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A Comprehensive Study of Carbonated Probiotic UF-Labneh Cheese as a Novel Product

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NEW dairy products in the markets have become an urgent need to attract the attention of the consumer and satisfy its passion about the novel products. Adding CO_2 on some dairy products has become modern trend. The production of carbonated probiotic UF-Labneh cheese was tested and evaluated. *Lactobacillus acidophilus* strain was encapsulated by two different methods (extrusion or spray drying method) in the presence of CO_2 (added 1.5 g solid CO_2 to 100 ml milk). All samples were inoculated with the traditional starter culture, and then divided to five treatments. Control sample inoculated with starter cultures only, while blank sample had no CO_2 gas. Treated sample (T_1) was inoculated with 2% free cells of *L. acidophilus*. The third part (T_2) was inoculated with 2% encapsulated *L. acidophilus* by spray drying method. The fourth part (T_3) was inoculated with 2% encapsulated *L. acidophilus* by extrusion method. All UF-labneh samples were evaluated for microbiological, chemical, rheological and sensory properties during 21 days of cold storage. The data indicated that the counts of *L. bulgaricus* and *S. thermophilus* were enhanced during the first 14 days and then a slight decrease was observed at the end of the storage of all treatments. The counts of *L. acidophilus* were developed during storage period until 14 days, and encapsulated *L. acidophilus* in T_2 and T_3 had the highest counts. In contrary, T_1 that fortified with free cells had the lowest counts. A little count of mold & yeast was detected in the T_1, T_2 and T_3 after two weeks. Also, there was a slight decrease in pH values in all treated samples during storage. Blank and control samples had higher pH values compared to treated samples. Additionally, the total solid content of blank samples were lower than the others without significant differences between all samples either fresh or during storage period in fat content. The most texture parameters (Hardness, Cohesiveness, Gumminess and Chewiness) of all labneh samples increased slightly during storage. On the other hand, samples which contained CO_2 and probiotic strains were more accepted. Adding CO_2 and probiotic bacteria to the labneh gave it a new desirable taste not familiar before to consumers.

Keywords: Carbon dioxide, Probiotics, Microencapsulation, Labneh, Sodium alginate, Spry drying, Extrusion method .

Introduction

The demand for unusual and distinctive dairy products among consumers grows as development accelerates. Gases addition as dissolved component of milk has been recorded since early 1900s. Carbon dioxide (CO_2) in the form of gas, supercritical fluid or solid (dry ice) is used in cheese manufacture, lactose, powder and probiotic milk products. The application of gases in dairy systems expands to butter, cheese and milk drinks as well. Many reviews have stated that blend of carbon dioxide can be considered as a cost effective method in improving sensory

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quality and rheological behavior of dairy products (Adhikari et al., 2018).

CO_2 is Generally Recognized as Safe (GRAS) (FDA 2011) and the dissolved gas is a natural component of freshly drawn milk that is subsequently lost during processing and transit. Adding CO_2 to both of raw milk and some dairy products controls the growth of psychrotrophic bacteria at refrigeration temperatures, increases the lag phase and the generation time of microorganisms (Ma et al., 2001). Improving the quality and safety of dairy products needs to control microbial growth. In addition, achieving economic benefits for the dairy industry comes from the flexibility in using and distributing milk and extending the shelf life, whether for raw or pasteurized milk. The attention of researches is now focusing on the effect of CO_2 on microbial growth as well the influence of CO_2 injection to milk on some of the important routine analytical test methods used in the dairy industry is not sufficiently clear (Ravindra et al., 2014).

Probiotics, which are live microorganisms, have a great role in human health when administrated in an appropriate amount (FAO/ WHO, 2002). Lately, it was observed the awareness of consumers for demanding probiotic fortified dairy products according to their advantages. It prohibit and cure some diseases like diabetes, gastric cancer, and inflammatory bowel disease (IBD) by enhancing the gut barrier function, producing antimicrobial compounds and immune-protective responses that cause elimination of pathogens such as rotaviruses, *Helicobacter pylori*, *Salmonella*, and modulation of host immunity (Razavi et al., 2021). Consumption of probiotics (as food products or as dietary supplements) is a major concern among consumers nowadays. *Lactobacillus* and *Bifidobacteria* groups are considered the most probiotic microorganisms used in food products. To obtain all or most of the benefits of these microbes, they must reach the stomach with a certain number of microbial loads 10^{6-7} CFU/ mL with an active situation in order to perform their functions optimally. The probiotics market is predicted to have a compound annual growth rate (CAGR) of 7.5 % from 2018 to reach USD 111.21 billion by 2030 (Liu et al., 2020). Consuming food fortified with probiotics is a natural alternative to medicines to eliminate a number of diseases, especially their impact on the Corona virus (Misra et al., 2021).

However, microencapsulation is gaining attention as it ensures the recommended level and target delivery of the core material. Encapsulation technique not only protects the probiotics from various harsh conditions, and it also maintains the therapeutic level of probiotics in food product. Microencapsulation of probiotics with compatible encapsulating material is the approach to protect live cells from adverse acidic conditions. Various encapsulating materials are Generally Recognized as Safe (GRAS) status is being used for encapsulation of probiotics on lab as well as industrial scale. The main type of materials includes proteins lipids polysaccharides and their derivatives. Sodium alginate and carrageenan are considered as effective encapsulating polymer among the hydrogel matrices. Sodium alginate is being used widely for encapsulation of probiotics due to its biocompatibility, versatility, heat, and acid resistance properties. Both, sodium alginate and carrageen wall materials are economical, nontoxic and easy to handle (Afzaal et al., 2020).

Uf-labneh, which known as a semi-solid food derived from yoghurt by draining away part of its whey and some water-soluble compounds, is more consumed in the Middle East Countries. Labneh cheese has several characteristics that distinguish it, including sour taste, cream color, smooth, pasty, semi-solid appearance, smooth consistency and good spreadability with creamy texture, and mild flavor. Existence of lactic acid bacteria which ferment the lactose to organic acids mainly lactic acid is one of its most important features. Its shelf life is short although in cold conditions (El-Sayed & El-Sayed, 2021). Many researches attempts to extend the shelf life of labneh cheese trying to utilize many ways. One of the novel ways is adding CO_2 in the process of labneh cheese at certain condition. Consequently, the main goal of this research is producing carbonated labneh cheese as a new dairy product, extend its shelf life and give it a special flavor. Chemical composition, rheological analysis, microbiological examination and sensory evaluation were also achieved.

Materials and Methods

Materials

The dry ice was purchased from DIFFCO Company in closed foam box to prevent it from the sublimation during transport. All microbial media and materials used in this study were obtained from Oxide. UF- retentate used in the manufacturing of UF labneh was obtained

from Dairy Industry Units, Animal Production Research Institute, Ministry of Agriculture, Giza, Egypt. The strains of *Lactobacillus acidophilus* CH-2, *Lactobacillus bulgaricus* Lb-12 DRI-VAC and *Streptococcus thermophilus* CH-1 were obtained from Dairy department at National Research Centre, Egypt.

