Effect of Spearmint Essential Oil on Chemical Composition and Sensory Properties of White Cheese

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Abstract: Spearmint (*Mentha spicata*) the most common herb in the Mediterranean region, widely used as a source of essential oil for flavoring, spearmint essential oil contains about 24 constituents representing 98.45 % of the total essential oil; the main compounds are carvone (68.58%) and limonene (16.42 %). Two lipid model systems (DPPH scavenging activity and β-carotene bleaching test) were used to determine the antioxidant activity of spearmint essential oil. White cheeses with different concentrations of spearmint essential oil (0.5 to 2.5 ml/kg retentate) were prepared and stored at (7 ^{0}C ± 2) for 5 weeks. The chemical composition and ripening index of spearmint white cheese were determined. Obtained results showed that lower concentrations of spearmint essential oil increased titratable acidity values significantly, while ripening index was increased significantly by increasing the concentration of essential oil. Prolonging the cold storage period for five weeks increased these values significantly. Panel study showed that lower concentration of essential oil got the highest total acceptability scores. [Journal of American Science 2010; 6(5):272-279]. (ISSN: 1545-1003).

Key words: white cheese, spearmint, essential oil, sensory evaluation, antioxidant activity, ripening index

1. Introduction

Spearmint is an herbal plant that is found abundantly in the Mediterranean region. Its byproduct, spearmint oil, provides plenty of health benefits. Obtained from the spearmint leaves, this oil is well known for its medicinal properties. This minty oil is used to treat a variety of ailments. Spearmint oil is loaded with nutrients such as vitamin A and vitamin C, and has a sweet taste. The characteristic feature of this oil is its refreshing fragrance that provides therapeutic benefits when inhaled. There are numerous health benefits of spearmint essential oil such as it can reduce fever, provide relief from depression and asthma (El-Moghazy, 2008).

Recently, there is a growing interest in substances exhibiting antimicrobial and antioxidant properties that are supplied to human and animal organisms as food components or as specific pharmaceutics. It has been well-known that essential oils have antimicrobial and antioxidant effects (Özer *et al.,* 2007). In addition to, common need is availability of natural extracts with a pleasant taste or smell combined with a preservative action, aimed to avoid lipid deterioration, oxidation and spoilage by microorganisms (Sacchetti *et al.,* 2005). The most common herb in the Mediterranean region is spearmint (*Mentha spicata*), widely used as a source of essential oils for flavoring and recently has been used as a valuable source of the potent antioxidant for the nutraceuticals and cosmetic industries (Wang and Weller, 2006). Proteolysis and lipolysis are the main biochemical reaction in the development of flavour in cheese during the ripening. Exogeneous and endogeneous enzymes of microflora contribute to cheese proteolysis and lipolysis during processing and ripening (Cinbas and Kilic, 2006). Lipolysis is an important biochemical event occurring during cheese ripening and varied from cheese type to another and Free Fatty Acids (FFA) are important precursors of catabolic reactions, while produce compounds that are volatile and contribute to flavor (McSweeney and Sousa, 2000). Spearmint essential oil was analyzed previously by the authors, results showed that it contained about 24 constituents were identified representing 98.45 % of the total essential oil; the main compounds, which identified by GC-MS spectrometer, were carvone (68.58%) and limonene (16.42 %) (Foda *et al.,* 2009 b).

The objectives of the present study were: evaluation of spearmint essential oil, study its effect on the chemical composition and consumers acceptability when applied to white cheese.

2. Material and Methods

Dried spearmint (*Mintha spicata* L.) was obtained from Medicinal and Aromatic Plant Research Dept., Agriculture Research Center, Giza, Egypt. Buffalo's milk retentate was purchased from Dairy Industry Unit, Animal Production Research Institute, Ministry of Agriculture, Cairo, Egypt. The milk retentate contained 64.0 % moisture content; 15.5 % fat; 10.5% total protein and 0.09% titrable acidity. Microbial rennet (*Mucor mehiei)* was obtained from Novo, Denmark.

