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# Novel Insights on the Functional Properties of Yoghurt Fortified with Tiger Nut Flour (*Cyperus Esculentus*) as Antioxidant, Antimicrobial and Antitumor

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## ABSTRACT

In this study, the yoghurt produced with tiger nut flour added in different ratios (1, 2 and 3%, w/v) was evaluated for its chemical, physical, total phenolic content, antioxidant, antibacterial, cytotoxic activity, texture, microbiological and sensory characteristics. Added tiger nut flour significantly increased ($P < 0.05$) protein, ash, carbohydrates, phenolic content, antioxidant, antibacterial, cytotoxic activity, adhesiveness, cohesiveness, gumminess, and chewiness of yoghurt treatments. There was a significant decrease in moisture content ($P < 0.05$). In addition, the fat content, fracturing, hardness, and springiness of the yoghurt were not significantly impacted by the addition of tiger nut flour. When tiger nut flour was added and refrigerated for up to 14 days, it significantly decreased ($P < 0.05$) syneresis and pH levels. Additionally, yoghurt treatments fortified with tiger nut flour were found to inhibit three human tumor cell lines, including colon cancer, breast cancer of the mammary gland, and cervix cancer of epithelioid carcinoma. Lactic acid bacteria's viability and texture were improved by adding tiger nut flour. The sensory properties (taste & flavor, and general appearance) of the yoghurt with additions of tiger nut flour were found to be lower than those without tiger nut flour. Still, the texture was higher, and the acidity did not change. Thus, we may conclude that tiger nut flour can be applied to produce a novel functional yoghurt

***Keywords*:** Tiger nut flour, functional food, total phenolic, antibacterial, cytotoxic.

## INTRODUCTION

One of the most critical methods to increase the nutritional content and highly qualified food is food fortification (Bakry *et al*., 2019). Yoghurt is a fermented dairy product that is produced when *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus* work together to ferment lactic acid (Zhang *et al*., 2019). One of the most popular dairy products, it has a high nutritional value and functions well as a matrix for preparing a range of health-promoting functional foods. It is high in calcium and protein and often belongs on lists of nutritious foods (Ladjevardi *et al*., 2015) & (Zhang *et al*., 2019).

The sedge family includes the tiger nut, scientifically known as *Cyperus esculentus*. It is a tuber that grows freely and is widely consumed throughout the Arabian Peninsula, Nigeria, other parts of West and East Africa, and parts of Europe, especially Spain. Tiger nuts, or chufa as they are known in Spanish, were formerly grown along the Nile River's borders in the region bounded by Sudan and Egypt (Malashree *et al*., 2021)

The tuber known as a tiger nut (*Cyperus esculentus*) can be eaten raw or used to make drinks. Its nutritional composition indicates a high starch content, like other tubers, and a considerable amount of fat and dietary fiber that is like nuts. In addition, fiber reduces cholesterol and body weight. The dietary fiber in this tuber helps protect against obesity, colorectal cancer and gastrointestinal disorders (Jing *et al*., 2016). Furthermore, rich in phosphorus, calcium, and phenolic components, tiger nuts improve their antioxidant activity (Roselló-Soto *et al*., 2019). Tiger nuts are a natural source of antioxidants and have good antioxidant qualities because of the flavonoid content (Jing *et al*., 2016).

The richness of fat, fatty acids, flavonoids, alkaloids and other phytochemicals of the plant supports some metabolic processes and has biological and therapeutic benefits that include aphrodisiac, anti-diabetic, anti-inflammatory, and antioxidant activities that improve general health and conditions (Edo *et al*., 2023).

The great nutty taste of chufa makes it a favorite snack and could have uses in food science. Moreover, it is a flavoring component for ice cream and a flour production ingredient. There is a variety of information regarding the health benefits of flour because it is a great substitute for dietetics and can be used in any type of flour because it is gluten-free. It is a great substitute for wheat flour because it does not contain gluten and is suitable for those who must avoid it. Tiger nut flour is milled without losing any of its nutritious properties (Gambo & Da'u, 2014).

Because tiger nut flour has several health benefits, this study aimed to create a functional food product by adding yoghurt with varying amounts of tiger nut flour. In other hand, during the storage period, the impact of the addition of tiger nut flour was examined on the physicochemical, rheological, textural, microbiological and sensory characteristics of the yoghurt. Evaluated the biological effects of yoghurt (including phenolic content, antioxidant, antibacterial, and antitumor activities).

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## MATERIALS AND METHODS

**Materials**

Fresh cow milk was supplied by the Faculty of Agriculture of Mansoura University in Egypt. In Mansoura, Egypt, a local market was where the tiger nut flour was bought. The yoghurt starter culture in commercial yoghurt culture consists of *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus* (YO-MIX 883 LYO 50 DCU Danisco, France SAS).

**Methods**

**Manufacturing of yoghurt fortified with various ratios of tiger nut flour**

Yoghurt was mixed with tiger nut flour in various percentages (1, 2 and 3% w/v), whereas the control sample did not receive additives. The milk- tiger nut flour mixture was continuously mixed while heated to 90°C for 30 minutes. After cooling to 42°C, a 3% yoghurt culture was added to the milk-tiger nut flour mixture and then incubated for three to four hours at 42°C. A pH of 4.6–4.7 was the indicator that fermentation was finished. After reaching a pH of 4.6, yoghurt samples were kept at 5°C for 14 days.

**Physicochemical evaluation**

According to the standards (AOAC, 2000); total solids, fat, ash, protein and dietary fiber were determined. Using an air oven to dry 2 to 3 g until a consistent weight reached 105°C, total solids (TS) were measured. The fat content was estimated using the Gerber method. To measure the ash content, each sample was dried for an hour at 105°C in an oven and then burned at 550°C in an electric muffle furnace. The Kjeldahl method was used to determine the total protein content of cow's yoghurt and tiger nut flour, and the results were expressed as the total nitrogen multiplied by factors 6.38 and 6.25; respectively (IDF, 1986). All the content of carbohydrates was determined by subtracting 100 from the total amount of ash, fat, water and protein. A pH meter (Adwa, waterproof pH meter, Romania) was used for measuring pH.

