



## PRODUCTION OF FETA LIKE CHEESE FORTIFIED WITH POMEGRANATE AND LEMON PEELS EXTRACT AS NATURAL ANTIOXIDANTS

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**ABSTRACT:** This study was carried out to clear the effect of addition of pomegranate and lemon peel extracts as natural antioxidant on Feta like cheese quality. These extracts were added to cheese during manufacture at a rate of 0.25 and 0.50% of each extract. Cheese treatments were analyzed for chemical analysis, oxidative stability, microbiological examination and organoleptic properties, when fresh and after 7, 14, 21 and 28 days of storage at refrigerator temperature. Results showed that these extracts have a high content of phenolic compounds, and gave high antioxidant activity. As well as the addition of these extracts to cheese did not significantly affect the chemical composition but affected the oxidative stability, bacteriological and organoleptic properties of cheese samples. A clear reduction in peroxide and acid values of cheese samples containing natural extracts during storage period than control cheese samples were observed. As well as total bacterial, coliform, as well as yeast and mould counts of cheese samples contain natural extracts did not detected during storage compared with control cheese samples. Also, results showed that organoleptic properties of all cheese treatments improved by progressed of storage period until the end of storage. Cheese containing lemon peels extracts showed lowest flavour intensity and body characteristics than other cheese. From the previous results, it could be recommended the use of some natural extracts in feta like cheese manufacture such as pomegranate and lemon peel extracts at a rate of 0.5%, where it improved the sensory and bacteriological characteristics of cheese samples and increased stability against oxidation.

**Key words:** Pomegranate peel extract, lemon peel extract, phenolic compounds, oxidative stability, microbiological examination.

## INTRODUCTION

There are increasing demand of consumers to use food products without preservatives or natural preservatives as possible has compelled the food industries for utilization of preservatives with herbal and microbial origins instead of artificial preservatives in their production (Khorshidian *et al.*, 2018).

Natural products and healthy foods have to be given a lot of interests for enhancing overall well-being, in the prevention of diseases and also in the incorporation of health-promoting substances into the diet as natural food additives. Newly, the valorization of

underutilized foods has more priority because of their antioxidant potential (Prasad *et al.*, 2012).

Consumers have more concerns to use natural antioxidants from food sources rather than synthetic antioxidants which have been restricted because of their toxic and carcinogenic effects (Abdel-Hameed *et al.*, 2014). Many epidemiological studies reported that the frequent consumption of high natural antioxidant containing foods could lower the incidences of particular types of cancers, hypertension, diabetes, and cardiovascular diseases, especially, in developing countries where most people have limited resources and access to modern treatments (Khorshidian *et al.*, 2018).

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Medicinal plants rich in natural antioxidants and phenolics are progressively applied in dairy foods manufacturing to improve nutritional and therapeutic properties (Martins *et al.*, 2014; Bertolino *et al.*, 2015).

Peels of some fruits have higher antioxidant activity than pulps. Pomegranate is a good example for this type of fruits wherein their peels constitute approximately 40% of the whole fruit and are rich in ellagic acid derivatives (El-Shourbagy and El-Zahar, 2014).

Citrus peels are rich in functional ingredients such as essential oils, fibers, phenols and vitamin C, flavanones and polymethoxylated flavones. Due to their antioxidant activity phenolic compounds are used in several applications such as formulation of healthy food, cosmetic, and pharmaceutical products (M'hiri *et al.*, 2017).

Soft cheese is the most commonly consumed in Egypt. It is produced by different procedures, *i.e.* traditional and ultrafiltration (UF) methods and stored at low temperature with or without brine. UF technology has many advantages in cheese making such as increasing cheese yield and nutritive values, decreasing the production cost and solving the environmental problems related to whey disposal. On the other hand, UF-soft cheese was characterized by slow flavour, which was attributed to the concentration of proteinase and peptidase inhibitors by UF (Abd El-Aziz *et al.*, 2012).

Recent advances in nutrition science have highlighted the contribution of UF- soft cheese to nutrition and health owing to the retention of whey proteins into intermediate concentrated retentate, which act as a cysteine delivery system in inhibiting tumor growth. Also, the focus of nutrition research has shifted towards specific food ingredients contributing to nutrition and health (El-Din *et al.*, 2010).

The interaction between food additives and nutrient within the food matrix is an important of future interest. Consequently the objectives of the present study were to develop UF- soft cheese, high in antioxidant activity and phenolic compounds using natural peels extracts.

## MATERIALS AND METHODS

### Materials

Plant biomass wastes, as a by-product of food industries, commonly found in Egypt, were used in this investigation. Retentate of buffalo's milk (40% solids) was obtained from Obour Land Company for Food Industries (Obour City, Cairo, Egypt). Pomegranate and lemon fruits were obtained from local market (Zagazig, Egypt), Then washed with distilled water and manually peeled. The starting materials were dried in an air draft drying oven (40°C). By-products were ground and sieved through 60 mesh sieve and finally cooled or kept at 4°C until the extractions were carried out. 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and microbial rennet powder (Marzyme-protease Rhizomucor miehei) has been obtained from Dsnisco France-2, Avenue Brun- Fauuier- 38470 VINAY (Franc) and used in cheese making after dilution (0.5 g/20 ml water/10 kg retentate. Gallic acid was purchased from Sigma (St. Louis, MO, USA). All other chemicals and reagents were of the highest purity available.