2. 1 Extraction of the essential oil

Essential oil was extracted according to Tepe *et al.*, (2005), by submitting dried spearmint to water distillation for 3 hrs using a Clevengar-type apparatus. The obtained essential oil was dried over anhydrous sodium sulphate and after filtration stored at 4°C for further analyzed. The yield of the essential oil was determined.

2.2 Evaluation of antioxidant activity:

Free radical scavenging activity was measured by 2,2-diphenyl-1 picryl-hydrazil (DPPH) according to the method which described by Tepe *et al.,* (2005). Essential oil concentration providing 50% inhibition (IC_{50}) was calculated using the graph by plotting inhibition percentage against extract concentration. Total phenolic compounds were determined with Folin-Cioalteu reagent according to Slinkard and Singleton (1977), the results were expressed as milligrams of gallic acid equivalent/g of dry extract. β–Carotene bleaching test was used according to Miller *et al.,* (1993), with modifications of Wanasundara *et al.,* (1994). Synthetic antioxidant reagent butylated hydroxy anisole (BHA) was used as a positive control. All determination was carried out in triplicate.

2.3 Cheese making:

White cheese was prepared according to Foda *et al.,* (2008). Milk retentate was divided into 7 portions; each portion was salted to a concentration of 3 %, well mixed and pasteurized at 73 °C for 15 sec. First portion was served as control and for the other six portions, different concentrations of spearmint essential oil (0.5, 0.75, 1.0, 1.5, 2.0, and 2.5 ml / kg retentate} were added at 40°C to prepare cheese treatments A, B, C, D, E and F respectively. Curds were hold at the same temperature after adding the rennet, cheeses samples were taken fresh and every week during stored under refrigerator temperature (5°C±1) for 5 weeks. Three replicates were prepared for each cheese to determine their chemical composition and organoleptic properties.

2.4 Chemical analysis

Cheese samples were analyzed for moisture and fat contents and titrable acidity according to Ling, (1963). Total nitrogen (TN %) was determined using Kjeldahl method according to AOAC, (2000). Protein content was obtained by multiplying the percentage of total nitrogen by 6.38. Water-soluble nitrogen (WSN) was extracted, Trichloroacetic acid soluble nitrogen (TCA-SN) and phosphotungstic acid soluble nitrogen (PTA-SN) were determined according to the method which described by Coskun and Tuncturk (2002). Ripening index (%) of white cheese was calculated according to the equation:

$$\text{Ripening index (\%)} = \frac{WSN}{TN} \times 100$$

Total volatile free fatty acids (TVFFA) were determined according to Kosikowski, (1978).

2.5 Sensory analysis:

Fifteen panelists (7 male and 8 female, aged between 25 and 45 years) who have experience with white cheese and regularly used its descriptive vocabulary, were participated. They scored the cheeses for appearance & color (20 points), body & texture (20 points), odor ((20 points), taste (20 points), and overall acceptability (20 points). Panel members were also instructed to report any defects or unpleasant flavor. Water and no salted crackers were provided to clean their palates between tasting.

2.6 Statistical analysis

Statistical analysis of experimental data was performed by analysis of variance (ANOVA) producers using SAS PROC GLM / STAT (1998). Differences among means were identified using Duncan multiple range test.

3. Results and Discussion

Yield of spearmint essential oil using Hydrodistillation was 2.0 (%). Edris *et al*., (2003) mentioned that yield of spearmint essential oil was varied from 1.28 – 3.9 % depending on cultivation locations.

3.1 Antioxidant properties

DPPH scavenging activity (%) of different concentrations of spearmint essential oil is shown in Table (1). It could be noticed that spearmint radical scavenging was increased significantly (P> 0.05) with increasing the essential oil concentrations from 5 to 25 μl/ml and maximum inhibition reached 71.07 %. The concentration of spearmint essential oil which provide 50% inhibition of DPPH (IC_{50}) was 10 780 μg/ml, which indicated that spearmint essential oil has very low inhibition effect compared with the synthetic antioxidant reagent (BHA). Also, total phenolic content in spearmint essential oil was very low (1.04 ± 0.02) mg (gallic acid/ g) dry extract.

