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Production of functional Pan Bread from Mixture of Tiger Nut Flour, Milk Permeate and Hard Wheat Flour

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Abstract

The effect of using tiger nut flour germinated (TNFG) and milk permeate supplement with probiotic bacteria to improve the functional properties of pan bread was explored. Hard wheat flour extraction 72% (HWF) in the pan bread formulation was replaced at three levels, 10, 20, and 30% with tiger nut flour germinated (TNFG). Pan bread containing only HWF or/ and milk permeate was used as control. Prepared Pan bread were analyzed for their rheological properties, proximate composition, physical properties (weight, volume and specific volume) color, sensory evaluation, texture and microbiological analysis. Water absorption, arrival time, dough-development time, mixing tolerance index and dough-weakening were significantly increased, however dough-stability was decreased parallel with an increment of the WF-substitution level using the TNFG and Milk Permeate. Incorporation of TNFG resulted in a significant increase in fiber and ash contents and in a decrease in protein content. Loaves volume and specific volume decreased as the TNFG level significantly increased and the significant decrease was observed at 30% replacement compared to the other levels. The crust and crumb of pan bread samples supplement with different levels of TNFG had lower L* and b* values and the reduction increased as the fortification level increased. With the increase in the level of GTNF in the formulation, the sensory scores for taste, aroma, Crumb grain texture, crust color, crumb color, symmetry of shape and Overall acceptability of pan bread decreased. Measurement of pan bread texture showed that Springiness, Cohesiveness and resilience values decreased when TNF content in the pan bread formulation increased. The total viable counts to all samples ranged from $6.0x10^2$ to $7.2\ x10^3$ cfu/g however, the total fungi counts ranged from $2.0\ x10^1$cfu/g to $2.2\ x10^2$cfu/g. The bacterial pathogens including (Coliforms group, *Salmonella typhimurium* and *Staphylococcus aureus*) were not detected in all treatments throughout the storage period.The results indicated that added10% TNFG was the most superior for production of Pan Bread with no adverse effect on the physical and sensory properties. The object of this study is to produce high nutritive probiotic bread supplement with different levels of TNFG and Milk Permeate.

Keywords: Bakery products, dairy products, rheological and physical properties, sensory evaluation.

1. Introduction

The tiger-nut has a great effect on reducing cholesterol, and minimizing the danger of coronary heart disease, arteriosclerosis and can be a good choice for those who have problems in digestion, cancer especially colon cancer ,flatulence and dysentery, [1]. Ultrafiltration (UF) of milk contains lactose as the most important ingredient, vitamins and soluble salts. That's why, the permeate may be regarded as a solution which is nutritionally important. Using the UF permeate in the food industry will minimize the environmental pollution and will be regarded as added value [2]. One of the most important research priorities is the reduction of food borne pathogens to be able to produce food that is safe for human consumption. *Staphylococcusaureus*, *Salmonella*, *Campylobacter*, etc. are among pathogens that may cause dangerous illnesses that can be fatal. [3].

Lactic acid bacteria (LAB) are a good solution for controlling and reduction of AFB1 and OTA from a contaminated medium, generally considered safe according to the USFDA; some of them also have many health benefits, which are called probiotic bacteria [4]. Screening of *Lactobacillus* strains, including commercial probiotics and *L. rhamnosus* GG, showed that all Lactobaccilli showed strong antibacterial activity towards the Gram-negative pathogen *Salmonella*enteri case rovar*typhimurium* SL1344 and safely prohibited the overrun of the pathogenin cultured cells of Caco-2 / TC7 similar to human enterocytes [5]. Many microorganisms, including bacteria, yeasts, and molds, can be able to minimize

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toxins in food and feed, so numerous studies made use of (LAB) to link toxins in lab and real life, [6]. Tiger-nuts are mainly eaten in a raw state and have undergone very little added value or product development.

Tubers contain a good quantity of protein, fat, minerals, fiber, ash, and vitamins [7-9], and thus they raise the nutrition and value of food. Besides, tiger-nut tubers can also be used in treating (can be used as a remedy for)flatulence, indigestion, diarrhea, dysentery, and severe thirst [10]. Compound flour is a type of flour that is prepared by blending cereals, roots, tubers, or legume flour in a special ratio. Improving nutritional value is an important reason for making blended food. we can have a doubled benefit from making food that consists of cereals and legumes: the first is raising the protein content of the food in comparison to when the cereal forms the base .The second is having a good balance of amino acids by the support of lysine, by legumes and methionine by cereals . Not only the composition has its effect on the nutritional value, but also the sensory, functional, and phytochemical value of the final food product [11].