Methods

Microencapsulation techniques

Growth conditions

Firstly, the strain *Lb. acidphilus* CH-2 was grown in MRS broth and incubated under anaerobic condition for 24 h at 37 ºC. The strain was sub-cultured two times in order to obtain high biomasses then the cell pellets were harvested by centrifugation at 5000 rpm, for 15 min at 4 ºC and washed by sterile saline solution. The obtained cell suspension was used for microencapsulation (Fareez et al., 2015).

Encapsulation of L. acidophilus by spray drying method

25 gram of freshly harvested cells pellets were mixed with 15% milk saturated with CO_2 (added 1.5 g solid CO_2 to 100 ml milk, 15% and 1.5 % dextrin) to obtain cells concentration 25%. The mixture solution was automized in hot steam of air at a co-current mode using Mini Spray Dryer B-290 (BÜCHI, Switzerland). The inlet air drying temperature of 150 ºC and outlet temperature of 60 ºC were used according to El-Sayed et al. (2020).

Encapsulation of L. acidophilus by extrusion method

Freshly harvested cells concentrates were mixed with 15% milk saturated with Co_2 (added 1.5 g solid Co_2 to 100 ml milk, 15%) and sodium alginate (3%), the mixture solution as (25 g cells/100 mL coating materials) to obtain cells concentration 25%. Sterilized syringe was used to drop-wise the mixture into calcium chloride solution (0.5 mmol). To obtain bacterial beads magnetic stirring was maintained during encapsulation process (Mahmoud et al., 2020).

Production of carbonated probiotic UF-Labneh cheese using encapsulated L. acidophilus

Carbonated probiotic UF labneh was prepared according to (El-Sayed et al., 2017). UF-retentate was heat treated at 72 °C for 5 min and cooled to 42°C. After that, the heat treated UF-retentate was divided to two parts one of them called blank sample without CO_2 addition. The other part was saturated with CO_2 with the concentration (1.5% w/w for full saturated with CO_2 gas). The saturated UF-retentate and the blank part (the first treatment as blank) were inoculated with *Lb. bulgaricus* and *S. thermophilus* with percentage (1:1 %). The saturated UF- retentate was divided into four portions as:

- The first part was served as the control
- The second part was inoculated with 2% free cells of *L. acidophilus*
- The third part was inoculated with 2% encapsulated *L. acidophilus* using spray drying method.
- The fourth part was inoculated with 2% encapsulated *L. acidophilus* using extrusion method.

All treatments were put individually in sterilized plastic cups (100 mL) and incubated at 42 ºC for 4 h. After that, all cups were stored at cold condition for 21 days for microbiologically and chemically analysis.

The microbiological evaluation of carbonated probiotic UF-Labneh cheese during storage period

10 grams of labneh treatments were homogenized with 90 ml of tri-sodium citrate (2%) as the first dilute and continuously using normal saline solution to prepare decimal dilutions. The *S. thermophilus* was enumerated using M17 agar and the plats were incubated at 37ºC/48 h aerobically (IDF, 1997). The *L. bulgaricus* was determined using MRS agar with pH 5.5 and the plates were incubated at 37º C/48 h anaerobically (IDF, 1997). The counts of *L. acidophilus* were enumerated with MRS agar supplemented with 0.15% bile salts and the plates were incubated at 37º C/48 h anaerobically (Vinderola & Reinheimer, 1999). The Psychrotrophic bacteria were enumerated using nutrient agar and the plates were incubated at 5 °C /7 days (Marshall, 1992). Yeasts and molds counts were detected by acidified potato dextrose agar pH 3.5 (APHA, 1994) and the plates incubated for 4 days at 25 ºC.

Physico-Chemical analysis of carbonated probiotic UF-Labneh treatments

The total solids (TS) and fat contents were measuring by methods described by **Ardo (1999).** pH value was measured using a digital pH-meter with combined glass-Calomel electrode (HANNA). Protein content was determined according to AOAC (1990).

Texture profile analysis of carbonated probiotic UF-Labneh treatments

The textural properties of labneh (hardness, cohesiveness, springiness, gumminess and chewiness) from different treatments were assessed using the textural analyzer (Multi test 1dMemesin, Food Technology. Corporation, Slinfold, W. Sussex, UK) equipped with a 25 mm diameter perplex conical shaped probe. The texture profile analysis (TPA) was done on Labneh samples using the double compression test, which generated a plot of force (Newton, N) versus time (second, s). Compression was done at five different points on the sample surface. In the 1st stage the sample was compressed by 30% of its original depth at a speed of 2 cm/min during the pretest, compression and the relaxation of the sample. The following parameters were calculated from the force-time curve according to the definition given by the International Dairy Federation IDF (1991):

Hardness (N) = maximum force of the 1st compression

Cohesiveness = area, A, under the 2nd compression/Area, A, under the 1st compression (A2/A1)

Springiness, mm = length, L, of the 2nd compression/Length, L, of the 1st compression (L2/L1)

Gumminess (N) = hardness × cohesiveness

Chewiness index (mJ) = gumminess × springiness

Sensory Evaluation of carbonated probiotic UF-Labneh treatments

The organoleptic properties of UF-Labneh were assessed by 20 panelists of the experienced staff members of Dairy Department National Research Center. Samples were evaluated periodically by regular-score panelists when fresh and after 7, 15 and 21 days of cold storage for flavor (50 points), body and texture (40 points) and appearance (10 points) as mentioned by Abbas et al. (2018).

Extraction of phenolic compounds

The phenolic compounds were extracted from the labneh samples according to the method described by Zoidou et al. (2014). Briefly, 1 g of labneh sample was diluted with 1 ml of distilled water, vortexed for 1 min, and sonicated in a water bath sonicator for 15 min. Then, the samples were centrifuged at 112 *g* for 5 min using a refrigerated centrifuge. The clear supernatant was separated and stored at −20°C for further use.

HPLC analysis for phenolic compounds

Determination and detection of polyphenol compounds in the labneh samples were carried out by HPLC, a system consisting of a quaternary pump coupled to a UV detector. A conventional reversed-phase C18 column (250 mm × 4.6 mm, 5 µm) was used as the stationary phase. The gradient elution program was implemented using a system of two solvents as follows: solvent A consisted of water containing 0.2% H_3PO_4 (v/v), and solvent B was the mixture of methanol and acetonitrile (50:50 v/v). The flow rate was constant at 1 ml/min and chromatographic analysis was performed at 25°C. Phenolic compounds were detected at 280 nm. The HPLC gradient program was as follows: 0 min, 96% A; 40 min, 50% A; 45 min, 40% A; 60 min, 0% A, 70 min, 0% A; 72 min; 96% A. Identification of the eluting peaks of phenolic compounds was performed by comparing their retention time (tR) values and the corresponding UV spectra.

