Evaluation of Salt-Free Labneh Quality Prepared Using Dill and Caraway Essential Oils

Wafaa M. Zaky, Jihan M. Kassem, Hayam M. Abbas and Sahar H. S. Mohamed

Dairy Department, National Research Centre, Dokki, Giza, Egypt
Prof.hayamabbas@yahoo.com

Abstract: Salt-free labneh samples were prepared from buffalo's milk using 2µL/100ml milk of both dill (T_1) and caraway (T_2) essential oils. Chemical, anti-oxidant and organoleptic properties of samples stored for 28 days at 7ºC±2 were investigated. Microbiological examinations were also periodically carried out until 14 days of storage. Data illustrated that adding essential oils had slight effect on titratible acidity contents; however their values were increased in all samples during storage. Dill and caraway essential oils encouraged the production of total volatile fatty acids (TVFAs) contents in treated samples which were also gradually increased during storage. Whereas their lowest values were recorded for control samples either fresh or during 28 days of cold storage. Acetaldehyde and diacetyl contents were reached to their maximum values in treated samples (T_1 & T_2) till 14 days of storage then they were decreased till the end of storage period. Tyrosine took the same trend of TVFAs, while tryptophan took the opposite trend. Obtained results showed that T_2 samples had the highest levels of tyrosine among all periods. On the other side; presence of dill and caraway essential oils enhanced the anti-oxidant activity and increased the scavenging of free radicals in treated samples comparing with control ones. Starter culture did not pronouncedly affected by using essential oils; while, total bacterial viable counts were increased for all samples till they reached their maximum counts after 7 days. Yeasts & molds as well as coliform bacteria were not detected in all samples through storage. Using dill and caraway essentials oils improved the sensory properties of salt-free labneh samples either fresh or during storage; where their body & texture became soft with desirable spreadability properties. Flavor of treated samples were also more pronounced and more favored. Essential oils enhanced the taste and odor of labneh also created refreshing mouth feeling. So, dill or caraway essential oil could be recommended for using to enhance the salt- free labneh quality as they are natural, cheap, healthy and medicinal materials.

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1. Introduction:

The World Health Organization has recently called for the incidence of Cardio-Vascular diseases by lowering the salt content in food (**WHO 2002).** If the level of salt is reduced; it is possible that food may be not safe. So, there is a scope or urgent need of new methods which make the food safe **(Burt 2004).** In the last decades consumer demands in the field of food production has considerably changed. Consumers more and more believe that foods contribute directly to their health (**Mollet and Rowland 2002).** Today, foods are not intended to only satisfy hunger and to provide necessary nutrients for humans but also to prevent nutrition-related diseases and improve physical and mental well-being of the consumers (**Menrad 2003 and Roberfroid 2000).** On the other hand; consumer demand for less use of synthetic chemical preservatives according to their dangerous side effects; led to search for-or- use of naturally food preservatives as green consumption(**Losasso *et al* 2012).** Herbs and Spices or their extracts and their essential oils (EOs) are historically used as flavoring agents(**Burt 2004)**; most essential oils are classified as Generally Recognized as Safe (GRAS) and have been approved for food and beverages consumption by **US Food and Drug Administration (2012).** Essential oils are aromatic, oily liquids obtained from plant materials. It has long been recognized that some essential oils can be used as food flavoring agents or preservatives and for medicinal purposes (**El–Nawawy *et al* 1998)**.Among different essential oils**;** dill *(Anethum graveolens L).* and caraway (*Carum caravi* L) essential oils have high antioxidant activity, therapeutic properties as well as anti-pathogenic effects (**Ahn *et al* 2012; Gwari *et al* 2012 and Sahar *et al* 2013).** The antioxidant effect of these EO_S improved the storage properties of food as well as serve as healthy functional materials. Furthermore, dill essential oil has hypolipidemic activity and could be used as a cardio protective agent (**Dahiya and Purkayastha 2012)**. Caraway plant is effective in the treatment and management of type II diabetes and cardiovascular diseases, and evokes beneficial effects on elevation of lipids in the bloodstream **(Bagdassarian *et al* 2013)**. Although of their beneficial effect, little studies were conducted in their application in dairy products **(Marhamatizadeh *et al***

2012). They produced probiotic milk and yoghurt by using different concentrations of dill extract.

