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# Phenolic compounds, Microbial content and Sensory evaluation of Synbiotic labneh containing Ginger and Probiotic

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**Abstract:** This study was evaluated the phenolic compounds and antimicrobial activity of dried ginger and fresh ginger extracts a gainst 7 strains of food borne pathogens , 8 strains of lactic acid bacteria and 6 strains of molds & yeasts. Therafter, fresh ginger extract and *Leuconostoc mesenteroides* were used to produce new synbiotic labneh. This study showed the variable antimicrobial activity of the ginger extracts against the all tested bacteria and fungi . Dried ginger extract showed highest zone of inhibition against *Staphylococcus aureus, Yersinia enterocolitica, Escherichia coli, Saccharomyces cerevisia* and *Aspergillus spp*. and lowest zone of inhibition against *Listria monocytogenes*. Low effect on lactic acid bacteria counts were observed .Total phenolic content of labneh samples either in fresh or during the cold storage were evaluated. Addition of ginger extract reduced the pathogens and fungal counts in labneh. Synbiotic labneh gave the highest scores for flavour, body and appearance than control labneh over storage period.
**Key words**: Synbiotic labneh -Anti microbial- Phenols- Probiotic - Ginger.

## Introduction

Ginger rhizome has been used for a long period of time as a popular spice as well as a medicinal herb due to its high content of antioxidants and anti-inflammatory properties[1]. In the last decades, ginger has been used in the medicinal application against fungi, bacteria, protozoa. Also, it has reported that ginger has antipyretic, analgesic and hypotensive property[2]. In addition, the rhizome of ginger posses many nutrients as fats, carbohydrates, protein, fiber and water. Also, it contains 1-2 % oil, which confers the unique flavor of the Spice. The Aliment and Drug Administration has given ginger, GRAS (generally apperceived as safe) status for utilize as a pabulum supplement. The pungency of fresh ginger results from a group of phenols, the gingerols, of which gingerol is most abundant[1].

Some studies as[3,4,5] are investigated the antimicrobial property of the volatile oil that extracted from ginger rhizomes. This essential oil extracted from ginger have antimicrobial activity against *Aspergillus niger, Saccharomyces cerevisiae*, and *Bacillus cereus*, which determined using disc diffusion method. Many reports studied ginger using to fortify dairy products. Okwute and Olafiaji,2013 [6] revealed that incorporation of ginger into Ogi (a Nigerian traditional fermented food) significantly reduced its microbial load during fermentation

which may lead to an improvement in its nutritional quality and the prevention of some food-borne diseases. David,(2016)[7] concluded that the ginger can be successfully used for the preparation of herbal ice-cream . Abd El-Aziz et al., (2012) [8] added ginger extract to soft cheese to produce functional dairy product. Ogunleke and Akinsoyinu, (2014) [9] studied The effect of ginger, (*Zingiber officinale*) Onion (*Allium cepa*) and bear berry (*Aframomum sceptrum*) on the chemical composition and microbial load of cheese.

Therefor,the current study was to assess the phenolic compounds and antimicrobial effect of both fresh and dried ginger and use of ginger extract as prebiotic with *Leuconostoc mesenteroides* as probiotic to produce a new synbiotic labneh(yoghurt cheese) .

## Materials and Methods :

### Pathogenic bacteria strains:

*Bacillus cereus* B-3711(G+), *Aspergillus flavus* 3357, *A. Parasiticus* and *Saccharomyces cerevisiae* Y-2223 were provided by the Northern Regional Research Laboratory Illinois, USA (NRRL). *Listeria monocytogenes* 598 was provided by the Department of Food Science, University of Massashusetts, Ambert MA, USA. *Yersinia enterocolitica, Salmonella typhi*, *Escherichia coli* 0157: H7 and *Staphylococcus aureus* were isolated and serologically identified by dairy microbiological Lab., National Research Center. *Aspergillus niger* and *Pseudomonas aeruginosa* , were obtained from Department of Microbiology, Swedish University of Agricultural Sciences.