The bakery products made using compound flour had a perfect quality, as they had some properties resembling wheat flour bread though the properties and texture of compound flour bakery differ from those made with wheat flour. With a better nutritional quality and shape .Beside being a source of calories and some other nutrients, wheat is regarded as poor in nutrition, because cereal proteins are poor in some necessary amino acids like lysine and threonine [12]. Some of the important quality criteria of bread are volume, texture and appearance, whereas smell and taste play a major role for producers and consumers. Around 300 volatile compounds can be identified in bread which is classified into different classes, like alcohols, esters, aldehydes, etc.

They are the result of many different interactions that happen between the type and concentration of ingredients in the time of processing, yeast activity during fermentation, and fermentation conditions (time, temperature, etc.) [13-14]. Thus, the present study aims to produce high nutritive probiotic bread supplementwith different levels of TNFG and Milk Permeate. Study of physicochemical, organoleptic and sensory properties of the final product was another goal of this work.

2. Materials and methods

Materials:

Tiger-Nut (*Cyperusesculentus*) was purchased from Al Azhar market, Cairo, Egypt. Strong wheat flour, Sugar, Corn oil, salt (sodium chloride) and dry yeast 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.

The probiotic bacterial strains, including *Lactobacillusrhamnosus*, *Bifidobacteriumbifidum* and *Streptococcusthermophilus* were obtained from the Laboratory of Microbiology of the National Research Centre, (Egypt).

Methods:

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 for72 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 [15]**.**

Blends preparation:

Hard wheat flour (HWF) was well blended with germinated tiger nut flour (GTNF) to produce individual mixtures containing 0, 10, 20and 30% GTNF. All samples were stored in airtight containers and kept at 3-5ºC till use.

Rheological properties:

Rheological properties of dough were evaluated using Farinograph and Extensograph apparatus according to the method described in AACC [16]. The Farinograph was utilized for estimating of arrival time, development time, stability time, weakening, and mixing tolerance index of pan bread dough.

Preparation and evaluation of baking quality and sensory properties of pan bread

Pan bread making was carried out at the pilot plant at the National Research Centre (NRC) in Dokki, Egypt according to AACC [16]. The pan bread ingredients were100 g mixed flour, 1.5g instant active dry yeast, 1g salt (sodium chloride), 5g sugar (sucrose) and 2g corn oil and water (an amount required to reach 500 Brabender Units of consistency). Pan bread was manufactured as follows: the dry ingredients were manually mixed in a wide bowel and then added to mixing bowl. Shortening and water were added to all ingredients .The components were thoroughly mixed with electric mixer for 2 min at low speed. The mixing speed was then changed to medium for 2 min and then at high speed for 8 min. The dough was divided into pieces rounded by hand and allowed to relax for 10 min. The dough were molded then panned and proofed (fermented) for 60 min at (30°C) in 86% relative humidity in a fermentation cabinet. The proofed pieces were baked at 250°C/21 min in an electric oven. Subsequently, the baked bread samples were then cooled for 1h at room temperature (30°C±2), packed in polyethylene bags and used for further analyses. Weight, volume, and specific volume of the bread samples were determined as described by AACC [16]. Sensory properties of pan bread samples were evaluated for taste (20), aroma (10), Symmetry(10), crumb grain texture (20), crumb colour (20), crust colour (20) and Overall acceptability (100).The bread formula composition of raw materials is tabulated as follow in table (1).

Table (1): Ingredients of Pan Bread formulas

Ingredients	Pan bread samples				
	Control (100% HWF)	Milk permeate	10% GTNF	20% GTNF	30%GTNF
Hard wheat flour (g)	1000	1000	900	800	700
Germinated tiger nut flour (g)	--	--	100	200	300
Permeate (ml)	--	560	560	560	560
Water(ml)	560	--	--	--	--
Dry yeast (g)	20	20	20	20	20
Corn oil (ml)	20	20	20	20	20
Sugar (g)	40	40	40	40	40
Salt (g)	10	10	10	10	10

HWF: Hard wheat flour, GTNF: Germinated tiger nut flour

Colour determinations

Objective evaluation of color for pan bread was measured. Hunter a*, b* and L* parameters were measured with a color difference meter using a spectro-colourimeter (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).

