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Production of High Nutritional Set Yoghurt Fortified with Quinoa Flour and Probiotics

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Abstract— *There has been highly attention in supplementation of yoghurt with probiotics and bioactive cereal compounds for enhancing their nutritional and therapeutic functions. So, the propose of this research was planned to develop novel set yoghurt supplemented with quinoa flour and probiotics. Four treatments (control, T1, T2 and T3) of yoghurt were manufactured buffalo's milk. Control treatment inoculated with Streptococcus thermophiles and Lactobacillus bulgaricus (1:1) without quinoa flour. The other treatments T1, T2 and T3 were manufactured with adding Streptococcus thermophiles and Lactobacillus brevis NRRLB-4527 (1:1), Streptococcus thermophiles and Lactobacillus reuteri NRRLB-14171 (1:1) and Streptococcus thermophiles and Lactobacillus curvatus NBIMCC-3452 (1:1) and quinoa flour at the level of 0.5, 1. and 1.5 % respectively. Yoghurt samples were analyzed when fresh and during cold storage at 5±2^o C., the results showed that the pH values took a reverse trend to acidity and gradually decreased by extending storage period. Moreover, fortification of yoghurt with quinoa flour leads to increase in viscosity because of the high starch contents and binding properties of quinoa flour. Furthermore, diacetyl contents were increased but acetaldehyde decreased by extending storage period. The high nutritive value of quinoa flour lead to improve the yoghurt starter and probiotic counts during all refrigeration storage time. The treatment supplemented with 1.5 quinoa flour had the higher counts of Streptococcus thermophiles and Lactobacillus strains. Organoleptic scores were 95, 96, 96 and 94 in treatments control, T1, T2 and T3 respectively. Coliforms were absent in all yoghurt samples either when fresh or during cold storage period. Finally, it can be recommended that yogurt can be fortified with quinoa flour without any defects and showed adequate potential for future dairy application.*

Keywords— ***Set yoghurt, quinoa flour, probiotics, diacetyl and acetaldehyde.***

I. INTRODUCTION

Functional foods are food products containing biologically active components that can improve human health and reducing the risk of diseases by beneficially affecting one or more target functions in the host [1, 2]. Many studies used several flours from different sources such as fruits, fruit skin/peel, different cereals for improving the growth of lactic and/or probiotic bacteria, sensory acceptance, rheological properties and nutritional values of functional foods [3]. Cereals and pseudocereals usually consumed in the form of fermented beverages, cakes, breads or porridges in developed countries. These kinds of food would increase consumption of cereals and contribute to increase probiotic intake, in case of using probiotic strain for fermentation process [4]. The uses of pseudocereals were increased not only in special diets of people allergic to cereals, but also in healthy diets functional foods [5]. Quinoa is an excellent example of pseudocereals rich in fibers, minerals, vitamins, fatty acids, antioxidants and phytonutrients, which contribute to human nutrition and improving neuronal functions [6]. Moreover, the recognition and diffusion of quinoa functional properties such as high water retention capacity, gelation and emulsification have allowed its increasing utilization in value-added products [7]. Quinoa flour used as a food supplement due the high amino acid contents, vitamins, high ratio of linoleic and linolenic acids, minerals and calcium. These characteristics provide the grain great advantage for human nutrition and health maintenance [8, 9, and 10]. Quinoa flour used in many food products such as cookies, biscuits, noodles, pasta, flat bread, white bread and pancakes [11, 12, 13, and 14]. Moreover, quinoa seeds can be fermented to make beer or a traditional ceremonial alcoholic beverage from South America called "chicha" [15]. Quinoa leaves eaten similarly to spinach [16]. The germinated quinoa seedlings quinoa sprouts incorporated in salads [17]. Yoghurt is one of the most popular fermented dairy products that consumed widely worldwide because of its therapeutic, nutritional benefits. This good reputation for yoghurt is due to the presence of many healthy nutrients [18]. In addition, many researchers have tried to add cereals to dairy products for improving the curd properties, sensory quality and nutritional characteristics [19]. The consumption of probiotic fermented dairy products in particular has reached in the last years a new dimension due to their beneficial effects on human health [20, 21]. Addition of various cereals into yogurt formulation is growing nowadays in many countries like lentil [22, 23], chickpea [24], bean [25] and quinoa [26, 27, and 28]. Also, quinoa was added to fermented dairy products such as quinoa-based yoghurt [29], fermented quinoa-based beverages [30], yogurt-like beverages [28], milk based fermented beverage from quinoa extract and buffalo's milk [32], synbiotic fermented beverage [33], processed cheese spread [34] and sprouted quinoa based-yoghurt beverage fermented with lactic acid bacteria [35]. The baby foods supplemented with quinoa capable to prevent malnutrition among kids [36]. Moreover, supplementation of diet with quinoa has been demonstrated to prevent cardiovascular disorders in healthy people [37]. Quinoa contains bioactive compounds that reduce the risk of different chronic disorders including cancer, cardiovascular diseases, diabetes, and aging [38]. The nutritional quality and protein value of quinoa is similar to casein from milk [8, 10]. From the previous information, it is clear that, the addition of quinoa flour in yogurt will improve the nutritional characteristics and quality of the product. Therefore, the aim of this study is to supplement set yoghurt with different levels of quinoa flour and probiotic cultures for enhancing the therapeutic nutrition of yoghurt and study the properties of resultant product during storage period.