Standard solutions

The stock solution of 1 mg/ml was prepared from the phenolic compound standard. Then, by diluting appropriate volumes of the stock solution with methanol, working standards were obtained at lower concentrations (0.02, 0.04, 0.1, 0.2, and 0.4 mg/ml). Stock solution and working standards were stored in a refrigerator at 4°C.

Results and Discussion

Microbiological evaluation of carbonated probiotic UF-Labneh treatments:

The counts of lactic acid bacteria of carbonated probiotic UF-Labneh treatments during storage:

The LAB counts in all labneh treatments were recorded in Fig. 1. Firstly, the counts of *L. bulgaricus* as starter culture were ranged between 8.84 and 9.00 log cfu/g at the fresh treatments without significant differences. During the storage periods, the counts of *L. bulgaricus* were enhanced for 14 days and after that slight decrease were observed at the end of storage period in all treatments. The counts of *L. bulgaricus* in T_2 & T_3 were slightly more than others treatments which recorded 9.10 and 9.73 log cfu/g for T_2 & T_3, respectively at the end of storage period. The slight declined in the progress counts of *L.*

bulgaricus in all treatments at 21 days may be related to development of acidity resulting from the metabolic activity of lactic acid bacteria as mentioned by others authors El-Shafei et al. (2011); Kavitake et al. (2018).

Secondly, the same trend of results was recorded for *S. thermophilus*. At fresh, the counts of *S. thermophilus* were ranged between 9.02 and 9.07 log cfu/g. During the storage periods all *S. thermophilus* in treatments were progressed for 14 days and after that slight decrease were observed. The counts of *S. thermophilus* were ranged between 8.7 and 9.10 log cfu/g and more counts were observed for T3 at the end of storage period. Similarly, the Co_2 that injected in retentate milk during manufacturing labneh was slightly enhanced the starter culture during storage as recorded in all treatments compared to blank (Ma et al., 2001).

Moreover, the counts of *L. acidophilus* either free or encapsulated form was ranged between 9.25 & 9.88 log cfu/g when fresh with no differences. While it was developed during storage period until 14 days, and the more counts was detected in encapsulated *L. acidophilus* in T_2 & T_3. By the end of storage period, *L.acidophilus* count was decreased in T1.

These results might be due to the protection action of encapsulation process and the effect of CO2 in inhancement the probiotic growth as mentione by Gueimonde & de los Reyes-Gavilan (2004).

The counts of psychrotrophic bacteria and mold & yeast counts of carbonated probiotic UF-Labneh treatments during cold storage period

Shelf life of labneh treatments was determined by the counts of mold & yeast, also psychrotrophic bacterial counts (Fig. 2). At the fresh time, no psychrotrophic bacteria was detected, but at the 7 days of storage period some psychrotrophic bacteria were found in all treatments where the psychrotrophic bacteria count ranged between 3.0 and 3.17 log cfu/g. As time pass, the counts of psychrotrophic bacteria were increased and more counts were detected in blank sample. At the end of storage, the psychrotrophic bacteria recorded 5.6, 4.47, 4.51, 4.47 and 4.59 log cfu/g for blank, control, T_1, T_2 and T_3, respectively. These results may be due to the preservative effect of injected CO_2 in the different treatments which is in accordance with that mentioned by Karagül-Yüceer et al. (2001); Hotchkiss et al. (2006) and Lo et al. (2016) that indicted that CO_2 had preservative effect and antimicrobial activity for contaminated microorganisms. So, our results recommended using CO_2 to preserve the dairy products from contamination during storage which had ability to delay appearing or spoilage and contaminated organisms.

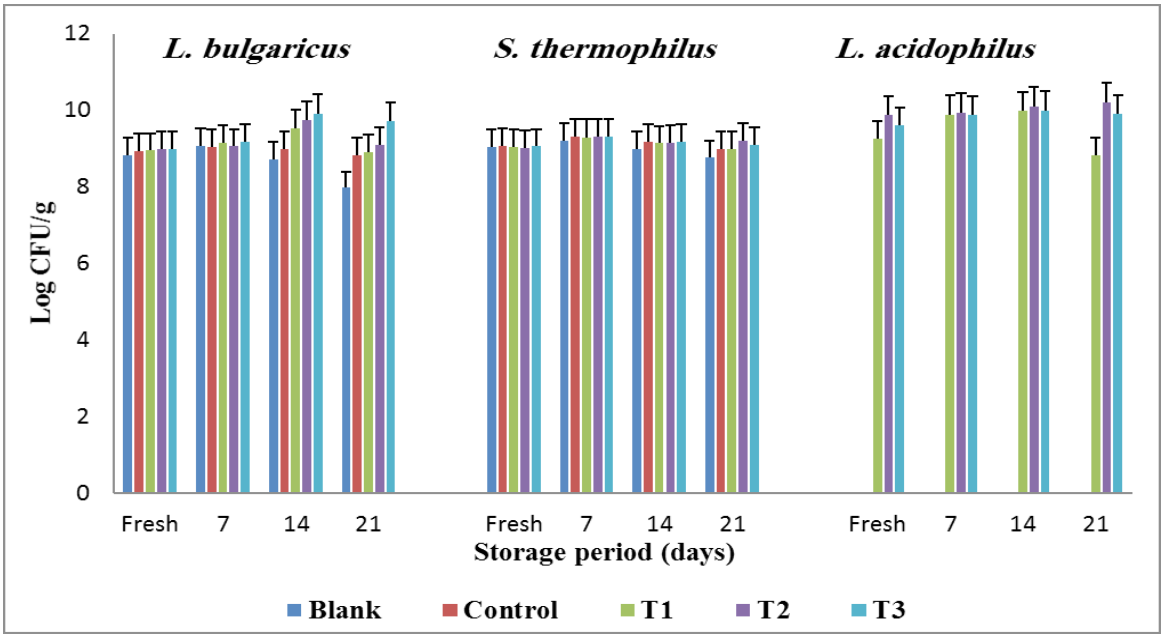


Fig. 1. Counts of LAB in carbonated probiotic UF-Labneh treatments during cold storage period.

The same trend of results was observed for the mold & yeast counts during storage as in Fig. (2). The obtained data revealed that there is no mold and yeast through the first week of storage but after 14 days, little mold & yeast counts were detected in the T_1, T_2 and T_3 which recorded 2.0, 2.47 and 1.77 log cfu/g, respectively. Moreover, at the end of storage, the counts of mold & yeast were recorded in blank (3.07 log cfu/g) than other treatments that saturated with CO_2. Accordingly, our result indicated that injection of UF-labneh during manufacturing with CO_2 didn`t prevent the appearing of mold and yeast counts, but it delayed their growth during storage period.

