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Production of pasta using tiger nut and fermented permeate with some probiotic bacteria

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Abstract

This study aimed to prepare pasta characterized with its higher nutritive value and higher sensorial properties by adding Germinated tiger nut flour (GTNF), Permeate and hard Wheat Flour (HWF) and their blends were at three levels (10, 20 and 30%) to enhance the nutritional value and function . Chemical composition, rheological properties, cooking quality, colour attributes, sensory properties and Microbiological Analysis of pasta were studied.The Microbiological examination of Pasta samples revealed that the total viable bacterial counts increased, a bit, throughout the storage period in all treatments. The same result was true with the control sample. The pasta manufactured with 20 and 30% of tiger nut delayed the appearance of yeasts and molds up to 30 days of storage compared to the control and the other treatments. Not detected pathogenic bacteria of coliform group, *Escherichia coli* O157: H7, *Bacillus cereus*, *Staphylococcus aureus*, *Listeria monocytogenes* and *Salmonella typhimurium* in all pasta treatments as well as control sample. Mixolab parameters showed that water absorption, dough stability and protein weakness were increased as the percentage of GTNF in blends increased. Cooking quality of pasta showed that, the optimum cooking time, volume increase and Nitrogen loss of formulated pasta with GTNF (10-30%) increased compared to control sample (pasta 100% HWF). Results also showed that Hunter colour parameters (L*, a* & b*) of pasta were darker as mixing level of GTNF increased. This result was confirmed with the obtained sensorial results. Moreover, sensory evaluation of pasta indicated that all samples were accepted, but all parameters of fortified pasta with 20 and 30% GTNF were significantly affected compared to control sample. Our study clearly demonstrated that of blended pasta with GTNF could ameliorate the nutritional value, sensory properties, cooking quality, and microbiological assay assured the safety of all pasta samples.

Key words: Germinated tigernut flour*;* wheat flour; pasta; cooking; sensory evaluation

Introduction

Italy is the first producer and consumer of pasta in the World; this is due, in large part, to the vast areas of cultivation of durum. In Campania the provinces with the largest area devoted to the cultivation of durum wheat are Scioscia et al. [1]. The pasta, on the contrary, suffers a milder heat treatment, the pasteurization, therefore has a high rate of humidity, at least 24% which makes it an appropriate growth substrate for bacteria and moulds. The pasteurization together with the quality of the raw material is the stage that most affects the quality of fresh pasta; in fact, many of the structural properties and cooking behavior of pre-packaged fresh pasta are definitely influenced by the intensity of the pasteurization process. As a basic food, pasta products have grown with the demands of modern life. In addition to the organoleptic properties that are widely accepted by consumers, compared with other foods, their low cost, easy cooking and long shelf life also make them popular. Pasta contains 74-77% carbohydrates [2]. Nowadays, consumers must not only satisfy hunger and provide necessary nutrition, but also prevent nutrition-related diseases and improve physical and mental health [3]. The attractiveness of pasta to consumers makes this food a potential promoter of functional food production. Aquatic resources contain important compounds, which are beneficial to health, have nutritional value, and can be used as functional ingredients. Ultrafiltration (UF) of milk contains lactose as the main ingredient as well as vitamins and soluble salts. Therefore, the permeate can be considered a nutritive important solution. The use of ultrafiltration permeate in the food industry will reduce environmental pollution and will consider added value [4].

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Reducing food-borne pathogens is a research priority to produce food that is safe for human consumption. Pathogens like *Staphylococcusaureus*, Salmonella, Campylobacter, etc. they cause serious illness and can be fatal in severe cases [5].

Lactic acid bacteria (LAB) are a good choice to control or reduce AFB1 and OTA in contaminated media, and are generally considered safe according to the US Food and Drug Administration (USFDA); some of them also have many health benefits, called probiotics [6-8]. Screening of *Lactobacillus* strains, including commercial probiotics and *L. plantaium* GG, showed that all Lactobaccilli showed strong antibacterial activity towards the Gram-negative pathogen *Salmonella typhimurium* [9]. Many microorganisms, including bacteria and yeasts are capable of reducing toxins in food and feed, so many studies used lactic acid bacteria (LAB) to bind toxins in vitro and in vivo [10].The aimed of study the effect of mixing Germinated tiger nut flour (GTNF), with some probiotic bacteria on the chemical composition, rheological properties, sensory properties, cooking quality and microbiological of pasta products.

