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Research Article

Impact of tiger nut flour as a prebiotic and thickener agent on bio-yoghurt drink

Magdy Shahein [1], Mohammed El-Ghandour [1] and Monira Basiony [2]*

[1] Food Science and Technology Department, Faculty of Agriculture, Tanta University, Tanta 31527, Egypt; magdrsh10@gmail.com
[2] Dairy Technology Department, Animal Production Research institute, Agricultural Research Center, Egypt; monira.basiony@arc.sci.eg
* Correspondence: monira.basiony@arc.sci.eg; (ORCID: 0000-0002-4685-1447)

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Abstract:

Bio- yoghurt drinks were prepared by mixing water (15%) with cow's milk yoghurt (85%) and sugar 6% . 0.15% of stabilizer (Unicream 300) was added only to the control treatment, while tiger nut flour was added to the other four treatments at levels of 0.5, 1, 1.5, and 2% (T1, T2, T3, and T4, respectively). The final bio-yoghurt drinks were stored in the refrigerator (4±1 °C) for 15 days and analyzed chemically, physically, microbiologically, and organoleptically when fresh and after 7 and 15 days of cold storage. Results showed that the addition of tiger nut flour increased the total solids, protein, fat, and acidity but decreased the pH value. An inverse relationship was observed between wheying off, viscosity, and the amount of tiger nut added, this mean that as the amount of tiger nut increased the previous parameters decreased. The addition of tiger nut flour also increased the counts of total viable bacteria, Lactic acid bacteria, and *Lactobacillus rhamnosus (L. rhamnosus)* (probiotic bacteria) during the storage period compared with the control treatment. All yoghurt drinks (including control treatment) had more than 1× 10^6 cfu / ml of probiotic bacteria after 15 days of cold storage. The sensory properties were improved by adding tiger nut flour to the yoghurt drink, especially body and texture and flavor.

1. Introduction

The manufacturing of dairy products with beneficial properties has become more popular recently because they provide additional health advantages or functionality besides their nutritional benefits. This is due to the awareness of the potential health benefits of some specific ingredients and the developments in food science and technology, **(**Kasote et al. 2021 and Topolska et al. 2021).

Fermented milk products such as yoghurt drinks are the most consumed functional food for their health-enhancing with functional ingredients. They have grown in popularity since customers frequently favor the things that are convenient and useful. They have fruit or fruit juice flavors, which increases their allure. Many yoghurt beverages are also enhanced, with necessary elements including vitamins, minerals, prebiotics, or probiotics (Basiony, 2017). Additional health advantages from these extra nutrients include helping digestion and enhancing the immune system. Some yogurt beverages are created to include a combination of probiotic bacteria in addition to the conventional yogurt-forming bacteria to satisfy this desire.

Probiotics are living microorganisms that, when taken in sufficient quantities, can improve the host's health by supporting the growth of good bacteria in the digestive tract (Suez et al., 2019). These probiotic strains were chosen after consideration of their potential benefits for immune function and gastrointestinal health. Furthermore, probiotics can aid in reducing the growth and activity of pathogenic bacteria in the digestive tract by taking up residence there and competing with dangerous bacteria (Hemarajata et al., 2013). *L. rhamnosus* is a species of beneficial bacteria commonly found in the human gut due to its capacity to thrive on bile-containing media, stomach acid pH, and enterocytes. In addition, *L. rhamnosus* can also release soluble substances that benefit the gut by promoting intestinal crypt survival, reducing intestinal epithelial apoptosis, and maintaining cytoskeletal integrity in addition to a biofilm that can mechanically protect the mucosa (Capurso, 2019). It also may be considered probiotic bacteria because it has been shown to confer health benefits when consumed in adequate amounts (Verdenelli et al., 2009) and is also available to use as a probiotic supplement in various forms, including capsules, powders, and probiotic blends.