### Lactic acid bacteria strains:

*Lactobacillus delbrueckii* subsp. *bulgaricus, L. casei , Streptococcus. lactis* and *L. acidophilus* obtained from Chr. Hansens's Lab., Denmark. *Lactobacillus plantarum, S.thermophilus and Leuconostocc mesenteroides* were isolated and identified by Dairy Science Dept.,(Dairy Microbiology Lab.),National Research Center[10,11]. *L. rhamnosus* B-445, *L. reuteri* B-14171, provided by the Northern Regional Research Laboratory. Illinois, USA.

### Preparation of ginger extracts:

Dried or fresh ginger was collected from local market for studied the effect of ginger extracts of lactic acid bacteria and pathogenic strains.

In dried ginger, 10gm of the ginger powder is mixed with 100 ml of cold water in a 250 ml conical flask and was stirred overnight by magnetic stirrer at (160 rpm/5ºC). The suspension was centrifugated at 5000 rpm for 10 min to obtain the clear ginger extract. The extract kept in cold storage.

Fresh ginger was washed, crushed finely. 25gm of the crushed fresh ginger was mixed with100ml hot water in a 250 ml conical flask and was stirred overnight by magnetic stirrer at (160 rpm/5ºC). The suspension was prepared as mentioned before in dried ginger. Also, the final extract was kept in cold storage.

### Effect of ginger extracts on lactic acid bacteria (LAB):

To determine the effect of ginger extracts on (Lactic Acid Bacteria), different strains of LAB were activated individually and grown in Elliker broth medium at 37ºC using 1% inoculums.

The molted sterilized Elliker agar medium was fortified with 0, 2.5, 5.0, 7.5 and 10.0% (v/v) of each ginger extract individually. 1ml of each LAB strain was added in Petri dish approximately $10^5$cfu/ml and the 15 ml molted Elliker medium contained different concentration of ginger extract added to each plates, and then mixed the inoculums with the agar medium. Left the plates to solidified and incubated at 37ºC for 48h.

### Effect of ginger extracts on pathogenic strains:

To determine the effect of ginger extracts on the pathogenic strains, the well diffusion method will be used according to Durairaj et al., (2009) [12]. Melted agar medium was transferred to the Petri dishes and allowed to solidify. An aliquot of 0.2 ml of each pathogenic strain suspension was transferred to plates and spreaded

uniformly over the agar surface with a sterile bent glass rod. Plates were dried at 37°C for 1 hr. A sterile cork borer (7mm) was used to make ditches in each plate for the extract. 0.5 ml of each ginger extract was dispensed into each ditch. The plates were left to allow for diffusion of extract before incubation at 37ºC for 24 hours. The zones of clearance produced around the ditches after incubation were observed, measured and recorded in millimeters.

**Preparation of labneh:**

Labneh were made using the method described by Mohamed et al.[13] with some modification. Fresh buffaloes milk was heated, (90°C/20 min.), cooled to 40°C, inoculated with 2% of yoghurt starter (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus).* The inoculated milk was divided into three equal portions. The first portion served as control (free from ginger extract), second fortified with 2.5% of ginger extract by hot water and third portions were fortified with 2.5% of ginger extract by cold water. All portions incubated at 40°C till complete coagulation. The plastic containers stored at (7°C±2) for 14 days. Samples of labneh were analyzed for Chemical, microbiological and sensory attributes, were determined when fresh and during storage.

**Treatments :**

T1: Control (*S. thermophilus* + *L. bulgaricus*).
T2: *S. thermophilus* + *L. bulgaricus* + *Leuc. mesenteroides*
T3: *S. thermophilus* + *L.bulgaricus* + *Leuc. mesenteroides* +2.5% cold ginger
T4: *S. thermophilus* + *L. bulgaricus* + *Leuc. mesenteroides* +2.5% hot ginger

**Microbiological analysis**:

*Lactobacillus bulgaricus* counts were enumerated using De Man-Rogosa- Sharpe (MRS) agar[14]. The plates were incubated at 37°C for 48h under anaerobic condition. *Streptococcus thermophilus* counts were enumerated aerobically at 35°C for 48 h using M17 agar[15]. Yeast and Mold counts were enumerated using potato dextrose agar acidified to pH 3.5 with sterile lactic acid solution (10% conc.) [16]. The plates were aerobically incubated at 25°C for 4 days.