Materials and methods

Materials:

Wheat flour (72% extraction rate) (WF) was purchased from the North Cairo Flour Mills Company, Egypt and salt were purchased from local markets in Giza, Egypt. All chemicals were analytical grade.

Tiger-Nut (*Cyperus esculentus*) was purchased from Al Azhar market, Cairo, Egypt. Strong wheat flour, Sugar, shortening, 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 *Lactobacillus plantaium*, *Bifidobacterium bifidum* and *Lactobacillus reuteri* were obtained from the Laboratory of Microbiology of the National Research Centre, (Egypt).

Methods:

Preparation of germinated tiger nut flour (GTNF)

Tiger nut 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 [11]**.**

Blends preparation:

Hard wheat flour (HWF) was well blended with germinated tigernut flour (GTNF) to produce individual mixtures containing 0, 10, 20 and 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 mixolab apparatus according to the method described in AACC [12].

Proximate Composition:

Moisture, ash, fat and protein contents of WF, SP and pasta samples were determined according to AACC [12]. Total carbohydrates were calculated by difference.

Colour measurement:

L*, a* and b* colour parameters were measured in pasta samples (raw and cooked) using Hunter colorimeter (Hunter Associates Lab Inc. (Model No: LabScan XE, USA).

Cooking quality of pasta:

Cooking quality of pasta were carried out by measuring the increases in weight, volume and cooking loss after cooking according to the methods of AACC [12].

Sensory evaluation of pasta:

Pasta samples were cooked in distilled water to optimum cooking time, and after draining for 2 min and then served to the panelists. The sensory test panel consisted of seven panelists who were trained academic staff. The panelists evaluated the products for colour, flavour, mouth feel, elasticity and overall acceptability using a 10-point hedonic scale ranging from 10-5 (like extremely) to 4-1 (dislike extremely) for each sensory characteristic [13].

Activation of the bacterial strains:

Bifidobacterium bifidum, *Lactobacillusreuteri*and *Lactobacillus plantaium* were activated individually bythree successive transfers in modified MRS followed by threesuccessive transfers in sterile 10% reconstituted

skim milkpowder and incubated at 37°C for 48 h under anaerobicconditions cultures were prepared 24 h before used[4,14-16]. Then 2.0% probiotic bacteria(*Bifidobacterium bifidum*, *Lactobacillus reuteri* and *Lactobacillus plantaium*1:1:1)were added to Pasteurized permeate.

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 Doha *et al*.[17]. 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 [18]. 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 [19]. 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 (Oxoid Ltd., England), media supplemented with egg yolk and potassium tellurite solution. Plates were incubated at 37ºC for 48 hrs [20]. 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 [18]. Enumeration of *Escherichia coli* O157: H7 in samples was carried out by spreading 0.l ml of each of sufficient (expected) dilution onto plates of sorbitol MacConkey agar medium, after 24 hrs at 35º C incubation. The growth of *E. coli* O157:H7 on MacConkey Agar with Sorbitol shows colourless colonies [15]. Each sample (25g) was homogenized and mixed with 225ml Listeria selective enrichment medium in 500ml flasks. Flasks were incubated at 30 ºC for 7 day. A plate of selective oxford agar base supplemented with Listeria supplement was streaked from each of an enrichment flask and incubated at 35ºC for 48 hrs. Typical colonies of *Listeria monocytogenes* will formblack zones around the colonies [19]. *Bacillus cereus* was determined by the surface plating technique onto the *Bacillus cereus* agar medium, supplemented with polymyxin B and egg yolk. The suspected colonies peacock blue-coloured and surrounded by precipitation zone were counted and tested for further specific identification [19]. The pasta samples were stored under ambient temperature and observed for 30 days.