Dairy products are important main categories of food classes. They are popular and nutritious meals. Generally, the use of plants extracts or their EOs had possessed great interest in cheeses (**Foda *et al* 2009a; Foda *et al* 2009b and Govaris *et al* 2011).** as well as in yogurt and fermented dairy products (**Ersöz *et al* 2011; Evrendilek & Balasubramaniam 2011; Moritz *et al* 2012; Shori** & **baba 2012 and Boroski *et al* 2012).** They used spearmint, oregano, thyme, clove and lemongrass essential o ils in these dairy products. On the other hand; Caraway and dill essential oils had been recently used as natural and safe anti pathogens materials in cheese yoghurt production **(Sahar *et al* 2013)**.

Labneh is a traditional fermented milk product. It is a popular food in various parts of the world especially in the Middle East, chiefly Turkey and Balkan regions where it plays a significant role in the family diet. Labneh has increased its popularity during the last years. The perceived nutritional benefits and storage characteristics have led to increase economic importance. However, much of the consumer acceptability is dependent on its sensory properties, which in turn, seem to be heavily dependent on the method of processing of the materials, (**Shamsia and El-Ghannam 2012).** No attempts have been investigated about the quality of labneh produced by using caraway or dill essential oils. Therefore, the aim of this work was the production of salt - free labneh using dill and caraway essential oils as natural medicinal materials also evaluation of their quality and their acceptability in the absence of salt.

2. Materials and Methods

Materials:

Essential oils source: Dill and caraway seeds oils were provided by the Medicinal and Aromatic Plants Department, National Research Centre, Dokki, Giza, Egypt.

Milk: Fresh buffalo's milk (6% fat and 16% TS) was obtained from Faculty of Agriculture, Cairo University, Giza, Egypt.

Starter culture: *Lactobacillus delbrueckii* subsp *bulgaricus* and *Streptococcus thermophilus* obtained from Chr. Hansen Denmark were used as starter culture for labenah preparation.

Methods:

Experimental study: Preliminary experiments of labneh were conducted to determine the adequate concentration of dill and caraway-EOs. These concentrations which will be used in this study to produce samples without any defects in their odor & taste according to the panels acceptability. The obtained results revealed that 2µL/100 ml milk was the favorite concentration which produced desirable flavor for both essential oils.

Labneh preparation: Labneh samples were prepared as mentioned by **Amer *et a1 (1*997)**. After partial removal of whey; the salt-free curd was divided into three equal parts; the first and second parts were fortified by 2µL dill-EO/100 ml milk (T_1) and 2µL caraway-EO/100 ml milk (T_2) respectively. They were gently well mixed into the coagula, packed in plastic containers and stored in refrigerator (7ºC±2) for 28 days. Sample without any oil was served as control .Three replicates were conducted.

Analytical procedures:

1- Chemical analysis:

Fresh labneh samples were chemically analyzed for TS, TP and fat (%) contents according to **AOAC (2007).** Stored samples were periodically analyzed after 0, 7, 14, 21 and 28 days for titratible acidity **(AOAC 2007**) and pH values. Acetaldehyde content (m mol/ 100 g) was estimated according to **Lee & Jago (1969)** and diacetyl content (m mol/ 100 g) was determined as reported by **Pack *et al* (1964).** Tryptophan and tyrosine contents (mg/100g) were estimated as described by **Vakalerise and Price (1959).** Total volatile fatty acids ($TVFA_S$) contents (0.1N NaOH/100g) were also determined according to **Kosikowiski (1986).**

2- Free radical scavenging activity measurements:

The DPPH (2, 2-diphenyl-1picryl-hydrazil) free radical scavenging activity was performed according to the method of **Locattili *et al.*, (2008).** 400 µL of sample or ethanol (blank) were added to 3600 µL of a 100 µm DPPH solution in ethanol; and well mixed. After 20 min; absorbance at 517 nm was measured. The DPPH radical scavenging activity (%) was calculated using the following equation:

Radical scavenging activity (%) = $(A_{control} - A_{sample}) / A_{control} \times 100$

Where: $A_{control}$ is absorbance of blank.