**Determination of total phenolic compounds (TPC)**

The total phenolic content determined by Folin-Ciocalteu Reagent[17], and calculated as Gallic acid.

The Folin Ciocalteu Reagent (FCR) method is the most widely used to determine the total phenolic content. In this method 20 µL aliquots of the diluted extracts were mixed with 100 µL of Folin-Ciocalteu phenol reagent and 300 µl of 20% $Na_2CO_3$. The absorbance of the supernatant was read with a SP-2000UV UV/Vis spectrophotometer at 765nm. Distilled water was used as a control in place of the plant extract. A standard curve of gallic acid was prepared with concentrations of 20, 40, 60, 80 and 100 mg/100g. The total phenolic content of each plant extract was calculated from the standard curve and expressed as gallic acid equivalent in (GAE) mg/100g extract.

## Results and Discussion

**Antimicrobial activity of ginger**

Antibacterial activities of dried and fresh ginger tested against 13 indicator pathogenic strains (bacteria, mold , yeast )are presented in Table (1) . The results showed that, there are differences were found between dried and fresh ginger against indicator pathogenic bacteria and molds. All indicator pathogenic strains inhibited by the crude dried ginger extract. The diameter zones are included between 2 to 10 mm and the biggest clear zones were recorded against *Staph.aureus, Sacch.cerevisiae, A.niger* and *A.parasiticus*, but the lowest clear zones were obtained against *L.monocytogenes* and *Sal.typhi*. A weak effect was observed by the 10% dried ginger and fresh ginger extracts. Our result also showed that crude extract of ginger was possessed more lethal substances which gave highest inhibition zone more than 10% of ginger extract.

**Table 1:The antimicrobial activity of dried and fresh ginger extracts against foodborne pathogens**

| Tested microorganisms | Zone of inhibition (mm) | | | |
|---|---|---|---|---|
| | dried ginger | | fresh ginger | |
| | 10% | crude | 10% | crude |
| *B. cereus* | -ve | 6 | -ve | -ve |
| *Staph. aureus* | 4 | 10 | -ve | 2 |
| *Y.enterocolitica* | 2 | 2 | -ve | -ve |
| *L.monocytogenes* | -ve | 2 | -ve | -ve |
| *E. coli* 0157: H7 | 2 | 8 | -ve | 2 |
| *P.aeruginosa* | -ve | 4 | -ve | -ve |
| *Sal. typhi* | -ve | 3 | -ve | -ve |
| *Sacch.cerevisiae* | 4 | 10 | 2 | 2 |
| *A. flavus* | 2 | 8 | -ve | -ve |
| *A.niger* | 4 | 10 | -ve | -ve |
| *A. parasiticus* | 4 | 10 | -ve | -ve |
| *A. ochracus* | 2 | 8 | -ve | -ve |

Onyeagba et al.[18] observed that the ginger extract using ethanol gave inhibition to wide range of bacteria such as, *B. spp*., *Staph. aureus, E. coli*, and *Sal. spp*. Additionally, the synergistic antimicrobial effect of soybean and ginger at boiling temperature against food borne pathogens indicates the thermostable antibacterial property of ginger extracts. Malik, (2015)[19] determined the antibacterial efficacy of methanol extract of ginger tubers using paper disc method against different gram negative bacteria and sensitivity in terms of zones of inhibition of all extract were also determined(Strains of *Staph. aureus, B. cereus* and *E.coli, Sal. enteritidis* and *vibrio parahemolyticus)*. The result shows that the methanol extract of ginger tubers was effective against all the bacteria tested. Helal et al.,(2014) [20] studied antimicrobial activities of the four aqueous extracts (two native and two sulfated) obtained from ginger . The results indicated that all extracts showed antibacterial activity against *E. coli*, but not against *Staph. aureus*. Water extracts of ginger showed effective antifungal activity. The results also showed moderate activity against yeast (*Candida albicans*) for all the extracts studied . On the other hand , the ginger volatile oil was examined for antibacterial activity against *E. coli, S. aureus* and *B. subtilis* and antifungal activity against *Aspergillus niger, A. fumigatus, A. flavus* and *Candida albicans.* The oil showed significant antimicrobial and antifungal activities in comparison to standard, Tetracycline and Fluconazole [21]. From the obtained results of Hasan et al.,(2012) [22] study ,they found that ginger extracts have exhibited wide spectrum of antimicrobial properties. Therefore, they can be used for preserving various foodstuffs against microbial spoilage and it can be incorporated into medications for topical antifungal or antibacterial therapy. Okwute and Olafiaji, (2013)[6] found that dry ginger had a greater inhibitory effect on pathogens than fresh. Also, they conclude that 5 % w/w concentration of both fresh and dry ginger considerably reduced and inhibited the growth of food pathogens. However, dry ginger had a greater inhibitory effect on pathogens than fresh. Therefore, the use of ginger in ogi and probably other food items would decrease the chances of food poisoning, reduce the risk of food contamination, protect the consumer from different food-borne diseases and improve health status by using a small quantity of it. Ogunleke and Akinsoyinu, [9]Ogunleke F.O. et al reported that addition of extract of ginger, onion and bear berry reduced the total coliform, fungal and Anaerobic counts in cheese. This might be due to the presence of antibacterial compounds such as gingerols, shogaols, vitamin A and B, paradol and zingerine in ginger[23].