**Syneresis index**

Yoghurt supplemented with tiger nut flour was measured for syneresis using the method described by Akalin *et al*., (2012). We put 100 g of yoghurt through a test sieve with a 0.5 mm mesh width. At 120 minutes, 10°C, the amount of whey that had been drained was measured. The formula to calculate syneresis was ml of drained whey/100g of yoghurt.

**Textural profile evaluation**

Using a QTS 20 texture analyzer (model QTS20, Brookfield Instruments, Harlow, UK) fitted with a 5-kg load cell, the texture profiles of the yoghurt sample were investigated (Hashim *et al*., 2009). The parameters of the set of evaluations of the textural profile were changed to test at 60 mm/s, 0.1N of the trigger force, 20% of the deformation and 2 s of holding time between cycles. Yoghurt was measured at a temperature of 5°C to evaluate the texture profile. Two further cycles (bite-sized increments) of compression-deformation were applied for every sample. The yoghurt samples' fracture, hardness, adhesiveness, cohesiveness, springiness, gumminess, and chewiness were examined using the program (Texture Pro software, Brookfield Instruments, UK).

**Analysis of phenolic content and antioxidant activity**

According to Attard (2013), using the Folin-Ciocalteu technique, the total phenolic content was determined. To perform the process, a 96-well microplate was filled with 10 µL of sample/standard and 100 µL of diluted Folin-Ciocalteu reagent (1:10). The mixture was then left to sit at room temperature (25°C) in the dark for 20 minutes after 80 L of 1M $Na_2CO_3$ was added. The resultant blue complex color was observed at 630 nm following the incubation time. The data are shown using means ± SD. Furthermore, according to the method (Chen *et al*., 2013), with the use of DPPH (2,2-diphenyl-1-picryl-hydrazylhydrate), a free radical experiment was carried out. In brief, a 96-well plate (n = 6) was filled with 100 µL of newly made DPPH reagent (0.1% in methanol) and 100 µL of sample. The reaction mixture was then let to stand at room temperature in the dark for half an hour. At 540 nm, the resulting decrease in DPPH color intensity was evaluated following the incubation time. Following the subsequent equation, the data are presented as means ± standard deviation: ((Average absorbance of blank-Average absorbance of the test)/ (Average absorbance of the test) / (average absorbance of the blank)) *100 is the formula for percentage inhibition.

**Antibacterial activity of yoghurt fortified with tiger nut flour supernatant**

To examine the antibacterial activity of the yoghurt supernatant, samples of fortified yoghurt with tiger nut flour were centrifuged at 4000 rpm for 30 minutes at 4°C using a 1-14K refrigerated centrifuge model. Using a Millipore membrane filter with a diameter of 0.45 µm diameter, the resulting supernatants were filtered. The antibacterial activity of the supernatant filtrates was evaluated using the agar diffusion method against the indicator strains of *Salmonella typhi* MSD-9331, *Bacillus cereus*, *Staphylococcus aureus* MSD-7447, *Enterococcus faecalis* and *Escherichia coli* MSD-10418, as reported by Hashemi *et al*. (2021). The strains were produced at Al-Azhar University in Cairo, Egypt, by the Fermentation Biotechnology and Applied Microbiology Centre (FERM-BAM). On tryptone soy agar (Oxoid), 0.5 ml of each bacterial culture was distributed. After aseptic drying of the plates, 100 µL supernatant filtrates were enclosed in wells (8 mm in diameter) punched from the agar using a sterile borer. Petri dishes were stored at 4°C for 4 hours before being incubated for 24 hours at 37°C to facilitate sample diffusion in agar. Antimicrobial activity was evaluated by measuring the diameter (mm) of the inhibitory zones surrounding the well.

**Cytotoxicity activity in vitro**

Cell lines for cervix epithelioid carcinoma (Hela), mammary gland breast cancer (MCF-7), and colon colorectal carcinoma (HCT-116) were acquired from ATCC through the Holding Company for Biological Products and Vaccines (VACSERA), Cairo, Egypt. According to Mosmann (1983), to investigate the potential growth-inhibiting effects of yoghurt fortified with tiger nut flour samples on these cells, the MTT test was used. Mitochondrial succinate dehydrogenase from surviving cells transforms yellow tetrazolium bromide (MTT) into a purple formazan derivative in this colorimetric experiment. RPMI-1640, a medium containing 10% foetal bovine serum, was used to cultivate cell lines. An incubator with 5% $CO_2$ at 37°C was filled with antibiotics (100 g/ml streptomycin and 100 units/ml penicillin). Following exposure to varying chemical concentrations, cells were grown for 24 hours. 20 µl of a 5-mg/ml MTT solution was added after the medicine had been given for 24 hours, and the mixture was then allowed to incubate for 4 hours. 100 µl of dimethyl sulfoxide (DMSO) is poured into each well to dissolve the purple formazan that forms. With the aid of a plate reader (EXL 800, USA), the colorimetric assay is measured and recorded at an absorbance of 570 nm. (A570 of treated samples/A570 of untreated samples) x 100 was the formula used to calculate cell viability, which was then expressed as a percentage.

**Microbiological evaluation**

A series of dilutions were made by dissolving each sample in 10 ml of saline solution. The microbiological enumeration (count) of each batch was then assessed in the manner described below: M17 agar (Oxoid) was used to count the *Streptococci*. Under an aerobic environment, the plates were incubated for 48 hours at 35°C (IDF, 1997). MRS agar (Oxoid) with a pH of 5.4 was used to determine *Lactobacilli* counts. In anaerobic conditions, plates were stored at 37°C for 48 hours (IDF, 1997). Throughout the 14-day storage period, once every seven days, all microorganisms found in the samples were tested. to a microbiological examination.

**Sensory properties**

A team of ten professionals from Mansoura University's Faculty of Agriculture assessed the sensory characteristics of yoghurt samples using the techniques described by Atwaa *et al.* (2022). Taste and flavor (45), general appearance (10), acidity (15) and textures (30) were the scores given for each characteristic.