### Methods

#### Preparation of Extracts

Dried materials were extracted with ethanol (70%), at a ratio of 10:1 (*V/W*, 10 ml solvent: 1 g raw material) in closed vessels by stirring at room temperature (25°C) for 4 hr., followed by filtration through Whatmann No. 1 filter paper. All vessels were wrapped with aluminum foil to prevent light degradation during extraction (Yu *et al.*, 2005). Ethanol extracts were evaporated in a rotary evaporator (Buchi-water bath-B-480, Switzerland) at 40°C, and freeze-dried (Thermo Electron Corporation- Heto Power Dry LL 300 Freeze Dryer, Czechoslovak). The dried extracts ,were weighed to determine the yield and stored at -20°C until used.

#### Determination of Total Phenolic Compounds (TPC)

The concentration of TPC in different extracts was measured using UV spectrophotometer (Jenway-UV-VIS Spectrophotometer), based on a colorimetric oxidation/reduction reaction, as described by Skerget *et al.* (2005) using Folin-

Ciocalteu reagent. Specifically, 0.5 ml of diluted extract (10 mg in 10 ml solvent) was mixed with 2.5 ml of Folin–Ciocalteu reagent (diluted 10 times with distilled water) and 2 ml of Na<sub>2</sub>CO<sub>3</sub> (75 g/l l). The samples were incubated for (5 min at 50°C) then cooled. For a control sample, 0.5 ml of distilled water was used. The absorbance was measured at 760 nm. Total phenolic content expressed as gallic acid equivalent (GAE) was calculated, and the results were expressed as a mg GAE g<sup>-1</sup> extract.

### Identification of Phenolic Acids Using HPLC

Phenolic acids of the dried extracts were identified according to the method described by **Mattila *et al.* (2000)**. HPLC (Hewlett Packard series 1050, USA) equipped with autosampling, injector, solvent degasser, UV detector set at 330 nm and quarter HP pump (series 1050) was used. Column (C18 hypersil BDS) with particle size 5 µl was used. The separation was carried out with methanol and acetonitrile as a mobile phase at flow rate of 1 ml/min. The column temperature was performed at room temperature (25°C) throughout the experiment. Identification and quantification were carried out based on calibrations of the standards prepared from phenolic acids dissolved in a mobile phase. Retention time and peak area were used for calculation of phenolic acid compounds by the data analysis of Hewlett Packard Software.

### Radical Scavenging Activity (RSA) of Extracts

The electron donation ability of the obtained extracts was measured by bleaching of the purple coloured solution of DPPH according to the method of **Hanato *et al.* (1988)**. One hundred µl of each extract (10 mg extract/10 ml solvent) was added to 3 ml of 0.1 mM DPPH dissolved in ethyl acetate, ethanol and hexane according to the solvent used for extraction. After incubation period of 60 min at room temperature, the absorbance was determined against a control at 517 nm (**Gulcin *et al.*, 2004**). Percentage of antioxidant activity of DPPH was calculated as follows.

DPPH scavenging effect (%) = [(A<sub>0</sub> - A<sub>1</sub>)/A<sub>0</sub>] × 100 where, A<sub>0</sub> is the absorbance of the control reaction and A<sub>1</sub> is the absorbance in the extract. Samples were analyzed in triplicate.

### Feta-Like Cheese Manufacture (Supplementation with Pomegranate and Lemon Peels Extract)

Buffalo's milk retentate was divided into five equal portions. One batch had no extract as a control. The latter batches were fortified with pomegranate and lemon peels extract at the rate of 0.25 and 0.50 g/kg retentate. UF-soft cheese was made according to the method described by **Renner and Abd El-Salam (1991)**. Retentate supplemented with pomegranate and lemon peel extracts well mixed with a blender with high speed. All treatments were heated at 65°C for 30 min and immediately cooled to 45°C. Calcium chloride and sodium chloride were added at the rate of 0.02% and 3%, respectively with stirring until completely dissolved then renneted at 37°C and stirring for 1 min, dispensed into plastic containers and kept at 37±2°C until a proper coagulum was formed after about one hour and then transferred to refrigerator at 5±2°C. Total phenolic, and antioxidant capacity, radical scavenging activity (RSA%) were determined in the resultant cheeses when fresh and after 7, 14, 21 and 28 days. In addition, the chemical composition, microbial examination and sensory evaluation of fresh and stored cheeses were carried out. All experiments were reported in triplicates and determinations in duplicated and average resultant were tabulated.

### Chemical Analyses

Feta like soft cheese was chemically analyzed for total solids, fat and titratable acidity as described by **AOAC (2007)**. pH value was measured in cheese samples using a digital pH meter. Total and soluble nitrogen percentages were determined by semi-micro kjeldhel method as described in the **AOAC (2007)**. Total volatile fatty acids (TVFA) of soft cheese and fermented artificial cream treatments were estimated according to **Kosikowski (1978)**.

### Oxidative Stability Indices

Fat was extracted from the cheese samples according to **Abd El-Fattah (2006)**. Cheese samples were dried at 40°C for 12 hr., in drying oven, ground and mixed with n-hexane as a solvent to extraction of fat. The solvent was removed at 40°C. Separated fat was immediately

analyzed for oxidative stability indices (peroxide and acid values). Peroxide and acid values of white soft cheese were determined according to AOAC (2007).

### Microbiological Analyses

Ten g sample were taken from cheeses at the age of 0, 7, 14, 21 and 28 days, then homogenized in sterile 90 ml of 0.1% peptone water. Serial 8 fold dilutions in sterile 0.1% peptone water were prepared for bacterial analysis. Total bacterial count (TBC) was determined using plate count agar according to Houghtby *et al.* (1992). Potato Dextrose Agar was used for yeast and mould enumeration. Plates were incubated at 25°C for 5 days, according to Marshall (1992). Violet Red Bile Agar was used for the enumeration of coliforms. Plates were incubated at 37°C for 24 hr., according to Marshall (1992).

