}±0.007	5.3^{c}±0	0.5^{b}±0	10.6^{ba}±0
CP	8.9^{a}±0.09	12.9^{b}±0.23	1.5^{a}±0.007	5.6^{a}±0.07	0.6^{a}±0	9.4^{c}±0
SWF +10%GTNF	9.12^{a}±0.09	13.8^{a}±0.03	1.5^{a}±0.004	5.56^{ba}±0.07	0.5^{b}±0	11^{a}±0.14
SWF +20%GTNF	9.8^{a}±0.3	13.8^{a}±0.3	1.4^{a}±0.007	5.45^{ba}±0.07	0.5^{b}±0	10.9^{ba}±0.14
SWF +30%GTNF	9.11^{a}±1.3	13.4^{ba}±0.12	1.49^{a}±0.2	5.4^{c}±0.14	0.5^{b}±0.04	10.1^{bc}±0.7

Where: SWF= Soft wheat flour; GTNF= Germinated Tiger Nut Flour; P= Permeate

Table 5. Chemical composition of biscuit

Samples	Approximate chemical composition of biscuit					
	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Fiber (%)	CHO (%)
Control	4.6±0.08	0.4±0.01	9.5±0.01	28± 0.3	0.25±0.04	56.8±0.47
CP	4.7±0.05	0.5±0.01	8.9±0.15	28±0.11	0.36±0.05	58.4±0.14
SWF +10%GTNF	4.7±0.05	0.5 ±0.01	8.9±0.15	28±0.05	0.36±0.05	57.4±0.16
SWF +20%GTNF	4.9±0.03	0.6 ±0.14	8.7±0.27	28.6±0.1	0.6±0.03	57±0.39
SWF +30%GTNF	4.9±0.03	0.73 ±0.014	8.7±0.04	29.2±0.02	0.8±0.02	55.6±0.18

Where: SWF= Soft wheat flour; TNF= Germinated Tiger Nut Flour; P= Permeate

3.4 Chemical Composition of Biscuits

Chemical composition of control and supplemented samples are presented also in Table 5. GTNF possessed a concerned quantity of fiber(5.41% along with ashes 3.24%) which is a source for minerals. Increasing addition of GTNF (10, 20 and 30%) has shown good enhancement in fiber and ashes in biscuits when compared to control (Table 5). Protein content was lower for supplemented samples in comparison to the control one with respect to its percentage on the raw materials, while fat content did not represent significant differences ($p>0.05$).

3.5 Color Parameters of Biscuits

Color is one of the most important sensory attribute that affect directly the consumer preference of any product. Special attention should be given to bakery products to attract the consumer attention. The color parameters of biscuit samples were evaluated using a Hunter laboratory colorimeter (Table 6). The L scale ranges from 0 black to 100 white; the a scale

Table 6. Color measurement of biscuit

Biscuit samples	L	a	b
Control	60.2^{e}±0.32	13.7^{a}±0.16	22.3^{b}±0.04
CP	58.2^{b}±0.014	15.2^{d}±0.06	26^{a}±0.02
SWF +10%GTNF	52.2^{c}±0.22	19.2^{c}±0.052	17.7^{c}±0.7
SWF +20%GTNF	48.6^{d}±0.41	22.3^{b}±0.12	15.4^{d}±0.007
SWF +30%GTNF	43.4^{e}±0.15	26.1^{a}±0.099	12.7^{e}±0.29

Where: SWF= Soft wheat flour; GTNF= Germinated Tiger Nut Flour; P= Permeate

Table 7. Organoleptic characteristics

Samples	Color (20)	Oder (20)	Taste (20)	Texture (20)	General appearance(20)	Overall acceptability (100)
Control	18.52a	17.59b	18.22a	17.41a	17.95a	89.69b
CP	18.77a	19.52a	18.59a	18.66a	19.34a	94.88a
SWF +10% TNF	17.63a	17.85b	17.62a	18.50a	17.04b	87.64b
SWF +20% TNF	16.85b	16.43b	17.64a	18.00a	16.55b	85.07b
SWF +30% TNF	15.22b	14.56c	16.74b	17.24b	15.30c	79.06c
LSD at 0.05	1.785	1.885	1.623	1.305	1.729	4.751

Where: SWF= Soft wheat flour; GTNF= Germinated Tiger Nut Flour; P= Permeate

extends from a negative value (green hue) to a positive value (red hue) and the b scale ranges from negative blue to positive yellow. Biscuit from SWF and GTNF it was darker than SWF and SWF with Permeate, where lightness (L^*) and redness values (b^*) decreased as a percentage of GTNF used in biscuit processing increased, while yellowness (a^*) of biscuit samples, where their values were getting higher in biscuits containing GTNF compared with control or biscuit containing Permeate. This result could be attributed to the darkness of GTNF (lower L^*) than SWF, so, darkness increased as a result of the presence of GTNF in biscuits. Such findings are in-agreement with Kim, Kordonowy &Young and Ramy [38,39,40].