**Statistical analysis**

The three replicates' mean ± standard deviation was used to express all the data. Using a one-way analysis of variance, the chemical composition, textural features and antibacterial activity were statistically examined (ANOVA). To find out if there were any significant differences between the treatment and storage period means, a two-way analysis of variance (ANOVA) statistical analysis was run on the remaining data using SPSS statistics (SPSS for Windows, Version 20). Tukey's test was used at a significance level of 5% ($P < 0.05$) to compare the data means.

## RESULTS AND DISCUSSION

**Chemical composition and pH value of cow milk and tiger nut flour used to make cow milk yoghurt**

The chemical composition of cow's milk and tiger nut flour (TNF) is shown in Table 1. The chemical composition of cow milk involved 86.81% moisture, 3.00% fat, 0.61% ash, 3.06% protein, 6.52% carbohydrates, 0.17% titratable acidity and a pH of 6.80. Badau *et al.* (2015) reported that cow milk contained 88.34% moisture, 3.35% fat, 0.72% ash, 4.56% carbohydrate, 3.88% protein and a pH of 6.44. Furthermore, Shahein *et al.* (2023), showed that fresh cow's milk with 2.9% protein, 3% fat and 11.6% TS was the chemical composition used for making bio-yoghurt drinks containing tiger nut flour. According to physicochemical analysis, tiger nut flour (TNF) has 4.69 % protein, 23.00% fat, 16.30% fiber and 64.00% total carbohydrate (Table 1). Moreover, Nina *et al.* (2019) presented that 6.23% protein, 22.06% fat, 6.04% fiber and 59.18% total carbohydrates in brown tiger nut flour. On the other hand, El-Hadidy *et al.* (2023), it is revealed that tiger nut flour comprises 7.00 % protein, 17.50 % crude fiber and 41.40 % carbohydrates. The many varieties of tiger nut flour might be the cause of the slight differences in several characteristics.

**Table 1. Chemical composition and pH values of cow milk and tiger nut flour powder**

| Parameters (%) | Cow milk |
|---|---|
| Moisture | 86.81±1.50 |
| Fat | 3.00±0.10 |
| Ash | 0.61±0.11 |
| Total protein | 3.06±0.06 |
| Total carbohydrate | 6.52±0.51 |
| Titratable Acidity | 0.17±0.02 |
| pH | 6.80±0.20 |
| Parameters (%) | Tiger nut flour (TNF) |
| Protein | 4.69±0.54 |
| Fats | 23.00±1.00 |
| Carbohydrates | 64.00±4.00 |
| Fiber | 16.30±0.30 |

**Each value was ± standard deviation of the average of three determinations.**
**Total carbohydrates (TC) = 100 − (protein + fat + ash + moisture).**

**Chemical composition of yoghurt (Y) fortified with tiger nut flour (TNF)**

The variations in the yoghurt's total chemical composition caused by the addition of tiger nut flour are displayed in Table 2. The fat content of the yoghurt was not significantly affected ($P > 0.05$) by the amount of tiger nut flour added. A total of 3% fat was included in the yoghurt treatments that were fortified with various ratios of tiger nut flour. In contrast, there was a significant ($P < 0.05$) impact of the amount of tiger nut flour added to the yoghurt's moisture, ash, total protein and total carbohydrate contents. The total protein and ash content of the yoghurt treatments fortified with different ratios of tiger nut flour increased significantly ($P < 0.05$) when tiger nut flour was added, but the moisture content of these treatments decreased. Furthermore, yoghurt treatments fortified with 3% tiger nut flour exhibited the greatest protein and ash contents, at 4.9 and 0.85%; respectively, when compared to other treatments. In addition, the moisture content of the yoghurt treatments without added tiger nut flour, the yoghurt treatments fortified with 1% tiger nut flour, the yoghurt treatments fortified with 2% tiger nut flour, and the yoghurt treatments fortified with 3% tiger nut flour was 87.09, 86.07, 85.25 and 83.29%; respectively. As shown in Table 2, the total carbohydrate content of the yoghurt samples ranged from 6.28 to 7.92%, when fortified with various amounts of tiger nut flour and without it. Ezeonu *et al.* (2016), indicated that using chufa milk increased the carbohydrate content of yoghurt from 6.85% for tiger nut yoghurt to 3.38 % for cow milk yoghurt (control). Moreover, Shahein *et al.* (2023), indicated that compared to the bio-yoghurt drink without tiger nut flour as a control, the treatments of the bio-yoghurt drink with tiger nut flour added had higher total solids and protein content.

**Table 2. Chemical composition of yoghurt (Y) fortified with tiger nut flour (TNF)**

| Properties | Treatments | | | |
|---|---|---|---|---|
| | Control (Y) | Y+ (1%TNF) | Y+ (2%TNF) | Y+ (3%TNF) |
| Fat% | $3.0±0.2^{a}$ | $3.0±0.1^{a}$ | $3.0±0.5^{a}$ | $3.0±0.3^{a}$ |
| Moisture % | $87.09±2.09^{a}$ | $86.07±3.03^{a}$ | $85.25±1.13^{b}$ | $83.29±3.15^{c}$ |
| Ash % | $0.76±0.05^{b}$ | $0.80±0.02^{ab}$ | $0.83±0.03^{ab}$ | $0.85±0.04^{a}$ |
| Total protein % | $2.87±0.52^{b}$ | $3.38±0.25^{b}$ | $4.32±0.31^{a}$ | $4.9±0.43^{a}$ |
| Total carbohydrate % | $6.28±1.14^{ab}$ | $6.75±0.73^{ab}$ | $6.60±0.60^{ab}$ | $7.92±0.92^{a}$ |

**Within a row, means with different superscript letters indicate a significant difference ($P < 0.05$).**

**pH values, and syneresis of yoghurt (Y) fortified with tiger nut flour (TNF)**

The pH values, and syneresis of the yoghurt treatments fortified with various ratios of tiger nut flour are shown in Table 3. One of the important factors determining the quality of yoghurt is whey separation or syneresis, which is an important problem that increases as storage time goes on. During this process, gel shrinkage causes water to be extracted or released from a gel. The addition of tiger nut flour and up to 14 days of refrigeration temperature storage had a significant ($P < 0.05$) impact on the pH and syneresis values of the yoghurt treatments supplemented with various tiger nut flour ratios. The results indicated that, adding tiger nut flour to yoghurt treatments and refrigerating them for up to 14 days caused decrease of the pH and syneresis values significantly ($P < 0.05$). Furthermore, the pH values of the yoghurt treatments with 3% tiger nut flour added were the lowest, whereas the pH values of the yoghurt treatments without tiger nut flour added were the highest (Table 3). Moreover, yoghurt treatments without added tiger nut flour had the highest syneresis values compared to the others. Wakil *et al.* (2014) found that the fermentation process of fermented nut milk resulted in a drop in pH and a rise in lactic acid content. In addition, Shahein *et al*. (2023) reported that the pH of the bio yoghurt drink decreased when tiger nut flour was added. However, the amount of tiger nut added to the bio yoghurt drink and the separated whey were shown to be inversely related; that is, the previous parameter was reduced as the amount of tiger nut flour increased.