Statistical analysis:

The obtained results were evaluated statistically using analysis of variance as reported by McClave& Benson [21].

Results and Discussion

Mixolab Parameters

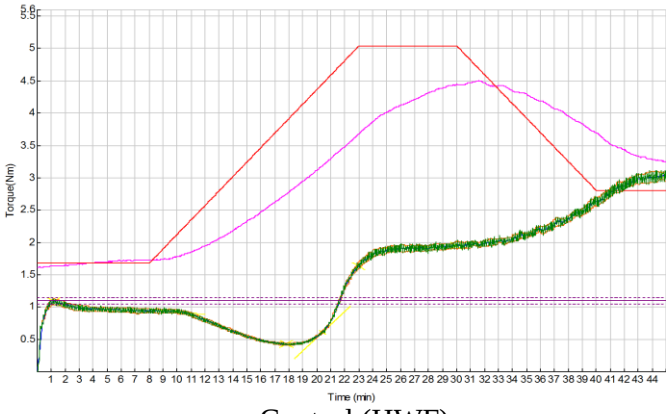
Mixolab parameters were used to identifycorrelations between thermo-mechanical behaviourof mixed HWF (72% extraction) withGTNF at different mixing rate (10%, 20% and30%). Table (1) and Fig (1) indicated that water absorptionof 72% HWF recorded lowered value (53.0%) compared to HWF mixed with at different mixingrate which recorded the highest value of waterabsorption (57.1%). This result agreed withAhmed & Hussein [22] and Elshenawy et al [23].They stated that water absorption of soft wheat flour mixed with GTNF was increased compared with control. On the other hand, dough stability was increased with increasing the levels of GTNF. They stated that water absorption of soft wheat flour mixed with GTNF was increased compared with control. On the other hand, dough stability was increased with increasing the levels of GTNF.Moreover, protein weakness values (C2) wereaffected by mixing levels of GTNF where C2values were increased lightly with increasing thelevel of GTNF. In spite of protein weakness, Izydorczyk et al[24] found that the presence of β-glucan in barley seems to override the negativeeffects associated with the dilution of wheatgluten upon mixing with fiber and starch, andleads to a strengthening of the dough. Theincrease in dough strength, due to β-glucanaddition, also depends on the quality of thewheat flour that is used, with a greater effect forpoor bread-making flour than

for good breadmakingflour. Also, Dhaka et al[25] indicated that thelower values of C2 led to produce dough's wereless tolerant to mixing as compared to the highervalues.

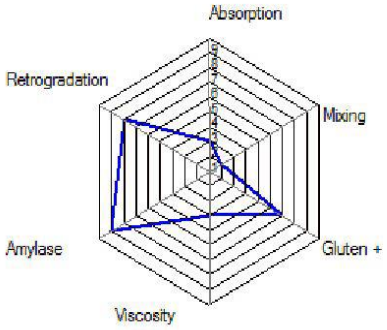
Table (1):Effect of adding of germinated tiger nut flour in wheat on rheological properties of pasta

Blends	Water absorption (%)	Dough Stability (min)	C1 (Nm)	C2 (Nm)	C3 (Nm)	C4 (Nm)	C5 (Nm)
HWF 72%	53.0	5.30	1.18	17.82	23.00	30.00	45.02
HWF +10% GTNF	53.8	6.18	1.23	17.02	25.48	33.60	45.02
HWF +20% GTNF	55.1	7.62	2.25	17.67	23.00	30.00	45.02
HWF +30% GTNF	57.1	11.02	3.50	16.40	25.20	32.83	45.02

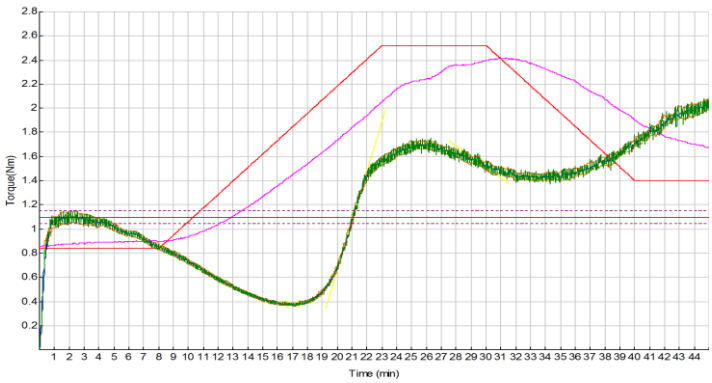
Where:C1: maximum torque, **C2:** minimum consistency, C3: pasting ability, C4: minimum torque and C5: final torque