Chemical composition of pan bread:

Moisture, ash, crude protein, fat and crude fiber contents were determined in pan bread according to the methods outlined in AOAC. [17]. Carbohydrates were calculated by difference as mentioned as follows: Carbohydrates = 100 – (% protein + % fat + % ash + % crude fiber). Soluble and insoluble dietary fibers were determined using a Total Dietary FiberAssay Kit (TDF100A, Sigma- Aldrich, St. Louis, Missouri, USA) according to the gravimetric enzymatic method [18]. Total dietary fiber was calculated as the sum of soluble and insoluble dietary fiber.

Texture properties of pan bread

Textural properties of pan bread were evaluated using texture meter, Brookfield model-CT3-10 kg, USA, equipped with Fixture (TA-SBA). Texture properties were conducted to determine hardness, deformation at hardness, hardness work, and load at target, deformation at target, peak stress, fracture ability and fracture load drop off. Trigger load and test speed were 9.00 N and 2.5 mm/sec, respectively.

Activation of the bacterial strains:

Strains of *Lactobacillus rhamnosus*, *Bifidobacterium bifidum* and *Streptococcus thermophilus*wereactivated individually by three successive transfers in the MRS, modified MRS and M17 followed by three successive transfers in thesterilePermeate. The cultures wereincubated at 37°C for 24 h under anaerobic conditions [19-20].

Bacteriological Analysis

Samples preparation

Twenty five grams 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-fold dilutions of homogenates samples were prepared and inoculated onto plates of selective media. 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 El-Kholy*et al*.[21]. 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 [22]. 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 blackcentres [23]. Enumeration of

Staphylococcus aureus in samples was carried out by spreading 0.l ml of each of sufficient (expected) dilution onto the surface agar medium Mannitol salt agar (OxoidLtd.,England), media supplemented with egg yolk and potassium tellurite solution. Plates were incubated at 37ºC for 48 hrs[24]. 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 [25].The bread samples were stored underambient temperature and observed for5 days.Visual observations for mould growth were carried out on the samples stored.

Statistical analysis:

The obtained results were evaluated statistically using analysis of variance as reported by McClave& Benson [26].

3. Results and discussion

Rheological properties

The rheological properties of the dough were evaluated using a farinograph (Table 2). Water absorption, arrival time, dough development time, mixing tolerance index, and dough weakening increased, but dough stability decreased with increasing WF replacement levels using TNFG and milk permeate. Compared with WF, this is related to the high fiber content of TNFG. Fiber tends to bind water and change rheological properties, which may be the result represented by Kim et al. [27]. These results are consistent with those obtained by adnađev et al. [28] and El-Shenawy et al.[29].

Table (2): Effect of addingGTNF with HWF on rheological properties in Pan bread samples

Samples	Water absorption (%)	Arrival time (min)	Dough development time(min)	Dough stability (min)	Mixing tolerance index (BU)	Dough weakening (BU)
HWF 72% Extraction	60.7	1.5	2.5	9.0	35	65
HWF +10% GTNF	62.1	1.5	2.5	7.5	40	90
HWF +20% GTNF	65.5	1.5	3.0	6.0	50	100
HWF +30% GTNF	66.0	2.0	3.0	5.0	65	120

Where: HWF=Hard wheat flour; GTNF= Germinated Tiger Nut Flour

Chemical Composition of Bread

In this study, then fortification of pan bread with TNFG to enhance its nutritional value (table 3). Thus, increasing the TNFG level up to 30% raised the nutrition value of pan bread. Fat, Protein, fiberand ash. Carbohydrate contents of pan bread ranged between 12.69-12.23, 3.10-8.44, 1.27- 2.61, 0.63-2.10 and 82.31-74.61%, respectively. From these results, it is clear that TNFG could be used to produce pan bread with lower content of carbohydrate and at the same time increasing its content of crude fiber, ash and fat. These findings showed some similarity with those previously reported by several authors [29-30].