**Table 3. pH value and syneresis of yoghurt (Y) fortified with tiger nut flour (TNF) during a storage period of 14 days at 4±1°C**

| Properties | Treatments | Storage period (days) | | | Means ± SD |
|---|---|---|---|---|---|
| | | Fresh | 7 | 14 | |
| pH | Control (Y) | 4.68±0.05 | 4.48±0.08 | 4.46±0.03 | $4.54±0.11^{A}$ |
| | Y+(1%TNF) | 4.65±0.03 | 4.44±0.02 | 4.36±0.04 | $4.48±0.13^{B}$ |
| | Y+(2%TNF) | 4.6±0.04 | 4.41±0.06 | 4.36±0.05 | $4.46±0.11^{B}$ |
| | Y+(3%TNF) | 4.59±0.07 | 4.39±0.10 | 4.33±0.01 | $4.44±0.13^{B}$ |
| | Means ± SD | $4.63±0.057^{a}$ | $4.43±0.07^{b}$ | $4.37±0.05^{c}$ | |
| Syneresis (%) | Control (Y) | 46.0±6.0 | 37.0±4.0 | 26.0±5.0 | $36.33±2.52^{A}$ |
| | Y+(1%TNF) | 40.67±2.52 | 14.0±2.0 | 13.0±2.0 | $22.56±13.72^{B}$ |
| | Y+(2%TNF) | 39.0±7.0 | 10.0±5.0 | 9.0±6.0 | $19.33±15.6^{BC}$ |
| | Y+(3%TNF) | 33.0±5.0 | 8.0±1.0 | 6.0±3.0 | $16.0±13.0^{C}$ |
| | Means ± SD | $39.67±6.67^{a}$ | $17.25±12.46^{b}$ | $13.75±8.55^{b}$ | |

**There was a significant difference ($P < 0.05$) between the means in the same row followed by different lowercase letters and the means in the same column that were followed by different uppercase letters.**

**Texture properties of yoghurt (Y) fortified with tiger nut flour (TNF)**

Some of the most important factors affecting the quality of yoghurt are its physical characteristics, including its hardness, fracture, cohesiveness, adhesiveness, springiness, gumminess and chewiness. For fermented dairy products, particularly yoghurt, these characteristics play a role in determining their quality. Table 4 indicates a significant change ($P < 0.05$) in the adhesiveness, cohesiveness, gumminess and chewiness values with an increase in the amount of tiger nut flour. The parameters adhesiveness, cohesiveness, gumminess and chewiness were highest in the yoghurt treatment fortified with 3% tiger nut flour, and lowest in the control sample. Additionally, when yoghurt fortified with 3% tiger nut flour was compared to other treatments, it had the highest adhesiveness, cohesiveness, gumminess, and chewiness evaluates (accordingly 3.28, 0.40, 0.40 and 3.67; respectively) in Table 4. In contrast, the characteristics of fracture, hardness, and springiness were not significantly affected by the addition of more tiger nut flour ($P > 0.05$). Alqahtani *et al*. (2021) found that the addition of oat flour caused a significant difference in the values of cohesion and chewiness, which increased in the following order: Goat milk yoghurt with 3% additional oat flour added (Y3) > 2% additional oat flour added (Y2) > 1% additional oat flour added (Y1) > 0% additional oat flour added goat milk yoghurt (Y0). Furthermore, goat milk yoghurt without oat flour added (Y0) had the lowest cohesiveness and chewiness values, while goat milk yoghurt with 3% added oat flour (Y3) had the highest values.

**Table 4. Texture profile parameters of yoghurt (Y) fortified with tiger nut flour (TNF)**

| Properties | Treatments | | | |
|---|---|---|---|---|
| | Control (Y) | Y+ (1%TNF) | Y+ (2%TNF) | Y+ (3%TNF) |
| Fracture (N) | $0.9±0.3^{a}$ | $0.9±0.05^{a}$ | $1.00±0.0^{a}$ | $1.03±0.15^{a}$ |
| Hardness (N) | $0.9±0.3^{a}$ | $1.00±0.0^{a}$ | $1.03±0.15^{a}$ | $1.06±0.11^{a}$ |
| Adhesiveness (mj) | $1.49±0.51^{b}$ | $2.78±0.15^{a}$ | $2.85±0.72^{a}$ | $3.28±0.22^{a}$ |
| Cohesiveness (Ratio) | $0.27±0.015^{b}$ | $0.28±0.025^{b}$ | $0.30±0.026^{b}$ | $0.40±0.025^{a}$ |
| Springiness (mm) | $6.96±2.53^{a}$ | $7.13±1.46^{a}$ | $8.96±0.24^{a}$ | $9.56±0.96^{a}$ |
| Gumminess (N) | $0.23±0.05^{b}$ | $0.27±0.05^{b}$ | $0.33±0.10^{a}$ | $0.40±0.00^{a}$ |
| Chewiness (mj) | $1.74±0.85^{b}$ | $2.15±0.77^{ab}$ | $2.96±0.47^{ab}$ | $3.67±0.23^{a}$ |