A_{sample} is absorbance of sample.

3- Microbiological examinations:

The count of starter culture was carried out on MRS medium for *Lactobacillus delbrueckii* subsp *bulgaricus* and M17 medium for *Streptococcus thermophilus*.

The total bacterial count was achieved by using Plate Count Agar medium. Coliform bacteria were detected by Violet Red Bile Agar medium (VRBA). Molds & yeasts were detected by Acidified Potato Dextrose Agar medium (APDA).

4 – Organoleptic properties evaluation:

Fresh and stored labneh samples were periodically evaluated for their organoleptic properties. Flavor (50 points), body & texture (40

points), and appearance (10 points) were evaluated every seven days till 28 days of storage at refrigerator temperature (7ºC±2) by 20 panelists according to score card suggested by **Keating and Randwhite (1990).**

3. Results and Discussion:

Gross chemical composition of fresh salt -free labneh:

Total solids, fat and total protein contents (%) were determined for fresh salt -free labneh samples. TS contents were 29.10, 22.89 and 25.33 % for control, T_1 and T_2 respectively. Fat contents were 14.40, 11.60 and 12.2 %, while TP contents were 10.0, 7.77 and 8.2 % for control, T_1 and T_2 respectively. These results were in the normal ranges and in accordance with **El-Samragy (1997).**

Titratible acidity and pH values of salt- free labneh samples during storage:

The change in titratible acidity is an important factor, since it affects the shelf life and acceptability of labneh (**Al-Otaibi and El-Demerdash 2008)**. Acidity contents (%) of fresh control & experimental labneh samples or during the storage periods were presented in Fig 1. The slightly high values were noticed for control labneh samples when fresh and they were increased up to the end of storage period. The presence of dill (T_1) and caraway (T_2) seeds essential oils were partially inhibited the growth of starter; so they had slightly affected the acidity values. The results of starter culture counts (Fig 7: A,B) confirmed these obtained data. **Burt (2004)** reported that the effect of the EOs on the viability of microorganisms depended upon the kind of microorganism, the type of essential oil and their concentrations. **Ersöz *et al* (2011)** indicated that natural phenolic compounds in the essential oils had a negative effect on the starter culture and total viable counts so affected the acidity content. **Taha *et al* (1997)** found that titratible acidity was slightly affected by the type of the used oil. The pH values (Fig 2) followed the opposite trend of titratible acidity. The pH values of control as well as T_1 and T_2 samples were decreased during storage period. These data were in accordance with **Ersöz *et al* (2011).**

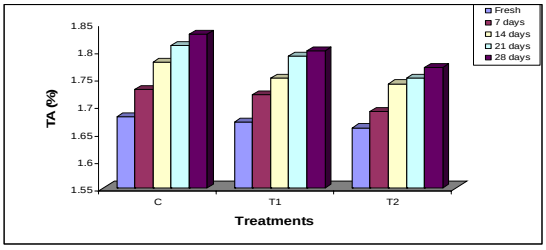


Fig (1): Titratible acidity Values (%) of salt -free labneh samples during 28 days of storage. C: Control T_1: Labneh fortified with dill oil T_2: Labneh fortified with caraway oil

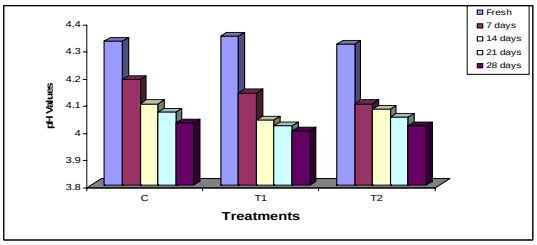
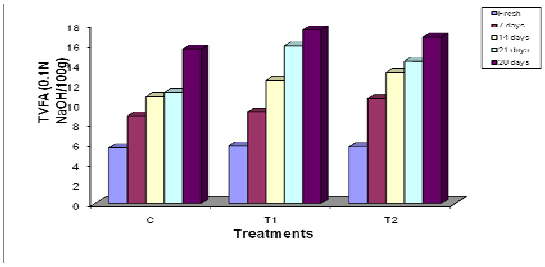