### Sensory Evaluation

Ten trained panelists from the staff members of Food Science Department Faculty of Agriculture, Zagazig University, Egypt used a quality rating score card for evaluation of appearance (10 points), flavour (30 points) and body and texture (60 points) (Bodyfelt *et al.*, 1988).

### Statistical Analysis

All the data of the present study were subjected to analyses of variance (ANOVA) using software (SAS, 2008). Differences between means were collected by the least significant differences (LSD) at  $p < 0.05$ . All measurements were carried out in triplicate.

## RESULTS AND DISCUSSION

### Yield, Total Phenolic Compounds (TPC) and Radical Scavenging Activity (RSA) of Plant Ethanolic Extracts

The yield of pomegranate (PEE) and lemon peel extracts (LPE) was varied from 19.32-28.24 g/100g (Table 1). Ethanolic LPE had higher yield (28.24g/100g) followed by ethanolic PPE (19.32g/ 100g). The variation in the extraction yield may be attributed to the content of total phenol compounds and the polarity of the compounds in plants. Such

differences have been reported by Prakasha *et al.* (2001).

Ethanolic LPE had the highest percentage of total phenols (220.30 mg/g) while the ethanolic PPE was 194.80 mg/g. Therefore, PPE and LPE are a good source of bioactive compounds which have high antioxidative properties.

The radical scavenging activity of the two studied materials showed high values (Table 1). It was 91.20% for LPE, and 88.65% for PPE.

The obtained results of yield extracts, total phenolic compounds of (TPC) and radical scavenging activity (RSA) for LPE are similar to those reported by M'hiri *et al.* (2017) and Elkhatim *et al.* (2018). While the results of yield extracts, total phenolic compounds (TPC) and radical scavenging activity (RSA) for PPE are similar to those reported by Mansour *et al.* (2013) and Brito *et al.* (2014).

### Identification of Phenolic Compounds by HPLC

Results in Table 2 show that LPE, recorded high level of some phenolic compound (caffic, coumaric acid, ferulic acid and sinapic acid) compared to PPE. While the remained phenolic compound (Gallic, Quercetin, Vanillic acid and Ellagic acid) were high in PPE compared to LPE.

Phenolic compounds identified in LPE ranged from 120.00 to 2014.00 mg/g. The obtained results are similar to those reported by M'hiri *et al.* (2017) and Elkhatim *et al.* (2018). While Phenolic compounds identified in PPE ranged from 1.36 to 124.00 mg/g (Mansour *et al.*, 2013; Brito *et al.*, 2014).

Gallic acid is phytochemicals that are considered a potential source of functional food ingredients for their high antioxidant capacity (Sethiya *et al.*, 2014). Quercetin another phytochemical is a flavonoid that has attracted great interest because it is a potent antioxidant with proven anticancer effects. Its structure contains a double bond in the C ring and a 4- oxo group, which enhance its antioxidant activity (Moskaug *et al.*, 2004).

**Table 1. Yield, total phenolic compounds (TPC) and radical scavenging activity (RSA) of plant ethanolic extracts**

Material	Yield (g/100g)	Total phenolic compounds (mg/g)	RSA (%)
Pomegranate peels	19.32	194.80	88.65
Lemon peels	28.24	220.30	91.20

**Table 2. Identification of phenolic compounds in ethanol extracts of pomegranate peels and lemon peels determined by HPLC**

Test item	Pomegranate peels extract (mg/100g)	Lemon peels extract (mg/100g)
Gallic	124.00	0.00
Caffic	24.68	120.46
Quercetin	2.40	0.00
Coumaric acid	4.6	312.20
Vanillic acid	1.36	0.00
Ellagic	36.24	0.00
Ferulic acid	0.00	2014.0
Sinapic acid	0.00	218.24

### Gross Chemical Composition of Cheese

Results presented in Table 3 show that control cheese had the lowest TS content. Cheese containing LPE and PPE at different concentrations (0.25 and 0.5 %) had the highest TS content. The TS content of all cheese type significantly ( $P \leq 0.05$ ) increased during storage period for 28 days at refrigerator temperature. The increase in TS content of cheeses along the storage period may be due to the curd concentration and whey expulsion resulting from acid development during the storage period (Salem *et al.*, 2010; Abd El-Aziz *et al.*, 2012).

From the same Table, it could be observed that the Fat/DM content of experimental cheese samples increased significantly ( $P \leq 0.05$ ) up to the end of storage period depending on the loss of moisture. The fat content of all treatments increased along the storage period up to the end of storage period. These results are in agreement with those reported by Abd El-Aziz *et al.* (2012) who manufacture UF soft cheese using ginger extract as natural antioxidants.

Results presented in Table 3 show the average total nitrogen/dry matter (TN/DM) of cheese from different treatments. Results showed

that TN/DM (%) content of cheese samples decreased gradually up to the end of storage period and there were no significant differences in TN(%) along of storage period due to high protein content and lower proteolysis in all treatments. Similar results were reported by Abd El-Aziz *et al.* (2012) and Omar *et al.* (2016).