**Within a row, means with different superscript letters indicate a significant difference ($P < 0.05$).**

**Antioxidant activity and total phenolic content of yoghurt (Y) fortified with tiger nut flour (TNF)**

The antioxidant activity and the total phenolic content are presented in Table 5. According to these results, antioxidant activity and total phenol content are significantly associated. Increased antioxidant activity is associated with increased values of total phenolic content. The findings shown in Table 5 demonstrate that the antioxidant activity of the yoghurt samples and the total phenolic content gradually increased when more tiger nut flour was added. Additionally, the yoghurt sample with 3% tiger nut flour showed the highest relative antioxidant activity and total phenolic (114.09 µM TE/g and 47.47 µg GA E/g; respectively) when compared to the other treatments. The polyphenols present in tiger nut flour may be the cause of yoghurt samples with increased antioxidant activity. Furthermore, the total phenolic content and antioxidant activity values of the yoghurt control without tiger nut flour were the lowest at 31.53 µg GA E/g and 94.89

µM TE/g; respectively. Shah (2000) reported that the phenolic compounds linked to the breakdown of milk proteins are shown by the total phenolic content values in the control yoghurt. Tyrosine is a phenolic side-chain amino acid that raises the Folin-Ciocalteu assay's TPC reading. Sánchez-Zapata *et al.* (2012) indicated that tiger nuts' cell walls contain a variety of monomeric antioxidant phenols that can contribute to increasing antioxidant activity. These include p-hydroxybenzoic acid, vanillic acid, p-hydroxybenzaldehyde, vanillin, p-trans-coumaric acid, trans-ferulic acid, p-cis-coumaric acid and cis-ferulic acid. Jing *et al*. (2016) found that the tiger nut has great antioxidant properties and can be utilized as a natural antioxidant source because it includes flavonoids. Roselló-Soto *et al.* (2019) reported that tiger nuts have strong antioxidant properties due to their high content of phenolic components.

**Table 5. Antioxidant activity and total phenolic content of yoghurt (Y) fortified with tiger nut flour (TNF)**

| Treatments | Antioxidant (µM TE/g sample) | Total phenolic (µg GA E/g) |
|---|---|---|
| Control (Y) | 94.89 ± 7.16 | 31.53 ± 5.48 |
| Y+(1%TNF) | 109.88 ± 6.98 | 41.69 ± 2.81 |
| Y+(2%TNF) | 113.28 ± 6.46 | 43.69 ± 3.39 |
| Y+(3%TNF) | 114.09 ± 6.30 | 47.47 ± 4.14 |

**Each value was ± standard deviation of the average of three determinations.**

### Antibacterial activity of yoghurt (Y) fortified with tiger nut flour (TNF)

Table 6 shows the antibacterial activity of the supernatant fortified with tiger nut flour for yoghurt treatment against *Salmonella typhi*, *Bacillus cereus*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Escherichia coli*. The yoghurt treatments fortified with various ratios of tiger nut flour exhibited increased antibacterial activity in the supernatant filtrates compared to the control yoghurt. Furthermore, the antibacterial activity of the yoghurt supernatant filtrates increased significantly ($P < 0.05$) as the amount of tiger nut flour increased. Moreover, yoghurt treatments fortified with 3% tiger nut flour showed significantly higher antibacterial activity against all pathogenic microorganisms examined, with inhibition zones of 17.33, 16.00, 11.33, 11.67 and 11.00 mm for *Salmonella typhi*, *Bacillus cereus*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Escherichia coli*; respectively, compared to yoghurt treatments fortified with various ratios of tiger nut flour and control yoghurt. On the other hand, no inhibitory effect against *E. coli was observed* in the supernatant filtrate of the control sample or the supernatant filtrate of the yoghurt treatment enriched with 1% tiger nut flour (Table 6). Asare *et al*. (2020) investigated *Cyperus esculentus* roots, flowers and shoots for antibacterial activity compared to common antimicrobial medications. The ethanol extract exhibited responses for *Salmonella typhi* therapy that were like those of ciprofloxacin. The study suggests that the plant extract's strong antibacterial properties could aid in the creation of novel antibiotic therapies for diseases associated with *S. typhi*

**Table 6. Antibacterial activity of yoghurt (Y) fortified with tiger nut flour (TNF) against pathogenic bacteria**

| Inhibition zone diameter (mm) | Treatments | | | |
|---|---|---|---|---|
| | Control (Y) | Y+(1%TNF) | Y+(2%TNF) | Y+(3%TNF) |
| *Salmonella typhi* | 16.00±0.0[ab] | 16.00±1.73[ab] | 16.67±10.58[ab] | 17.33±1.15[a] |
| *Bacillus cereus* | 12.67±1.15[b] | 13.67±2.08[ab] | 15.33±1.15[ab] | 16.00±1.73[a] |
| *Staphylococcus aureus* | 4.33±7.50[c] | 5.33±9.23[c] | 9.33±8.81[b] | 11.33±9.81[a] |
| *Enterococcus faecalis* | 9.00±7.81[b] | 9.67±8.39[b] | 10.67±9.29[a] | 11.67±10.12[a] |
| *Escherichia coli* | ND* | ND | 4.67±8.08[b] | 11±9.54[a] |

**Within a row, means with different superscript letters indicate a significant difference ($P < 0.05$).**
**ND*: Not detected**

### Evaluation of cytotoxicity and in vitro anticancer of yoghurt (Y) fortified with tiger nut flour (TNF)

Using MTT-based assays against three standard cell lines for mammary gland cancer (MCF-7), epithelioid carcinoma of the cervix cancer (Hela), and colon colorectal carcinoma (HCT-116) the in vitro cytotoxicity of yoghurt fortified with tiger nut flour was assessed. One of the best anticancer medications, doxorubicin was employed as a positive control. The results were expressed in contrast to untreated controls using growth inhibitory concentration (IC50) values, which show the treatment concentrations required to produce a 50% inhibition of cell growth after 72 hours of incubation (Table 7). The results showed that the fortified yoghurt with tiger nut flour inhibited three of the human tumor cell lines tested to varying degrees. When additional tiger nut flour was added, the cytotoxic activity of the yoghurt samples gradually increased (Table 7). When tested against mammary gland breast cancer cell lines (MCF-7), colorectal colon cancer (HCT-116), epithelioid carcinoma cervix cancer (Hela), and yoghurt samples containing 3% tiger nut flour showed moderate cytotoxic activity, with percentage viability IC50 values of 25.17, 31.83 and 29.75 µg /ml; respectively. On the other hand, the yoghurt sample without tiger nut flour exhibits weak activity (IC50 = 66.23 µg and 71.38 µg) against the MCF-7 and HCT116 cell lines; respectively (Table 7). Gambo & Da'u (2014), demonstrated that it has been suggested that tiger nuts (*Cyperus esculentus*) may help lower the probability of colon cancer. Malashree *et al.* (2021) reported that one of the many incredible advantages of tiger nuts is that they are a resistant starch food. Resistant starch (RS), compared to regular starch, has various health benefits, one of which is a reduced risk of colon cancer. Moreover, adding more tiger nut flour to the yoghurt samples decreased the relative viability of the three human tumor cell lines examined (Figure 1). Furthermore, yoghurt samples containing 3% tiger nut flour had a stronger effect on the reduction in viability of the three human tumor cell lines investigated than did the control sample without tiger nut flour and other treatments. According to Adenowo & Kazeem (2020), regular consumption of tiger nuts may not cause medical problems, including prostate cancer or colon cancer.