Fig (2): pH Values of salt -free labneh samples during 28 days of storage. C: Control T_1: Labneh fortified with dill oil T_2: Labneh fortified with caraway oil

Total volatile fatty acids contents of salt-free labneh samples during storage:

Fig (3) reflected the total volatile fatty acids (TVFAs) contents of labneh samples prepared with the respective two essential oils without adding salt. The highest values of TVFAs were recorded for labneh manufactured with the essential oils (T_1 and T_2), whereas the lowest values were recoded for untreated control either fresh or after 28 days of storage. The TVFAs contents were gradually increased in all samples with extended storage period. These results are in agreement with **Al-Otaibi and El- Demerdash (2008),** they illustrated that TVFAs contents for labneh manufactured with marjoram essential oil gained the highest value comparing with untreated samples. These results were also in agreement with those reported by **Taha *et al* (1997)**. The increases in TVFAs could be explained on the basis that the increase in the cheese proteolysis as amino acids can serve as precursors for the development of certain volatile fatty acids **(Salem *et al* 2007).** Similar results were also reported for Edam cheese **Kebary *et al* (2002)** and for Tallaga cheese by **Badawi and Kebary(1998).**

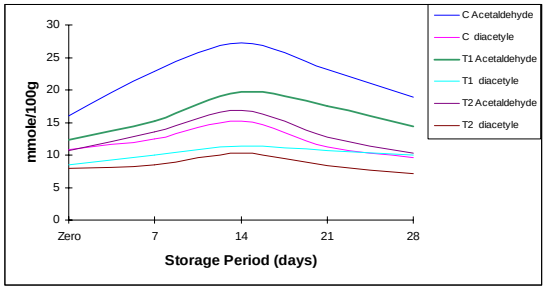


Fig(3): Total Volatile Fatty Acids (TVFAs) contents of salt- free labneh samples during 28 days of storage. C: Control T_1: Labneh fortified with dill oil. T_2: Labneh fortified with caraway oil.

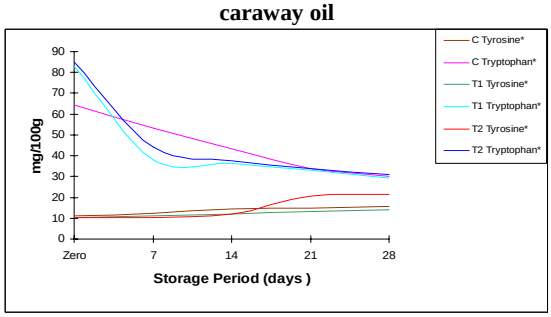
Acetaldehyde and diacetyl contents of salt-free labneh samples during storage:

The acetaldehyde and diacetyl are the main flavor compounds produced by the starter organisms and impart the characteristic flavor of fermented milk and labneh. The acetaldehyde contents of labneh were

higher than the developed diacetyl contents, and the ratio of acetaldehyde to diacetyl was almost unchanged throughout storage **(Abd El-Salam *et al* 2011).** Fig (4), showed the contents of both acetaldehyde and diacetyl of different labneh samples when fresh and after 28 days of storage. It was obvious that both acetaldehyde and diacetyl contents were increased to reach their maximum values till 14 days of storage then decreased until the end of storage period (28days) in all treatments. On the other side, T_1 samples had the higher values of both acetaldehyde and diacetyl than T_2 samples. **Taha *et al* (1997)** reported that the acetaldehyde content of labneh made with plant oils were gradually increased and reached the maximum values after 7 days of storage and then decreased to reach a minimum value after 28 days. Similar data were also reported by **Salem *et al* (2007),** they indicated that both acetaldehyde and diacetyl contents; in labneh samples; were increased to reach their maximum values after 10 days and then decreased until the end of storage in all treatments. They added that concentration of acetaldehyde and diacetyl can differ to a great extent, depending on the medium composition, growth conditions and the specific activity of the bacteria and their enzymes.