Physico-chemical properties and pH values of carbonated probiotic UF-Labneh cheese during storage period

Total solid, total protein and fat contents of UF-labneh were evaluated periodically every week until 3 weeks and presented in Fig. 3. It was observed that there were little differences in total solid contents in the periodical time of the same sample. However, there were differences ($p<0.05$) between each treated sample. Total solid content of blank sample was significantly ($p<0.05$) lower than the others. Total solid contents at fresh time were 26.70, 27.01, 27.0, 29.38 and 26.97% for blank, control, T_1, T_2 and T_3 treatments respectively. It could be due to that the blank sample did not contain injected CO_2 and *L. acidophilus* either free or capsulated. According to addition of encapsulated *L. acidophilus* to T_2 and T_3; total solids of both samples were significantly higher than that of blank and control samples either fresh or during cold storage. As mentioned lately, microbiological examinations had proven that the counts of lactic acid bacteria (LAB) were enhanced within the first 14 days of storage then slightly decreased. Both encapsulated treatments (T_2, T_3) had more LAB counts than blank and control and T_1. During cold storage there were slight increases in total solid contents in all samples due to moisture loss according to evaporation process as stated by El-Sayed & El-Sayed (2021). The changes in total solids of control and treated samples related to blank sample has been presented in Table (1). It was clear from this Table that the total solids in T2 increased by almost 9% when compared with other treatments. This could be explained on the fact that CO2 promoted the growth of starter culture bacteria and encapsulated one in this treatment, which led to a sharp reduction in the pH value (Fig. 4), and as a result a shrinkage of the curd occurred and thus the expulsion of a part of Labneh whey therefore the total solids increased.

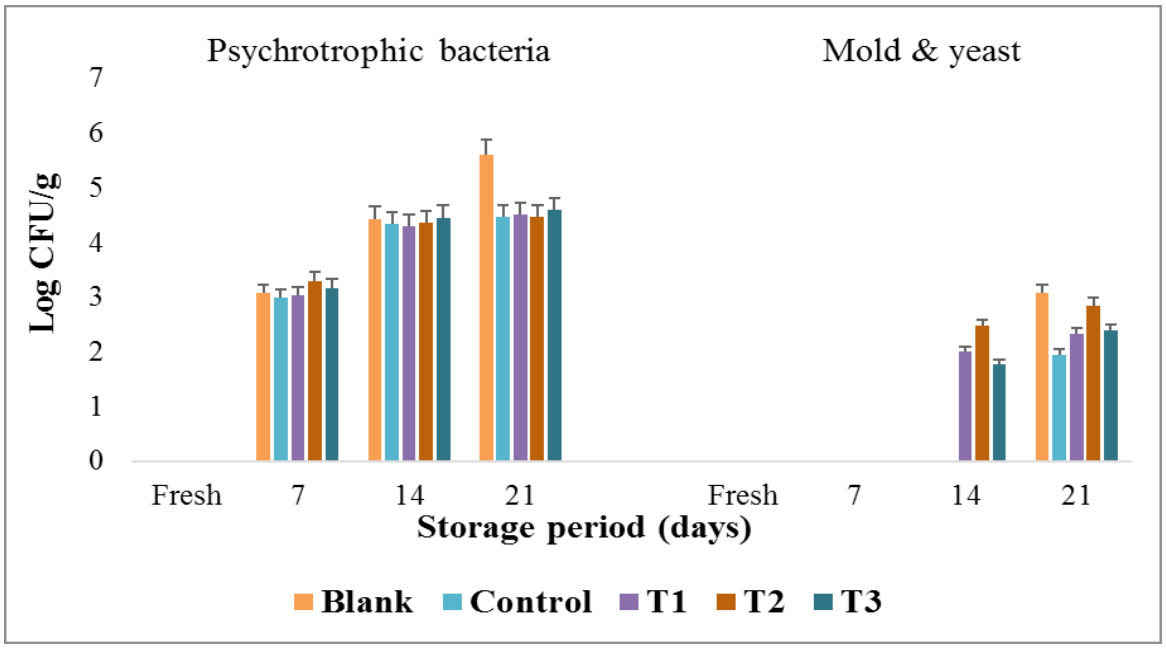


Fig. 2. The counts of psychrotrophic bacteria and mold & yeast counts in carbonated probiotic UF-Labneh treatments during cold storage period.

Fat contents had different direction in all samples during storage period of carbonated probiotic UF-labneh. There were no significant differences between all samples either fresh or during storage period. As stated the results revealed that there were no differences in fat contents if injected with CO2 or not, free *L. acidophilus* cells or capsulated. The results were in harmony with these found by El-Shafei et al. (2011) and lower than the results which had been shown by Ali (2020) due to the addition of avocado fruit to Labneh cheese.

Furthermore, pH values of carbonated UF-labneh samples were illustrated in Fig. 4 during three weeks of storage period. There was a slight decrease in pH values in all treated samples during storage. Blank and control samples had higher pH values compared to treated samples. It could be due to the summation of injected CO_2 and probiotic bacteria (*L. acidophilus*) either free or capsulated. Many studies had been clarified that adding CO_2 to fermented dairy products invigorated the growth of starter culture bacteria **(**Hotchkiss et al., 2006). These results were in harmony with the microbiological examination which induced that both of starter cultures were enhanced in the first 14 days then slightly decreased. Also, it was found that the capsulated treated samples had more counts of *L. acidophilus* compared to free or samples without probiotic because of the existence of CO_2 that stimulated probiotic bacteria. Values of pH at fresh time were 4.77, 4.72, 4.40, 4.29 and 4.39 for blank, control, T1, T2 and T3 respectively. While, pH values were 4.40, 4.50, 4.26, 4.20 and 4.28 at the end of the trail at the same order. Our data were in line with those reported by Ibrahim et al. (2008).

Texture profile analysis of carbonated probiotic UF- labneh cheese

Textural properties are essential for determining the acceptance and quality of dairy products. It has been claimed that, the rheological properties of labneh cheese has been affected by the manufacturing techniques and the total solids and total protein of Labneh (Kebary et al., 2021; Nsabimana et al., 2005). The changes in the textural parameters of carbonated probiotic UF-labneh cheese during cold storage for 21 days are presented in Table 1. The obtained results of texture parameters of fresh UF-labneh and after 3 weeks of cold storage indicated that the texture parameters (Hardness, Cohesiveness, Springiness, Gumminess and Chewiness) followed almost similar trends. From the table it is evident that hardness (force necessary to attain a given deformation) is a commonly evaluated parameter when determining yogurt and labneh texture. Hardness values of all treatments of UF-Labneh cheese (blank, control, T_1, T_2 and T_3 treatments) were differences. There were significantly increasing in hardness values either fresh or during cold storage.