### Acidity (%)

Results presented in Table 4 show the average total acidity of cheese from different treatments. Results showed that there was a significant increasing trend ( $P \leq 0.05$ ) in acidity of all cheese treatments throughout the storage period. Titratable acidity increased gradually in all cheese samples with the progress of the storage period. An important observation that control cheese had higher acidity than other treatments, Except T4 cheese containing natural antioxidants had lower acidity. This may be due to higher antimicrobial activity of these extracts (Brito *et al.*, 2014; Elkhatim *et al.*, 2018). The trend of the change in pH values of all treatments was opposite to that of acidity (Table 4). pH values decreased in all treatments with the progress of the storage period. Similar results were obtained by Abd El-Aziz *et al.* (2012) and Olmedo (2013).

**Table 3. Chemical analyses of feta like cheese as affected by adding different natural antioxidants during storage at refrigerator temperature for 28 days**

Treatment	Storage period (day)														
	Total solids(TS) (%)					Fat/DM (%)					Total nitrogen TN/DM (%)				
	Fresh	7	14	21	28	fresh	7	14	21	28	fresh	7	14	21	28
C	40.53 <sup>B</sup>	39.24 <sup>C</sup>	40.16 <sup>D</sup>	41.06 <sup>C</sup>	41.44 <sup>CD</sup>	39.95 <sup>B</sup>	40.16 <sup>B</sup>	40.87 <sup>C</sup>	41.05 <sup>B</sup>	41.60 <sup>C</sup>	5.51 <sup>BC</sup>	5.24 <sup>C</sup>	5.19 <sup>C</sup>	5.15 <sup>C</sup>	4.18 <sup>BC</sup>
T1	40.33 <sup>B</sup>	41.05 <sup>BC</sup>	41.49 <sup>CD</sup>	41.79 <sup>BC</sup>	42.54 <sup>CD</sup>	39.99 <sup>B</sup>	40.13 <sup>B</sup>	40.61 <sup>C</sup>	41.28 <sup>B</sup>	41.64 <sup>C</sup>	5.67 <sup>B</sup>	5.64 <sup>B</sup>	5.46 <sup>AB</sup>	5.42 <sup>B</sup>	4.24 <sup>BC</sup>
T2	40.54 <sup>B</sup>	41.32 <sup>B</sup>	41.74 <sup>C</sup>	41.92 <sup>B</sup>	42.92 <sup>C</sup>	40.22 <sup>AB</sup>	41.20 <sup>AB</sup>	41.70 <sup>B</sup>	42.36 <sup>AB</sup>	42.76 <sup>B</sup>	5.74 <sup>B</sup>	5.82 <sup>A</sup>	5.58 <sup>A</sup>	5.60 <sup>A</sup>	4.38 <sup>B</sup>
T3	41.63 <sup>A</sup>	42.27 <sup>A</sup>	42.36 <sup>B</sup>	43.29 <sup>AB</sup>	43.58 <sup>B</sup>	40.42 <sup>A</sup>	41.63 <sup>A</sup>	42.47 <sup>A</sup>	42.94 <sup>A</sup>	43.41 <sup>A</sup>	6.13 <sup>A</sup>	5.60 <sup>B</sup>	5.41 <sup>AB</sup>	5.16 <sup>C</sup>	5.05 <sup>A</sup>
T4	41.96 <sup>A</sup>	42.58 <sup>A</sup>	43.54 <sup>A</sup>	43.88 <sup>A</sup>	44.26 <sup>A</sup>	40.64 <sup>A</sup>	41.26 <sup>AB</sup>	41.64 <sup>B</sup>	42.28 <sup>AB</sup>	42.64 <sup>B</sup>	6.22 <sup>A</sup>	5.76 <sup>AB</sup>	5.52 <sup>A</sup>	5.42 <sup>B</sup>	5.18 <sup>A</sup>

Means with the same letter are not significantly different

C: control Feta like cheese, T1: Feta like cheese treated with 0.25% lemon peel extract, T2: Feta like cheese treated with 0.50% lemon peel extract, T3: Feta like cheese treated with 0.25% Pomegranate peel extract and T4: Feta like cheese treated with 0.50 % Pomegranate peel extract.

**Table 4. Acidity (%) and pH values of feta like cheese as affected by adding different natural antioxidants during storage at refrigerator temperature for 28 days**

Treatment*	Storage period (day)									
	Acidity (%)					pH				
	Fresh	7	14	21	28	Fresh	7	14	21	28
C	0.180 <sup>A</sup>	0.190 <sup>A</sup>	0.220 <sup>A</sup>	0.250 <sup>A</sup>	0.270 <sup>A</sup>	6.30 <sup>b</sup>	6.15 <sup>B</sup>	5.69 <sup>b</sup>	5.41 <sup>B</sup>	5.36 <sup>AB</sup>
T1	0.135 <sup>D</sup>	0.150 <sup>c</sup>	0.200 <sup>B</sup>	0.220 <sup>b</sup>	0.250 <sup>B</sup>	6.70 <sup>A</sup>	6.36 <sup>A</sup>	6.14 <sup>A</sup>	5.94 <sup>A</sup>	5.72 <sup>A</sup>
T2	0.162 <sup>C</sup>	0.166 <sup>B</sup>	0.212 <sup>AB</sup>	0.232 <sup>B</sup>	0.266 <sup>AB</sup>	6.40 <sup>b</sup>	6.28 <sup>AB</sup>	6.12 <sup>A</sup>	5.86 <sup>A</sup>	5.64 <sup>A</sup>
T3	0.142 <sup>CD</sup>	0.180 <sup>AB</sup>	0.210 <sup>AB</sup>	0.240 <sup>AB</sup>	0.260 <sup>AB</sup>	6.77 <sup>A</sup>	6.17 <sup>B</sup>	6.00 <sup>A</sup>	5.50 <sup>B</sup>	5.44 <sup>AB</sup>
T4	0.174 <sup>B</sup>	0.194 <sup>A</sup>	0.224 <sup>A</sup>	0.254 <sup>A</sup>	0.276 <sup>A</sup>	6.46 <sup>b</sup>	6.04 <sup>B</sup>	5.94 <sup>b</sup>	5.42 <sup>B</sup>	5.28 <sup>B</sup>