Table (3): Chemical composition of pan bread samples

Samples	Approximate chemical composition of pan bread					
	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Fiber (%)	CHO (%)
Control	24.17^{d}	1.27^{e}	12.69^{bc}	3.10^{d}	0.63^{d}	82.31^{a}
CB	25.05^{c}	1.83^{d}	13.50^{a}	3.14^{d}	0.61^{d}	80.91^{b}
10% GTNF	26.13^{b}	2.07^{c}	13.08^{b}	4.97^{c}	1.10^{c}	78.87^{c}
20% GTNF	26.99^{a}	2.39^{b}	12.57^{cd}	6.65^{b}	1.55^{b}	76.83^{d}
30% GTNF	27.23^{a}	2.61^{a}	12.23^{d}	8.44^{a}	2.10^{a}	74.61^{e}
LSD at 0.05	0.578	0.172	0.422	0.438	0.181	0.818

Where:CB: control with milk permeate

Mean values in each column having different superscript (a, b, c, d and e) are significantly different at $P < 0.05$.

Physical properties of pan bread

Results presented in Table 4 showed the weight(g), volume (cm3) and specific volume (v/w) of pan bread. On the other hand, loaves volume decreased as the TNFG level increased and the significant decrease was observed at 30%

replacement compared to the other levels. Consequently, specific volume of TNFG containing bread, which is a reliable quality indicator, had lower values in comparison with to the control sample. It is thought that this effect was made by high fiber content in TNFG and its higher water holding capacity. The deformation of gluten network led starch granules to aggregate resulting in faster Co2 loss and finally compact crumb structure. Such results were in agreed with the results those gained by Masoodi et al. [31], Sharma and Chauhan [32], Gómez et al. [33], Kohajdová et al. [34] and Zhou et al.[35].

Table (4): Physical properties of bread

Samples	Physical properties of pan bread			
	Weight (g)	Volume (cm^3)	Specific volume (cm^3/g)	Increase of specific volume (%)
Control	39.75^{b}	197.50^{b}	4.97^{ab}	--
CB	40.65^{b}	223.50^{a}	5.54^{a}	11.46
10% GTNF	42.00^{ab}	189.00^{b}	4.50^{bc}	-9.45
20% GTNF	43.42^{ab}	196.00^{b}	4.63^{bc}	-6.84
30% GTNF	45.94^{a}	195.00^{b}	4.24^{c}	-14.68
LSD at 0.05	5.242	15.028	0.649	--

Mean values in each column having differentsuperscript (a, b, c, d and e) are significantly different at $P < 0.05$.

Colour attributes of pan bread

Colour is a quality indicator that influences theconsumer decision for the selection of bakery products. The results of the colour parameters are written in Table (5). The crust of pan samples of bread supplement with different levels of TNFG had less L*and b* values and the reduction increased as thefortification level increased. On contrary, a* values of bread crust showed a reversed trend to L* and b*values. The crumb of supplement bread samples showedlower a* and b* values compared to the controlbread. In general, all supplement bread samples haddarker crust and crumb compared to the control.These results could be due to the high fiber and phenolic acid contents of TNFG that accelerate the formation of Maillard reactionproducts during the baking process [36].The results obtained are consistent with those of Kim et al. [27] With Lamy et al. [37].Using bran in baked products s causes darkening the colour of products, that's why either its concentration has to be controlled or suitable additives have to be used to lessen this browning.

Table (5): Color measurement of pan bread samples

Bread samples	Crust color			Crumb color		
	L	a	b	L	a	b
Control	52.07^{b}	14.81^{d}	30.72^{b}	72.58^{b}	1.48^{d}	19.89^{d}
CB	56.19^{a}	11.57^{e}	30.62^{b}	76.93^{a}	1.35^{e}	20.95^{c}
10% GTNF	50.77^{c}	15.44^{c}	31.27^{a}	71.91^{c}	2.76^{c}	22.28^{a}
20% GTNF	46.84^{d}	16.14^{b}	30.36^{c}	67.63^{d}	3.16^{b}	21.55^{b}
30% GTNF	39.80^{e}	16.69^{a}	25.85^{d}	63.01^{e}	3.47^{a}	20.84^{c}
LSD at 0.05	0.0655	0.075	0.142	0.0418	0.0418	0.118

Mean values in each column having differentsuperscript (a, b, c, d and e) are significantly different at $P < 0.05$.