Blank and Control samples had been scored the lowest values of hardness than other treatments. Where, treatment T_2 exhibited the highest. It has been attributed to the significant decreased in total solids of both samples (blank & control) than other treatments and the significant increasing in TS ratios between T_2 and the rest of samples. Changes in hardness can be attributed mainly to the changes in the total solids and total protein contents of Labneh as close relation was apparent between hardness and total solids. Nsabimana et al. (2005); Saad et al. (2015) and El-Sayed et al. (2017), who stated that the rheological behavior of Labneh depended on the protein concentration. Also, the hardness of all Labneh cheese samples were increased significantly with the progress of storage period up to 3 weeks due to moisture loss according to evaporation process.

On the other hand, the results presented in Table 1 indicated that, most texture parameters (Hardness, Cohesiveness, Gumminess and Chewiness) of all Labneh samples increased slightly. On contrast, springiness (The rate at which a deformed material returns to its original shape on removal of the deforming force). Szczesniak et al. (1963) and Bourne, (1978) found decreased slightly during the storage period. Similar trends were obtained by El-Sayed et al. (2017) and Ali (2018). From the foregoing results it can be concluded that the most obvious changes in the textural properties of Uf-labneh cheese as affected by addition CO_2 and *L. acidophilus* either free or capsulated occurred in the hardness or treatment of Labneh cheeses containing CO_2 and encapsulated *L. acidophilus* with spray drier method (T_2) was high hard than other samples.

Fig. 3. Chemical Composition (%) of carbonated probiotic UF-labneh cheese during cold storage period (a: total solids, b: total protein and c: fat).

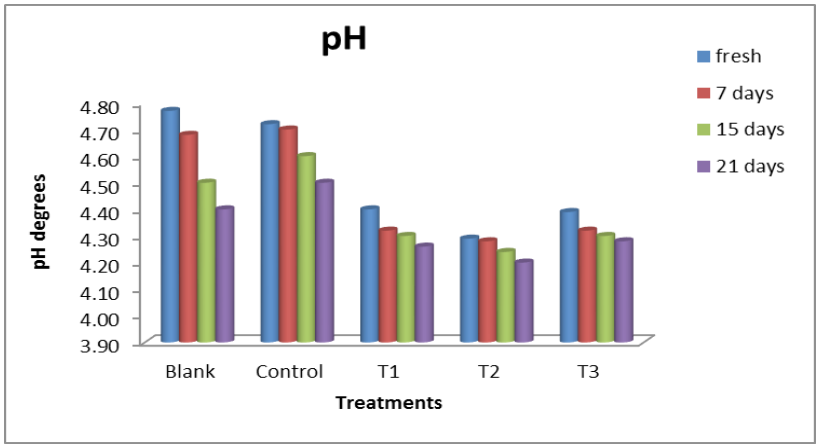


Fig. 4. pH values of carbonated probiotic UF-labneh cheese during cold storage period.

TABLE 1. Rheological properties of carbonated probiotic UF-labneh during cold storage period.

Samples	Hardness N	Cohesiveness (B/A area)	Springiness mm	Gumminess N	Chewiness N/m
Fresh					
1	8.90^{cC}	0.54^{cB}	0.892^{aA}	4.806^{cC}	4.286^{bC}
2	9.00^{cC}	0.58^{cB}	0.870^{aA}	5.220^{cC}	4.541^{bB}
3	11.85^{bD}	0.63^{bC}	0.829^{bA}	7.466^{bD}	6.189^{aB}
4	14.79^{aD}	0.70^{aC}	0.766^{cA}	10.353^{aD}	7.930^{aC}
5	12.10^{bC}	0.65^{Bb}	0.800^{Ba}	9.680^{aC}	7.744^{Ab}
1 Week					
1	9.30^{cC}	0.59^{cBA}	0.861^{aB}	5.487^{cB}	4.724^{cC}
2	9.61^{cC}	0.58^{cB}	0.840^{aB}	5.568^{cC}	4.677^{cB}
3	12.35^{bC}	0.67^{bC}	0.784^{bB}	8.274^{bC}	6.486^{bB}
4	16.02^{aC}	0.77^{aB}	0.620^{cB}	12.335^{aC}	7.647^{aB}
5	12.50^{bC}	0.68^{bB}	0.772^{bB}	8.500^{bC}	6.562^{Bb}
2 Weeks					
1	10.27^{eB}	0.60^{cBA}	0.867^{aB}	6.180^{cB}	5.358^{bBC}
2	10.80^{dB}	0.62^{cB}	0.861^{aA}	6.696^{cB}	5.765^{bA}
3	15.54^{cB}	0.73^{bB}	0.760^{bB}	11.344^{bB}	8.621^{aA}
4	18.85^{aB}	0.80^{aB}	0.587^{dC}	15.08^{aB}	8.851^{aA}
5	15.80^{bB}	0.75^{abA}	0.745^{Cc}	11.852^{bB}	8.828^{aA}
3 Weeks					
1	11.70^{cA}	0.62^{cAB}	0.830^{aC}	7.254^{cA}	6.021^{cA}
2	12.00^{cA}	0.65^{cAB}	0.802^{bC}	7.800^{cA}	6.256^{cA}
3	18.30^{bA}	0.79^{bA}	0.570^{cC}	14.457^{bA}	8.240^{bA}
4	20.74^{aA}	0.91^{aA}	0.493^{dD}	18.873^{aA}	9.304^{aA}
5	18.62^{bA}	0.80^{bA}	0.556^{cD}	14.896^{bA}	8.282^{bA}

Means with small letter superscripts (effect of treatments) and means with capital letter superscripts (effect of storage) are not significantly different ($P< 0.05$).

Sensory evaluation of carbonated probiotic UF-Labneh cheese during storage period:

The sensory aspects of carbonated UF-labneh samples was evaluated and exhibited in Fig. (5-a, b, c). Data revealed that there were no differences during storage period in the same sample except after 15 days the difference was observed. Blank and Control samples had higher scores compared to treated samples; it could be due to the addition of free and capsulated *L. acidophilus* which made some difference in appearance.

As for body & texture evaluation, the addendum of CO_2 did not influence on the texture compared to control sample either fresh or during storage period. Free cells in T_1 sample gained more body & texture scores than both of T_2 and T_3. It could be due to the existence of capsulated clusters of probiotic bacteria.

