

QUALITY APPRAISAL OF ULTRA-FILTERED SOFT BUFFALO CHEESE USING BASIL ESSENTIAL OIL

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ABSTRACT

Background. Preparing and producing a healthy new dairy product that attracts consumers is an important issue. Assessing the effects of supplementation of milk with an essential oil to improve the quality of ultra-filtered soft buffalo cheese was the main target of this research.

Materials and methods. UF soft buffalo cheese was traditionally prepared to achieve four treatments: control samples (C) had no additives and (T_1 , T_2 and T_3) were supplemented with extracted basil essential oil (E-BO) at concentrations of 0.025, 0.050 and 0.075% v/v respectively. The microbiological, chemical and sensory properties of the resultant samples were determined at intervals (fresh, 15, 30 and 60 days).

Results. Results revealed that a high ratio of E-BO (0.075%) promoted the growth of starter culture followed by 0.050% and 0.025%. At the same time E-BO inhibited mold and yeast growth in all soft UF cheese samples. The pH values decreased during the storage period as a result of starter activity. Adding E-BO improved the sensory properties of UF soft buffalo cheese samples compared with control.

Conclusion. Fortification of UF soft buffalo cheese with extracted basil essential oil enhanced the growth of starter culture and inhibited mold and yeast growth. It also improved the sensory properties of the final product.

Keywords: buffalo milk, UF-cheese, Basil essential oil

INTRODUCTION

Cheese is one of the most common and well-loved dairy products, which has a specific role in human nutrition. Ultra-filtered (UF) soft cheese is one of the most widely consumed types of cheese in the world (Alizadeh et al., 2006). It has nutritional and health benefits owing to the retention of whey proteins in the concentrated retentate (El Din et al., 2010; Shojaei and Sani, 2015).

The quality of UF buffalo cheese is lower than the quality of that manufactured using cow's milk. There are great differences in the nature and structure of

casein in buffalo and cow's milk, which result in poor degradation of buffalo casein. Also, one of the greatest challenges that UF-cheese manufacturers face is the plain taste and lack of flavor due to the absence of lactose, and its poor and weak texture. Another challenge is mold spoilage, which is commonly attributed to the increased surface area of the cheese shreds and the extra handling and exposure that the shreds experience in the cutting and packing facility (Shojaei and Sani, 2015).

The use of plant additives has been increasing mainly because of their health benefits, flavor enhancements

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and antimicrobial activities. Therefore, there is great potential for their application in the food industry (Pereira et al., 2006; Vasek et al., 2015).

Essential oils which have been isolated from plant parts are famous for their biological behaviors and are also considered to be GRAS (Generally Recognized as Safe) (Branciari et al., 2015; Sajadi and Bahramian, 2015). Thus, various research has been focused on the application of essential oils (EO) as effective agents in food safety and preservation (Burt, 2004; Fisher and Phillips, 2006; Zaky et al., 2013). Essential oils have been shown to possess potential antibacterial properties against many species of pathogenic bacteria (Sahar et al., 2013). Other studies have found that essential oils modified and enhanced the growth and metabolism of different species of bacteria (Wallace, 2004). However, Jamroz et al. (2005) also illustrated that the number of lactobacillus-colony-forming-units was significantly raised after using a blend of plant extracts. In the same way Fatemeh et al. (2015) showed that the application of some essential oils (mint, bee balmand, zizaphora) increased the viability of the probiotic bacteria in samples of drinking yoghurt.

Among the most popular herbs, basil (*Ocimum basilicum* L.) is an annual herb which grows in several regions around the world. There are more than 65 species of the genus *Ocimum*. Basil is the master essential oil crop which is cultivated commercially in favorable countries (Sajjadi, 2006). It has a characteristic odor and sharp taste. Traditionally, it has been extensively utilized in the food sector as a flavoring agent, and in the perfume and medical industries (Telci et al., 2006). However, there are more potential uses of *Ocimum basilicum* essential oil, particularly as the antimicrobial agent has also been examined (Wannissorn et al., 2005). Riyazi et al. (2015) reported that an increased level of basil essential oil led to fewer *E. coli* colonies and boosted the number of lactobacillus colonies when this oil was used as a broiler feed additive. The prior studies had shown that linalool is the main active agent responsible for antibacterial activity (Abbas et al., 2017; Moghaddam et al., 2011; Politeo et al., 2007).