Means with the same letter are not significantly different

\*See under Table (3)

### Nitrogen fractions

Table 5 shows SN/TN and NPN/TN of cheese containing different natural antioxidants extracts. Results indicated that both SN/TN and NPN/TN contents of cheese from different treatments gradually increased with the advanced of the storage period. It could be also noticed that there were insignificant differences in the nitrogen fractions of cheese made from the different treatments. These results indicated that addition of the different antioxidants extracts did not show remarkable effect on proteolysis during cheese ripening. **Abd El-Aziz *et al.* (2012) and Omar *et al.* (2016)**, showed similar general trend of these results.

### Total volatile fatty acids

Results presented in Table 5 show the average Total volatile fatty acids (TVFA) contents of cheese made from different treatments. Results indicated that there were

significant differences in TVFA content of cheese as compared with control when fresh and during the storage period. Control cheese had the lowest TVFA contents after 28 days of storage. Similar results were reported by **Ozer *et al.* (2003) and Abd El-Aziz *et al.* (2012)**.

### Oxidative stability and acid values

Results presented in Table 6 show the development of peroxide value (PV) and acid value (AV). Values of cheese from different treatments up to the end of storage period. Results indicated that control cheese (C1) had the highest values for PV and AV. than cheese made from natural antioxidants during storage period. However, it could be noticed that cheese containing 0.5% LPE showed significant ( $P \geq 0.05$ ) lower values of PV and AV than other cheese treatments. Addition of LPE and PPE to cheese significantly ( $P \geq 0.05$ ) reduced the development of PV and AV in compare with

control cheese. Moreover, it could be observed that the most effective extract in this respect was which showed the lowest values for PV and AV levels. Similar results were reported by **Abd El-Aziz et al. (2012)**.

#### Microbial examination

Table 7 shows the differences in total bacterial counts of cheese made with some natural antioxidants at different concentrations. There were significant differences in viable bacterial count between control cheese and other cheese samples made with natural antioxidant. The results indicated that total bacterial count increased gradually throughout the storage period until the end of storage period. The obtained results also showed that control cheese had the highest counts of total bacterial count's. LPE fortified cheese had the lowest counts of total bacterial count followed by PPE extract fortified cheese, respectively. This might be due to the antimicrobial activity of LPE (**Elkhatim et al., 2018**) and PPE (**Brito et al., 2014**). Similar results were reported by **Olmedo (2013)**.

Yeast and mould were not detected in all cheese treatments up to 21 days of storage expect control cheese which contained the highest yeast and moulds counts. While LPE and PPE fortified cheeses had the lowest yeast and mould counts at the end of storage period. The general trend of these results agreed with those reported by **Al-Jasser and Al-Dogan (2009)**.

Coliforms were not detected in all cheese treatments up to 28 days of storage expect

control cheese which contained coliform bacteria at fresh and at 7 days of storage. The general trend of these results agreed with those reported by **Olmedo (2013)**.

#### Organoleptic properties

Results in Table 8 show the average score points given for appearance, body of texture, and flavour of feta like cheese as affected by adding natural antioxidants. These results showed that there were significant differences between the control cheeses (C) and all experimental cheeses when fresh and during ripening period up to 28 days. Cheese containing with PLE and PPE at ratio of 0.5% (T2 and T4) showed the lowest score for organoleptic properties than other cheese. Control cheese showed the highest scores for a organoleptic properties. All cheese treatments were acceptable by panelists All additives improved cheese properties and over all acceptability.

Also, organoleptic properties of all cheese treatment improved by progressed of storage period until the end of storage. These results are in agreement with the results obtained by **Azzam (2007) and El-Din et al. (2010)**.

#### Conclusion

It is noticed that PLE and PPE had a strong antioxidant capacity. Therefore, it can be used as natural antioxidants in manufacturing Feta like cheese to improve its healthy value and oxidative stability during storage.

**Table 5. Proteolysis and lipolysis of feta like cheese as affected by adding different natural antioxidants during storage at refrigerator temperature for 28 days**