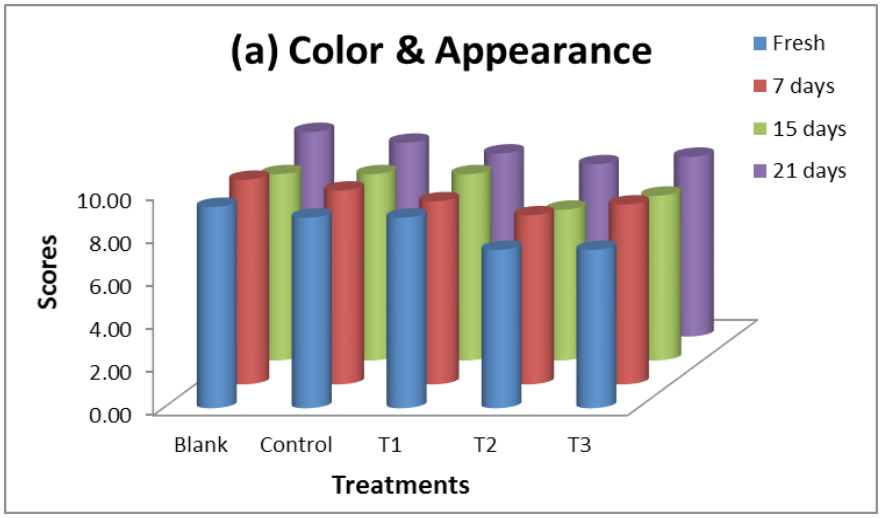
With regard to flavor evaluation of carbonated UF-labneh cheese during cold storage period as illustrated in Fig. (5-c) the results were distinct. It was remarked that the samples which more liked according to the judges were T_1, control, blank, T_3 and T_2 in descending order at fresh time. On time, two encapsulated samples (T_2 and T_3) have gained more desirable flavor than samples contained free cells of probiotics. While flavor scores for blank and control samples were slightly increased during storage periods. Adding CO_2 and probiotic bacteria to the labneh gave it a new taste not familiar to consumers and it need sometime to be familiar.

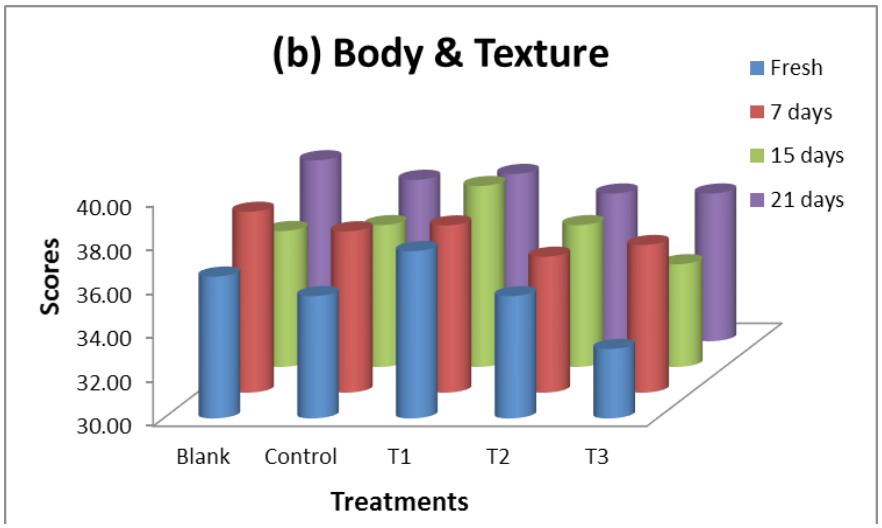
Polyphenol compounds of carbonated probiotic UF-Labneh cheese during storage period:

Polyphenol compounds of carbonated probiotic UF-labneh cheese were analyzed when fresh and at the end of the experimental time. The results at fresh time had been exhibited in Fig. 6 and Table 2. The data revealed that gallic, chlorogenic, coumaric, cinnamic and daidzein acids were the most plenteous phenol compounds detected by HPLC in carbonated probiotic UF-labneh cheese. It was found that the sum of polyphenols of all treatments at fresh time were (9.2, 6.17, 3.68, 3.81 and 3.28 µg/mL) for blank, control, T_1, T_2 and T_3 respectively. While, it was found that ellagic acid had been only detected in blank sample compared to others. On the other hand, gallic acid gained no significant ($p \leq 0.05$) difference between all samples at fresh time. Gallic acid can prohibit the oxidation and rancidity of fat and oils owing to their scavenging of free radicals and antioxidant nature (Kahkeshani et al., 2019). As time passes the polyphenol compounds are missing as a result of storage period, acidity and other factors. The polyphenol compounds of carbonated probiotic UF-labneh cheese at the end of the trail were shown in Fig. 7 and Table 3. The loss ratio (%) of polyphenol compounds was great in blank and control samples compared to the rest of the treatments. The loss ratio was 4.26, 20.7, 29.03, 54.62 and 59.8% for T_3, T_2, T_1, control and blank samples in an ascending order. This reduction in phenolic compound may be due to the activity of starter culture which utilizes these components as carbon or energy source. The existence of probiotic bacteria protected polyphenols from lost. It was observed that treatments which contained capsulated probiotic bacteria (T_2 & T_3) had the lowest loss in polyphenols after 21 days of storage period. Furthermore, some of phenol compounds had been disappeared or did not detect by HPLC analysis. It could be as a reason of producing lactic acid in fermentation process which leads to degradation of unsettled polyphenols at acidity environment. The results were in harmony with Pourghorban et al., (2022) who demonstrated that phenolic compounds were reduced during storage period of yogurt enriched with olive leaf powder.

Conclusion

The aim of this study was to produce a new healthy product that attracts the attention and interest of the consumer. At the same time, to be an acceptable healthy product with beneficial health effects for the health of consumers. The new carbonated probiotic UF-labneh cheese had gained attention from the judges due to it considered a modernistic dairy product. Using of two different encapsulation methods (spray drying and extrusion methods) gave many advantages for this product. The encapsulated process protected probiotic bacteria from the surrounding environment such as high acidity. As well the encapsulated treated samples had gained more polyphenol compounds than other treatments during the storage period. The probiotic counts in encapsulated treatments were higher than treatment with free cells either fresh or during storage period. The injection of carbon dioxide gas (CO_2) provided the product a novel taste and protected the product from psychotrophic bacteria and mold& yeast due to its antimicrobial effect. Thus, it could be concluded that it is an advantage of encapsulated probiotic bacteria to get its sufficient counts and to reach the consumer stomach in appropriate ratios. Adding CO_2 to UF-labneh cheese was a good alternative to produce many modern dairy products in order to fulfill the consumer requests.