The fundamental target of this study is to improve the quality of UF-soft buffalo cheese and limit the microbial growth by using basil essential oil. Evaluating the resultant UF-soft cheese from microbiological, chemical and sensory aspects through 60 days of cold storage were also a considerable aim.

MATERIALS AND METHODS

Materials

- Full fat buffalo milk retentate (TS 33.67%, fat 12%) was obtained from Animal Production Research Institute, Ministry of Agriculture, Giza, Egypt.
- Calf rennet powder (HALA) and starter cultures (*Lactococcus lactis* ssp. *Lactis* and *Lactococcus lactis* ssp. *Cremoris* 1:1) were obtained from Chr. Hansen's Lab., A/S Copenhagen; Denmark.
- Basil oil was extracted and identified in the laboratory using GC-MC by Dr. Reham M. Sabry; Department of Medicinal and Aromatic Plants Research, National Research Centre, Giza, Egypt. The first major oil constituent was linalool with a mean relative percentage of 37.18. The second main compound was 1.8 cineole with 16.4% followed by α -bergamotene (14.71%) as well as the fourth and fifth compounds, Υ cadinol and α -cadinol, which had mean relative percentages of 6.82 and 3.53 respectively (Reham et al., 2016).
- **Media:** Plate count agar (PCA), M17 agar and potato dextrose agar (PDA) were obtained from (Hi-MEDIA laboratories Pvt. Limited, A-406 Bhaveshwar plaza, Mumbai-400086, India).

Methods

UF-soft cheese process. UF-soft buffalo cheese samples were manufactured using the method of Renner and Abd El-Salam (1991): milk retentate \rightarrow heat treatment (75°C/10 min) then cooled to 38°C \rightarrow starter culture (2%) left to stand for 30 min \rightarrow then added salt (2%) and calf rennet \rightarrow classified to four portions to add Basil essential oil \rightarrow packaging and left at 38°C till coagulum formed \rightarrow stored at 5°C for 60 days.

Basil essential oil was added to the cultured milk samples at three different ratios (0.025, 0.050 and 0.075% v/v) served as T_1 , T_2 and T_3 respectively. Another sample without any oil served as the control (C). The resultant cheese samples were stored at 5 \pm 2°C and periodically analyzed for fresh, 15, 30 and 60 days, as recommended by Egyptian standards of UF-soft cheese.

Microbiological examination. Total bacterial counts of all cheese samples were determined on plate count agar and incubated at 37°C for 24 hrs. Starter cultures

were also counted on M17 agar and incubated at 30°C for 24 hrs, while mold and yeasts were detected using Potato Dextrose Agar (PDA) and incubated at 25°C for 5 days.

Analytical procedures. Total solids (TS) and soluble nitrogen (SN) contents of cheese samples were determined according to AOAC (2012). The pH values were measured using a digital pH meter (HANNA, Instrument, Portugal) with a glass electrode. The spectrophotometric method of Vakaleris and Price (1959) was used to measure the tyrosine content in cheese samples. Total volatile fatty acids (TVFAs) were also determined as described by Kosikowski (1986).

Sensory evaluation. The sensory properties of the resultant cheese samples were evaluated periodically by regular-score panelists when fresh and after 15, 30 and 60 days of cold storage for flavor (50 points), body and texture (40 points) and appearance (10 points).

Statistical analysis was performed by using the User's Guide of SAS (2004), and a probability of $p < 0.05$ was chosen to test significance.

RESULTS

Microbiological examination

The effect of adding basil essential oil (0.025, 0.050 and 0.075% v/v) on starter culture, total bacterial count, mold and yeasts in UF buffalo cheese samples during storage periods (fresh, 15, 30 and 60 days) was studied. Figure 1a illustrated that the presence of 0.025% of basil essential oil in cheese samples led to an increased number of starter culture bacteria during 15 and 30 days of storage periods (8.08 and 8.4 log cfu/g respectively). This increase was not significant ($p > 0.05$) if compared with the control. In contrast, using 0.050% and 0.075% significantly ($p < 0.05$) affected the growth of starter culture bacteria, particularly after 15 days of storage (8.5 log cfu/g) against the control (Fig. 1a).