II. MATERIALS AND METHODS

2.1. Yoghurt starter and probiotic origin

The yoghurt starter containing *Streptococcus thermophiles* and *Lactobacillus delbrueckii* subsp. *bulgaricus* were obtained from dairy science dept., (microbiology lab) National Research Centre. *Lactobacillus brevis* NRRLB-4527, *Lactobacillus reuteri* NRRLB-14171 were given from Northern Regional Research Laboratory, Illinois USA (NRRL). *Lactobacillus curvatus* NBIMCC-3452 were obtained from National Bank for Industrial Microorganisms and Cell Cultures, Sofia, Bulgaria (NBIMCC). All strains were activated by successive transfers in sterilized skim milk (12 % TS) for 24 h for preparing fresh mother culture before yoghurt manufacture.

2.2. Yoghurt manufacture

Yoghurt was manufactured from fresh buffalo's milk (6.5 % fat). The milk heated at 90^{o} C for 10 min then cooled to 42^{o} C and inoculated with previously activated yogurt starter and probiotic cultures at the level of 2 %. The treatments were manufactured as follows; control treatment without quinoa flour, only added *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (1:1); treatment T1 supplemented with quinoa flour at the level of 0.5 % and *Streptococcus thermophilus* + *Lactobacillus brevis* NRRLB 4527 (1:1); treatment T2 enriched with quinoa flour at the level of 1 % and *Streptococcus thermophilus* + *Lactobacillus reuteri* NRRLB-14171 (1:1). Finally, treatment T3 enriched with quinoa flour at the level of 1.5 % and *Streptococcus thermophilus*+ *Lactobacillus curvatus* NBIMCC 3452 (1:1). Inoculated portions mixed well then incubated at 42^{O} C until the fermentation was completed when pH reached to 4.6-4.7. Yoghurt analyses (acidity, pH values, diacetyl and acetaldehyde, apparent viscosity, *Streptococcus* and *Lactobacilli* counts and sensory evaluation) were carried out an overnight after production and each week of refrigeration storage at 5 ± 2^{o} C.

2.3. Yoghurt analysis:

2.3.1. Acidity development

The titratable acidity % (expressed as lactic acid) of yogurt samples were determined by titration method using 0.1M sodium hydroxide solution and 0.3 ml of phenolphthalein to noticeable pink color as an end point. The titratable acidity percentage = (Volume of NaOH x N/9 x 90 / weight of sample x 100) x 100

2.3.2. pH analysis

The pH values of yoghurt samples were measured using a digital pH meter (Adwa, AD1000, Romania) by immersing the electrode in the yogurt samples.

2.3.3. Acetaldehyde and Diacetyl

Acetaldehyde and diacetyl contents of yoghurt samples were determined according to [39, 40] respectively.

2.3.4. Apparent viscosity (cPs) of set yoghurt

The apparent viscosity of fortified set yoghurt was measured at room temperature using a Brookfield digital viscometer (Middleboro, MA 02346, U.S.A). The sample was subjected to shear rates ranging from 0.3 to 100 S^{-4} for an upward curve. Viscosity measurements expressed as centipoise (cP.s) according to [41].