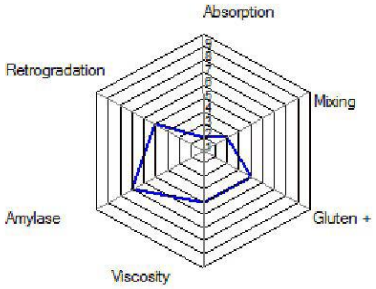
Control (HWF)



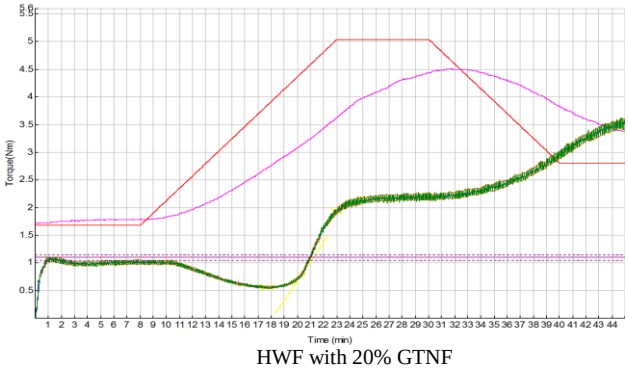
Control (HWF)



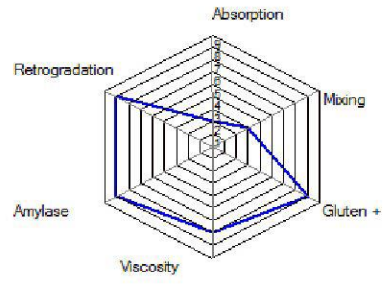
HWF with 10% GTNF



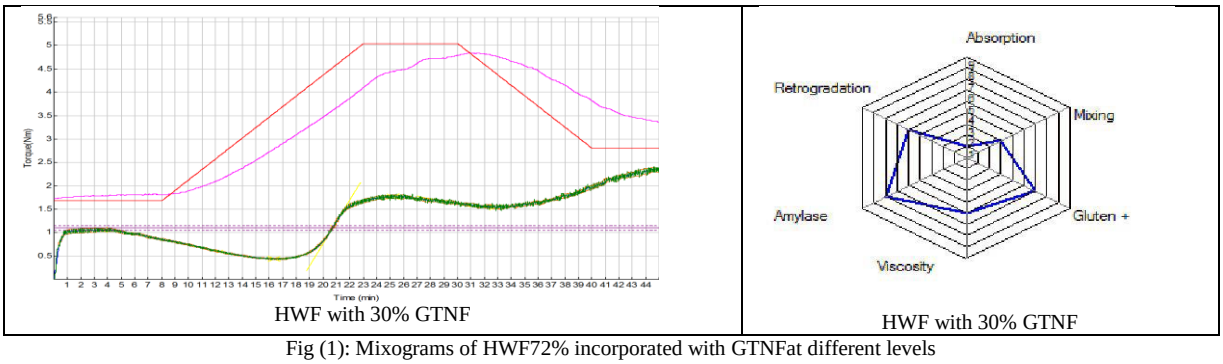
HWF with 10% GTNF



HWF with 20% GTNF



HWF with 20% GTNF



HWF with 30% GTNF

HWF with 30% GTNF

Fig (1): Mixograms of HWF72% incorporated with GTNFat different levels
HWF=Hard wheat flour; G**TNF=** Germinated Tiger Nut Flour

Proximate composition of pasta

Proximate composition of Pasta and pasta of different mixing level with GTNF (10, 20 and 30 %) were presented in Table (2).Therefore, increasing mixing level of GTNF (10 to 30%) with HWF led to increase the nutritional value of pasta for ash**,** fat and fiber while moisture, protein and carbohydrate decreased where ranged between (1.20-8.76%), (0.55- 1.67%), (0.58- 2.28 %), (12.30- 12.10), (13.47- 12.13) and (84.21-75.17%), respectively. It can also be observed that the pasta mixed with GTNF and HWF shows some similarities with the pasta discovered by several authors[23, 26].