Organoleptic properties of pan bread

Sensory evaluation is considered one of thelimiting factors of consumer acceptability fororganoleptic properties including taste, aroma, Crumb grain texture, crust color, crumb color, symmetry of shape and approval is general. The influence of Permeate and TNFG on characteristics of senses of pan bread is presented in Table (6). When the level of GTNF increasedin the formulation, thescores of sensoryforaroma, taste,crust color, crumb color, Crumb grain texture, symmetry of shape and Overall acceptability of pan bread decreased. Data indicated that a significant ($P<0.05$)changes were found in all properties for all experimental products. Data in Table (6) showed that pan bread made from mixture containing 30% GTNF had lower scores for most properties compared to the other tested products. Besides itshowed the lowest score for overall acceptability(66.25) for pan bread. The highest overall acceptability scores of biscuit were registered forcontrol (100% HWF plus milk permeate). From the sensory approval rating, we can concluded that pan bread were made from TNFG could be incorporated at 10%level used in this study to HWF in the formation of pan bread without influencing quality of senses is a significant.

Table (6): Scores for sensory attributes of pan bread samples from the composite flour

Samples	Sensory attributes						
	Crust color (20)	Crumb color (20)	Crumb grain texture (20)	Symmetry of shape (10)	Taste (20)	Aroma (10)	Overall acceptability (100)
Control	17.58^{a}	18.54^{a}	19.50^{a}	9.50^{a}	18.43^{a}	8.90^{a}	92.45^{a}
CB	18.23^{a}	18.10^{a}	19.21^{a}	9.50^{a}	19.50^{a}	9.10^{a}	93.64^{a}
10% GTNF	16.26^{b}	16.45^{b}	18.17^{ab}	9.10^{a}	18.92^{a}	8.50^{a}	87.4^{b}
20% GTNF	14.35^{c}	13.28^{c}	16.98^{bc}	8.14^{b}	17.18^{b}	7.85^{b}	77.78^{c}
30% GTNF	12.18^{d}	11.74^{d}	12.84^{c}	8.00^{c}	14.33^{c}	7.16^{b}	66.25^{d}
LSD at 0.05	1.854	1.104	1.524	1.035	1.874	0.927	5.981

Mean values in each column having differentsuperscript (a, b, c, d and e) are significantly different at $P < 0.05$.

Texture profile of pan bread

In table 7, the results are shown for the texture distribution analysis (TPA) made for bread samples. It can be noticed that the adding of TNFG and milk permeate to the formula of the flat bread sample will affect the texture characteristics of the produced bread. When being compared with bread made from wheat flour, bread samples rigidity increased by 72 % when the level ofTNFG supplementation increased. Bread rigidity (hardness) is an essential factor which affects consumer's conception of bread freshness. [38]. Feili et al [39] reported that bread hardness is caused by the interaction between gluten and fiber materials.Elasticity can be regarded as a way to measure how much crumbs of bread rebound after being compressed .It is the elasticity of the crumbs ofbread. The degree of bread staleness can also be determined by its elasticity [40-41].

The springiness of bread samples made from wheat flour 72% supplemented with TNFG and Milk Permeate were slightly low between 18.61and 17.95 mm compared to control sample (19.28 mm). Supplementation of all tested materials decreased the springiness values of the produced bread. According to Hoseney [42]. One of the factors that make the dough more elastic is the gelatinized starch and the gluten dough. It can also make structure of bread sponge-like after its heating. Therefore, the lower elasticity value may be due to the dilution of gluten, resulting in a lower gas retention capacity, which leads to a decrease in bread elasticity. Cohesiveness values of bread samples made from wheat flour 72% supplemented with TNFG bread decreased from 0.73 to 0.65. This decrease shows that the bread formulated with TNFG has a little resistance to the bread structure beneath the teeth before it deforms.

Chewiness can be regarded as a texture parameter that is easily correlated with sensory analysis [43]. It is related to the work required to chew solid samples (like bread) to a stable swallowing state. The chewiness of bread crumbs is the result of crumb hardness, cohesion and elasticity, but it depends more on hardness. Therefore, the chewiness value of bread generally follows a trend similar to hardness. Generally, all the tested samples proved to be higher in chewiness if compared to the control samples. Bread samples with 10% TNFG were the highest in chewiness value. This study stresses the importance of using TNFG instead of wheat flour to raise the nutritional value and fiber content of wheat.