Antioxidant activity (%) of spearmint essential oil was determined at intervals of incubation time by β-carotene test compared with BHA is shown in Table (2). It can be seen that spearmint essential oil did not exhibit any antioxidant activity at the concentrations of 50 or 100 μg/ml during the experimental period (100 min), only the concentration of 200 µg/ml showed 1.04 % inhibition of the linoleic acid oxidation. This study showed that spearmint essential oil had poor free radical scavenging compared with the synthetic antioxidant BHA, while previous antioxidant activity results showed that spearmint water and ethanolic extracts ranged from 59 – 62 % and 70 – 37 % respectively with 200 µg/ml during the same incubation period 20 - 100 min. (Foda *et al.,* 2009a). Ollanketo *et al.,* (2002) reported that the antioxidative performance of plant extracts depends not only on the extraction method, but also on the quality of the original plant, its geographic origin, the harvesting date, its storage and processing prior to extraction.

3.2 Effect of spearmint essential oil on chemical composition of white cheese

The changes of chemical composition of control and white cheese with different concentrations of spearmint essential oil (0.5, 0.75, 1.0, 1.5, 2.0, and 2.5 ml / kg retentate) which ordered as treatment A, B, C, D, E, and D respectively, are shown in Table (3). It could be noticed that although there is revealed variations in moisture content in white cheese made by essential oil compare to control samples, increasing the essential oil concentrations did not affect the moisture content significantly. While, significant (P<0.05) differences were appeared after two weeks of cold storage compared with fresh cheese. Increasing the concentrations of essential oil increased the protein content of white cheese significantly (P<0.05). Also, protein content increased significantly after 4 weeks of cold storage.

It could be noticed that essential oil has no significant effect on cheese fat content, while prolonging the cold storage affected this content significantly. Spearmint essential oil had no significant effect on the moisture of white cheese; this contradicts earlier findings by Ayar, (2002) while the results of fat and protein content were matched by his finding. Juven *et al.,* (1994) and Ultee *et al.,* (1998) reported that protein content in the food may also have an influencing factor in the effectiveness of the essential oils, bovine serum albumin neutralized the antimicrobial action of thymol (a major component of oil of thyme), and form a complex between phenolic components and proteins or other components of cell envelope especially the cell membrane which is widely regarded as one of the primary target sites for plant essential oil. Titratable acidity values (TA) in Table (3) shows that lower concentration of essential oil (0.5, 0.75 or 1.0 ml) caused significant increasing (P<0.05) in acidity values compare with control cheese. While higher oil concentrations had no effect on titratable acidity values (TA) this could be due to its antimicrobial activity (Foda *et al.,* 2009b). Similar results were observed in Feta cheese by Vafopoulou, (1989) and Ayar (2002) reported that pH and acidity values varied due to moisture and microbial activity of cheese samples. Cold storage increased the titratable acidity values significantly (P<0.05).

3.3 Effect of spearmint essential oil on biochemical characters of white cheese

Water Soluble Nitrogen (WSN) fraction is commonly used in cheese as an index of ripening (Lopez-Fandino *et al.,* 1994), it contains small & medium sized peptides and free amino acids separated from proteins and large peptides mainly as a result of the activity of chymosin and a lesser extent plasmin (McSweeney and Fox, 1997). Figure 1(a-c) shows the effect of different concentrations of spearmint essential oil on the ripening index of white cheese. It could be noticed that the WSN/TN ratio was increased by increasing the concentrations of spearmint essential oil. Higher concentrations (≤1.0 ml) increased this ratio significantly, while lower concentration 0.5 ml caused significant reduction (P<0.05) compared with control sample (Table-4). Prolonging the cold storage more than two weeks increased the WSN/TN ratio significantly (P<0.05). Also, increasing the essential oil concentrations increased the trichloroacitic acid soluble nitrogen (TCA-SN**),** and higher concentration (≤1.5 ml) caused significant increase compared with control cheese. TCA-SN values increased significantly (P< 0.05) only after 4 weeks of cold storage.

