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ANTIOXIDANT ACTIVITY OF DRIED SPEARMINT AND ITS USE IN WHITE CHEESE

Foda, Mervat I.[1] ; M. M. A. El-Sayed[1]; Marwa M. Al-Moghazy[1]; Amal, A. Hassan[2] and Nagwa M. Rasmy[2]

[1]Dairy Science Dept., National Researh Center, El-Behoos, Dokki, Cairo, Egypt.

[2]Food Science and Technology Dept., Faculty of Agric. Ain Shams Univ., Shubra El-Khema, Egypt.

ABSTRACT

Recently, spearmint (*Mentha spicata*) has been used as a valuable source of the potent antioxidant for the nutraceuticals and cosmetic industries**.** So, antioxidant activity of spearmint extracts, ethanolic or water, were determined using two lipid model systems (DPPH scavenging activity and β-carotene bleaching test) and compare with a synthetic one, phenolic compounds were also determined. UF-white cheese was manufactured with adding different concentrations of dried spearmint. Chemical composition and consumer's acceptability of produced white cheese were studied. The results show that DPPH scavenging activity was 77 % and 87 % for ethanolic and water extract respectively and lower concentrations of spearmint showed the highest overall acceptability.

*Corresponding author: mervat1m@yahoo.com, Dairy Science Dept., National Research Centre, El-Behoos, Dokki, 12622 Cairo, Egypt, tel: + 012 2536 503, Fax: + 202 337 0931

INTRODUCTION

Foods contain factors that promote the oxidation of fatty acids, which is the major cause of chemical spoilage, resulting in rancidity and/or deterioration of the nutritional quality, colour, flavour texture and safety of foods (Antolovich *et al.,* 2002). There is an increasing interest in both industry and scientific research for spices and aromatic herbs because of their strong antioxidant and antimicrobial properties. These properties are due to many substances, including some vitamins, flavonoids, terpenoids, carotenoids, phyto- estrogens, minerals (Calucci *et al.,* 2003 and Suhaj, 2006). Antioxidant may be present as endogenous factors or may be added to preserve food lipid components from quality deterioration. Synthetic antioxidants such as butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT) and propyl gallate (PG) are commonly used in food formulations. However, due to safety concerns, interest in natural antioxidants has intensified, to address the demand by consumers (Shahidi, 2000). Spearmint, as a plant, contains a board range of bioactive compounds from the secondary metabolism such as lipids, phytochemical, pharmaceutics, flavor, fragrances and pigments. Extraction techniques have been widely investigated to obtain such valuable natural compounds for commercialization. Spearmint (*Mentha spicata*) is originally a native of the Mediterranean region, belonging to the family Labiatae (Lamiaceae), and the most common one (Wang & Weller, 2006). Plants belonging to the Lamiaceae family are very rich in Phenolic

compounds which have been shown to have antioxidant activity. Furthermore, these compounds are very attractive not only in modern phytotherapy but also in food industry (Kanatt *et al.,* 2007). Previous studies found that spearmint water extract inhibited the mutagenic activity of the parent compound and other heterocyclic amines through inhibition of carcinogen activation and via direct effects on the activated metabolites (Vu *et al.,* 2004). On the other hand, white cheese is a widespread cheese group produced in many countries and the most popular and economically the most important variety of traditional soft or semi hard cheese. In Egypt, more than 70% of milk produced is used in cheese making especially white cheese, statistics show that there is increase in local consumption of white cheese with the rate of 1.8 % annually (CAPMS, 2004). So, the aim of the present work was to study the antioxidant activity of spearmint extracts and applied for the production of white cheese.

MATERIALS AND METHODS

Buffalo's milk retentate was obtained from Dairy Industry Unit, Animal Production Research Institute, Ministry of Agriculture, Cairo, Egypt. The chemical composition of milk retentate was 29.2% total solids; fat 15.5 %; total protein 12.5% and Titratable acidity 0.09%. Dried spearmint (*Mintha spicata* L.) was obtained from Medicinal and Aromatic Plant Research Dept., Agriculture Research Centre, Giza, Egypt. Microbial rennet (*Mucor mehiei)* was obtained from Novo, Denmark.