3.6 Sensory Properties of Biscuit

Sensory evaluation is considered one of the limiting factors of consumer acceptability for organoleptic properties including color, odor, taste, texture, appearance and overall acceptability. The effects of Permeate and GTNF on sensory characteristics of biscuits are presented in Table (7). With the increase in the level of GTNF in the formulation, the sensory scores for color, taste, odor, texture, appearance and overall acceptability of biscuits decreased. Data indicated that a significant ($P<0.05$) changes were found in all properties for all experimental products. Data in Table (7) showed that biscuit made from mixture containing 30% GTNF had lower scores for most properties compared to the other tested products. Besides it showed the lowest score for overall acceptability (79.06) for biscuit. The highest overall acceptability scores of biscuit were registered for control (100% SWF). Finally, the results showed that in all the sensory qualities that increase the proportion of GTNF about 30% less than in the sensory qualities, especially color and appearance. Also these results were confirmed by the results of the physical properties of biscuits. From the sensory acceptability rating, it was concluded that biscuits were made from GTNF could be incorporated all levels used in this study to SWF in the formation of biscuits without significantly affecting on sensory quality.

3.7 The Bacteriological Analysis of Biscuit

The bacteriological analysis of all the manufactured biscuits, during storage period of a year, are shown in Table 8.

Table 8. Microbial analysis of control and different treatment of biscuitcfu/g

Storage period	Treatments	T.C	Coliform	*S. typhimurium*	*E. coli*	*S. aureus*	*B. cereus*	M.Y
Zero	**Control**	32 $x10^2$	Nil	Nil	Nil	Nil	Nil	Nil
	CP	38 $x10^2$	Nil	Nil	Nil	Nil	Nil	Nil
	SWF +10% GTNF	42 $x10^2$	Nil	Nil	Nil	Nil	Nil	Nil
	SWF +20% GTNF	48 $x10^2$	Nil	Nil	Nil	Nil	Nil	Nil
	SWF +30% GTNF	51 $x10^2$	Nil	Nil	Nil	Nil	Nil	Nil
3 months	**Control**	69 $x10^2$	Nil	Nil	Nil	Nil	Nil	Nil
	CP	74 $x10^2$	Nil	Nil	Nil	Nil	Nil	Nil
	SWF +10% GTNF	84 $x10^2$	Nil	Nil	Nil	Nil	Nil	Nil
	SWF +20% GTNF	87 $x10^2$	3$x10^1$	Nil	Nil	Nil	Nil	Nil
	SWF +30% GTNF	91 $x10^2$	5 $x10^1$	Nil	Nil	Nil	Nil	Nil
6 months	**Control**	94 $x10^2$	Nil	Nil	Nil	Nil	Nil	Nil
	CP	10 $x10^3$	7 $x10^1$	Nil	Nil	Nil	Nil	Nil
	SWF +10% GTNF	18 $x10^3$	10 $x10^1$	Nil	Nil	Nil	Nil	Nil
	SWF +20% GTNF	22 $x10^3$	16 $x10^1$	Nil	Nil	Nil	Nil	Nil
	SWF +30%	30 $x10^3$	22 $x10^1$	Nil	Nil	Nil	Nil	2

Storage period	Treatments	T.C	Coliform	*S. typhimurium*	*E. coli*	*S. aureus*	*B. cereus*	M.Y
	GTNF							$x10^1$
9 months	**Control**	12 $x10^3$	Nil	Nil	Nil	Nil	Nil	Nil
	CP	54 $x10^3$	15 $x10^1$	Nil	Nil	Nil	Nil	Nil
	SWF +10% GTNF	63 $x10^3$	26 $x10^1$	Nil	Nil	Nil	Nil	Nil
	SWF +20% GTNF	71 $x10^3$	31 $x10^1$	Nil	Nil	Nil	Nil	Nil
	SWF +30% GTNF	87 $x10^3$	39 $x10^1$	Nil	Nil	Nil	Nil	3 $x10^1$
12 months	**Control**	46 $x10^3$	6 $x10^1$	Nil	Nil	Nil	Nil	Nil
	CP	91 $x10^3$	24 $x10^1$	Nil	Nil	Nil	Nil	Nil
	SWF +10% GTNF	99 $x10^3$	38 $x10^1$	Nil	Nil	Nil	Nil	Nil
	SWF +20% GTNF	24 $x10^4$	51 $x10^1$	Nil	Nil	Nil	Nil	Nil
	SWF +30% GTNF	48 $x10^4$	63 $x10^1$	Nil	Nil	Nil	Nil	10 $x10^1$

C = control; P= Permeate; SWF= Soft wheat flour; GTNF= Germinated Tiger Nut Flour, T.C: Total bacterial count, Coliform: coliform bacterial group, S. typhimurium:Salmonella typhimurium, E. coli: Escherichia coli: S. aureus: Staphylococcus aureus, B.cereus: Bacillus cereus, M.Y: moulds and yeasts, Nil: not detected

The total viable counts of the samples ranged from $3.2x10^3$ to 4.8 $x10^5$cfu/g. However, the counts gradually increased till end of storage time. Also, the total coliform counts ranged from 3 $x10^1$cfu/g to 6.3 $x10^2$cfu/g. no growth in the first 3 months days. On the 6 month of storage, the mould count was 2x 10^1cfu/g. The growth observed could be due to post-processing contamination. These results are in agreements with Oladipo and Agu&Okoli [41,42] who stated that the presence of the microbial count of the best biscuit after 20 days of storage was 4.0 x 10^3cfu/g for bacteria and mould contained 5.0 x 10^4cfu/g, while bacterial pathogens were not detected in all treatments (*Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus)*.

4. CONCLUSION

For consumers, who prefer unique and desirable products, a remarkable biscuit fortified with germinated tiger nut flour was presented. Consequently, the chemical, microbiological and sensory evaluation indicated that biscuit with 10% of germinated tiger nut flour had the highest scores compared to other treatments.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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