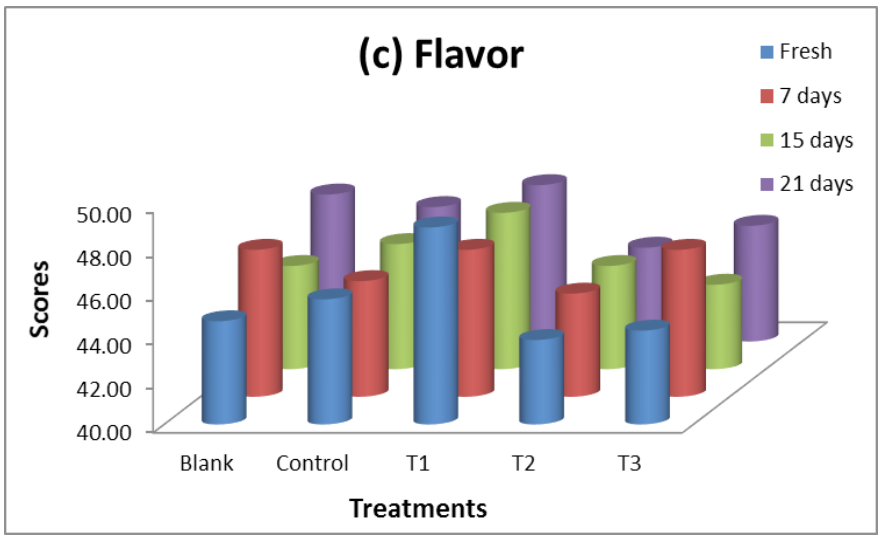


Fig. 5. Sensory evaluation of carbonated probiotic UF-labneh cheese during cold storage period (a) color & appearance (10), (b) body & texture (40) and (c) flavor (50).

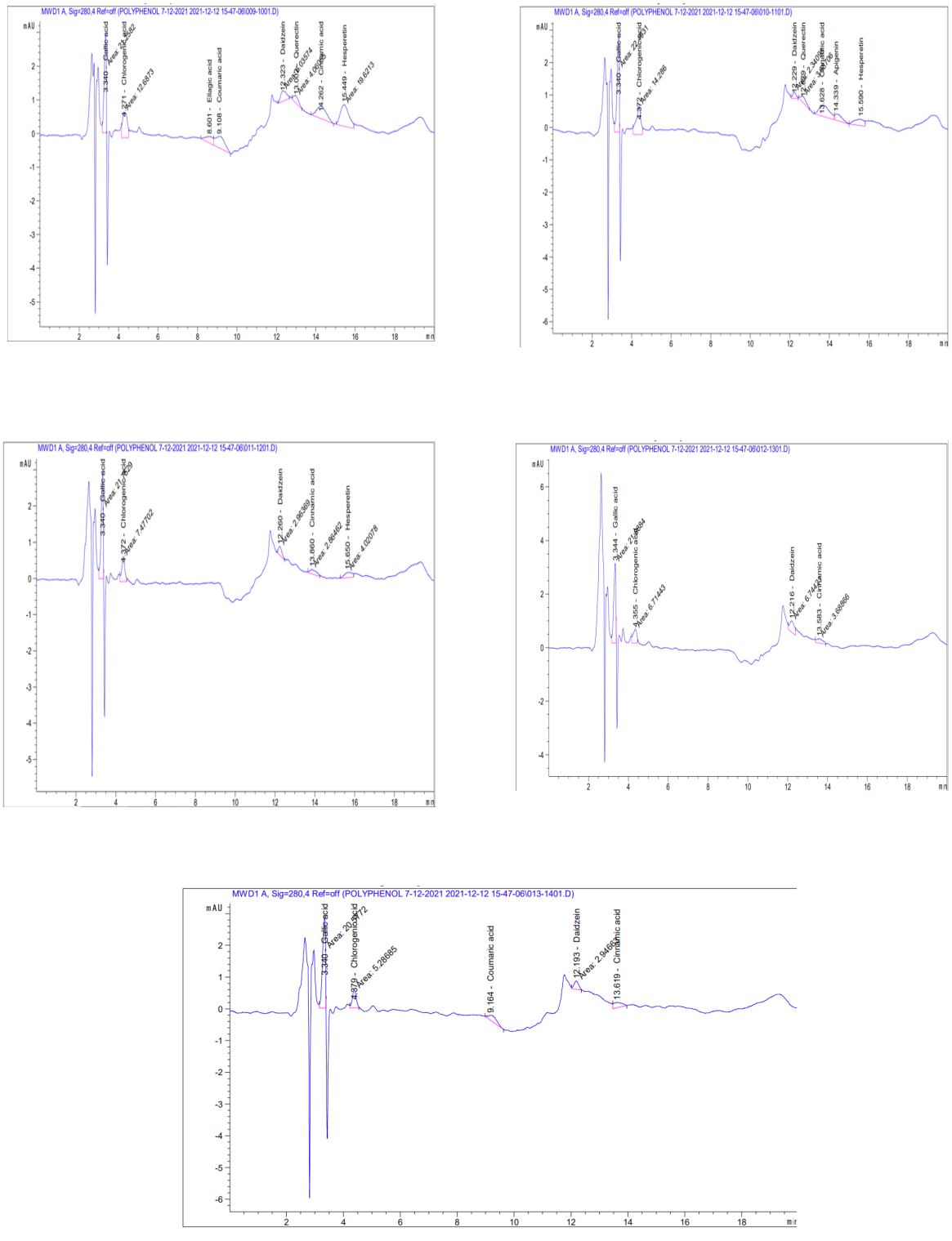


Fig. 6. Pholyphenol compounds of carbonated probiotic UF-labneh cheese at fresh time. From left to right (blank, control, T_1, T_2 and T_3).

TABLE 2. Pholyphenol compounds of carbonated probiotic UF-labneh cheese at fresh time.

Phenolic compounds	Area	Concentration (µg/ml)	Concentration (µg/g)
		Blank Sample	
Gallic acid	21.26	2.11	8.44
Chlorogenic acid	12.69	2.05	8.20
Ellagic acid	5.21	2.51	10.03
Coumaric acid	11.68	0.31	1.25
Daidzein acid	6.04	0.43	1.71
Querectin	4.06	0.48	1.93
Cinnamic acid	12.95	0.3	1.18
Hesperetin	19.62	1.01	4.02
Sum. of Polyphenol compounds		**9.2**	**36.76**
		Control Sample	
Gallic acid	22.49	2.23	8.93
Chlorogenic acid	14.29	2.31	6.23
Daidzein acid	2.34	0.17	0.66
Querectin	3.69	0.44	1.75
Cinnamic acid	12.78	0.29	1.17
Hesperetin	6.31	0.35	1.40
Apigenin	5.07	0.38	1.51
Sum. of Polyphenol compounds		**6.17**	**21.65**
		T_1 Sample	
Gallic acid	21.75	2.16	8.64
Chlorogenic acid	7.48	1.21	4.83
Daidzein acid	2.96	0.21	0.84
Cinnamic acid	2.86	0.07	0.26
Hesperetin	4.02	0.21	0.82
Sum. of Polyphenol compounds		**3.86**	**15.39**
		T_2 Sample	
Gallic acid	21.89	2.17	8.69
Chlorogenic acid	6.71	1.08	4.34
Daidzein acid	6.74	0.48	1.91
Cinnamic acid	3.69	0.08	0.43
Sum. of Polyphenol compounds		**3.81**	**15.37**
		T_3 Sample	
Gallic acid	20.58	2.04	8.17
Chlorogenic acid	5.29	0.85	3.42
Coumaric acid	3.65	0.1	0.39
Daidzein acid	2.95	0.21	0.83
Cinnamic acid	3.37	0.08	0.31
Sum. of Polyphenol compounds		**3.28**	**13.12**