Preparation of the extracts:

Alcoholic and water extracts were prepared according to the method which described by Pautanachokchai *et al.*, (2002) and Kansoh *et al.,* (2001) respectively. The extracts were decanted and the supernatants were filtered through filter paper Whatman No.1, and lyophilized by a lyophilizer (Snijders type 2040, Netherlands). The Yield of each extract was determined.

Evaluation of antioxidant activity of spearmint extracts

Antioxidant activity of spearmint extracts were characterized individually by two complementary test systems, using 2,2-diphenyl-1 picryl-hydrazil, DPPH (Tepe *et al.,* 2005) and *β–carotene/*linoleic acid (Miller *et al.,* 1993) with modifications by Wanasundara *et al.,* (1994), and compared with the stable synthetic antioxidant, Butylated Hydroxy Anisole (BHA). Spearmint extracts concentration which providing 50% inhibition (IC_{50}) was calculated using the graph by plotting inhibition percentage against extract concentration. Total phenolic compounds were determined according to Zheng & Wang (2001), and the results were expressed as milligrams of gallic acid equivalent/g of dry extract. All tests were carried out in triplicate.

Cheese making:

Control and spearmint white cheese were prepared according to Foda *et al.,* (2006) as follows, milk retentate was divided into 4 portions; the first was served as control. Three different levels {0.25, 0.5 and 0.75% (w/v)} of ground dried spearmint were added to the other three portions. The mixture was salted to a concentration of 3%, mixed well and pasteurized at 73 °C for

15 sec., curds were hold at 40°C after adding the rennet, then distributed in plastic containers. All cheese samples were stored under refrigerator temperature (5±2°C) for 5 weeks; samples were taken fresh and every week for different analysis. Three replicates were prepared for each cheese to determine their chemical composition and sensory analysis.

Chemical analysis

Cheese samples were analyzed for moisture**,** fat contents and Titratable acidity according to Ling, (1963), while total nitrogen (TN %) was determined by Kjeldahl method (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 & Tuncturk (2000). Total volatile free fatty acids (TVFFA) were determined according to Kosikowski, (1978).

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 cheese for appearance & colour (20), body & texture (20), odour (20), taste (20), and overall acceptability (20). Panel members were also instructed to report any defects or unpleasant flavour. Water and no salted crackers were provided to clean their palates between tasting.

Statistical analysis

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

RESULTS AND DISCUSSION

Yield of spearmint extracts

Ethanolic extract of dried spearmint obtained higher yield (24 %) compared with water extract (5.4 %). Similar results were reported by Kanatt *et al.,* (2007) who found the yield of water extract was 6.1 %.

DPPH radical scavenging activity

DPPH radical scavenging activities (%) were increased with increasing the spearmint extracts from 10 to 250 µg / ml, as shown in Fig.1, with both ethanolic and water extract. Higher concentration (≤ 20 µg/ml) of water extract reduced the DPPH free radical significantly, with an efficacy much higher than the reference (BHA). Concentration of spearmint providing 50 % inhibition of DPPH (IC_{50}) is shown in Table (1). IC_{50} of water extract was (7.7 l µg/ml); lower than ethanolic extract and close to BHA value. Kanatt *et al.,* (2007) found the IC_{50} of spearmint water extract was 25.8 µg/ml, this difference could be due to the spearmint type, the extraction method and/or the quality of the original plant, its geographic origin, the harvesting date, storage and processing prior to extraction (Ollanketo *et al*., 2002). Phenolic compound is one of the major groups acting as primary antioxidants, is shown in Table (1). Total phenolic compounds in spearmint ethanolic extract

were significantly higher than in water extract. Similar result was found by Kaur & Kapoor (2002).