Table (7): Texture profile analysis of pan bread samples

TPA	Control	Pan bread with			
		Milk permeate	10% TNFG	20% TNFG	30% TNFG
Hardness cycle 1 (N)	6.23	6.21	10.66	9.92	27.51
Resilience	0.25	0.26	0.21	0.21	0.13
Adhesiveness (mJ)	0.10	0.10	1.00	0.10	1.30
Hardness cycle 2 (N)	5.82	43.90	9.39	8.98	0.00
Cohesiveness	0.73	0.74	0.63	0.65	0.00
Gumminess (N)	4.56	4.61	6.72	6.42	0.00
Chewiness (mJ)	87.90	89.80	125.10	122.70	0.00
Springiness (mm)	19.28	19.48	18.61	19.12	17.95

The Microbiological Analysis of Pan Bread

The microbiological analysis of all the manufactured pan bread, during storage period of 5 days, is shown in Table 8.The total viable counts of the samples ranged from 6.0x10^2 to 7.2 x10^3 cfu/g. However, the counts gradually increased a bit till end of storage time. Also, the total fungi counts ranged from 2.0 x10^1cfu/g to 2.2 x10^2cfu/g. no growth in the first day for all treatments. After3 days of storage, the mould ount was 2x 10^1cfu/g. The growth observed could be due to post processing contamination. These results are in agreements with Ijah et al. [44], who stated that the fungal counts ranged from 8.0 × 10^1cfu/g to 1.20 ×10^3cfu/g of the sample andcoliforms were not detected in the bread.While bacterial pathogens were not detected in all treatments (Coliforms, *Salmonella typhimurium*and*Staphylococcus aureus*).

4. Conclusion

The present study emphasizes that high nutritive probiotic bread can be produced by replacing wheat flour with 10% TNFG and Milk Permeate without affecting the physicochemical, organoleptic and sensory properties of the final product. Consequently, this product of bread can be used by nutrition experts to treat some malnutrition diseases, as it is high in nutritional value and fiber.

Table (8): Microbial examination of control and different treatment of pan bread cfu/g

Samples	Total bacterialcount cfu/g			Mould &Yeast count		
	Fresh	3 days	5 days	1	3	5
Control	23x10^2	41 x10^2	59 x10^2	Nil	12×10^1	18×10^1
CB	45 x10^2	55 x10^2	72 x10^2	Nil	15×10^1	22×10^1
10% GTNF	18 x10^2	26 x10^2	32 x10^2	Nil	7×10^1	13×10^1
20% GTNF	13 x10^2	19 x10^2	27 x10^2	Nil	5×10^1	9×10^1
30% GTNF	6 x10^2	12 x10^2	18 x10^2	Nil	2×10^1	7×10^1

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References

1. El-ShenawyMoustafa A., Rashed A. Zaghloul, Ibrahim H. Abbass, Amira I. Esmail and Mohamed T. Fouad (2016). Incidence of some epidemiologically relevant food-borne pathogens in street-vended sandwiches. Journal of Pharmaceutical, Biological and Chemical Sciences 7 (2), 468-474.
2. El-ShenawyMoustafa, Mohamed T. Fouad, Laila Hassan, FatenSeleet and Mahmoud Abd El-Aziz (2019). A probiotic beverage made from Tiger-nut extract and milk permeate. Pakistan Journal of Biological Sciences. 22(4): 180-187.
3. Ghazya, O.A., M.T. Fouad, H.H. Saleha, A.E. Kholif, T.A. Morsy. (2021). Ultrasound-assisted preparation of anise extract nanoemulsion and its. Food Chemistry. 341:1-7.
4. Fouad Mohamed T., Moustafa El-Shenawy and Tarek A. El-Desouky. (2020). Efficiency of Selected Lactic Acid Bacteria Isolated from some Dairy Products on Aflatoxin B1 and Ochratoxin A. J. Pure Appl. Microbiol., 1-8.
5. Fouad M.T, AmrMoustafa, Laila Hussein, RamyRomeilah, Mostafa Gouda. (2015). In-Vitro Antioxidant and Antimicrobial Activities of Selected Fruit and Vegetable Juices and Fermented Dairy Products Commonly Consumed in Egypt. Research Journal of Pharmaceutical, Biological and Chemical Sciences 6 (2): 541-550.
6. Fouad Mohamed T. and El-DesoukyTarekA..(2020). Anti-Toxigenic Effect of Lactic Acid Bacteria against Aspergillus spp Isolated from Wheat Grains. The Open Microbiology Journal. 14, 252-259.
7. Ade-Omowaye BIO, Akinwande BA, Bolarinwa IF, Adebiyi AO. (2008). Evaluation of tigernut (Cyperusesculantus) wheat composition flour and bread. Afr J of Food Sci. 2(1):087-091
8. Alobo AP and Ogbogo PO (2007). Selected properties of tigernut starch as affected by physical and chemical modification. Proceeding of the 31st Annual NIFST conference, Abuja, Nigeria.
9. Oladela AK and Aina JO. (2007). Chemical composition and functional properties of flour produced from two varieties of tiger nut (Cyperusesculantus). Afr J of Bio. 6(21):2473-2476.
10. Chavallier A. (1996).The encyclopedia of medicinal plants. Dorling Kindersley London
11. Gyebi DK and Minnaar A (2011). Legume composite flours and baked goods: Nutritional, functional, sensory and phytochemical qualities. Flour and Breads and their fortification in Health and Disease prevention. Elsevier Inc.; 193-194.
12. Dhingra, S. and Jood, S. (2001). Organoleptic and nutritional evaluation of wheat breads supplemented with soybean and barley flour. Journal of Food Chemistry 77: 479–488.