Generally, at the end of the storage period (60 days), starter culture bacteria decreased. The same results were observed when determining the total bacterial count (Fig. 1b). The greatest increment of the total bacterial count was after 15 days of storage (8.7 log cfu/g) for

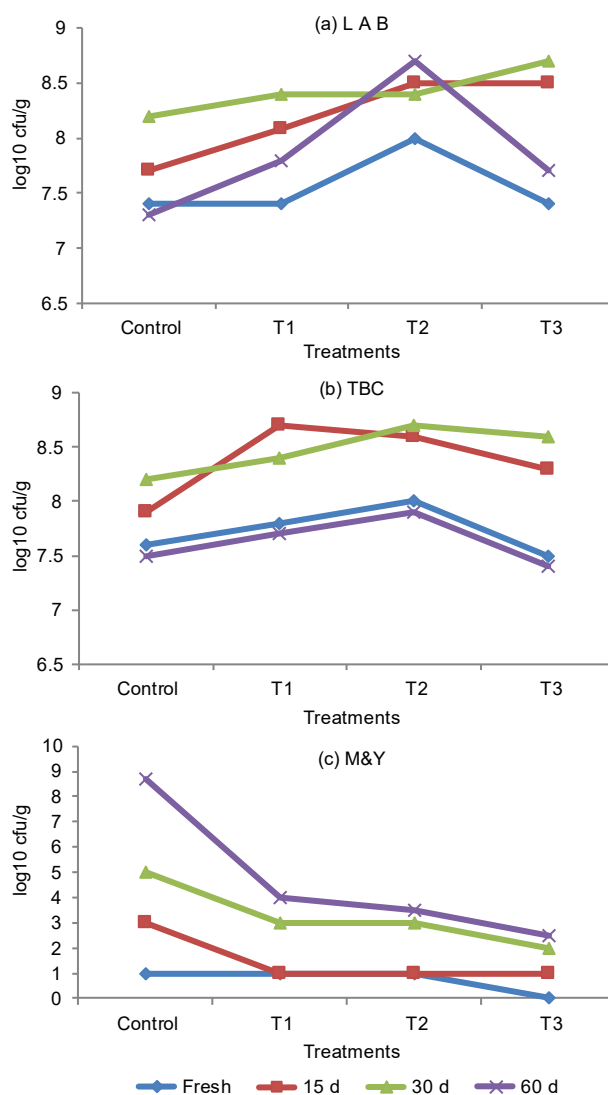


Fig. 1. Microbiological examination of UF-soft buffalo cheese samples fortified with E-BO when fresh and after cold storage period: T_1 – 0.025% E-BO, T_2 – 0.050% E-BO, T_3 – 0.075% E-BO; a – lactic acid bacteria counts (LAB), b – total bacterial counts (TBC), c – mold and yeast counts (M&Y)

cheese samples with 0.025% ratio (Fig. 1b), while it was 8.7 and 8.6 log cfu/g for T_2 and T_3 respectively after 30 days of storage (Fig. 1b).

Cheese samples containing 0.025, 0.050 and 0.075% E-BO had lower counts of molds and yeasts compared to control samples (Fig. 1c).

Chemical analysis

Total solid (TS) contents. Total solid contents of all cheese samples are shown in Table 1. It was observed that control samples tended to increase significantly ($p < 0.05$) during the storage period, especially after 30 days. The opposite trend was observed in treated samples (T_1 , T_2 and T_3). Treated sample T_3 had significantly ($p < 0.05$) lower total solid contents than other treatments (T_1 and T_2).

Table 1. Total solid contents of UF-soft buffalo cheese samples fortified with E-BO when fresh and after cold storage period

Cold storage period, days	Control	T_1	T_2	T_3
Fresh	33.60 ^{Ac}	33.65 ^{Aa}	33.63 ^{Aa}	33.60 ^{Aa}
15	34.21 ^{Acb}	32.59 ^{Bb}	32.27 ^{Bb}	32.02 ^{Bb}
30	34.99 ^{Aab}	32.21 ^{Bc}	31.303 ^{Cc}	30.04 ^{Dc}
60	35.30 ^{Aa}	30.35 ^{Bd}	29.90 ^{Bd}	29.16 ^{Cd}

E-BO – extracted basil oil. T_1 – 0.025% E-BO, T_2 – 0.050% E-BO, T_3 – 0.075% E-BO.