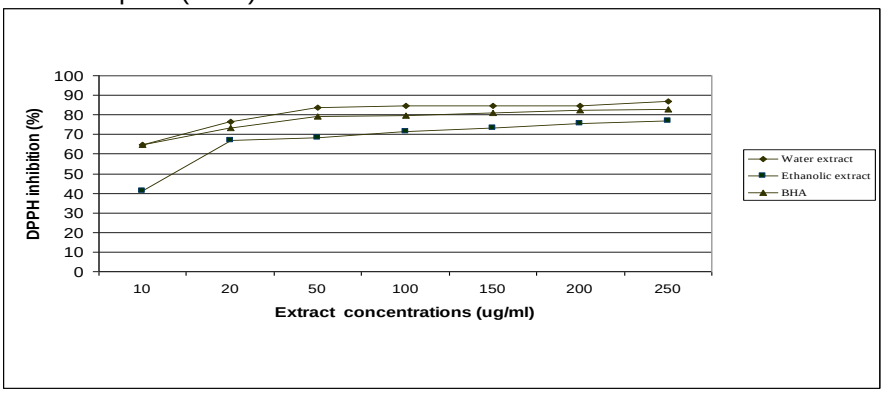


Fig.1: DPPH radical scavenging activity of different concentration of spearmint extracts compared with BHA

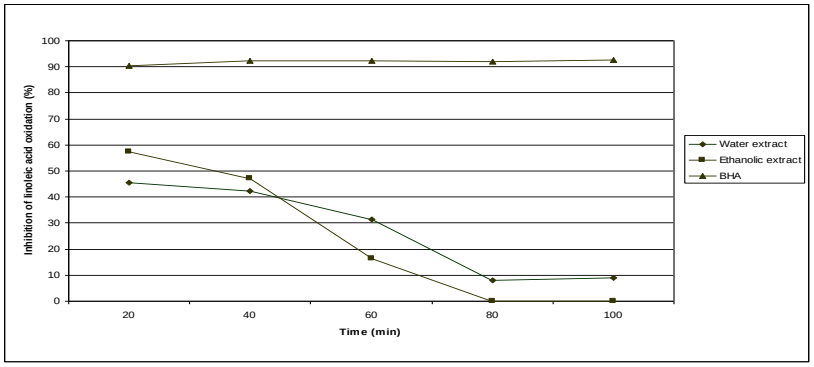
Table (1) Spearmint extracts providing 50% inhibition of DPPH (IC_{50}) and their total phenolic compounds

Concentration	Water extract	Ethanolic extract	BHA
IC_{50}	7.7± 0.91 ^{B}	13.5± 1.71 ^{A}	5.0± 0.47 ^{C}
Total Phenolic*	39.77 ± 0.20	117.37 ± 3.47	ND
*Amount of Phenolic compounds (mg gallic acid/ g dry extract)			
Means in the same raw showing the same capital letters are not significantly different (p≤ 0.05)			
ND = not detected			

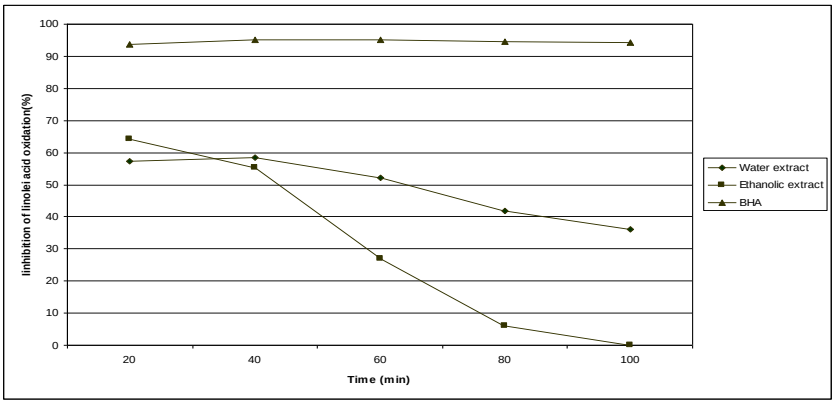
β-carotene bleaching test

β-carotene bleaching assay, has a high specificity for lypophilic compounds and in this assay antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation. The inhibition of linoleic acid oxidation (%) by different concentrations (50, 100 and 200 µg/ml) of spearmint extracts determined at 20,40,60, 80 and 100 min incubation time are shown in Fig.2 a-c. The antioxidant power decreased with increasing the incubation time. Ethanolic extract did not show any antioxidant activity at both concentrations 50 and 100 µg/ml, while with increasing its concentration to 200 µg/ml the activity reached 36.56%, it is considered as low antioxidant activity (<60%). Water extract showed higher antioxidant activity after 40 min of incubation time with lower concentration (50 µg/ml), and after 60 min with higher concentration 100 or 200 µg/ml, reached 62.28 %, considered as moderate antioxidant activity (60- 70 %) as mentioned by Kaur & Kapoor (2002). These results are in agreement with Tepe *et al.,* (2005) who reported that polar extract exhibited stronger activity than non-polar extracts, indicating that polyphenoles or flavonoids may play an important role in the activity. BHA being a synthetic antioxidant cannot be