Fig. (1-c) shows effect of essential oil on the change of phosphotungestic acid soluble fraction (PTA-SN) in fresh cheese and during the cold storage period for 5 weeks. It could be noticed that lower concentration of essential oil (≥ 1.0 ml) caused significant decrease in PTA-SN, while higher concentration (≤1.5 ml) increased these values significantly (P< 0.05) compared with control sample (Table- 4). Yvon *et al.,* (1989) reported that trichloroacitic acid soluble nitrogen (TCA-SN**)** expresses small molecules of peptides (lower than 20 amino acid residue) and free amino acid its levels regarded to ripening depth index. Also, the phosphotungestic acid soluble fraction (PTA-SN) contains tri-, di,- peptides and free amino acids are soluble state (Fialaire and Postaire, 1994). The changes of total volatile free fatty acids (TVFFA) in control and spearmint cheeses are shown in Fig.2. Adding essential oil with any concentrations affected the TVFFA values significantly (P<0.05) compared with control sample (Table-4). Moreover, increasing essential oil concentration, more than 1.0 m/kg retentate, increased these values significantly. Data indicated also, that prolonging the cold storage for 5 weeks increased (TVFFA) values significantly compared with fresh cheese. These results in agreement with those obtained by Abdel-Salam *et al.,* (1993) who reported that most of the changes in (TVFFA) occur during the first 15-30 days of storage which coincide with maximum bacterial growth and high concentration of total volatile free fatty acids in cheese would contribute significantly to the total acidity.

3.4 Sensory analysis:

Effect of different concentrations of spearmint essential oil on the organoleptic properties of white cheese during cold storage for 5 weeks is shown in Table (5). Increasing spearmint essential oil from 0.5 to 2.5 ml/ kg retentate did not affect the appearance of white cheese during cold storage, while after 4 weeks body & texture scores decreased significantly (P<0.05). Cheese odor, taste and overall acceptability scores decreased significantly (P<0.05) by higher concentration of essential oil (≤ 1.5 ml), while prolonging the cold storage had no significant effect. Similar results were obtained by Ayar, (2002) and Hussein (2004).

Table (1): DPPH scavenging activity (%) by different concentrations of spearmint essential oil

Spearmint essential oil (µl/ml)	DPPH scavenging activity (%)
5	32.5 ± 1.9 [d]
10	46.4 ± 0.29 [c]
15	53.4 ± 0.4 [c]
20	59.6 ± 0.87 [b]
25	71.07 ± 2.9 [a]
*IC_{50}	10 780 ± 95
BHA	5 ± 0.47
**Total phenolic	1.04 ± 0.02

Means ± Stander Deviation
Means with the same letter are not significantly different (P<0.05)
*IC_{50} values of DPPH assay as µg/ml; ** Given as mg gallic acid/g dry extract

Table (2): Antioxidant activity (%) of different concentration of essential oil by β-carotene test compared with BHA

Time (minutes)	Inhibition of linoleic acid oxidation (%)					
	BHA (µg/ml)			Essential oil (µg/ml)		
	50	100	200	50	100	200
20	90.39	93.73	97.22	0.0	0.0	0.0
40	92.19	95.02	97.25	0.0	0.0	0.0
60	92.19	95.03	97.37	0.0	0.0	0.0
80	91.85	94.58	96.70	0.0	0.0	0.0
100	92.47	94.39	96.89	0.0	0.0	1.04 ± 0.02

Table (3): Effect of spearmint essential oil on the gross chemical composition of white cheese during cold storage period.