Small letters (a–d) between storage periods. Capital letters (A–D) between treatments. Significant ($p < 0.05$).

pH values. Using basil essential oil had a clear effect on pH values of cheese samples. A significant reduction in pH values ($p < 0.05$) was observed in all treated samples through cold storage when compared with control samples as shown in Table 2. The influence

Table 2. pH values of UF-soft buffalo cheese samples fortified with E-BO when fresh and after cold storage period

Cold storage period, days	Control	T_1	T_2	T_3
Fresh	6.48 ^{Aa}	6.46 ^{Aa}	6.45 ^{Aa}	6.44 ^{Aa}
15	6.09 ^{Aa}	5.21 ^{Bb}	5.04 ^{Bb}	4.96 ^{Bb}
30	5.36 ^{Ab}	5.11 ^{Bb}	4.78 ^{Cc}	4.51 ^{Dc}
60	4.75 ^{Ac}	4.50 ^{ABc}	4.28 ^{ABd}	4.12 ^{Bd}

E-BO – extracted basil oil. T_1 – 0.025% E-BO, T_2 – 0.050% E-BO, T_3 – 0.075% E-BO.

Small letters (a–d) between storage periods. Capital letters (A–D) between treatments. Significant ($p < 0.05$).

of adding oil in T_2 and T_3 was more pronounced than in T_1 samples.

Soluble nitrogen contents. Importantly, there was a significant increase ($p < 0.05$) in soluble nitrogen contents in both control and treated UF-soft buffalo cheese samples during cold storage (Table 3). This augmentation was more pronounced in treated samples than control samples. Data presented in the same Table reflect a significant increase ($p < 0.05$) in the tyrosine contents of all UF-soft buffalo cheese samples during cold storage. This rise was obvious after 30 days of storage till the end of the experiment. The difference between treated samples was clear and significant after 30 days in T_3 , T_2 and T_1 respectively.

Table 3 also shows the content of TVFAs of all cheese samples. A significant ($p < 0.05$) increase

Table 3. Soluble nitrogen (SN), tyrosine and TVFAs contents of UF-soft buffalo cheese samples fortified with E-BO when fresh and after cold storage period

Treatments	Cold storage period, days	SN %	Tyrosine mg/100 g	TVFAs (0.1 N NaOH/100 g)
Control	fresh	0.22 ^{Ac}	37.08 ^{Ac}	14.5 ^{Bd}
	15	0.24 ^{Bc}	38.02 ^{Cc}	16.2 ^{Cc}
	30	0.27 ^{Cb}	40.90 ^{Db}	20.9 ^{Db}
	60	0.31 ^{Ca}	42.58 ^{Da}	30.0 ^{Ca}
T_1	fresh	0.24 ^{Ac}	39.34 ^{Ac}	14.8 ^{Bd}
	15	0.29 ^{Bc}	42.29 ^{Bc}	20.05 ^{Bc}
	30	0.35 ^{Bb}	45.87 ^{Cb}	25.3 ^{Cb}
	60	0.45 ^{Ba}	50.83 ^{Ca}	36.0 ^{Ba}
T_2	fresh	0.25 ^{Ac}	41.66 ^{Ac}	15.09 ^{Bd}
	15	0.38 ^{Ab}	44.95 ^{Ac}	25.0 ^{Ac}
	30	0.49 ^{Aa}	49.09 ^{Bb}	30.11 ^{Bb}
	60	0.55 ^{Aa}	56.00 ^{Ba}	38.02 ^{Ba}
T_3	fresh	0.26 ^{Ad}	41.69 ^{Ac}	15.90 ^{Ad}
	15	0.40 ^{Ac}	45.19 ^{Ac}	27.01 ^{Ac}
	30	0.52 ^{Ab}	53.90 ^{Ab}	35.07 ^{Ab}
	60	0.60 ^{Aa}	60.79 ^{Aa}	42.08 ^{Aa}

E-BO – extracted basil oil. T_1 – 0.025% E-BO, T_2 – 0.050% E-BO, T_3 – 0.075% E-BO.

Small letters (a–d) between storage periods. Capital letters (A–D) between treatments. Significant ($p < 0.05$).