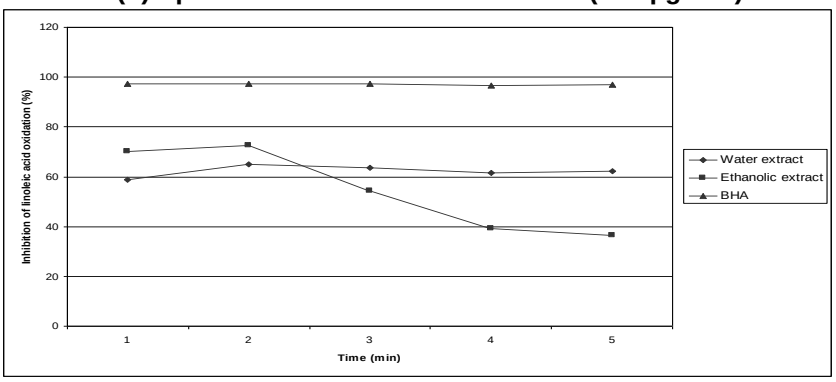
used beyond a concentration of 200 ppm, while, for antioxidant from natural sources there is no such limit (Suja *et al*., 2005).



(a) Spearmint extracts concentration (50 µg / ml)



(b) Spearmint extracts concentration (100 µg / ml)



(c) Spearmint extracts concentration (200 µg / ml)

Fig. 2: Antioxidant activity of spearmint extract concentrations determined by β-carotene test compared with BHA

Physiochemical properties of white cheese with dried spearmint:

Table (2) shows that increasing spearmint concentration from 0.25 to 0.75 % increased the moisture contents of white cheese insignificantly. This could be due to the ability of the herb for absorbing more water and keep it into the curd. Also, prolonging the cold storage for 2 weeks decreased the moisture content significantly. However, the reduction of moisture content in all samples was slowly, this is due to the fact that cheese were packaged and stored in plastic containers with low moisture permeability. These results are in contrarily with previously obtained by Coskun & Tuncturk (2000), Tarakci & Kucukoner (2006) and Foda *et al.,* (2006). Different concentrations of spearmint didn't affect fat or protein contents, but significant effect ($P<0.05$) was observed in fat content during the cold storage for 5 weeks, while with protein content only after 2 weeks. These results are similar to those obtained by Tarakci *et a*l., (2004), Tarakci & Kucukoner (2006)) and Foda *et al.,* (2006). Titratable acidity (TA) values were changed insignificantly by increasing the spearmint concentrations and increased significantly ($P<0.05$) by prolonging the storage period. Similar results were previously reported by Foda *et al.,* (2006) and in contrarily with Coskun, & Tuncturk (2000).

Table (2): Effect of dried spearmint on physiochemical properties of white cheese during cold storage period

Cheese samples	Moisture (%)	Fat (%)	Protein (%)	Acidity
Dried spearmint (%)*				
Control	**68.22 ± 0.59^{BC}**	**15.22± 0.27^{A}**	**10.52±0.14^{BC}**	**0.22±0.10^{B}**
0.25	**68.77 ± 0.64^{AB}**	**15.25 ±0.23^{A}**	**10.47 ±0.14^{D}**	**0.22 ±0.1^{B}**
0.50	**68.80 ± 0.77^{AB}**	**15.24 ±0.34^{A}**	**10.52 ±0.21^{D}**	**0.23 ±0.1^{B}**
0.75	**69.08 ± 0.63^{A}**	**15.23± 0.19^{A}**	**10.58 ±0.40^{DC}**	**0.22 ±0.1^{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}**

Different letters are significantly different (p< 0.05) in each group (cheese samples or storage period).*Each value represents 18 values, ** Each value represents 30 values.