Treatment*	Storage period (day)															
	SN/TN (%)					NPN/TN (%)					TVFA					
	Fresh	7	14	21	28	Fresh	7	14	21	28	Fresh	7	14	21	28	
C	17.62 <sup>C</sup>	21.40 <sup>A</sup>	22.22 <sup>BC</sup>	24.62 <sup>B</sup>	30.67 <sup>C</sup>	7.97 <sup>B</sup>	8.12 <sup>C</sup>	9.05 <sup>B</sup>	11.50 <sup>C</sup>	14.75 <sup>C</sup>	1.82 <sup>B</sup>	1.94 <sup>C</sup>	2.18 <sup>D</sup>	2.52 <sup>C</sup>	2.76 <sup>C</sup>	
T1	19.15 <sup>A</sup>	20.33 <sup>C</sup>	22.95 <sup>B</sup>	25.58 <sup>A</sup>	31.35 <sup>B</sup>	7.41 <sup>B</sup>	8.94 <sup>B</sup>	10.55 <sup>AB</sup>	14.55 <sup>A</sup>	16.94 <sup>B</sup>	2.92 <sup>B</sup>	2.80 <sup>B</sup>	2.92 <sup>D</sup>	3.20 <sup>B</sup>	3.44 <sup>B</sup>	
T2	19.42 <sup>A</sup>	20.74 <sup>BC</sup>	23.24 <sup>A</sup>	25.80 <sup>A</sup>	32.14 <sup>A</sup>	8.12 <sup>AB</sup>	9.20 <sup>A</sup>	10.94 <sup>A</sup>	14.98 <sup>A</sup>	17.20 <sup>AB</sup>	3.14 <sup>A</sup>	3.95 <sup>A</sup>	4.12 <sup>A</sup>	4.46 <sup>A</sup>	4.92 <sup>A</sup>	
T3	17.77 <sup>BC</sup>	20.62 <sup>BC</sup>	21.65 <sup>C</sup>	24.83 <sup>B</sup>	30.40 <sup>C</sup>	8.24 <sup>A</sup>	8.76 <sup>B</sup>	10.48 <sup>AB</sup>	13.50 <sup>B</sup>	17.50 <sup>B</sup>	2.66 <sup>BC</sup>	2.90 <sup>B</sup>	3.20 <sup>C</sup>	3.66 <sup>B</sup>	3.84 <sup>B</sup>	
T4	17.96 <sup>B</sup>	20.84 <sup>B</sup>	21.90 <sup>C</sup>	25.12 <sup>AB</sup>	31.22 <sup>B</sup>	8.32 <sup>A</sup>	9.04 <sup>A</sup>	10.86 <sup>A</sup>	13.80 <sup>B</sup>	17.64 <sup>A</sup>	2.98 <sup>B</sup>	3.62 <sup>A</sup>	3.98 <sup>B</sup>	4.14 <sup>A</sup>	4.32 <sup>A</sup>	

Means with the same letter are not significantly different

\*See under Table (3)

**Table 6. Oxidative stability indices of feta like cheese as affected by adding different natural antioxidants during storage at refrigerator temperature for 28 days**

Treatment*	Storage period (day)									
	Peroxide value (meq O <sub>2</sub> /kg)					Acid value (mg KOH/g)				
	Fresh	7	14	21	28	Fresh	7	14	21	28
C	0.58 <sup>A</sup>	0.64 <sup>A</sup>	0.82 <sup>A</sup>	1.04 <sup>A</sup>	1.28 <sup>A</sup>	0.50 <sup>A</sup>	0.56 <sup>A</sup>	0.64 <sup>A</sup>	0.80 <sup>A</sup>	0.92 <sup>A</sup>
T1	0.52 <sup>A</sup>	0.58 <sup>AB</sup>	0.65 <sup>C</sup>	0.76 <sup>C</sup>	0.98 <sup>B</sup>	0.45 <sup>AB</sup>	0.50 <sup>AB</sup>	0.58 <sup>AB</sup>	0.70 <sup>BC</sup>	0.78 <sup>BC</sup>
T2	0.44 <sup>AB</sup>	0.52 <sup>B</sup>	0.60 <sup>C</sup>	0.66 <sup>D</sup>	0.78 <sup>D</sup>	0.40 <sup>C</sup>	0.44 <sup>C</sup>	0.50 <sup>C</sup>	0.62 <sup>C</sup>	0.68 <sup>c</sup>
T3	0.54 <sup>A</sup>	0.62 <sup>A</sup>	0.76 <sup>B</sup>	0.94 <sup>B</sup>	1.02 <sup>B</sup>	0.48 <sup>A</sup>	0.52 <sup>A</sup>	0.62 <sup>A</sup>	0.75 <sup>B</sup>	0.84 <sup>B</sup>
T4	0.48 <sup>AB</sup>	0.58 <sup>AB</sup>	0.72 <sup>B</sup>	0.78 <sup>C</sup>	0.86 <sup>C</sup>	0.44 <sup>AB</sup>	0.50 <sup>AB</sup>	0.60 <sup>AB</sup>	0.72 <sup>BC</sup>	0.80 <sup>BC</sup>

Means with the same letter are not significantly different

\*See under Table (3)

**Table 7. Microbiological examination of feta like cheese as affected by adding different natural antioxidants during storage at refrigerator temperature for 28 days**

Treatment*	Storage period (day)														
	Total count (cfu/g) 10 <sup>4</sup>					<i>E. coli</i> count (cfu/g)10 <sup>1</sup>					Yeast and mould (cfu/g) 10 <sup>1</sup>				
	Fresh	7	14	21	28	Fresh	7	14	21	28	Fresh	7	14	21	28
C	52	65	104	123	130	2	1	ND	ND	ND	2	6	8	12	15
T1	12	7	2	0.92	0.25	ND	ND	ND	ND	ND	ND	ND	ND	ND	8
T2	9	6	2	0.9	0.11	ND	ND	ND	ND	ND	ND	ND	ND	ND	2
T3	45	20	12	4	1	ND	ND	ND	ND	ND	ND	ND	ND	ND	10
T4	14	12	8	2	0.82	ND	ND	ND	ND	ND	ND	ND	ND	ND	6

\*See under Table (3)

ND = Not detected

**Table 8. Organoleptic properties of feta like cheese as affected by adding different natural antioxidants during storage at refrigerator temperature for 28 days**