Tiger nut (*Cyperus esculentus L.*) is a species of herbaceous plant that yields a delicious sweet tuber, and it is commonly farmed in Spain, Burkina Faso, Mali, Niger, and Nigeria. Despite its name, tiger nut is not a nut but rather a small root vegetable. They have been used for centuries in various cultures and cuisines and have been used to make the milky beverage Orchata (Pascual et al., 2000). Tiger nut powder is a type of flour made from the dried and ground tubers of the tiger nut plant and has health benefits in addition to nutritional benefits. As for nutritional benefits, it is abundant in iron, magnesium, good fats, fiber, vitamin E, vitamin C, and omega-6 fatty acids. These elements may be crucial in preventing certain disorders such as coronary heart disease, colon cancer, diabetes, and obesity (Martín-Esparza et al., 2018a). It might aid intestinal health, be a good source of energy, and be suitable for people with dietary restrictions or allergies because it is gluten-free, nut-free, and grain-free (Gasparre and Cristina, 2019). Tiger nut has a slightly sweet and nutty flavor, which makes it a versatile ingredient in cooking, baking, as well as using as a natural thickening agent in soups, sauces, stews, because it adds a subtle sweetness and helps to create a creamy texture, sprinkled over oatmeal, yogurt, or mixed with other grains to create a nutritious and flavorful breakfast cereal. Moreover, it may be combined with other ingredients like nuts, dried fruits, and sweeteners to make homemade energy bars, granola bars, or snack balls. The chemical composition of dried Tiger nut (brown variety) was 3.78 % moisture, 9.70 % crude protein, 35.41% lipid, 5.62% crude fiber, 41.22 % carbohydrates, 4.25% ash, and 1511 energy (KJ) (Gambo and Da'u, 2014). Salama et al. (2013) mentioned also that available carbohydrates of tiger nut flour were 70.11 %, crude fibers 13.87 %, and crude protein 10.75%.

The aim of this work was to study the effect of adding tiger nut flour to bio-yoghurt drink on its properties and quality, during cold storage.

2. Materials and Methods

Materials

Fresh cow's milk was obtained from Al-Gemmeiza Station, Animal Production Research Institute, Agricultural Research Center, Egypt. Its chemical composition was 11.6%TS, 3% fat and 2.9% protein. Tiger nut flour and sugar were obtained from the local market. A commercial stabilizer named Unicream 300 was obtained from Misr Food Additives Co. Egypt.

Yoghurt culture (*Lactobacillus delbrueckii subsp. bulgaricus* and *S. thermophiles*) and *L. rhamnosus LGG* were obtained from Chr. Hansen's Lab A/S Copenhagen, Denmark. The preparation and activation of these cultures were described by Sezer et al. (2020).

Methods

Bio-yoghurt drink preparation

Bio-yoghurt drinks were prepared as described by Tamime and Robinson, (1999) with some modifications. Cow's milk was heat-treated at 90 °C for 15 min., cooled to 40–42 °C, inoculated with 2% yoghurt culture plus 2 % activated probiotic bacteria (*L. rhamnosus*), and incubated at 42°C for a fully coagulation. The resultant bio-yoghurt was kept at 4±1°C in the refrigerator. Bio- yoghurt drinks were prepared by mixing water (15%) with cow's milk yoghurt (85%) and sugar (6%) in all treatments. 0.15% of Unicream 300 (stabilizer) was added only to the control treatment, while tiger nut flour was added to the other four treatments at levels of 0.5, 1, 1.5, and 2% (T1, T2, T3, and T4, respectively). The final drinks were filled into well-clean glass bottles, stored in the refrigerator (4±1 °C) for 15 days, and analyzed chemically, microbiologically, physically, and sensory properties.

Tiger nut flour preparation

Tiger nut flour preparation was done as described by Akajiaku et al. (2018)

Methods of analysis

The pH values of bio-yoghurt drink samples were determined using a digital pH meter (Jenway electrode no. 3505, Dunmow, England). All bio-yoghurt drink samples were analysed chemically for acidity, total solids, fat, and total protein contents according to the methods described by AOAC (2000). Soluble nitrogen contents were estimated according to Ling (1963) and Total volatile fatty acids according to Kosikowiski (1978).