13. Birch A.N., Petersen M.A., and Hansen A.S. (2012). The aroma profile of wheat bread crumb influenced by yeast concentration and fermentation temperature. LWT – Food Science and Technology, 50 (2): 480–488.
14. Birch A.N., Petersen M.A., Arneborg N., and Hansen A.S. (2013). Influence of commercial baker's yeasts on bread aroma profiles. Food Research International, 52(1):160–166.
15. Chinma CE, Adewuyi O, Abu JO. (2009). Effect of germination on the chemical and pasting properties of flour from brown and yellow varieties of tiger nut (*Cyperusesculentus*). Food Res. Intern. 42:1004–1009.
16. AACC. (2000). Approved Methods of Analysis. 10thEdn., American Association of Cereal Chemists, St. Paul, MN., USA., ISBN-13: 9781891127120;1200.
17. AOAC, Official methods of analysis, 18th Ed. Association of Official Analytical Chemists AOAC International, Gaithersburg, MD, USA. (2005).
18. Hasmadi M., Noorfarahzilah M., Noraidah H., Zainol M.K. and Jahurul M.H.A. (2020). Functional properties of composite flour: a review. Food Research 4 (6) : 1820 - 1831
19. Abosereh N.A., Salem Abd El Ghani, Rasha S. Gomaa and Mohamed T. Fouad (2016). Molecular Identification of Potential Probiotic Lactic Acid Bacteria Strains Isolated from Egyptian Traditional Fermented Dairy Products. Biotechnology 15 (1-2): 35-43.
20. Hussein Ahmed Mohamed Saied, Mohamed TawfeekFouad, Mahmoud Abd El-Aziz, Nagah El NoemanyAshour and Esam Ahmed Mohamed Mostafa (2017). Evaluation of Physico-chemical Properties of Some Date Varieties and Yoghurt Made with its Syrups. J. Biol. Sci., 17:213-221.
21. El-Kholy, W., Azzat B. Abd El-Khalek, Sahar H.S. Mohamed, Mohamed T. Fouad and Jihan M. Kassem (2016). Tallaga Cheese as a New Functional Dairy Product. American J. Food Technology, 11(5):182-192.
22. Mohamed Doha A., Minar Mahmoud Hassanein, Tamer M. El-Messery, Mohamed T. Fouad, Marwa M. El-Said, KaremFouda and Adel Gabr Abdel-Razek. (2017). Amelioration of Type 2 Diabetes Mellitus by Yoghurt Supplemented with Probiotics and Olive Pomace Extract. J. Biol. Sci., 17:320-333.
23. El-Shenawy, Moustafa, Mahmoud Abd El-Aziz, WagihElkholy and Mohamed T. Fouad (2016). Probiotic Ice Cream Made with Tiger-nut (Cyperusesculentus) Extract. American J. Food Technology, 11(5):204-212.
24. Hussein H. A. & Mohammed T. Fouad&Khaled A. Abd El-Razik&Amal M. Abo El-Maaty& C. D'Ambrosio4 & A. Scaloni& A. M. Gomaa. (2020). Study on prevalence and bacterial etiology of mastitis, and effects of subclinical mastitis and stage of lactation on SCC in dairy goats in Egypt. 52:3091–3097.
25. Abbas, H. M., Nadia M. Shahein, Nabil S. Abd-Rabou, Mohammed T. Fouad and Wafaa M. Zaky (2017). Probiotic-fermented Milk Supplemented with Rice Bran Oil. International Journal of Dairy Science, 12, 204-2010.
26. McClave J.T. and Benson P.G. Statistics for Business and Economics. USA, San Francisco: Dellen Publ., 1991.
27. Kim, Y.S., Ha, T.Y., Lee, S.H.; Lee, H.Y. (1997). Effect of rice bran dietary fiber on flour rheology and quality of wet noodles. Korean J. Food Sci. Technol., 20: 90–95.
28. Hadnađev T.D., Torbica A., Hadnađev M. (2011). Rheological properties of wheat flour substitutes/alternative crops assessed by Mixolab, Procedia Food Science 1:328-334.
29. El-ShenawyMoustafa, Hussien Ahmed M. S. and Fouad Mohamed Tawfeek (2020). Production of Biscuits from Mixture of Tiger NutFlour, Milk Permeate and Soft Wheat Flour. Asian Food Science Journal 18(2): 11-21.
30. Ahmed Zahra S, Hussein AMS (2014). Exploring the Suitability of Incorporating Tiger Nut Flour as Novel Ingredient in Gluten-Free Biscuit. Pol. J. Food Nutr. Sci. 64(1):27-33.
31. Masoodi F., Bhawana S., and Chauhan G.(2002). Use of apple pomace as a source of dietary fiber in cakes. Plant Foods for human Nutrition. 57(2):121–128.
32. Sharma H. and Chauhan G. (2002). Effect of stabilized rice-fenugreek blends on the quality of bread and cookies. Journal of Food Science and Nutrition39 (3): 223–333.
33. Gómez M., Ronda F., Blanco C.A., Caballero P.A. and Apesteguía A. (2003). Effect of dietary fibre on dough rheology andbread quality, European Food research and technology 216(1):51-56.
34. KohajdováZ.,KarovičováJ., MagalaM. And KuchtováV. (2014). Effect of apple pomace powder addition on farinographic properties of wheat dough and biscuits quality, Chemical papers 68(8): 1059-1065.
35. ZhouJ.,LiuJ. andTangX. (2018). Effects of whey and soy protein addition on bread rheological property of wheat flour, Journal of texture studies 49(1): 38-46.
36. Martins S.I., Jongen W.M., Van Boekel M.A. (2000). A review of Maillard reaction in food and implications to kineticmodelling, Trends in food science & technology 11(9-10): 364-373.
37. Ramy A., Salama M.F., and Shouk A.A.(2002). Pollards a potential source of dietary fiber for