Effect of dried spearmint on biochemical characters of white cheese:

Water soluble nitrogen (WSN) in herby white cheese, Fig. 3, were increased significantly (P<0.05) by increasing the concentrations of spearmint compared with control cheese. These results could be due to higher content of proteolytic bacteria in herby cheese. Also, significant increased (P<0.05) was observed after 2 weeks of cold storage (Table, 3). Similar results were obtained by Coskun& Tuncturk (2000), Agboola & Tesic (2002), Tarakci, *et al.*, (2004) and Foda *et al.,* (2006).

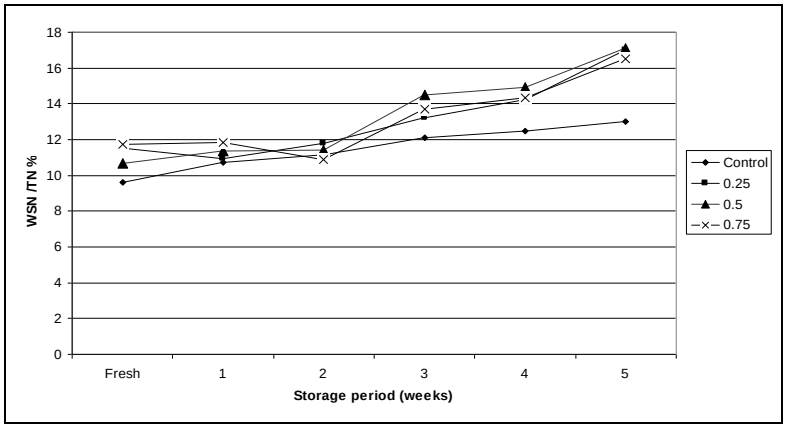


Fig.3: Effect of dried spearmint concentrations on WSN content of white cheese during cold storage period

Fig.4 shows insignificantly increasing in TCA-SN content between spearmint cheese and control sample. While, significant increased (P<0.05) was observed after 4 weeks of cold storage (Table, 3). Fig.5 shows that dried spearmint increased PTA-SN content, but there is no significant increase presented between the different concentrations. While, significant increase (P<0.05) was observed after 2 weeks of cold storage (Table 3). Similar results were obtained by Coskun, & Tuncturk, (2000), Tarakci *et al.,* (2004), in contrarily with those obtained by Agboola & Tesic (2002). Previous study showed that different values of TCA-SN and PTA-SN were found in 20 samples of commercial fresh herby cheese related to the environmental conditions such as storage temperature and humidity, in addition to cheese making conditions such as pH, salt, different herbs, etc., (Tarakci & Kucukoner, 2006).

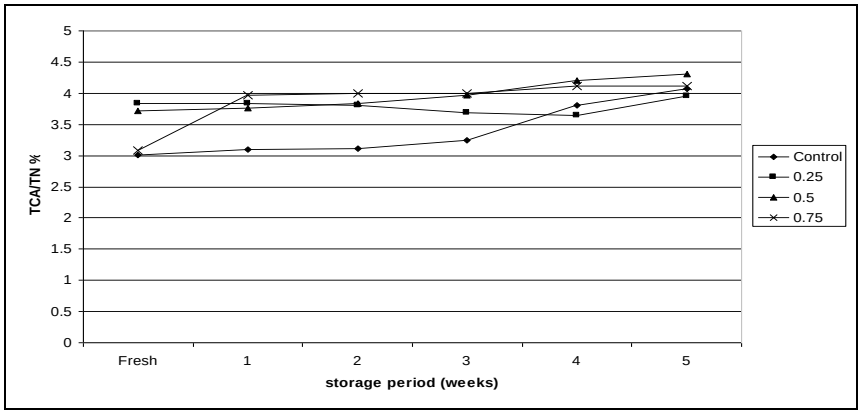


Fig.4: Effect of dried spearmint concentrations on TCA-soluble nitrogen content of white cheese during cold storage period