The amount of separated whey from bio- yoghurt drink samples was measured after keeping the bottles for 7 and 15 days in the refrigerator. The separated upper layer of whey was collected using syringe, and the amounts of collected whey were measured using a graduated cylinder as given by Hatem (1996).

Viscosity of the bio- yoghurt drinks were measured at 20°C using a Höppler falling ball viscometer (BH2 NO. 17377, Germany).

The total bacterial count was determined using a Nutrient agar medium according to Houghtby et al., (1992). Lactic acid bacteria on MRS agar medium according to Frank et al. (1992) and *L. rhamnosus* (which counted on MRS agar medium supplemented with 0.05% L – cysteine and 1% caco3,) according to Rajoka et al. (2017). Yeast and molds were counted according to IDF (1985 b).

The sensory evaluation of bio-yoghurt drink samples was done by a taste panel of 10 experienced panelists from the Dairy Technology Department, Al-Gemmaiza Station, Animal Production Research Institute. The samples were evaluated for appearance (15 points), body & texture (35 points) and flavor (50 points) out of 100 score points (Nelson and Trout, 1964).

Data were subjected to statistical analysis using general liner model procedure adapted by SPSS 10 for windows (SPSS, 1999) with one-way ANOVA and Duncan test was done.

3. Results and Discussion

Results of titratable acidity (TA) and pH values of fresh and cold stored bio- yoghurt drinks are illustrated in Fig. (1). It is common knowledge that the trend of acidity and pH values was the opposite in all treatments during the storage. Furthermore, it was observed that the pH values of the tiger nut treatments were significantly decreased as the levels of tiger nut flour increased. On the other hand, the increase in acidities of the tiger nut treatments, compared to the control one, was due to the high level of carbohydrates (glucose) and protein in the flour added (Salama et al., 2013). This means that, there was a positive relationship between the increase in acidities and the levels of tiger nut flour added. These findings are in line with those found by Bristone et al. (2015), who demonstrated that increasing tiger nut raised the acidity of tiger nut treatments from 1.09% in cow-tiger nut yoghurt 50:50 treatment to 1.13% in cow-tiger nut yoghurt 20:80

treatment. Overall, TA of all samples was generally increased during 15 days of cold storage. The pH values in the control treatment and tiger nut treatments were in the range of 4.55 to 3.03. The lowest pH value and highest acidity (%) were found in the sample made with 2% tiger nut flour on day 15.

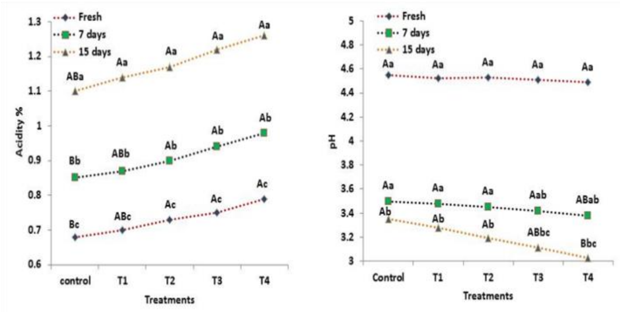

Figure 1. Influence of using different ratios of tiger nut flour on acidity (%) and pH values of fresh and cold stored bio- yoghurt drink.

ABCD Letters indicate significant differences between yoghurt drink treatments

abcd Letters indicate significant differences between storage times

C Control bio-yoghurt drink.

T1 Bio- yoghurt drink + 0.5 % tiger nut flour.

T2 Bio- yoghurt drink + 1.0 % tiger nut flour.

T3 Bio- yoghurt drink + 1.5 % tiger nut flour.

T4 Bio-yoghurt drink + 2.0 % tiger nut flour.