**Table 7. Cytotoxic activity of yoghurt (Y) fortified with tiger nut (TNF)**

| | In vitro cytotoxicity $IC_{50}$ (µg) | | |
|---|---|---|---|
| **Treatments** | **HCT116** | **Hela** | **MCF-7** |
| Doxorubicin | 5.23±0.3 | 5.57±0.4 | 4.17±0.2 |
| Control (Y) | 71.38±3.8 | 49.17±2.5 | 66.23±3.5 |
| Y+(1%TNF) | 57.61±3.1 | 42.57±2.4 | 61.92±3.4 |
| Y+(2%TNF) | 46.95±2.6 | 35.05±2.1 | 38.19±2.3 |
| Y+(3%TNF) | 31.83±1.9 | 29.75±2.0 | 25.17±1.8 |

**IC50 (µg/ml): 1 – 10 (very strong). 11 – 20 (strong). 21 – 50 (moderate). 51 – 100 (weak) and above 100 (noncytotoxic)**

### Microbiological properties of yoghurt (Y) fortified with tiger nut (TNF)

Table 8 shows viable counts of *S. thermophilus* and *Lactobacillus delbrueckii subsp. Bulgaricus* in yoghurt fortified with different amounts of tiger nut flour during storage periods at refrigerator temperature up to 14 days. The addition of tiger nut flour to yoghurt treatments and storage for up to 14 days at refrigerator temperature significantly ($P < 0.05$) impacted the *S. thermophilus* numbers. The yoghurt production process was improved by adding more tiger nut flour, which promoted the growth of *S. thermophilus*. Additionally, the yoghurt treatments without tiger nut flour had higher *S. thermophilus* counts at the beginning of storage than the yoghurt sample that contained tiger nut flour. The increase in *S. thermophilus* numbers was generally significant ($P < 0.05$) for 7 days and then decreased at 14 days (Table 8). Furthermore, the storage period of the treatments had a significant impact on the count of *L. delbrueckii subsp. bulgaricus* ($P < 0.05$). Additionally, the yoghurt treatments without tiger nut flour had a lower *L. delbrueckii subsp. bulgaricus* counts at the beginning of storage than the yoghurt sample that contained tiger nut flour. *L. delbrueckii subsp. bulgaricus* numbers generally increased significantly ($p < 0.05$) for 7 days and then decreased at 14 days. On the contrary, the growth of *L. delbrueckii subsp. bulgaricus* was not significantly affected ($P > 0.05$) by adding tiger nut flour to yoghurt treatments (Table 8). Shahein *et al.* (2023) found that when comparing the bio yoghurt drink treated with tiger nut flour to the control treatment, there was a noticeable rise in the number of lactic acid bacteria during the storage period.