Cheese samples*	Moisture (%)	Fat (%)	Protein (%)	Acidity
Control	68.22 ± 0.59 [BC]	15.22±0.27 [A]	10.52±0.14 [BC]	0.22±0.10 [B]
A	67.68 ±0.26 [E]	15.22 ±0.36 [A]	10.98 ±0.04 [AB]	0.26 ±0.14 [A]
B	67.16 ±1.01 [DE]	15.18 ±0.32 [A]	11.03 ±0.16 [AB]	0.28 ±0.15 [A]
C	68.35 ± 0.16 [BC]	15.15 ±0.40 [A]	11.13 ±0.16 [A]	0.28 ±0.15 [A]
D	68.60 ±0.93 [A-C]	15.18 ±0.30 [A]	10.46 ±0.34 [D]	0.21 ±0.10 [B]
E	68.11 ±0.47 [CD]	15.17 ±0.28 [A]	10.50 ±0.15 [D]	0.22 ±0.11 [B]
F	68.40 ±0.53 [BC]	15.12 ±0.29 [A]	10.57 ±0.19 [DC]	0.20 ±0.11 [B]
Storage period (weeks)**				
Fresh	68.86 ±0.58 [A]	14.75 ±0.58 [E]	10.67 ±0.28 [B]	0.12 ±0.04 [F]
1	68.91 ±0.71 [A]	15.07 ±0.71 [D]	10.61 ±0.26 [B]	0.14 ±0.03 [E]
2	68.63 ±0.82 [A]	15.15 ±0.82 [C]	10.58 ±0.28 [B]	0.19 ±0.04 [D]
3	67.94 ±0.66 [B]	15.22 ±0.66 [C]	10.61 ±0.31 [B]	0.26 ±0.06 [C]
4	67.94 ±0.53 [B]	15.35 ±0.53 [B]	10.72 ±0.33 [B]	0.31 ±0.07 [B]
5	67.62 ±0.63 [B]	15.63 ±0.63 [A]	11.03 ±0.27 [A]	0.38 ±0.06 [A]

A= 0.5 ml essential oil, B= 0. 75 ml, C= 1.0 ml, D = 1.5 ml, E = 2.0 ml, and F = 2.5 ml. Means ± Standard Division, different letters are significantly different ($P< 0.05$). *Each value represents 18 values, **Each value represents 30 values.

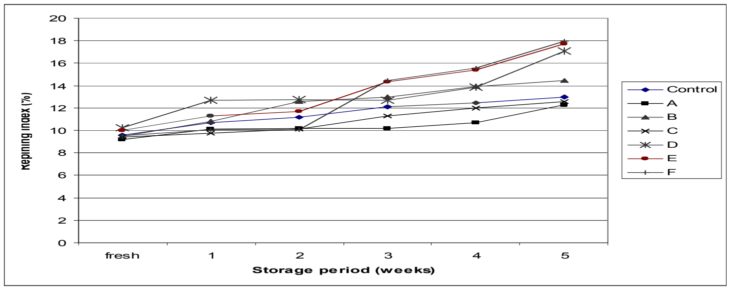
Table (4): The changes of biochemical characteristics of control and whit cheese with spearmint essential oil during cold storage

Cheese samples	WSN/TN%	TCA-TN%	PT-TN%	TVFFA
Treatment *				
Control	11.51 ± 1.26^{C}	3.44 ± 0.25^{B}	2.35 ± 0.17^{B}	2.41 ± 0.40^{D}
A	9.19 ± 0.06^{D}	3.91 ± 0.39^{AB}	1.97 ± 0.09^{C}	2.26 ± 0.19^{E}
B	12.36 ± 1.90^{B}	3.40 ± 0.30^{C}	1.89 ± 0.08^{C}	2.18 ± 0.24^{E}
C	10.86 ± 1.29^{C}	3.35 ± 0.05^{C}	1.79 ± 0.03^{C}	2.24 ± 0.74^{E}
D	13.72 ± 1.72^{A}	3.70 ± 0.20^{B}	3.12 ± 0.30^{A}	2.88 ± 0.47^{B}
E	13.57 ± 2.7^{A}	3.87 ± 0.59^{AB}	3.20 ± 0.46^{A}	2.57 ± 0.26^{C}
F	12.94 ± 3.54^{AB}	4.08 ± 0.14^{A}	3.17 ± 0.41^{A}	2.58 ± 0.32^{C}
Storage period (weeks)**				
Fresh	10.53 ± 1.34^{D}	3.56 ± 0.46^{C}	2.44 ± 0.50^{D}	2.12 ± 0.48^{F}
1	10.88 ± 1.00^{D}	3.63 ± 0.37^{BC}	2.48 ± 0.46^{CD}	2.21 ± 0.46^{E}
2	11.15 ± 1.14^{D}	3.62 ± 0.30^{BC}	$2.62\pm0.55^{B-D}$	2.37 ± 0.43^{D}
3	12.83 ± 1.66^{C}	3.65 ± 0.38^{BC}	$2.77\pm0.68^{A-C}$	2.60 ± 0.41^{C}
4	13.59 ± 1.94^{B}	3.84 ± 0.43^{AB}	2.88 ± 0.78^{AB}	2.81 ± 0.43^{B}
5	15.25 ± 2.88^{A}	3.96 ± 0.41^{A}	3.02 ± 0.89^{A}	3.05 ± 0.40^{A}