Fig(4): Acetaldehyde and Diacetyl contents of salt- free labneh samples during 28 days of storage. C: Control T_1: Labneh fortified with dill oil T_2: Labneh fortified with caraway oil



Fig(5): Tyrosine and tryptophan contents of salt -free labneh samples during 28 days of storage. C: Control T_1: Labneh fortified with dill oil. T_2: Labneh fortified with caraway oil.

Tyrosine and tryptophan contents of salt-free labneh samples during storage:

Tyrosine and tryptophan contents of labneh samples had been illustrated in Fig (5). Comparing with control and dill labneh samples; caraway samples had the highest levels for tyrosine contents among all periods. During storage, the tyrosine content increased in all samples indicating a continuous breakdown of the proteins. In contrary view, tryptophan contents were decreased as the storage periods increased. Labneh samples (T_2) had the highest contents of tryptophan compared to control and T_1 samples either when fresh or during storage periods. These data were in accordance with **Taha *et al* (1997)**. Similar trends were also reported by **Amer *et al* (1997); Kebary *et al* (2002) and Salem *et al* (2007)**.These observations might be due to the increase in moisture content that enhances proteolysis (**Banwart 1981).** Proteolysis that is an important indicator of tyrosine content is usually not an important factor in flavor or defects in fermented milks but, it is important factor in cheese texture and flavor development during ripening. Since, the enzymes activity by the starter culture may be able to produce peptides and amino acids that could be used as growth factors. The restricted level of bitterness in fermented milk products is 0.5 mg/ml of tyrosine content as mentioned by **Misirlilar *et al* (2012)**.

Free radicals scavenging activity of salt -free labneh samples during storage:

In the DPPH assay, the ability of any essential oil to act as a donor of hydrogen atoms or electrons in the transformation of DPPH into its reduced form DPPH-H was investigated. The samples contained the two essential oils (dill & caraway) were able to reduce the stable, purple-colored radical DPPH˙ into yellow-colored DPPH **(Boroski *et al.*, 2012).** Fig.(6) depicted the two essential oils required to scavenge free radicals; and the scavenging activity as inhibition percentage at various samples. In this Fig; It could be observed that there were great differences between control and the essential oils-contained samples. Presence of dill or caraway essential oils increased the scavenging of free radicals. Caraway- labneh samples had more free radicals-scavenging activity followed by dill- labneh samples. **Sahar *et al* (2013)** recorded that caraway- EO had 74% carvone (as phenolic compounds); so, acts as anti-oxidants material. **Marhamatizadeh *et al* (2012)** mentioned that, significant amount of antioxidants activity had been observed from dill extract, and it has phytochemicals which act as free radical scavenging materials. **Bagdassarian *et al* (2013)** indicated that the extracts of dry seeds from Apiaceae family to which belong: fennel, dill, anise, caraway and coriander, are rich in phytochemical contents which

possessed high antioxidant and antimicrobial activities. They added that polyphenolic compounds have been found to protect erythrocytes from oxidative stress or increase their resistance to damage caused by oxidants. Essential oils are able to act as antioxidants in a number of ways, mainly as reducing agents, hydrogen donors, single oxygen quenchers, and metal chelating agents.