Table (2): Gross chemical composition of control **pasta** and pasta with different levels of germinated tigernut flour**(g/100 g on dry basis)**

Samples	Moisture	Ash	Protein	Fat	Fiber	CHO
Pasta produced from different levels of GTNFand HWF						
Control 100% HWF	12.30±0.19	0.55±0.02	13.47±0.11	1.20±0.0.3	0.58±0.01	84.21±0..35
Control (100% HWF) +Permeate	12.20±0.22	0.75±0.07	13.62±0.13	1.22±0.01	0.57±0.03	83.83±0.44
90% HWF+ 10%GTNF	12.16±0.16	1.07±0.01	13.12±0.15	3.82±0.07	1.18±0.02	80.81±0.53
80% HWF+ 20% GTNF	12.12±0.13	1.33±0.05	12.61±0.10	6.39±0.09	1.69±0.05	77.97±0.60
70% HWF+ 30% GTNF	12.10±0.15	1.67±0.03	12.13±0.13	8.76±0.11	2.28±0.04	75.17±0.52

Where: HWF:Hard Wheat Flour, GTNF: germinated tigernut flour

Cooking Quality of Pasta

The results obtained for the cooking quality of pasta added with permeate and GTNF at different levels are shown in Table 3.Pasta quality could be estimated from cooking attributes such as optimum cooking time, volume increase and Nitrogen loss. The control pasta made with 100% hard wheat flourcould be observed that, the highest value of optimum cooking time **(**min) was observed by the pasta supplemented with 30% GTNF**(**16.63 min) with significant **(**$p<0.05$) compared to the other samples and control sample.The results showed that an increase in volume was observed with different levels of GTNFcompared to the control sample (Table 3).

Nitrogen loss one of the important factors of pasta quality and expressed as loss percentage of total nitrogen after cooking compared to the same sample before cooking. Where, the results referred to great loss in nitrogen content of pasta samples which containing 30% GTNF with significant compared to the control sample and the others containing 10 or 20% GTNF. The Nitrogen loss in all the pasta samples was below the technologically acceptable limit (≤8%). There were significant differences in optimum cooking time, volume increase and Nitrogen lossamong the groups (P >0.05); therefore, it can be concluded that adding GTNFin these levels affect the cooking quality of Pasta

Table (3): Effect of adding different levels of germinated tiger-nut flourto pasta on cooking quality

Sample	Optimum cooking time (min)	Volume (cm^3)			Nitrogen loss
		uncooked	Cooked	Cooking yield (%)	
Pasta produced fromdifferent levels of germinated tiger-nut flour					
Control 100% HWF	15.15^{c}±0.12	83^{b}±1.16	208^{d}±3.15	125^{e}±2.66	4.50^{d}±0.10
Control (100% HWF) +Permeate	14.82^{c}±0.32	87^{a}±2.11	222^{c}±4.22	135^{d}±3.19	7.0^{c}±0.29
90% HWF+ 10%GTNF	14.61^{d}±0.26	85a±2.19	230^{b}±5.19	145^{c}±2.65	7.30^{b}±0.22
80% HWF+ 20% GTNF	16.00^{b}±0.22	85a±1.96	236^{b}±3.16	151^{b}±4.19	7.50^{b}±0.19
70% HWF+ 30% GTNF	16.63^{a}±0.38	86a±2.09	249^{a}±7.15	163^{a}±1.33	9.40^{a}±0.33
LSD at 0.05	0.425	2.984	8.974	6.847	0.385