Chemical composition

The effect of using different levels of tiger nut flour on the total solids (%), fat (%), and total protein (%) of fresh and stored bio- yoghurt drinks were tabulated in Table (1). The amount of tiger nut flour and storage time had significant effects on the total solids content of bio- yoghurt drink. Results also showed that the control treatment had lower total solids (12%) compared with fresh tiger nut bio- yoghurt drink treatments, which were 12.95%, 13.8%, 14.65 and 15.47%, for T1, T2, T3, and T4, respectively. The total solids in all treatments increased during storage period; this may be attributed to the loss of some moisture content consumed by the flora as well as the biochemical reactions that took place during the cold storage.

Fat contents were 2.93%, 3.28%, 3.42%, 3.56 and 3.73% in control, T1, T2, T3, and T4 of fresh treatments, respectively. No significant differences were found in fat contents among all treatments during storage. Increasing the fat content in tiger nut treatments was due to the high-fat content in tiger nut flour (35.41% lipid) (Gambo and Da'u, 2014).

Table (1) moreover, shows that the protein content takes the same trend of total solids, this mean that it increases in the tiger nut flour treatments than in the control one; owing to the high percent of protein (approximately 10.75 %, Salama et al., 2013) in the tiger nut flour. Protein contents of all treatments were not increased significantly during the storage period.

Wheying off and viscosity

The amount of separated whey is measured by a process called syneresis, which is an important factor in consumer acceptance of the product. In general, it is preferable if the finished product has a small amount of whey (Lafta, 2019). Figure (2) shows the amount of separated whey and the viscosity of the bio-yoghurt drinks. All treatments had no whey separation in fresh samples, while after 7 days, the amount of separated whey recorded 2.2, 1.95, 1.6, 1.43, and 1.12 ml in control, T1, T2, T3, and T4, respectively. This shows that the tiger nut flour (which acts as a thickening agent) added to the bio-yoghurt drink led to a decrease in whey separation compared to the control treatment and gave it thickness. The amounts of whey significantly increased in all treatments, during the storage period.

It was also, noticed in Table 2 that adding tiger nut flour caused a significant increase in the apparent viscosity of bio-yoghurt drink in comparison to control yoghurt; this may be due to the increase in the total solids because tiger nut flour contained 70.11% carbohydrates, crude fibers 13.87 % and crude protein 10.75% (Salama et al., 2013).

In general, Increasing the total solid content in the product leads to an increase in the viscosity in the final one (Tamime and Robinson, 1985). This can also be explained by the fact said that, tiger nut flour acts as a prebiotic, which is a water structuring-agent and can create complexes (H-bridge formation) with protein aggregates in yogurt, thus acting as a thickener (Tesfaye, 2013). The viscosity values of all the samples were increased gradually during the storage period.

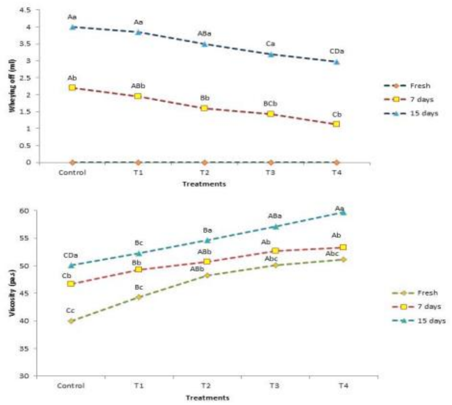

Figure 2. Influence of using different ratios of tiger nut flour on wheying off (ml) and viscosity (pa.s) of fresh and stored bio- yoghurt drinks stored in refrigerator.

ABCD Letters indicate significant differences between yoghurt drink treatments

abcd Letters indicate significant differences between storage times

C Control bio-yoghurt drink.

T1 Bio- yoghurt drink + 0.5 % tiger nut flour.

T2 Bio- yoghurt drink + 1.0 % tiger nut flour.

T3 Bio- yoghurt drink + 1.5 % tiger nut flour.

T4 Bio-yoghurt drink + 2.0 % tiger nut flour.