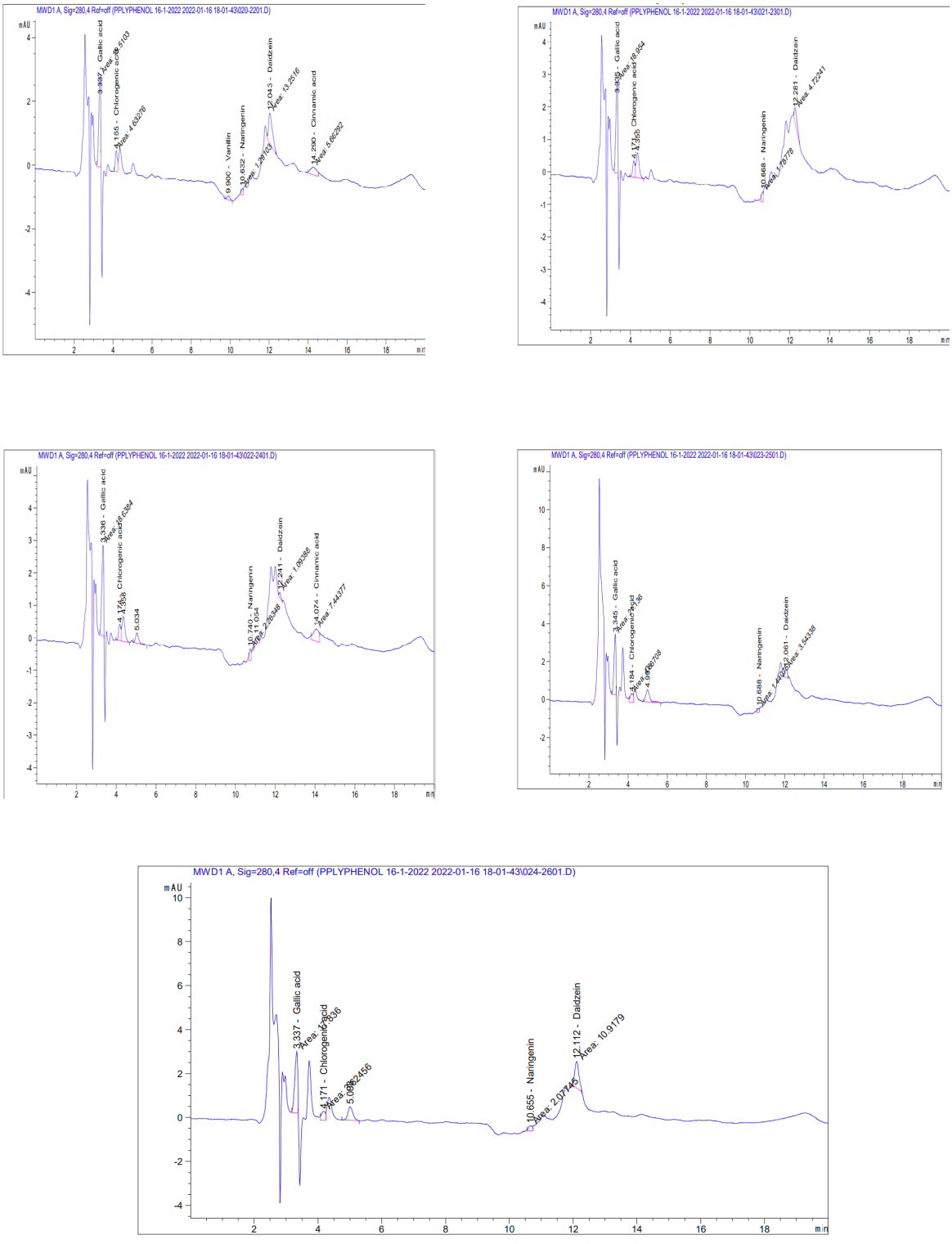


Fig. 7. Pholyphenol compounds of carbonated probiotic UF-labneh cheese at the end time. From left to right (blank, control, T_1, T_2 and T_3).

TABLE 3. Pholyphenol compounds of carbonated probiotic UF-labneh cheese at the end time.

Phenolic compounds	Area	Concentration (µg/ml)	Concentration (µg/g)
		Blank Sample	
Gallic acid	19.51	1.72	5.17
Chlorogenic acid	4.63	0.68	2.05
Vanillin	1.20	0.05	0.14
Naringenin	1.29	0.15	0.46
Daidzein acid	13.25	0.97	2.90
Cinnamic acid	5.66	0.13	0.38
Sum. of Polyphenol compounds		**3.7**	**11.1**
The loss ratio (%) of polyphenol		**59.8**	**69.8**
		Control Sample	
Gallic acid	18.95	1.67	5.02
Chlorogenic acid	3.93	0.58	1.73
Daidzein acid	4.72	0.34	1.03
Naringenin	1.79	0.21	0.63
Sum. of Polyphenol compounds		**2.8**	**8.41**
The loss ratio (%) of polyphenol		**54.62**	**61.15**
		T_1 Sample	
Gallic acid	18.64	1.65	4.94
Chlorogenic acid	3.86	0.57	1.70
Daidzein acid	1.09	0.08	0.24
Cinnamic acid	7.44	0.17	0.51
Naringenin	2.26	0.27	0.80
Sum. of Polyphenol compounds		**2.74**	**8.19**
The loss ratio (%) of polyphenol		**29.02**	**46.78**
		T_2 Sample	
Gallic acid	21.41	1.87	5.60
Chlorogenic acid	4.78	0.72	2.15
Daidzein acid	3.54	0.26	0.77
Naringenin	1.44	0.17	0.51
Sum. of Polyphenol compounds		**3.02**	**9.03**
The loss ratio (%) of polyphenol		**20.7**	**41.25**
		T_3 Sample	
Gallic acid	17.84	1.57	4.72
Chlorogenic acid	3.62	0.53	1.60
Daidzein acid	10.92	0.80	2.39
Naringenin	2.08	0.24	0.73
Sum. of Polyphenol compounds		**3.14**	**9.44**
		4.26	**28.05**

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