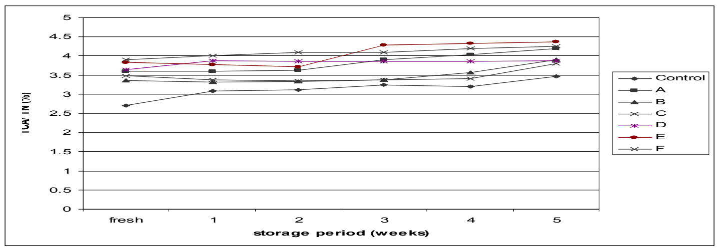
Table (5): Effect of spearmint essential oil on some organoleptic properties of white cheese during cold storage

Cheese samples	Organoleptic properties of white cheese				
	Appearance & color (20)	Body &Texture (20)	Odor (20)	Taste (20)	Overall acceptability (20)
Treatment *					
Control	19.06 ± 0.76^{A}	18.61 ± 0.35^{A}	18.15 ± 1.34^{AB}	17.72 ± 0.99^{AB}	18.59 ± 1.23^{A}
A	18.89 ± 0.62^{A}	18.65 ± 0.53^{A}	18.54 ± 0.68^{A}	18.72 ± 0.86^{A}	18.72 ± 0.76^{A}
B	19.00 ± 0.39^{A}	18.78 ± 0.21^{A}	18.46 ± 0.31^{A}	18.15 ± 0.38^{AB}	18.59 ± 0.41^{A}
C	19.09 ± 0.21^{A}	18.46 ± 0.51^{A}	17.85 ± 0.76^{AB}	17.52 ± 1.25^{B}	18.00 ± 0.96^{A}
D	19.07 ± 0.67^{A}	18.30 ± 0.45^{A}	16.20 ± 0.76^{C}	14.02 ± 1.25^{D}	14.37 ± 1.04^{C}
E	19.00 ± 0.80^{A}	18.20 ± 0.52^{A}	15.30 ± 0.93^{C}	12.72 ± 1.00^{E}	12.81 ± 1.39^{D}
F	19.07 ± 0.68^{A}	18.37 ± 0.52^{A}	14.28 ± 0.86^{D}	11.85 ± 1.27^{E}	11.67 ± 0.94^{E}
Storage period (weeks)**					
Fresh	17.50 ± 3.65^{A}	18.31 ± 0.79^{A}	17.07 ± 1.49^{AB}	16.08 ± 2.72^{A}	16.04 ± 2.90^{A}
1	17.41 ± 3.36^{A}	18.24 ± 0.73^{A}	$16.84\pm1.75^{A-C}$	16.27 ± 2.22^{A}	16.19 ± 2.89^{A}
2	17.24 ± 3.49^{A}	18.12 ± 0.64^{A}	$16.81\pm1.72^{A-C}$	16.06 ± 2.43^{A}	15.93 ± 2.64^{AB}
3	17.58 ± 3.06^{A}	18.20 ± 1.04^{A}	17.48 ± 1.38^{A}	16.29 ± 1.81^{A}	16.43 ± 1.95^{A}
4	17.32 ± 1.70^{A}	17.74 ± 0.76^{AB}	16.14 ± 1.76^{C}	15.40 ± 2.68^{AB}	15.69 ± 2.26^{AB}
5	17.50 ± 1.85^{A}	17.43 ± 1.00^{B}	16.58 ± 1.62^{BC}	15.02 ± 3.16^{B}	15.14 ± 3.05^{B}