Treatment*	Storage period (day)																			
	Appearance (10)					Flavour (30)					Body and texture (60)					Total (100)				
	Fresh	7	14	21	28	Fresh	7	14	21	28	Fresh	7	14	21	28	Fresh	7	14	21	28
C	9	9	9	8	8	28	29	29	28	28	58	58	59	60	60	95 <sup>A</sup>	96 <sup>A</sup>	97 <sup>A</sup>	96 <sup>A</sup>	96 <sup>A</sup>
T1	8	8	8	7	7	25	26	26	24	24	56	58	59	60	60	89 <sup>B</sup>	92 <sup>B</sup>	93 <sup>B</sup>	91 <sup>B</sup>	91 <sup>B</sup>
T2	6	6	5	5	5	23	23	24	22	22	58	59	59	60	60	87 <sup>BC</sup>	88 <sup>C</sup>	88 <sup>D</sup>	87 <sup>C</sup>	87 <sup>C</sup>
T3	6	6	6	5	5	27	27	28	26	26	54	56	58	58	59	87 <sup>BC</sup>	89 <sup>C</sup>	90 <sup>C</sup>	87 <sup>C</sup>	88 <sup>C</sup>
T4	5	5	5	5	4	24	25	25	23	23	56	58	58	59	59	85 <sup>C</sup>	88 <sup>C</sup>	88 <sup>D</sup>	87 <sup>C</sup>	86 <sup>D</sup>

Means with the same letter are not significantly different

\*See under Table (3)

## REFERENCES

- Abd El-Fattah, A.M. (2006). Lipolysis and oxidative stability of fat in ultrafiltrated white soft cheese containing vegetable oils. J. Agric. Sic., Mansoura Univ., 31: 5193-5200.
- Abd El-Aziz, M., H.S.S. Mohamed and F.L. Selet (2012). Production and evaluation of soft cheese fortified with Ginger extract as functional dairy food. Poi. J. Food Nutr. Sci. 62, 2, 77-83.
- Abdel-Hameed, E.S., M.A. Nagaty, M.S. Salman and S.A. Bazaid (2014). Phytochemicals, nutritionals and antioxidant properties of two prickly pear cactus cultivars (*Opuntia ficus indica* Mill.) growing in Taif, KSA. Food Chem., 160: 31-38.
- Al-Jasser, M.S. and A.A. Al-Dogan (2009). Properties of white soft cheese produced using rennet substitute extracted from solanum seeds. Egypt. J. Dairy. Sci., 37: 237-243.



- AOAC (2007). Association of Official Analytical chemists- Official Method of Analysis (18<sup>th</sup> Ed.), Benjamin Franklin Station Washington, DC, USA.
- Azzam, M.A. (2007). Effect of partial replacement of milk fat with vegetable oils on the quality of processed cheese spreads. Egypt. J. Dairy Sci., 35: 87-95.
- Bertolino, M., S. Belviso, B. Dal Bello, D. Ghirardello, M. Giordano, L. Rolle and V. Gerbi (2015). Influence of the addition of different hazelnut skins on the physicochemical, antioxidant, polyphenol and sensory properties of yogurt. LWT- Food Sci. Technol., 63: 1145–1154.
- Bodyfelt, F.W., J. Tobias and G.M. Trout (1988). Sensory Evaluation of Butter. PP 377–417 in The Sensory Evaluation of Dairy Products. Van Nostrand Reinhold, New York, NY.
- Brito, A., J.E. Ramirez, C. Areche, B. Sepúlveda and M.J. Simirgiotis (2014). HPLC-UV-MS profiles of phenolic compounds and antioxidant activity of fruits from three Citrus Species Consumed in Northern Chile. Molecules, 19: 17400-17421.
- El-Din, M.F., I. Ghita, M.A. Badran, A.S. Gad, and M. El-Said (2010). Manufacture of low fat UF-soft cheese supplemented with Rosemary extract (as natural antioxidant). J. Ame. Sci., 6 (10): 570 – 579.
- Elkhatim, K.A., R.A.A. Elagib and A.B. Hassan (2018). Content of phenolic compounds and vitamin C and antioxidant activity in wasted parts of Sudanese citrus fruits. Food Sci. Nutr., 6 : 1214–1219.
- El-Shourbagy, G.A. and K.M. El-Zahar (2014). Oxidative stability of ghee as affected by natural antioxidants extracted from food processing wastes. Annals of Agric. Sci. 59 (2), 213–220.
- Gulcin, I., O.I. Kufrevioglu, M. Oktay and M.E. Buyukokuroglu (2004). Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle (*Urtica dioica* L.). J. Ethnopharmacol, 90: 205–215.
- Hanato, T., H. Kagawa, T. Yasuhara and T. Okuda (1988). Two new flavonoids and other constituents in licorice root: their relative astringency and radical scavenging effects. Chem. Pharm. Bull., 36: 2090–2097.
- Houghtby, J.H., G. Brenda and E.K. Koenig (1992). Microbiological Count Methods. in Standard Methods for the Examination of Dairy Products. 16<sup>th</sup> TR Marshall, Ed. Ame. Public Health Assoc., Washington, DC., 213-246.
- Khorshidian, M., M. Yousefi, E. Khanniria and A.M. Mortazavian (2018). Potential application of essential oils as antimicrobial preservatives in cheese. Innovative Food Sci. and Emerg. Technol., 45: 62–72.
- M'hiri, N., M.I. Ghoul and M. Boudhrioua (2017). Phytochemical characteristics of Citrus peel and effect of conventional and non-conventional processing on phenolic compounds: A review. Food Rev. Int., 1- 48.
- Mansour, E., A. Ben Khaled, B. Lachiheb, M. Abid, Kh. Bachar and A. Ferchichi (2013). Phenolic compounds, antioxidant, and antibacterial activities of peel extract from tunisian pomegranate J. Agric. Sci. Technol., 15: 1393-1403.
- Marshall R.T. (1992) Standard Methods for the Examination of Dairy Products. 1992, 16<sup>th</sup> Ed. American Public Health Association (APHA), Washington, DC, USA, 158.
- Martins, A., L. Barros, A.M. Carvalho, C. Santos-Buelga, I.P. Fernandes, F. Barreiro, and I.C.F.R. Ferreira (2014). Phenolic extracts of *Rubus ulmifolius* Schott flowers: characterization, microencapsulation and incorporation into yogurts as nutraceutical sources. Food Funct., 5: 1091–1100.
- Mattila, P., J. Astola and J. Kumpulainen, (2000). Determination of flavonoids in plant material by HPLC with diode-array and electro-array detections. J. Agric. Food Chem., 48: 5834-5841.
- Moskaug, J., H. Carlsen, M. Myhrstad and R. Blomhoff (2004). Molecular imaging of the biological effects of quercetin and quercetin-rich foods. Mechanisms of Ageing and Develop., 125 (4) :315-324.