Mean values in each column having different superscript (a, b, c, d and e) are significantly different at P < 0.05. , HWF:Hard Wheat Flour, GTNF: germinated tigernut flour

Colour parameters

Since colour is related to product freshness and flavour expectations, colour is one of the most important quality characteristics of food acceptability and therefore has a direct effect on consumers' perceptions. A Hunter laboratory colorimeter was used to evaluate the colour parameters of pasta with penetrant and GTNF samples (Table 4). The L scale ranges from 0 black to 100 white; a scale extends from negative (green hue) to positive (red hue), and b scale ranges from negative blue to positive yellow. Pasta fromHWF and GTNF it was darker than HWF andHWF with Permeate, where lightness (L*) and yellowness (b*) of pasta decreased as a percentage of GTNF used in Pasta processing increased,while redness values (a*) of pasta samples, wheretheir values were getting higher in pasta containing GTNF compared with control orPasta containing Permeate. This result could beattributed to the darkness of GTNF (lower L*)than HWF, so, darkness increased as a result of the presence of GTNF in Pasta. Such findings are in agreement with Kim, Kordonowy & Young and Ramy [27-29].

Table (4): Effect of germinated tiger-nut flour addition on colour measurements of control and Pasta samples

Samples	L*		a*		b*	
	Raw	Cooked	Raw	Cooked	Raw	Cooked
Pasta produced fromdifferent levels of germinated tiger-nut flour						
Control 100% HWF	62.70^{b}±1.25	73.30^{a}±1.29	1.80^{c}±0.03	1.08^{a}±0.001	9.12^{b}±0.13	12.10^{b}±0.17
Control +Permeate	67.80^{a}±1.35	73.18^{a}±1.32	1.03^{d}±0.05	1.71^{d}±0.03	10.52^{a}±0.19	14.18^{a}±0.10
10%GTNF	60.30^{b}±1.72	68.00^{b}±0.77	2.90^{b}±0.07	2.70^{c}±0.09	8.40^{c}±0.16	10.90^{c}±0.08
20% GTNF	57.60^{c}±1.56	63.31^{c}±1.17	3.03^{b}±0.13	3.61^{b}±0.32	7.05^{d}±0.13	9.22^{d}±0.09
30% GTNF	55.06^{d}±0.46	58.92^{d}±1.23	3.83^{a}±0.22	5.43^{a}±0.26	6.01^{e}±0.10	8.03^{e}±0.11
LSD at 0.05	2.142	3.915	0.382	0.345	0.688	0.684

Mean values in each column having different superscript (a, b, c, d and e) are significantly different at P < 0.05. , HWF:Hard Wheat Flour , GTNF: germinated tigernut flour

Sensory properties

The organoleptic properties of pasta produced from HWF and HWF supplemented with permeate and GTNF at different levels (10, 20 and 30%) were evaluated for taste, colour, appearance, tenderness, flavour, moistness and overall acceptability in Table (5). Table (5) revealed that, pasta colour significantly decreased in pasta of different GTNF mixing levels, this result is confirmed with the previous colour parameter (L, a and b) where darkness was increased with increasing replacement of GTNF. TendernessandMoistnessof

control pasta wassignificantly affected with increasing replacing levels of GTNF up to 30%. The obtained sensorial results indicated that tasteand appearance, overall acceptability of pasta fortified with 10, 20 and 30 % GTNF were significantly affected compared to control sample. From the presented results in Tables (5) it could be noticed that the sensory characteristics were decreased with increasing the level addition of GTNF. Pasta may be enriched with GTNF at levels 10 or 20% without any reverse effect on sensory acceptance of the product.