**Table 2:The effect of dried and fresh ginger extracts against lactic acid bacteria**

| Tested microorganisms | Zone of inhibition (mm) | | | | |
|---|---|---|---|---|---|
| | | dried ginger | | fresh ginger | |
| | Control | 10% | crude | 10% | crude |
| *L. plantarum* | 2.4 | 2.3 | 2.3 | 2.3 | 2.3 |
| *L. casei* | 2.2 | 1.2 | ND | 2.1 | 1.8 |
| *L. bulgaricus* | 2.2 | 2.1 | 1.9 | 2.1 | 2.1 |
| *L. acidophilus* | 2.1 | 1.4 | 1.2 | 1.4 | 1.3 |
| *L. rhamnosus* | 2.2 | 2.2 | 1.7 | 1.5 | 1.2 |
| *L. reuteri* | 2.2 | 1.2 | ND | 1.3 | 1.2 |
| *Leuc. mesenteroides* | 2.1 | 1.6 | 1.4 | 1.8 | 1.6 |
| *S. thermophilus* | 2.2 | 2.1 | 2.1 | 2.1 | 2.1 |

The results of the effect of ginger against lactic acid bacteria are presented in Table (2).

The results revealed that the effect of the dried and green ginger varied depending on the ginger concentration and bacterial species used. Similar changes in counts (log cfu/ml)of lactic acid bacteria strains in both ginger extract,showed lower sensitivity to ginger extract except highest effect was found against *L.casei* and *L.acidophilus* compare to the control . These results are in accordance with those reported by Okwute & Olafiaji ,(2013) and Adeniran et al. (2010) [6,24].

## Evaluation of synibiotic labneh treatments

### Microbiological properties of labneh

Viable counts of *L.bulgaricus*, *S.thermophilus* and *Leuc.mesenteroides* in labneh during storage period s are presented in Figures ( 1 – 3 ).