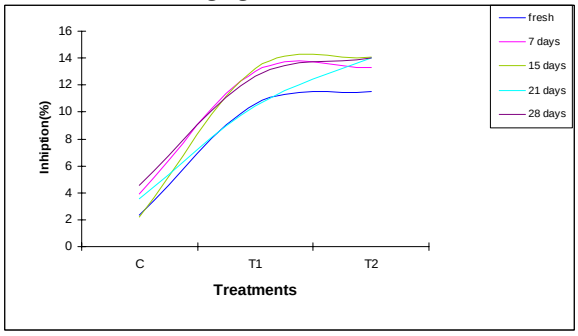


Fig (6): Free radicals scavenging activity of salt-free labneh samples during 28 days of storage. C: Control T_1: Labneh fortified with dill oil T_2: Labneh fortified with caraway oil

Microbiological examinations of salt- free labneh samples during storage:

The effect of dill and caraway- EOs on viability of starter culture and total bacterial viable counts (TVCs) in salt -free labneh stored at 7°C±2 during 14 days were showed in Fig 7 (A, B and C). The results revealed that essential oils slightly affected the growth of starter culture (*Lactobacillus delbrueckii* subsp *bulgaricus* and *Streptococcus thermophilus*) in salt-free labenah compared with control. For T_1 or T_2, there were decreases of 0.3 and 0.8 Log in *Streptococcus thermophiles* counts respectively after 7 days when compared with the control, and these decreases reached to 1.9 and 1.3 Log at the end of storage period, (Fig 7 A). While the counts of *Lactobacillus delbrueckii* subsp *bulgaricus* increased by 0.1 Log for T_1 and T_2 then decreased to 0.2 Log for T_2 after 7 days comparing with control. At the end of storage; the counts of *Lactobacillus delbrueckii* subsp *bulgaricus* were decreased to 0.6 and 0.7 Log in T_1 and T_2 respectively when compared with control. (Fig7 B).

These data were in agreement with **Abbasifar *et al* (2009)** and **Cristiane *et al* (2012)** who reported that using essential oil in white brined cheese or fermented milk had slight effect on the growth of starter culture. However, these results were comparable to those obtained by **Simsek *et al* (2007)** who used mint, thyme and garlic; also for those belongs to *Ziziphorus* which used by **Khodaparast (2007)**. **Bayoumi (1992**) reported that essential oils of clove, cinnamon, cardamom and peppermint reduced the final population of lactic acid cultures 1.5-3 log cycles in flavored yoghurt. On the contrary, oregano and its essential oil inhibited growth of *Lactobacillus plantarum* and *Leuconostoc mesenteroides* in a liquid medium **(Govaris *et al* 2011)**.

Regarding to TVCs which presented in Fig (7 C); small difference in their counts were observed between the control and treated samples. Their counts had slightly increases in control samples than treated samples. The population of TVCs were gradually increased in all cases; up to 7th day of storage and then decreased thereafter till the end of storage. **Al-Otaibi and El- Demerdash (2008)** reported that TVCs increased in the treated samples and reached to a maximum population after 7 days of storage then, they decreased till the end of storage.