Figure (3) showed that control treatment had low contents of both soluble nitrogen (S.N) and total volatile fatty acids (TVFA) compared with tiger nut treatments, when fresh and after 7 and 15 days of storage. As the rate of tiger nut added increased the contents of the former detections were increased. The main reason for the presences of S.N and TVFA in the drinks may be due to the growth of psychrotrophic bacteria, during cold storage.

Psychrotrophic bacteria predominate the milk microflora depending on the duration of the refrigerated storage. Psychrotrophs are microorganisms that can grow at temperatures between 0 and 7ºC and produce visible colonies or turbidity within 7–10 days. One of the major characteristics of psychrotrophic bacteria in milk is their ability to produce extracellular enzymes that can attack milk constituents. Bacteria of this group are in general inactivated by heat treatments applied to market milk products; however, their enzymes may be extremely heat resistant remaining active in the products, decreasing thus their shelf-life resistant remaining active in the products, decreasing thus their shelf-life. In fact, an initial count as little as 10^2 cfu ml–1 can spoil raw milk during cold storage within five days (Frank and Hassan 2002). Moreover, some thermoduric bacteria that can survive pasteurization and are able to grow during refrigerated storage of dairy products belong to this group. Psychrotrophic bacteria found in raw milk belong mainly to Gram negative genera Pseudomonas, Enterobacter, Flavobacterium, Klebsiella, Aeromonas, Acinetobacter, Alcaligenes, Achromobacter and Serrtia Pseudomonas spp. and Enterobacteriaceae are the most abundant microorganisms in cold stored raw milk; (Griffiths et al. 1987, Champagne et al. 1994, Stepaniak 2003). Both initial level of bacterial counts and storage temperature affect psychrotrophic counts and, therefore, the storage life of raw milk under refrigerated conditions. P. fluorescens is found in soil and water but a major source for milk is the milking utensils, the storage tanks and the transport equipment. As mentioned earlier, the major characteristic of psychrotrophs is the production of extracellular enzymes which play active role in degradation of certain milk compounds resulting in flavor defects. Extracellular thermostable proteinases, lipases, phospholipases, exopeptidases and glycosidases are produced by psychrotrophic bacteria in milk.

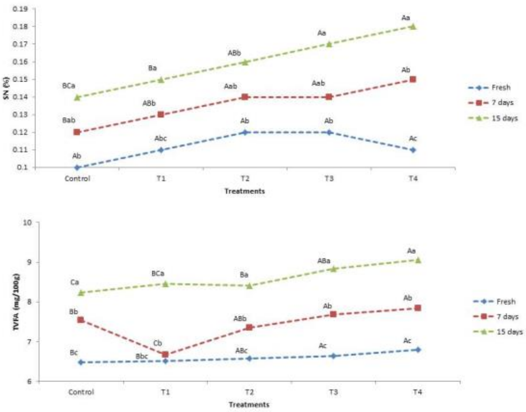

Figure 3. Influence of using different ratios of tiger nut flour on SN (%) and TVFA (mg/100g) of fresh and stored bio-yoghurt drinks stored in refrigerator.

ABCD Letters indicate significant differences between yoghurt drink treatments

abcd Letters indicate significant differences between storage times

C Control bio-yoghurt drink.
T1 Bio- yoghurt drink + 0.5 % tiger nut flour.
T2 Bio- yoghurt drink + 1.0 % tiger nut flour.
T3 Bio- yoghurt drink + 1.5 % tiger nut flour.
T4 Bio-yoghurt drink + 2.0 % tiger nut flour.