pasta manufacture. Egyptian Journal of Food Science30: 313–330.

38. Onyango, C.; Mutungi, C.; Unbehend, G.; Meinolf, G. and Lindhaue, r M. G. (2010). Rheological and baking characteristics of batter and bread prepared from pregelatinised cassava starch and sorghum and modified using microbial transglutaminase. Journal of Food Engineering, 97, 465–470.
39. Feili, R.; Zzaman, W.; Abdullah, W. N. and Yang, T. A. (2013). Physical and sensory analysis of high fiber bread incorporated with jackfruit rind flour. Food Science and Technology 1, 30-36.
40. Karaoglu, M. M.; Kotancilar, H. G. and Gerçekaslan, K. E. (2008). The effect of par-baking and frozen storage time on the quality of cup cake. International Journal of Food Science and Technology, 43, 1778–1785.
41. Tian, Y. Q.; Li, Y.; Jin, Z. Y.; Xu, X. M.; Wang, J. P.; Jiao, A. Q.; Yu, B. and Talba, T. (2009). β-cyclodextrin (β-CD): A new approach in bread staling. ThermochimicaActa, 489, 22–26.
42. Hoseney, R. C. (1994). Principles of cereal science and technology. 3rd edition, United Kingdom: American association of cereal chemists, Inc. P. 203-206.
43. Gomez, M.; Ronda, F.; Caballero, P. A; Blanco, C. A. and Rosell, C. M. (2007). Functionality of different hydrocolloids on the quality and shelf-life of yellow layer cakes. Food Hydrocolloids, 21, 167–173.
44. Ijah, U.J. J., Helen ShnadaAuta, Mercy OluwayemisiAduloju, and SesanAbiodunAransiola (2014). Microbiological, Nutritional, and Sensory Quality of Bread Produced from Wheat and Potato Flour Blends. International Journal of Food Science.1,1-6.