Table (5): Sensory evaluation of control pasta and pasta with different levels of germinated tiger-nut flour

Samples	Appearance (10)	Colour (10)	Taste (10)	Tenderness (10)	Moistness (10)	Flavour (10)	Overall acceptability (10)
Pasta produced fromdifferent levels of germinated tiger-nut flour							
Control 100% HWF	8.8^{a}	8.7^{a}	8.7^{a}	8.5^{a}	8.9^{a}	9.2^{a}	8.9^{b}
Control +Premeate	9.1^{a}	9.0^{a}	9.1^{a}	8.9^{a}	9.1^{a}	9.6^{a}	9.5^{a}
10%GTNF	8.1^{b}	7.9^{b}	8.0^{b}	7.9^{b}	7.8^{b}	8.2^{b}	7.8^{c}
20% GTNF	7.6^{c}	7.1^{c}	7.5^{c}	7.0^{c}	6.9^{c}	7.6^{b}	7.3^{c}
30% GTNF	6.9^{d}	6.6^{d}	6.8^{d}	6.3^{d}	6.5^{c}	6.8^{c}	6.4^{d}
LSD at 0.05	0.339	0.494	0.406	0.581	0.494	0.698	0.536

Mean values in each column having different superscript (a, b, c, d and e) are significantly different at P < 0.05., HWF:Hard Wheat Flour , GTNF: germinated tigernut flour

The Microbiological Examination of Pasta

The counts of the examined microbial parameters (Total viable bacterial counts, coliform group, mould and yeast, *Escherichia coli* O157: H7, *Bacillus cereus*, *Staphylococcus aureus*, *Listeria monocytogenes* and *Salmonella typhimurium*) of the processed pasta.Figure 2 shows the counts of pasta (log cfu/g), the control, and the changes in the total number of bacteria during treatment with different levels of GTNF. The result of Pasta made with 10, 20 and 30% GTNFhad slight increase in total viable bacterial counts (log10 CFU 4.15, 4.11 and 4.04 respectively) if compared with control (log10 CFU 4.18).The obtained data showed that the total viable bacterial counts increased throughout storage period in all treatments and control.

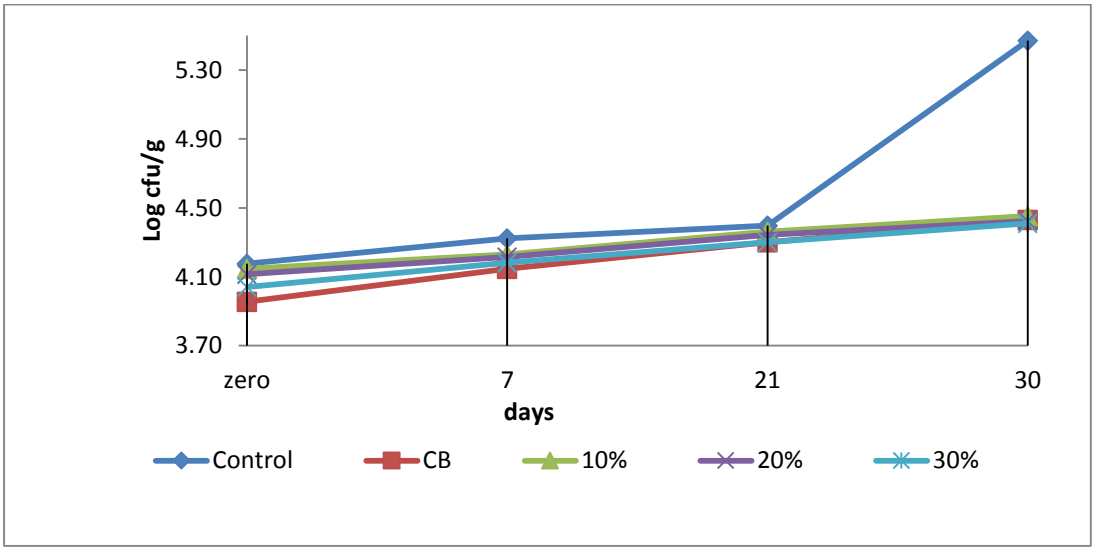


Fig. 2: Total bacterial counts (CFU/ g) in pasta fortified with 10, 20 and 30% of tiger nut during storage period for 30 days, C: Control, CB: control + permeate, T1: 10% tiger nut , T2: 20% tiger nut and T3: 30% tiger nut .