Table 1. The chemical composition of bio-yoghurt drink supplemented with tiger nut flour during 15 days of cold storage

Properties	Storage Period (days)	Treatments				
		Control	**T_1**	**T_2**	**T_3**	**T_4**
Total Solids (%)	**Fresh**	12.00 ± 0.15^{Da}	12.95 ± 0.11^{Ca}	13.80 ± 0.012^{BCa}	14.65 ± 0.13^{Ab}	15.47 ± 0.15^{Aa}
	7	12.08 ± 0.11^{Ea}	13.05 ± 0.10^{Da}	13.86 ± 0.12^{Ca}	14.71 ± 0.09^{Ba}	15.53 ± 0.12^{Aa}
	15	12.14 ± 0.12^{Da}	13.12 ± 0.11^{CDa}	13.92 ± 015^{Ca}	14.78 ± 0.12^{Ba}	15.60 ± 0.13^{Aa}
Fat (%)	**Fresh**	2.93 ± 0.08^{BCa}	3.28 ± 0.09^{Ba}	3.42 ± 0.11^{ABa}	3.56 ± 0.12^{Aa}	3.73 ± 0.09^{Aa}
	7	2.87 ± 0.10^{Ca}	3.30 ± 0.12^{Ba}	3.44 ± 0.08^{ABa}	3.58 ± 0.07^{Aa}	3.75 ± 0.09^{Aa}
	15	2.79 ± 0.12^{Ca}	3.31 ± 0.11^{BCa}	3.45 ± 0.09^{Ba}	3.60 ± 0.08^{Aa}	3.76 ± 0.08^{Aa}
Total Protein (%)	**Fresh**	2.70 ± 0.03^{Ca}	2.76 ± 0.08^{BCa}	2.82 ± 0.07^{Ba}	2.88 ± 0.08^{Aba}	2.95 ± 0.08^{Aa}
	7	2.72 ± 0.07^{BCa}	2.78 ± 0.09^{Ba}	2.85 ± 0.10^{ABa}	2.90 ± 0.13^{Aa}	2.98 ± 0.07^{Aa}
	15	2.76 ± 0.09^{BCa}	2.80 ± 0.07^{Ba}	2.89 ± 0.09^{ABa}	2.92 ± 0.09^{Aa}	3.00 ± 0.09^{Aa}

ABCD Letters indicate significant differences between yoghurt drink treatments

abcd Letters indicate significant differences between storage times

C Control bio-yoghurt drink.
T1 Bio- yoghurt drink + 0.5 % tiger nut flour. T2 Bio- yoghurt drink + 1.0 % tiger nut flour.
T3 Bio- yoghurt drink + 1.5 % tiger nut flour. T4 Bio-yoghurt drink + 2.0 % tiger nut flour.

Figure (4) shows the counts of total viable bacteria (TC), Lactic acid bacteria (LAB), *L. rhamnosus* and yeast and molds of all treatments, along the cold storage period. Results revealed that the addition of tiger nut flour resulted in an increase in the counts of TC, LAB and *L. rhamnosus*, along the storage period compared with control treatment. This increase may be due to the presence of some ingredients in the tiger nut flour, such as resistant starch and dietary fiber, act as a growth factors. Results also indicated that there is a direct relationship between the rate of flour added and the counts of the former flora. Treatment 4 recorded the highest counts of these flora while control treatment recorded the lowest ones. Moreover, it was noticed that the counts of the probiotic L. rhamnosus were above 1× 10^6 cfu / ml in all treatments, and this count will confer the health benefits in the bio- yoghurt drink. This finding was coincided with (Verdenelli et al., 2009) who said that probiotic bacteria confer health benefits when consumed in adequate amounts (not less than 1× 10^6 cfu / ml). As the storage period progressed the counts of all treatments were decreased owing to the increase in the rate of acid production and metabolite by-products.

Concerning the yeast and molds, it appeared at the day 15 of storage with low numbers. Control treatment recorded the highest counts and T4 treatment recorded the lowest ones, through the storage period.