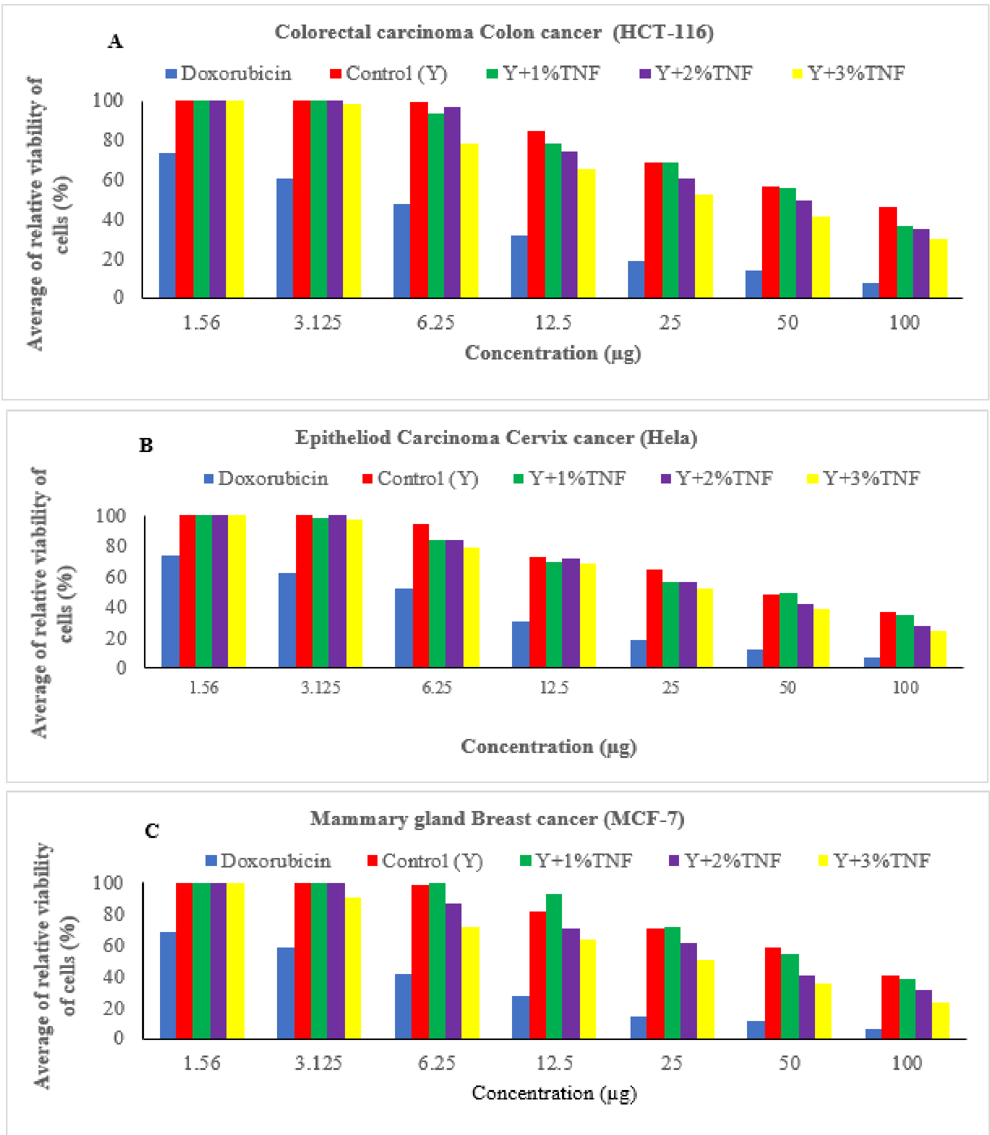


**Figure 1. The study evaluated the average relative viability of several cancer cell lines, including plain yoghurt (Y) as a control, tiger nut flour (TNF), {Y + 1% TNF, Y + 2% TNF and Y + 3% TNF}, as well as colorectal carcinoma (HCT-116) of the cell line (A),epitheliod carcinoma cervix cancer (Hela) (B) and mammary gland breast cancer cell line (MCF-7) and at different concentrations of doxorubicin.**

**Table 8. Microbiological properties of yoghurt (Y) fortified with tiger nut (TNF) during a 14-day storage period at 4±1 °C.**

| Microbial Type (Counts) | Treatments | Storage period (days) Fresh | 7 | 14 | Means ± SD |
|---|---|---|---|---|---|
| *Streptococcus thermophiles* count (Log cfu/ml) | Control (Y) | 9.77±0.52 | 10.95±0.85 | 10.19±0.09 | 10.3±0.72$^{A}$ |
| | Y+(1%TNF) | 8.81±0.8 | 10.03±0.03 | 9.09±0.09 | 9.31±0.87$^{B}$ |
| | Y+(2%TNF) | 9.52±0.61 | 10.49±0.44 | 9.82±0.72 | 9.98±0.67$^{A}$ |
| | Y+(3%TNF) | 9.78±0.73 | 10.71±0.31 | 10.03±0.6 | 10.17±0.64$^{A}$ |
| | Means ± SD | 9.47±0.70$^{b}$ | 10.54±0.55$^{a}$ | 9.78±0.76$^{b}$ | |
| *Lactobacillus delbrueckii subsp. Bulgaricus* count (Log cfu/ml) | Control (Y) | 8.75±0.75 | 10.14±1.0 | 9.96±1.90 | 9.62±1.31$^{A}$ |
| | Y+(1%TNF) | 9.67±0.55 | 10.39±0.26 | 10.1±0.06 | 10.05±0.43$^{A}$ |
| | Y+(2%TNF) | 9.73±0.40 | 10.51±0.11 | 10.26±0.25 | 10.16±0.42$^{A}$ |
| | Y+(3%TNF) | 9.75±0.44 | 10.53±0.20 | 10.30±0.25 | 10.19±0.44$^{A}$ |
| | Means ± SD | 9.48±0.64$^{b}$ | 10.39±0.47$^{a}$ | 10.15±0.86$^{a}$ | |

**There was a significant difference ($P < 0.05$) between the means in the same row followed by different lowercase letters and the means in the same column that were followed by different uppercase letters.**

## Sensory properties of yoghurt (Y) fortified with tiger nut flour (TNF)

Consumer acceptance of food products is greatly influenced by their sensory properties. Figure 2 presents the sensory evaluation results for yoghurt treatments fortified with different ratios of tiger nut flour during storage periods about taste & flavor, texture, acidity and general appearance. Yoghurt treatments fortified with varying ratios of tiger nut flour did not show any significant variations ($P > 0.05$) in taste & flavor, texture, acidity, or general appearance after up to 14 days of storage at refrigerated temperatures. On the other hand, when tiger nut flour was added, it significantly ($P < 0.05$) impacted the yoghurt treatments' general appearance, texture, and taste & flavor; however, the acidity of the yoghurt treatments fortified with different ratios of tiger nut flour was not significantly ($P > 0.05$) influenced. When the amount of tiger nut flour in the yoghurt treatments increased, there was a significant decrease ($P < 0.05$) in its general appearance and taste & flavor. Furthermore, compared to other samples, yoghurt treatments that did not have added tiger nut flour had the highest degree of taste & flavor and a general appearance. On the contrary, the more tiger nut flour added to the yoghurt treatments, the more significantly the texture increased ($P < 0.05$). Compared to other samples, the yoghurt treatments fortified with 3% tiger nut flour had the highest texture score (Figure 2). Shahein *et al.* (2023) found that tiger nut flour was added to the yoghurt drink to enhance its sensory properties, particularly its body and texture.