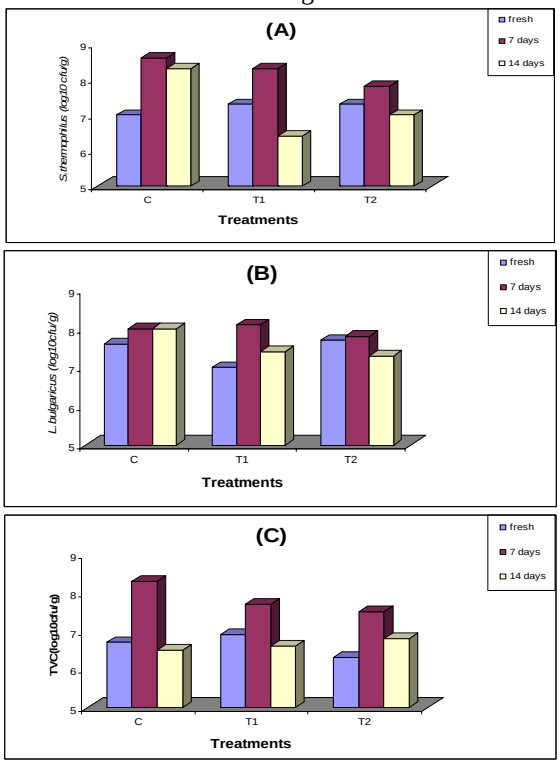


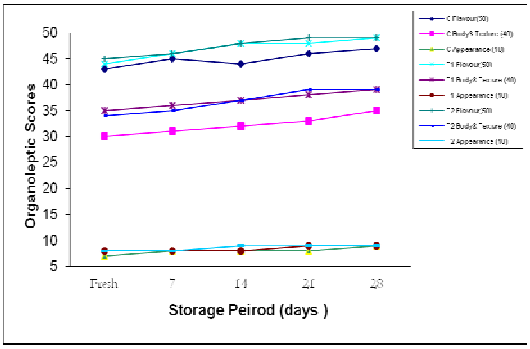
Fig (7): Effect of essential oils on starter culture (A, B) and TVCs counts (C) of salt -free labneh samples during 14 days of storage
C: Control T_1: Labneh fortified with dill oil. T_2: Labneh fortified with caraway oil.

Coliform bacteria were not detected in any of the salt-free labneh samples prepared by using the respective essential oils in spite of absence of salt (data not showed). On the other hand, yeasts and molds counts are considered indicative of the quality and the shelf life of labneh. In his regard, yeasts & molds were also not detected in salt free labneh

samples containing the two essential oils throughout the storage period, while they were detected in the untreated control only after 14 days of storage (2×10^3 cfu/g,) (data not showed). These results were in agreements with **Senatore *et al* (2000); Schelz *et al* (2006) and Hassan *et al* (2001)** who reported that essential oils had antifungal and antimicrobial activities.

Organoleptic properties of salt -free labneh samples during storage:

Organoleptic properties are important parameters to determine the quality and shelf life of labneh. The organoleptic properties of labneh samples were investigated and the results were presented in Fig (8). There were considerable differences in the sensory properties of the experimental samples as compared with untreated control. It could be noticed that labneh samples contained dill or caraway; were more acceptable than control. The addition of essential oils produced more soft and spreadable body, had further acceptable body and texture properties. Flavor of T_1 as well as T_2 samples were also more pronounced where adding caraway and dill essential oils enhanced the taste and odor of the experimental samples and created preferable refreshing mouth feeling as recorded by 90% of panelists. There were no clear differences in the appearance of control and all treated samples. The Organoleptic properties of salt-free Labneh were still acceptable till 28 days of storage without any defects in spite of the absence of salt.



Fig(8): Organoleptic properties of salt- free labneh samples during 28 days of storage. C: Control T_1: Labneh fortified with Dill oil T_2: Labneh fortified with Caraway oil.

Al-Otaibi and El-Demerdash (2008) investigated the effects of essential oils (thyme, marjoram and sage) in production of labneh. They found that the untreated control labneh was preferred compared to treated samples. Furthermore, in all cases the total scores of the sensory evaluation decreased gradually during storage **(Al-Otaibi and El-Demerdash 2008**). **Metry *et al* (2007)** indicated that the use of spices essential oils (cardamom, thyme and clove) in white soft cheese improved the flavor; and the scores were affected by the type and concentration of spice essential oils. **El-Turki *et al* (2008)** used oregano, marjoram, sage and licorice essential oils in production of labneh. They reported that, in general, labneh produced with herbal extracts were accepted by the panelists, with exception to the color of labneh .The rest of the sensorial properties, appearance, flavor and overall acceptability, showed no significant difference between labneh produced with oregano and marjoram extracts compared to the control and were preferred over those produced with sage and licorice.

Conclusion:

The previous results confirmed that, using dill and caraway essential oils enhanced the labneh quality and successfully improved the organoleptic properties especially the taste & odor. Using dill or caraway essential oils extended the shelf life of labneh to 28 days in spite of the manufacture of labneh without adding salt with any sensorial defects. So, dill and caraway essential oils; which did not commonly used in dairy products; can be recommended as natural, cheap and safe sources of antioxidant and antimicrobial agents in manufacture of labneh and other dairy products.

Corresponding author:
Prof. Dr. Hayam M. Abbas
Dairy Department, National Research Centre, Dokki, Giza, Egypt
Email: prof.hayamabbas@yahoo.com.

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