2.3.5. *Streptococcus* and *Lactobacilli* counts

Ten grams of resultant set yoghurt samples were homogenized in 90 ml of sterile physiological saline (0.85 % NaCl w/v) then; the homogenate was serially diluted up to 10^{-8} [42]. One milliliter from each dilution plated onto sterile petri dishes in duplicate after that, M17 agar and de Mann Rogosa Sharpe (MRS) agar were poured for *Streptococcus thermophilus* and *Lactobacilli* respectively [43]**.** The plates were incubated anaerobically at 37°C for 48 h for *Lactobacillus* strains and aerobically at 37°C for 48 h for *Streptococcus thermophilus*.

2.3.6. Coliform counts

Coliform counts were determined on violet red bile agar according to the method of **APHA (2001)**.

2.3.7. Sensory Evaluation:

Yoghurt fortified with different concentrations of quinoa flour were sensory evaluated when fresh and after 21 days of storage by staff members of dairy department at Food Industry and Nutrition Research Division, National Research Centre, using the score sheet according to [44].

III. RESULTS AND DISCUSSION

3.1. Titratable acidity (%)

Figure (1) shows the changes in titratable acidity of set yoghurt fortified with quinoa flour during storage period at 5° C for 21 days. The titratable acidity in fresh samples recorded 0.81 in control treatment, 0.86 in treatments T1, T2 and 0.88 in T3. The titratable acidity were increased with extending refrigeration storage period reached to 1.17, 1.14, 1.25 and 1.28 in control, T1, T2 and T3 respectively. The continuous development in titratable acidity of all yoghurt treatments by extending the storage time could be attributed to the high metabolic activities of added starters and conversion of residual lactose into lactic acid [45, 46]**.** In addition, the addition of quinoa flour up to 1 % resulted in an increase in the yoghurt acidity with a reduction in pH values. Our findings agree with the data recorded by [26, 27]**.**

3.2. pH values

The pH values of set yoghurt fortified with quinoa flour illustrated in **Figure 2** when fresh and along storage time at 5° C for 21 days. As can be seen, the pH values of fortified set yoghurt ranged from 4.65 to 4.68 at zero time. Meanwhile, the pH values of the examined set yoghurt took a reverse trend to acidity and gradually decreased by extending storage period. The decreasing in pH values is most evident in yoghurt samples with the highest concentration of the quinoa flour and recorded 4.31, 4.23, 4.19 and 4.13 in control, T1, T2 and T3 respectively at the end of storage period. These data suggest that quinoa flour enhance the growth of probiotic and yogurt starter cultures due to high amino acids and minerals [47, 48]**.**

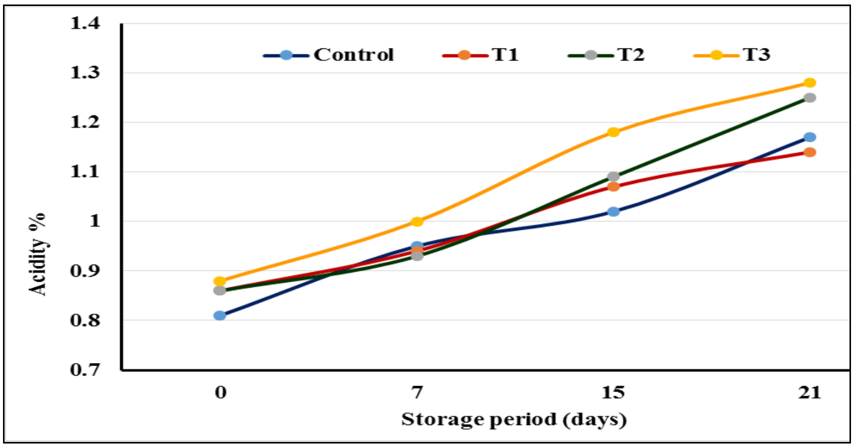

Fig- 1 Titratable acidity of set yoghurt fortified with quinoa flour during refrigerated storage.

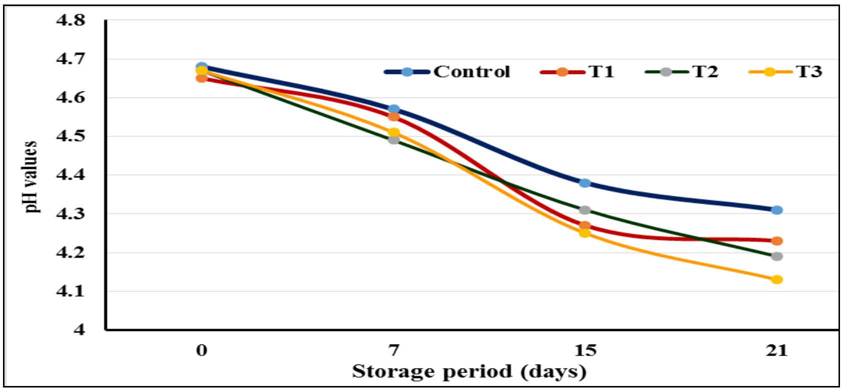

Fig- 2 pH values of set yoghurt fortified with quinoa flour during refrigerated storage.