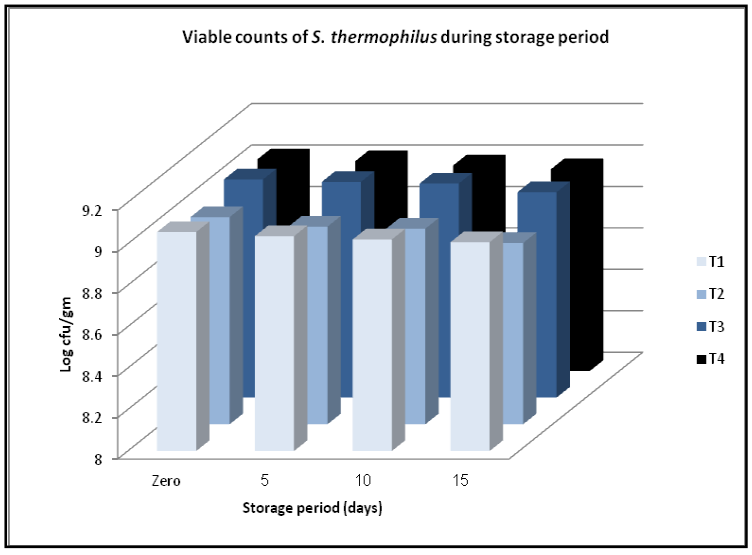


**Fig.(1) Viability of *S. thermophilus* in labneh during storage period**

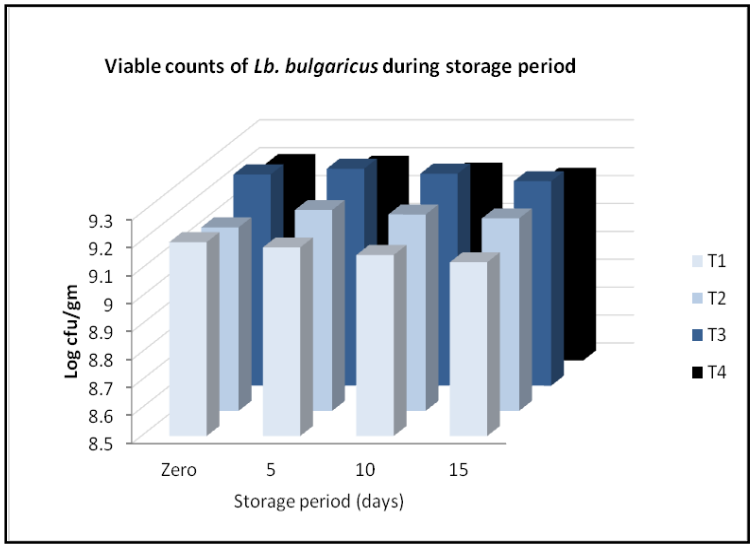


**Fig.(2) Viability of *L. bulgaricus* in labneh during storage period**

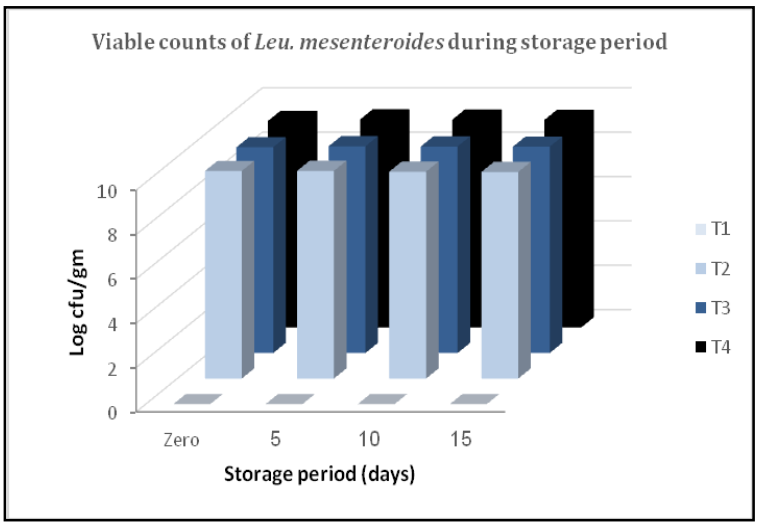


**Fig.(3) Viability of *Leuc. mesenteroides* in labneh during storage period**

Results revealed that the log counts of *S.thermophilus* in the labneh treatments T1,T2,T3&T4 were 9.0 , 8. 9, 9.0 and 9.0 cfu/gm, Log count of *L.bulgaricus* also were 9.1to 9.2, 9.2 and 9.2 cfu/gm respectively after 5days of storage. Kindred trend of viability was found at the end of storage. The initial probiotic counts of ginger labneh (T3 and T4) inoculated with *Leuc.mesenteroides* were 9.3 and 9.3 log cfu/gm and after 15 days storage were 9.1 and 9.0 log cfu/gm respectively.

**Viability of molds and yeasts in labneh during storage period**

**Table 3: Log viable counts of molds and yeasts( cfu/gm) during storage period:**

| Treatments | ZERO | 5 | 10 | 15 |
|---|---|---|---|---|
| T1 | 1.3 | 1.5 | 1.8 | 2.1 |
| T2 | - | - | - | - |
| T3 | - | - | - | - |
| T4 | - | - | - | - |

Molds &yeasts were not detected in treatments T2, T3 and T4 of labneh either when fresh or during the storage period ( Table 3) .