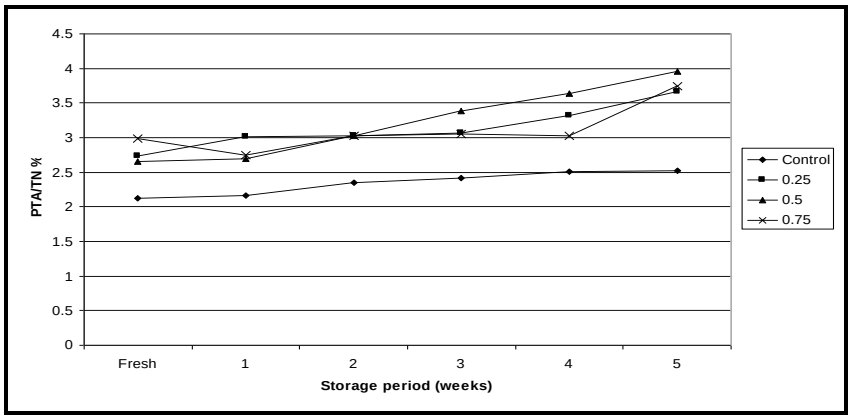


Fig.5: Effect of dried spearmint concentrations on PTA-soluble nitrogen content of white cheese during cold storage period

The changes of total volatile free fatty acids (TVFFA) regard to spearmint concentration are shown in Fig.6. The highest spearmint concentration (0.75%) caused significant increase in the TVFFA, this value significantly increased after one week of cold storage (Table, 3). Lipolysis of control semi hard cheese and which prepared with 1g mint / kg cheese showed no increase in lipolysis value between days 7 and 30 (Agboola & Tesic, 2002). These differences could be due to different type of cheese (soft and semi –hard).

Cheese samples	WSN/TN%	TCA-SN	PTA-SN	TVFFA
Dried spearmint* (%)				
Control	11.51±1.26^{C}	3.44 ± 0.25^{B}	2.35±0.17^{B}	2.31± 0.40^{D}

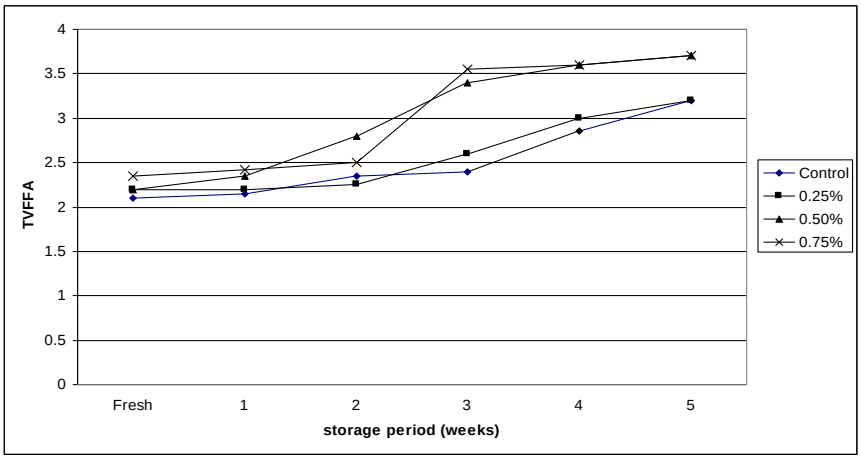


Fig.6: Effect of dried spearmint concentrations on Total Volatile Free Fatty Acids (TVFFA) content of white cheese during cold storage period

Table (3): Effect of the concentrations of dried spearmint on iochemical characters of white cheese during cold storage.

0.25	**12.95±2.38^{AB}**	**3.79 ± 0.11^{AB}**	**3.29 ±0.32^{A}**	**2.37±0.68 ^{C}**
0.50	**13.32 ±2.55^{A}**	**3.79 ± 0.38^{AB}**	**3.14±0.59^{A}**	**2.52 ±0.18^{CD}**
0.75	**13.31±1.92 ^{A}**	**4.05 ± 0.06 ^{A}**	**3.10±0.34^{A}**	**3.02 ±0.12^{A}**
Storage period (weeks) **				
Fresh	**10.53±1.34^{C}**	**3.56±0.46 ^{C}**	**2.44±0.50^{D}**	**2.12±0.48^{F}**
1	**10.88±1.00^{C}**	**3.63±0.37^{BC}**	**2.48±0.46^{CD}**	**2.21±0.46^{E}**
2	**11.15±1.14^{C}**	**3.62±0.30^{BC}**	**2.62±0.55^{B-D}**	**2.37±0.43^{D}**
3	**12.83±1.66 ^{B}**	**3.65±0.38^{BC}**	**2.77±0.68^{A-C}**	**2.60±0.41^{C}**
4	**13.59±1.94^{AB}**	**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}**