3.3. Viscosity (cP) of set yoghurt

The data in Figure (3) presents the apparent viscosity of set yoghurt fortified with different concentrations of quinoa flour. There was high viscosity observed in treatments supplemented with quinoa flour. In addition, we found that increasing of quinoa flour levels lead to increase the viscosity of set yoghurt. The highest viscosity values were recorded in T3 and this may be due to the high total solids in set yoghurt fortified with 1.5 % quinoa flour comparing with other treatments. The increase in apparent viscosity is due to the high binding properties of quinoa flour and high starch granules rich in amylopectin used as thickener in frozen and fermented foods [49]. In addition, quinoa flour is good for enhancing viscosity, stability and water absorption capacity in yogurt [27, 10]. In addition, quinoa flour used as stabilizing and emulsifying agents because of high protein and starch contents [50].

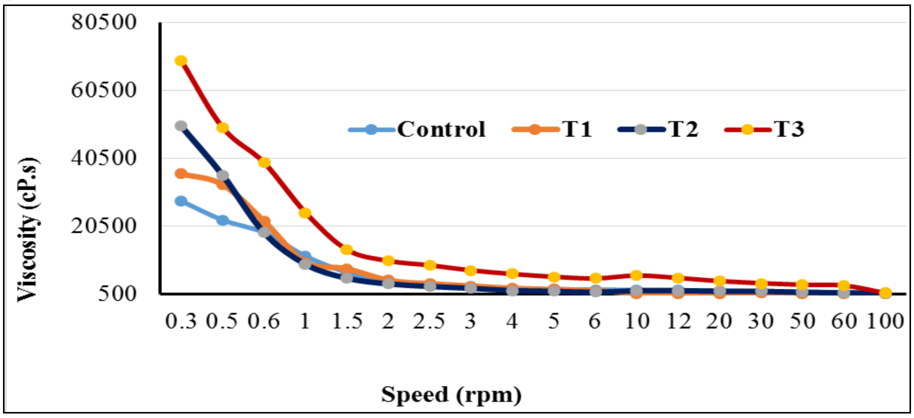

Fig- 3 Effect of quinoa flour levels on apparent viscosity of set yoghurt.

3.4. Acetaldehyde and Diacetyl Contents

The results of acetaldehyde and diacetyl contents of set yoghurt fortified with quinoa flour are presented in Figures (4 and 5) respectively. The diacetyl contents were increased in all treatments with extending storage period. At the end of storage period the diacetyl contents reached to 272.8, 414.5, 416 and 448 microgram/ 100g of the product in treatments control, T1, T2 and T3 respectively. Diacetyl contents increased by increasing the level of quinoa flour addition. On the other hand, acetaldehyde contents were decreased and recorded 25.44, 26.4, 19.82 and 18.39 microgram/100g in treatments control, T1, T2 and T3 respectively. From the results as prolonging the cooled storage of yoghurt samples, the acetaldehyde contents of all treatments were decreased and diacetyl contents took an opposite trend to acetaldehyde. This may be due to the activation of lactic acid starter cultures and their ability to convert the acetaldehyde to ethanol and diacetyl. The results were similar with those obtained by [51, 52].

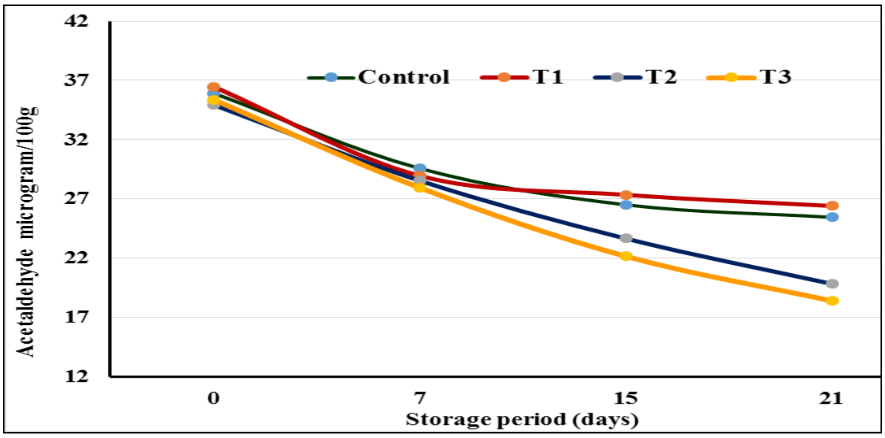

Fig -4 Acetaldehyde contents of set yoghurt fortified with quinoa flour during refrigerated storage.