Abd El-Aziz et al., [8]evaluated soft cheese fortifi ed with ginger extract as a functional dairy food ,they found that ginger extract enhanced growth of *Lactococcus* strains, flavour compounds and exhibited antioxidant activity. They added that the increase in counts during storage due to the ginger protease can degrading protein to peptides and amino acids that enhanced the growth of the *Lactococcus* in the cheese. Yeast and mould were detected only in cheese control sample after 2 weeks. Such an effect has been reported by Abd El-Aziz et al.,[25] in white cheese pickled in brine solution containing ginger extract (aqueous ginger extract and ethanol ginger extract) and Singh &Kumar, (2013) [26] in yoghurt . Adesokan et al.[27] found that addition of ginger to Ogi , led to a relatively reduced microbial load during storage and hence an improvement in the shelf stability of the product.

**Total Phenolic Content (TPC)**

Phenolic compounds have an important role in human health because of their activity as antioxidant by donating hydrogen atom from the aromatic hydroxyl group to free radicals[28].

**Table (4) Total phenolic content in ginger**

| | **Total phenolic content (mg Gallic cid/gm)** |
|---|---|
| Dried ginger | 7.291 |
| Fresh ginger (cold) | 4.93 |
| Fresh ginger (hot) | 5.1585 |

Total phenolic content of dried ginger, fresh ginger (cold) and fresh ginger (hot) were presented in Table (4).Also, total phenolic content of labneh samples either in fresh or during the cold storage were evaluated in Fig.(4).

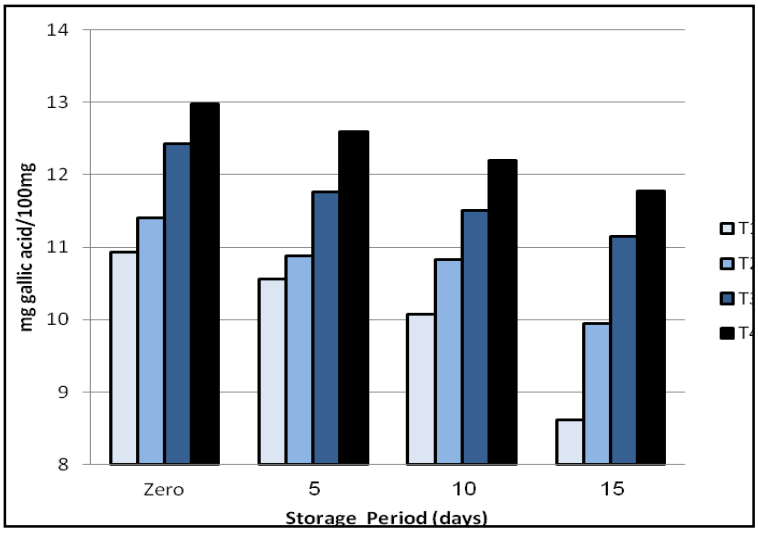


**Fig. 4: Total Phenolic Content of labneh treatments either in fresh or during the cold storage .**

It is obvious that using *Leuc.mesenteroides* with the traditional starter (*S.thermophilus* and *L. bulgaricus*) $T_2$ in the manufacture of labneh increased its TPC by 4.3% in respect to $T_1$. Also, supplementing labneh with fresh ginger extract $T_3$ and $T_4$ increased its TPC. This attributed to the TPC in ginger extract such as engenol, shogoal, zingerone, gingerol and their derivatives[29,30,31,32]. Lee et al.[33] determined phenolic compounds of ginger roots by spectroscopic analysis and identified the structure of these compounds. Moreover, from the same figure, it can be noticed that labneh supplemented with hot fresh extraction ($T_4$) had TPC more than $T_3$ (cold extraction). $T_3$ had 13.4% TPC more than $T_1$ while $T_4$ had 18.7% TPC in respect to $T_1$. This finding was in accordance with El-Din et al[34], they pointed that heating the hearbal extracts increased its content of phenolic compounds. Stewart et al[35] found that heat treatment increased the level of free flavonols.