)ifferent letters are significantly different (p< 0.05) in each group (cheese samples or ɔrage period).*Each value represents 18 values, ** Each value represents 30 values. 'FFA = (Total Volatile Free Fatty Acids) 0.1N of Na OH /10g cheese

Sensory evaluation

Sensory scores of white cheese contained different concentrations of dried spearmint during cold storage are presented in Table (4). Dried spearmint with different concentration decreased the appearance & colour score significantly (P<0.05). While, prolonging the cold storage for 5 weeks showed insignificant effect on these scores. Increasing dried spearmint from 0.25 to 0.50 decreased body & texture, odour and taste significantly (P<0.05), while, prolonging the cold storage didn't affect these parameters. Similar results were previously obtained by Tarakci & Kucukoner (2006) and Foda *et al.,* (2006).

Table (4): Effect of dried spearmint concentrations on some organoleptic properties of white cheese during cold storage.

Conclusion

Dried spearmint and its extracts (ethanolic or water) are recommended to produce white cheese with high antioxidative activity. The recommended concentrations of dried spearmint can vary based on the consumer's acceptability.

Cheese samples	**Appearance & colour (20)**	**Body &Texture (20)**	**Odour (20)**	**Taste (20)**	**Overall acceptability (20)**
Dried spearmint* (%)					
0.25	**16.20 ±0.31^{B}**	**16.83 ±0.46^{B}**	**17.46 ±0.76^{B}**	**17.02 ±0.99^{B}**	**16.85 ±0.56^{B}**
0.50	**13.22 ±1.22^{C}**	**17.15±0.40^{B}**	**16.13 ±1.00^{C}**	**15.91 ±1.06^{C}**	**15.07 ±0.64^{C}**
0.75	**11.39 ±2.22^{D}**	**16.74 ±0.58^{B}**	**15.83 ±1.02^{C}**	**14.89 ±0.96^{CD}**	**14.37 ±0.49^{C}**
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 ±1.75^{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 ±1.72^{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}**

Different letters are significantly different (p< 0.05) in each group, cheese samples or storage period. *Each value represents 18 values, ** Each value represents 30 values.

Further research should be done to study the effect of cheese processing (milk pasteurization and homogenization) on the antioxidant activity of herby cheese.

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تقييم النشاط المضاد للأكسدة للنعناع البلدي الجاف واستخدامة في الجبن الابيض

ميرفت ابراهيم فوده [١]، مجدي محمد أحمد السيد[١] ، مروة محمد المغازي[١] ، امال احمد حسن[٢] ، نجوي موسي رسمي[٢]

[١] قسم الألبان – المركز القومي للبحوث – الدقي – القاهرة

[٢] قسم علوم الأغذية – كلية الزراعة – جامعة عين شمس – شبرا الخيمة - القاهرة

يعتبر النعناع البلدي مصدر غني بمضادا ت الاكسدة لذا فهو يستخدم حديثا في الصناعات الغذائية والدوائية. وفي هذا البحث تم تقدير المركبات الفينولية والنشاط المضاد للاكسدة لكلا من المستخلص المائي والكحولي للنعناع الجاف بطريقتي (DPPH scavenging activity and β-carotene bleaching test).

وقد استخدم تركيزات مختلفة من النعناع الجاف مع اللبن المركز لانتاج جبن ابيض وقد تم تقدير التركيب الكيماوي و التحكيم الحسي للجبن الأبيض الناتج الطازج و المخزن في الثلاجة لمدة ٥ أسابيع.

أظهرت النتائج المتحصل عليها أن النشاط المضاد للأكسدة المستخلص الكحولي (٧٧%) ونشاط المستخلص المائي كان (٨٧%) كما أظهر التحكيم الحسي للجبن الأبيض الناتج أن التركيز المنخفض للنعناع الجاف أكثر قبولاً من التركيز المرتفع.