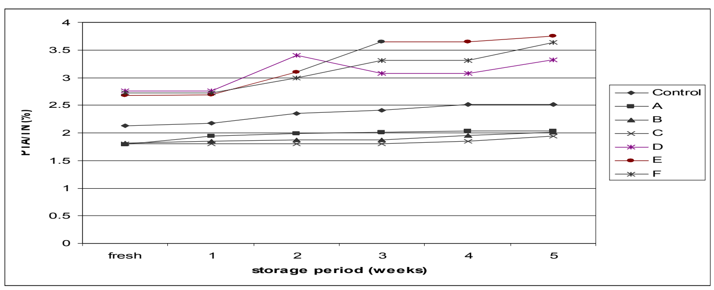
A= 0.5 ml essential oil, B= 0. 75 ml, C= 1.0 ml, D = 1.5 ml, E = 2.0 ml, and F = 2.5 ml.
TVFFA = (Total Volatile Free Fatty Acids) 0.1N of Na OH /10g cheese. Means ± Standard Division, different letters are significantly different ($P< 0.05$). *Each value represents 18 values, **Each value represents 30 values.
A= 0.5 ml essential oil, B= 0. 75 ml, C= 1.0 ml, D = 1.5 ml, E = 2.0 ml, and F = 2.5 ml. Means ± Standard Division Different letters are significantly different ($p< 0.05$). *Each value represents 18 values,
** Each value represents 30 values.



(a) Water-soluble nitrogen (WSN/TN)



(b) Trichloroacetic acid-soluble (TCA-SN)



(c) Phosphotungstic acid-soluble nitrogen (PTA-SN)

Fig. 1: Effect of spearmint essential oil on biochemical characters of white cheese during cold storage 5 weeks. A = 0.5 ml essential oil, B= 0.75 ml, C= 1.0 ml, D= 1.5 ml, E= 2.0 ml, F= 2.5 ml.

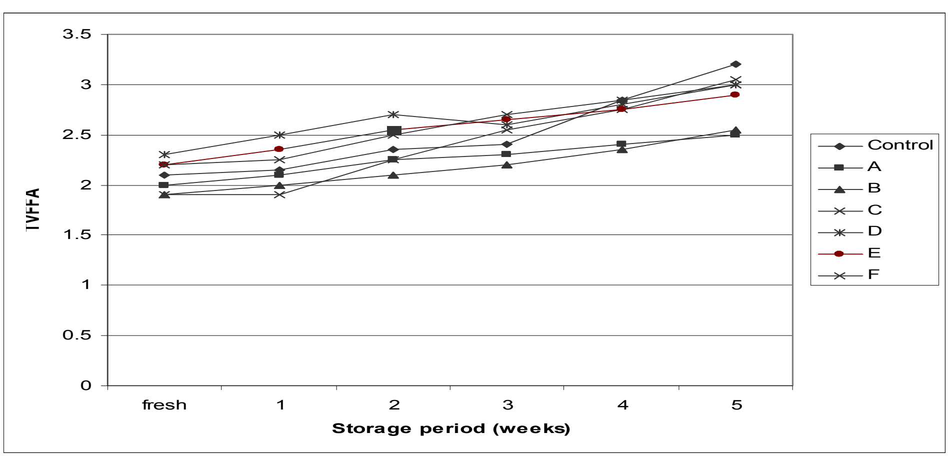


Fig. (2): Effect of spearmint essential oil on Total Volatile Free Fatty Acids (TVFFA) of white cheese during cold storage 5 weeks. A = 0.5 ml essential oil, B= 0.75 ml, C= 1.0 ml, D= 1.5 ml, E= 2.0 ml, F= 2.5 ml.

4 Conclusions

Lower concentration of spearmint essential oil would be recommended for white cheese because the use of spearmint essential oil at high concentration, required to be effective in cheese quality, could raise concerns regarding changes in the organoleptic properties. A number of options can be considered to overcome this problem, such as to view the essential oil not only as a preservative but also as a flavour component. Alternatively, it could be incorporated into products which already have a strong flavour, or to use the most active components instead of the whole oil. This would hopefully reduce the changes in the organoleptic properties, whilst retaining antimicrobial activity.

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