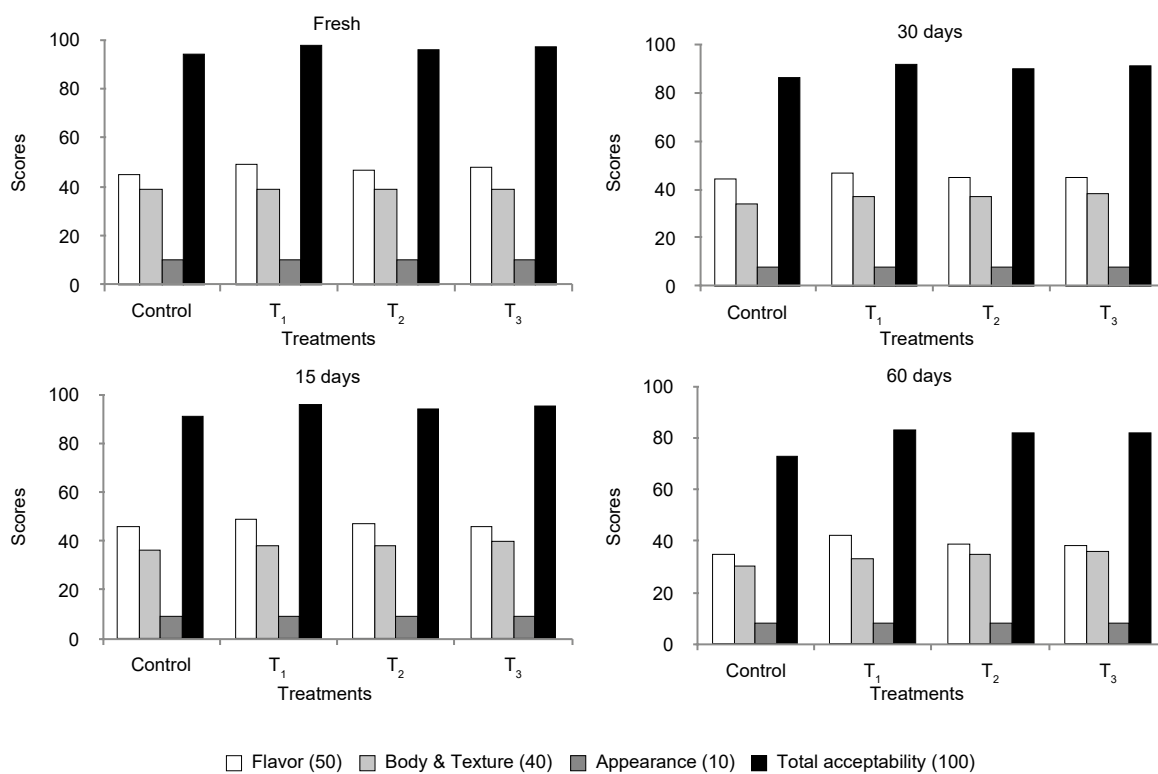


Fig. 2. Sensory evaluation of UF-soft buffalo cheese samples fortified with E-BO when fresh and after cold storage period: T_1 – 0.025% E-BO, T_2 – 0.050% E-BO, T_3 – 0.075% E-BO

in TVFAs values was evident in all treated samples kept fresh until the end of the storage period. The addition of basil essential oil had a clear effect on TVFAs values when compared to the control samples. Comparing the treated samples with each other revealed a remarkable increase in T_3 and T_2 values over those of T_1 .

Sensory evaluation. Sensory evaluation of UF-soft buffalo cheese samples is shown in Figure 2, when fresh and after 15, 30 and 60 days of cold storage. At the beginning of the trial there were no significant differences ($p > 0.05$) between all samples in appearance and body and texture values. During the storage period, the results showed that E-BO had a positive effect on the body and texture of cheese samples, where they were softer than control samples, especially after storage. Treated sample T_3 had higher scores for body and texture followed by T_2 then T_1 . Also, the data showed a significant ($p < 0.05$) influence of E-BO on the flavor of treated-UF cheese samples; as

was expected of this oil, since it is a flavoring agent. The control cheese samples had the minimum flavor scores, while the treated ones had higher scores. It was observed that T_1 were the most favorable of all the treated samples.

On the other hand, no significant difference ($p > 0.05$) was recognized in the appearance of UF-soft cheese in control or treated samples during storage.

DISCUSSION

The addition of basil essential oil (E-BO) to UF-soft buffalo cheese at three ratios (0.025, 0.050 and 0.075%) caused an enhancement of starter cultures. Both high concentrations of 0.05 and 0.075% significantly ($p < 0.05$) affected the growth of starter culture (Lactic Acid Bacteria). At the same time, the three ratios inhibited mold and yeast contents. On the other hand, it was noticed that there was no significant increase ($p > 0.05$) in starter culture bacteria at the end of