Also this may be attributed to the effect of heat on the hydrogen bonds between the phenolic hydroxyl group[36]. Mukherjee et al.[37] suggested that extraction of polyphenols from ginger is affected by the solvent time and temperature of extraction. Adel and Prakash[38] found that total polyphenols, tennin and flavonoids of ginger roots were more in 100$^{O}$C water extract than other solvents and revealed that, this can be related to the high solubility of these compounds in hot water. During cold storage, TPC gradually decreased for all sample (the same figure). The decreased rate in TPC of $T_1$ after 15 days was 21.2% while it was 12.7%, 10.3% and 9.3% for $T_2$, $T_3$ and $T_4$ respectively, this may be occurred during storage which the highly unstable phenolic compound can transformation and undergo numerous enzymatic and chemical reactions as studied by Cheynier 2005, Legrand and Es-Safi et al. [39,40,41].

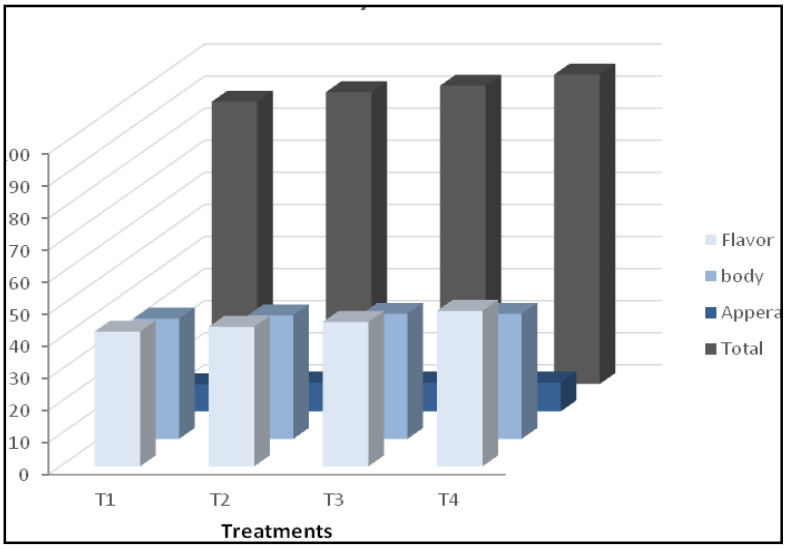


**Fig.5: sensory evaluation of labneh treatments**

**Sensory Evaluation of synbiotic labneh**

The sensory evaluation of the labneh treatments are presented in Fig.(5). Treatment 4 containing 2.5% of hot ginger extract was rated best in all parameters tested which included flavor, body, appearance and total acceptability. Our results in accordance with those obtained by Abd El-Aziz et al.[8] found that Ginger extract- -fortifi ed cheese gained the highest scores and became more acceptable to panelists than control cheese during storage. Also, Adesokan et al.[27] revealed that incorporation of 5% ginger into Ogi significantly improved its sensory attributes, led to a relatively reduced microbial load during storage and hence an improvement in the shelf stability of the product. David[7] prepared herbal ice-cream with addition of ginger (*Zingiber Officinale*) juice. The treatments containing 6% level of ginger juice score the highest value. He added that the ginger can be successfully used for the preparation of herbal ice-cream, without sacrificing its palatability and therapeutic values. Abd El-Aziz et al[25] manufactured white cheese pickled in brine solution containing ginger extract . The results revealed that ginger extract enhances the sensory properties of white brined cheese compared with control cheese.

Adeniran et al[24] found that isolated *L.plantarum* and *L. bulgaricus* were able to survive and still active during storage to four weeks in ginger and ginger with garlic beverages at both ambient and refrigeration temperatures. Also, beverages that containing probiotics or without probiotic strains have ability to inhibit the growth of *E. coli* and *Staph. aureus*.

## Conclusion:

The results of this study revealed that ginger possesses some antimicrobial properties as antibacterial and antifungal and phenolic compounds, therefore it can be used as a potential source of active ingredients for food, and incorporated into dairy products as therapeutic products.

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