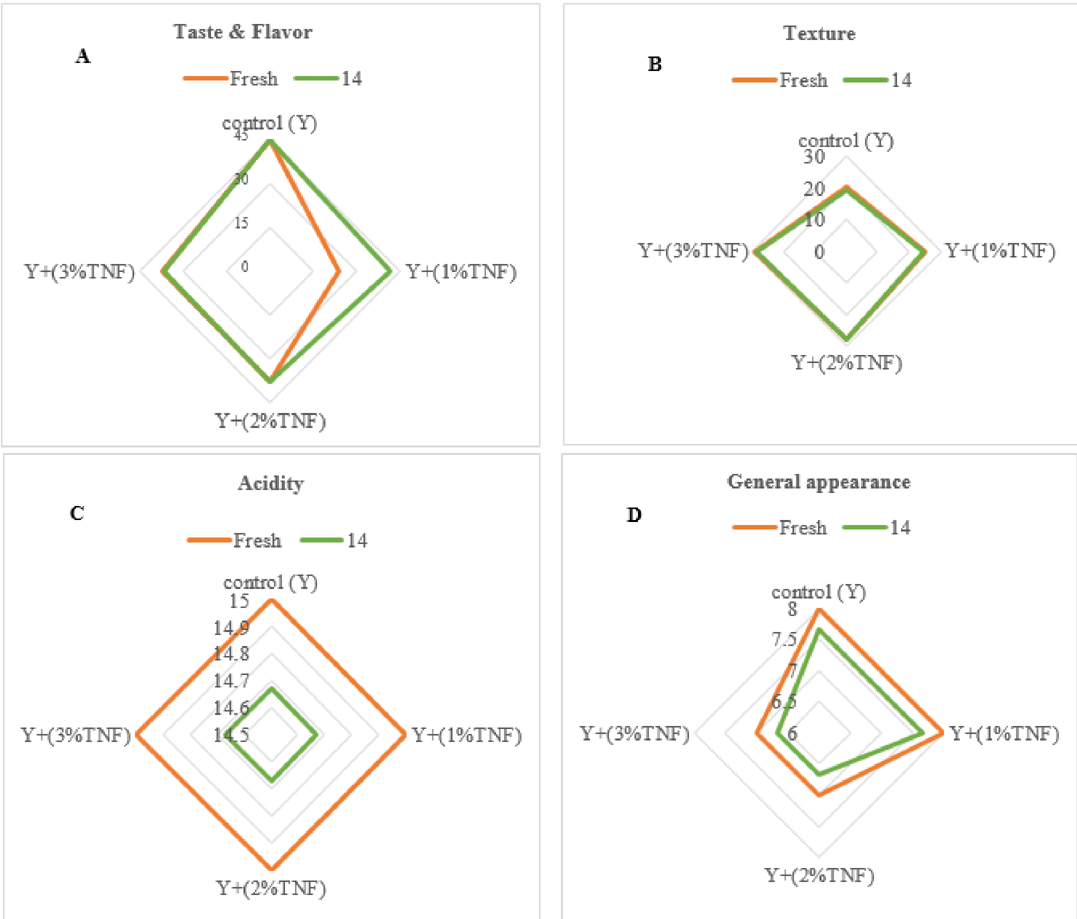


**Figure 2. Sensory properties of yoghurt (Y) fortified with tiger nut flour (TNF) during a 14-day storage period at 4±1°C.Plain yoghurt [control (Y)] and other samples of yoghurt fortified with tiger nut flour (TNF), [ Y + 1% TNF), Y + 2% TNF), and Y + 3% TNF)]. $^{*}P \leq 0.05$ is presented by means ± standard deviations of triplicates (n = 3).**

## CONCLUSION

According to the results of this study, it is possible to produce special functional yoghurt with the desired texture using tiger nut flour. The resulting yoghurt's phenolic content and antioxidant activity were enhanced by the functional component of tiger nut flour, which allows it for use in anticancer diets. Additionally, yoghurt with tiger nut flour demonstrated significantly higher antibacterial activity against all examined bacteria when compared to the control yoghurt. Furthermore, compared to other samples, yoghurt samples containing 3% tiger nut flour showed a greater effect on the decline in the viability of the three human tumor cell lines under investigation. As fermentable substrates for lactic acid bacteria, the fiber and phytochemical components included in these tiger nut flour can increase the viability of yoghurt cultures. Yoghurt treatments supplemented with 3% tiger nut flour had the most desirable textures and slightly lower taste & flavor and general appearance than other treatments. Thus, new fermented milk products with various functional, nutritional, and health benefits can be made with tiger nut flour.

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# رؤى جديدة حول الخصائص الوظيفية للزبادي المدعم بدقيق حب العزيز (*Cyperus Esculentus*) كمضاد للأكسدة ومضاد للميكروبات ومضاد للأورام

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## الملخص

في هذه الدراسة، تم تقييم الزبادي المنتج بإضافة دقيق حب العزيز بنسب مختلفة (1، 2، و3٪، وزن/حجم) من حيث محتواه الكيميائي والفيزيائي ومحتواى الفينولات الكلية ومضادات الأكسدة ومضادات البكتيريا والنشاط السام للخلايا والملمس والخصائص الميكروبيولوجية والحسية. أدى إضافة دقيق حب العزيز إلى زيادة كبيرة ($P < 0.05$) في البروتين والرماد والكربوهيدرات ومحتوى الفينولات ومضادات الأكسدة والمضادات البكتيريا والنشاط السام للخلايا والقدرة على الالتصاق والتماسك والصمغ والمضغ لمعاملات الزبادي. كان هناك انخفاض كبير في محتوى الرطوبة ($P < 0.05$). بالإضافة إلى ذلك لم يتأثر محتوى الدهن والكسر والصلابة ومرونة معاملات الزبادي بشكل كبير بإضافة دقيق حب العزيز. عندما تمت إضافة دقيق حب العزيز وحفظ المعاملات بالتبريد لمدة تصل إلى 14 يومًا، فقد أدى ذلك إلى انخفاض كبير ($P < 0.05$) في مستويات التشريش والرقم الهيدروجيني. بالإضافة إلى ذلك، وجد أن معاملات الزبادي المدعمة بدقيق حب العزيز أدت الى تثبيط ثلاثة خطوط من خلايا السرطانية البشرية، بما في ذلك سرطان القولون وسرطان الغدة الثديية وسرطان عنق الرحم. تم تحسين قابلية بكتيريا حمض اللاكتيك للبقاء والملمس بإضافة دقيق حب العزيز. ووجد أن الخصائص الحسية (الطعم والنكهة والمظهر العام) لمعاملات الزبادي المضاف اليه دقيق حب العزيز أقل من تلك التي لا تحتوي على دقيق حب العزيز. بينما كان التركيب أعلى، ولم تتغير الحموضة. وبالتالي، يمكننا أن نستنتج أنه يمكن استخدام دقيق حب العزيز لإنتاج زبادي وظيفي جديد.

**الكلمات الدالة**: دقيق حب العزيز ، الغذاء الوظيفي، إجمالي الفينولات، مضاد للبكتيريا، سام للخلايا.