the storage period (60 day) in both samples containing 0.050% and 0.075% essential oil. In fact, the inhibitory effect of E-BO might be due to the constituents of this essential oil. Essential oils (EOs) have several targets in the cell, such as degradation of the cell wall and weakening of the membrane, causing enhanced permeability, leading to the loss of intracellular components. EOs, such as juniper, lemon, marjoram, clary sage, basil, ginger or lemon balm, containing non-phenolic main compounds have also been found to show high toxicity against yeasts (Tserennadmid et al., 2011). Many researchers have demonstrated that basil essential oil is rich in chemical compounds such as linalool, estragole, methyl eugenol and methyl chavicol (Ababutain, 2017; Joshi, 2014). Other studies have confirmed that the inhibitory effect of basil is due to the presence of linalool. Eugenol was also identified as an active compound that can inhibit microorganisms (Ababutain, 2017; Araújo et al., 2016). Several studies suggested that basil can be used as a natural food preservative to reduce microbial growth (Gyawali and Ibrahim, 2014; Ko et al., 2015; Riyazi et al., 2015). Also, Akarca et al. (2016) found that the Lactococcus content of mozzarella cheese samples supplemented with different spices (thyme, mint, cumin, bay and basil) clearly decreased during the storage period.

The presented data agreed with Holley and Patel (2005), Rodríguez et al. (2009) and Angienda and Hill (2012), who reported that lactic acid bacteria can be more resistant to the cytotoxic effects of essential oils because lactic acid bacteria exist and grow on phenol containing plants, and thus have adapted in order to successfully colonize such antagonistic substrates and also have been shown to have stimulated growth in the presence of some oils. Also, Jamroz et al. (2005) suggested that an increased level of basil essential oil had reduced the number of *E. coli* colonies and increased the number of *Lactobacillus* colonies. Ouwehand et al. (2010), mentioned that the growth of *Lactobacillus fermentum* and *Lactobacillus reuteri* increased when using some essential oil up to 500 mg/l due to the presence of carvacrol and thymol.

However, a number of studies have reported that basil essential oil can completely inhibit the growth of 22 species of molds, including the aflatoxigenic strains *Aspergillus parasiticus* and *A. flavus* at concentrations of 1.5 mL/L, and also the growth of *Candida albicans*

and *A. flavus* were completely inhibited at concentrations of 5000 ppm during 7 days of incubation (Sani and Shojaei, 2015). However, Soliman and Badeaa (2002) found that basil oil acts as a fungistatic agent against *F. verticillioides* at a concentration of 2000 ppm, and as a fungicide agent at a concentration of 3000 ppm. The results which were presented by Fandohan et al. (2004) also showed a complete growth inhibition of *F. verticillioides* at concentrations higher than 2.7 µL/ml.

An increase of the total solids content in control samples is due to the effect of storage and loss of water. On the other hand, adding basil essential oil prevented water draining which made treated cheese samples softer than control ones. This data is in agreement with El-Din et al. (2010) and Sahar et al. (2013).

The reduction in pH values in treated samples means an increase in acidity. The addition of basil essential oil to UF-soft cheese caused two remarkable results: an enhancement in starter culture activity and inhibition of undesirable microbes. Consequently, the quality of the UF-soft cheese was improved. This data was confirmed by the microbiological analysis (Fig. 1). The same trend was recognized by El-Din et al. (2010) and Sahar et al. (2013).

On the other hand, the increase in soluble nitrogen (SN), tyrosine and total volatile fatty acids (TVFAs) values was the result of an enhancement in starter culture activity, as also shown in the microbiological data. This increase also referred to the proteolysis of cheese protein which leads to the production of more tyrosine. This data was in accordance with Al. Otaibi and El. Demerdash (2008). Similar results were obtained by Zaky et al. (2013), who stated that the addition of dill and caraway essential oils caused more TVFA contents than in control samples.

Sensory evaluation is an important aspect of the consumer's tendency to buy a specific product. It is also the main indicator of the success of any new product. The addition of basil essential oil to UF-soft buffalo cheese positively affected the body & texture and flavor values of all cheese samples where treated samples had a softer body & texture, and a refreshing and acceptable flavor. Zaky et al. (2013) and Abbas et al. (2017) confirmed these results

The panelists stated that basil essential oil highly enhanced the flavor of the cheese and gave a good acceptability as well as a softer body & texture.

CONCLUSION

Using basil essential oil with levels of 0.050 and 0.075% improved the quality of UF-soft buffalo cheese by enhancing culture activity. Increasing culture viability lead to increase in the proteolytic and lypolytic rates and produced more TVFAs, SN and tyrosine. Consequently, the quality of UF-buffalo soft cheese was enhanced. Adding E-BO markedly improved the plain taste of this kind of cheese. In general, extracted basil essential oil can be used as an antifungal agent to extend the shelf life of UF-soft cheese.

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