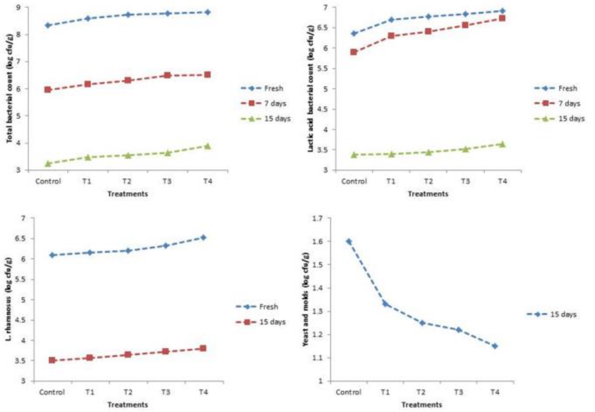

Figure 4. Effect of using different levels of tiger nut flour on the counts of total viable bacteria, lactic acid bacteria, *L rhamnosus* and yeast and molds (log cfu/g) of bio-yoghurt drinks, during cold storage

C Control bio-yoghurt drink. T1 Bio- yoghurt drink + 0.5 % tiger nut flour. T2 Bio- yoghurt drink + 1.0 % tiger nut flour. T3 Bio- yoghurt drink + 1.5 % tiger nut flour. T4 Bio-yoghurt drink + 2.0 % tiger nut flour.

Scoring points of the organoleptic properties of all drinks are shown in Table (2). Control treatment gained the highest scoring points of appearance either in fresh or after 7 and 15 days of the cold storage. When tiger nut flour was added, the bio-yogurt drink's hue turned light brown, which somewhat lowered its evaluation grade for attractiveness. In contrast to the appearance evaluation, body and texture of bio-yoghurt drinks were slightly improved by adding tiger nut flour, which act as a thickening agent. T4 received the highest body and texture evaluation from the panelists because to its significant good thickness. Control treatment gained the lowest scores of flavor. The sensory evaluation scores were gradually decreased in the different bio- yoghurt drink treatments during the storage period.

4. Conclusions

Generally, supplementing yoghurt drink with tiger nut flour is a great interest to create functional foods with health benefits. Incorporation of tiger nut flour increased viability of probiotic bacteria. This might be as a result of knowledge on the composition of tiger nut especially the protein and fiber, the protein content may be up to 9.70% depending on the variety which helps in body development and repair of tissues, the fiber also helps in reducing cholesterol and body weight. Addition of tiger nut flour to bio-yoghurt drinks led to improve the chemical, physical, microbiological and sensory properties of the resultant products compared with control drink. Moreover, this addition increased, also, the nutritional value and the health benefits. All drinks had counts of probiotic bacteria not less than one million per ml at the end of storage period. Generally, we can consider the resultant tiger nut drinks, as a functional beverages yoghurt drink.

Table (2): Organoleptic properties of fresh and cold stored bio-yoghurt drinks as affected by using different ratios of tiger nut flour, during cold storage.

Properties	Storage period	Treatments				
		control	T_1	T_2	T_3	T_4
Appearance (15)	fresh	14.00	12.50	12.00	11.75	11.33
Body& texture (35)		34.50	33.33	33.50	33.66	34.00
Flavor(50)		48.00	48.5	48.50	48.60	48.80
Total scores (100)		94.50	94.03	94.00	94.01	94.13
Appearance (15)	7 days	12.50	12.00	11.00	11.33	11
Body& texture (35)		32.25	31.00	31.75	31.75	32.00
Flavor(50)		46.00	46.30	46.70	46.80	47.00
Total scores (100)		90.75	89.30	89.45	89.88	90.00
Appearance (15)	15 days	12.00	11.75	11.50	11.00	11.00
Body& texture (35)		28.75	27.50	27.80	28.00	28.00
Flavor(50)		45.20	45.30	45.30	45.60	45.70
Total scores (100)		85.95	84.55	84.60	84.6	84.70

C Control bio-yoghurt drink. T1 Bio- yoghurt drink + 0.5 % tiger nut flour. T2 Bio- yoghurt drink + 1.0 % tiger nut flour. Bio- yoghurt drink + 1.5 % tiger nut flour. T4 Bio-yoghurt drink + 2.0 % tiger nut flour.

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