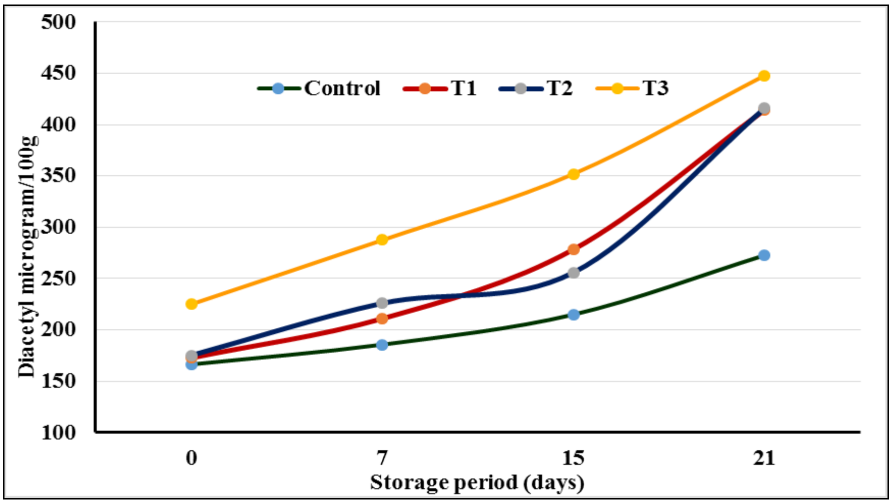

Fig-5 Diacetyl contents of set yoghurt fortified with quinoa flour during refrigerated storage.

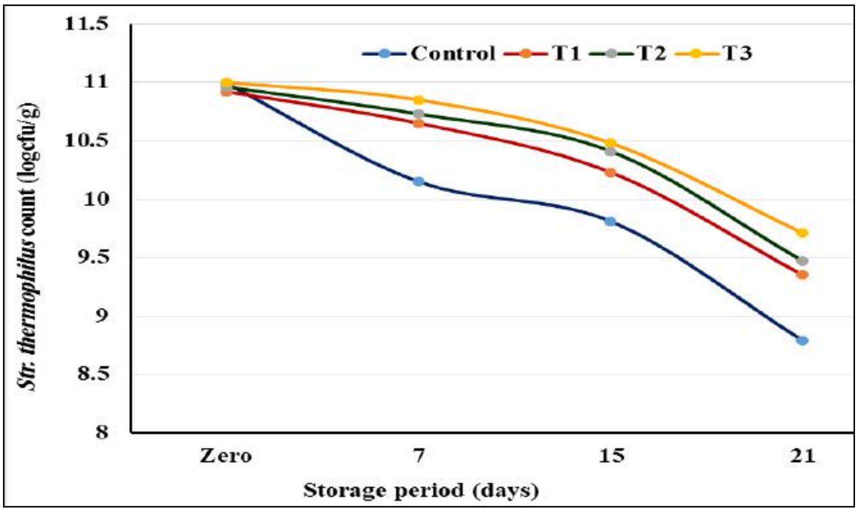

Fig- 6 Streptococcus thermophilus count (Log cfu/ml) in set yoghurt fortified with quinoa flour during refrigerated storage.