Our obtained results are in agreement with Scioscia *et al*. and Ricci *et al*., [1, 30]. They found that the total bacterial count of the samples ranged from $10^3$to 10^7 cfu/g. Figure 3 shows that moulds and yeasts were present in all controls and pasta treatments during freshness and storage. During storage, the mould and yeast in the pasta treatment gradually increase and the hardness increases.

The mould and yeast were undetected in 10, 20 and 30% GTNF at the21, 30 and 30 days from storage period respectively. Our findings are in accordance with Scioscia *et al*. [1], who stated that the total mould and yeast count of the samples ranged from 10^1 to 10^3cfu/g. The growth observed could be due to post processing contamination. These results are in agreements with Ijah *et al.* [31], who stated that the fungal counts ranged from 8.0×10^1cfu/g to 1.20×10^3cfu/g of the sample however coliforms were not detected in the pasta. All bacterial pathogens were not detected in all treatments including coliform group, *Escherichia coli* O157: H7, *Bacillus cereus*, *Staphylococcus aureus*, *Listeria monocytogenes* and *Salmonella typhimurium*.

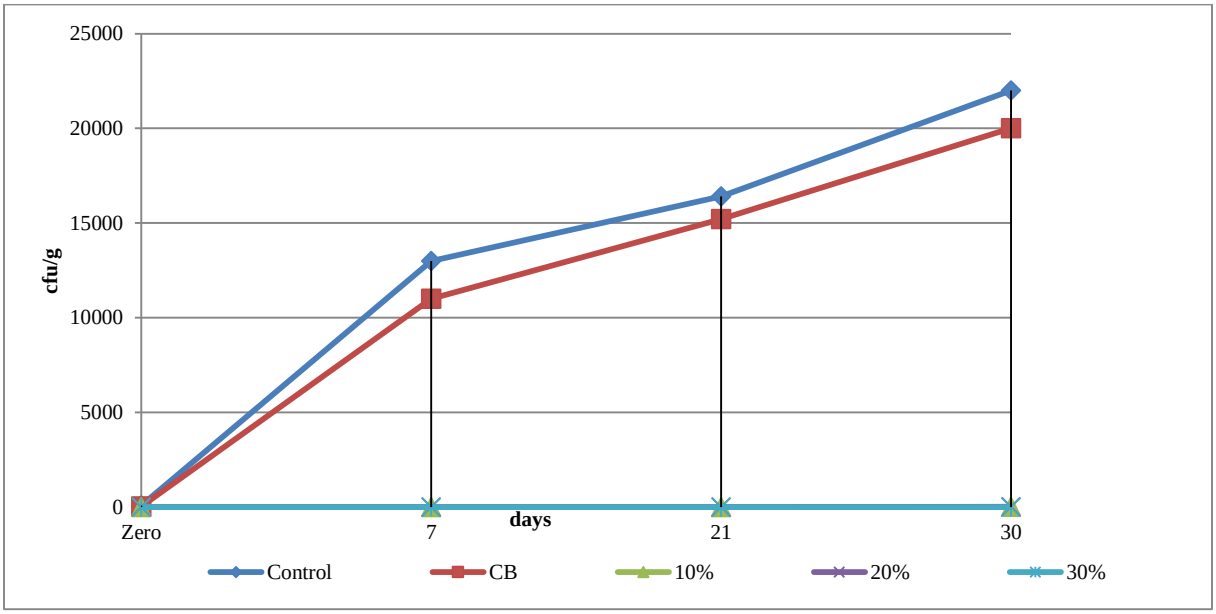


Fig. 3: Mould and yeast counts (CFU/ g) in pasta fortified with 10, 20 and 30% of tiger nut duringstorage period for 30 days, C: Control, CB: control + permeate, T1: 10%tiger nut, T2: 20% tiger nut and T3: 30% tiger nut.

Conclusion

From the results obtained, it can be concluded that permeate and GTNF can be used together with HWF to prepare pasta with good process and microbiological properties and higher nutritional value. In addition, they have a positive effect on rheological properties and can be used as pasta. The results show that adding 20% or 30% GTNF can be used as a good fortifier for nutritional and therapeutic pasta production, because it will not affect the chemical and sensory properties, but will improve the rheological properties of the final product.

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