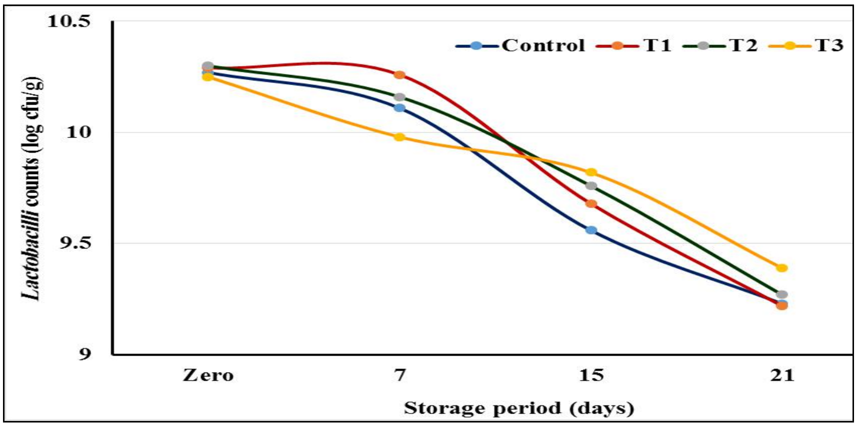

Fig-7 Lactobacilli counts (Log cfu/ml) in set yoghurt fortified with quinoa flour during cooled storage.

3.5. Viable counts of streptococci and lactobacilli

Generally, as shown in Figures (6, 7) the counts of all added starters streptococci and lactobacilli were slightly increased at the first week of storage time then, started to decrease until the end of storage period. The counts of all strains still above the recommended therapeutic level and *Lactobacillus curvatus* NBIMCC 3452 recorded the highest count (9.39) log cfu/g in treatment (T3) supplemented with 1.5 quinoa flour. In addition, *Streptococcus thermophilus* recorded (9.71) log cfu/g in the same treatment. The high ingredients and nutritive value of quinoa flour may be lead to improve the survival and high counts of probiotics in the product. The obtained results were coincide with the reported by **[**26, 53]**.** They reported that the fermented milk supplemented with quinoa, oat, rice and barley flours promoted the growth of *L. rhamnosus* GR-1 (10^8 cfu/mL) up to the 14^{th} day of storage and resulted in significantly higher population of *Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* ssp. *lactis* BB-12 in fermented milk.

3.6. Organoleptic properties

Organoleptic scores of set yoghurt fortified with quinoa flour are shown in Figure (8). The yoghurt samples were evaluated when fresh and after 21 days of storage period and the mean organoleptic scores showed that the adiition of quinoa flour had no effects on the acceptability of set yoghurt when fresh and at the end storage. The total organoleptic scores ranged from 95 to 98 at zero time but at the end storage recorded 94, 95,96 and 96 in treatments T3, control T1 and T2 respectively. The Sensory analyses indicated that the incorporation of quinoa flour in set yoghurt improve the quality, organoleptic profile and nutritional value, which may increase the appeal of the product to consumers. The results are agreement with those reported by [26, 27, 54] they mentioned that, the addition of quinoa flour had desirable effects on yoghurt stability and increases the consumer acceptability. Finally, the addition of quinoa malt or powder into milk makes it more nutritious and increase the acceptability of milk by many people [55].

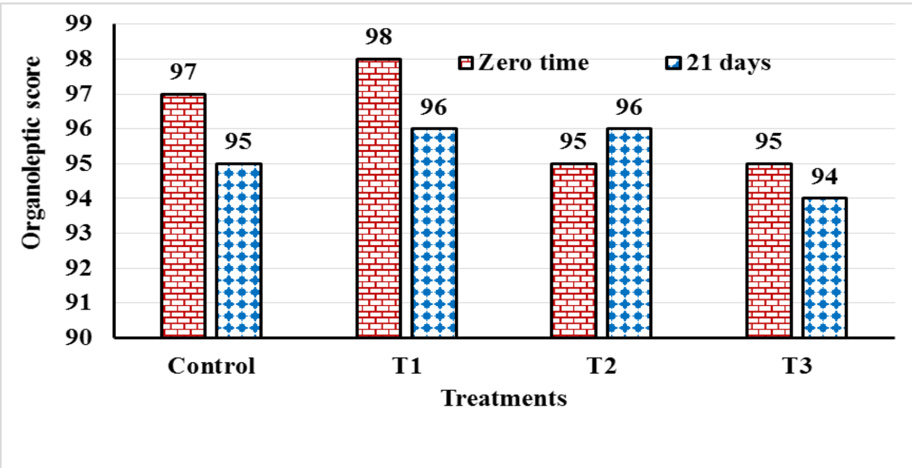

Fig 8- Organoleptic acceptability of set yoghurt fortified with quinoa flour when fresh and after 21 days of refrigerated storage.

IV. CONCLUSIONS

This study concluded that, the use of quinoa flour and probiotics for producing functional set yoghurt gavin positive properties to the product and showed adequate potential for future dairy application.

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