- Olmedo, R.A., V. Nepote and R. Nelson (2013). Preservation of sensory and chemical properties in flavoured cheese prepared with cream cheese base using oregano and rosemary essential oil. *LWT. Food Sci. and Technol.*, (53): 409 – 417.
- Omar, H.H., A.M. El-Nimer and M.A. Ahmed (2016). Improving the Quality of Low fat Uf-white soft pickled cheese. *J. Food and Dairy Sci., Mansoura Univ.*, 7 (2): 59 -70.
- Ozer, H., R.K. Robinson and A.S. Grandison (2003). Textural and microstructural properties of UF cheese (a white-brined Turkish cheese) *Dairy Technol.*, 56: 3:171 – 176.
- Prakasha, G.K., R.P. Singh and K.K. Sakariah (2001). Antioxidant activity of grape seeds extracts on peroxidation models *In vitro*. *Food Chem.*, 73: 285-290.
- Prasad, K.N., K.W. Kong, N.S. Ramannan, A. Azrina and I. Amin (2012). Selection of experimental domain using two level factorial design to determine extract yield, antioxidant capacity, phenolics and flavonoids from *Mangifera pajang* Kosterm. *Sep. Sci. Technol.*, 47: 2417–2423.
- Renner, E. and M.H. Abd El-Salam (1991). Application of Ultrafiltration in the Dairy Industry. El-Sevier Science publishing, Co., Inc. London.
- Salem, S.A., I.E. Gad El-Ral and W.S. El-Sharaihy (2010). Improving the quality of fat soft cheese (Domiaty) by incorporation whey protein concentrate. *Proc. 11<sup>th</sup> Egypt. Conf. Dairy. Sci. and Technol.*, 145-162.
- SAS, S. (2008). *STAT 9.2 User's Guide. Version 9.2.* Cary, NC: SAS Institute. Inc.
- Sethiya, N., A. Trivedi and S. Mishra (2014). The total antioxidant content and radical scavenging investigation on 17 phytochemical from dietary plant sources used globally as functional food. *Biomed. and Prev. Nutr.*, 4 (3): 439-444.
- Skerget, M., P. Kotnik, M. Hadolin, A. Rižner-Hraš, M. Simonič and Z. Knez (2005). Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. *Food Chem.*, 89: 191–198.
- Yu, J., M. Ahmedna and I. Goktepe (2005). Effects of processing methods and extraction solvents on concentration and antioxidant activity of peanut skin phenolics. *Food Chem.*, 90: 199-206.

## إنتاج جبن شبيه الفيتا المدعم بمستخلصات قشور الرمان والليمون كمضادات أكسدة طبيعية

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أجريت هذه الدراسة بهدف تقدير النشاط المضاد للأكسدة ومحتوى المواد الفينولية لمستخلصات كل من قشور الرمان والليمون ودراسة تأثير إضافة هذه المستخلصات الطبيعية على جودة جبن شبيه الفيتا، حيث تم إضافة هذه المستخلصات عند صناعة الجبن بمعدل ٠,٢٥ و ٠,٥% إلى مركز اللبن الناتج من الترشيح الفائق مستخلص وتم تحليل الجبن من حيث التركيب الكيماوي، الثبات ضد الأكسدة، الفحص البكتيريولوجي والخواص الحسية وذلك بعد التصنيع مباشرة وبعد مرور ٧ و ٢١ و ٢٨ يوماً من التخزين على درجة حرارة التلاجة، وأوضحت النتائج أن هذه المستخلصات ذات محتوى عالي من المواد الفينولية ولها نشاط عالي مضاد للأكسدة، كذلك إضافة هذه المستخلصات إلى الجبن لم يمكن لها تأثير معنوي على التركيب الكيماوي ولكنها أثرت معنوياً على الثبات ضد الأكسدة والفحص البكتيريولوجي والخواص الحسية لعينات الجبن، حيث لوحظ انخفاض واضح في قيم رقم البيروكسيد ورقم الحموضة في عينات الجبن المحتوية على المستخلصات الطبيعية وذلك خلال فترة التخزين عن عينات الجبن الكنترول، ومن حيث الخواص البكتيريولوجية لوحظ انخفاض في أعداد البكتيريا وبكتيريا الكوليفورم والفطريات والخمائر في عينات الجبن المحتوية على المستخلصات الطبيعية وذلك خلال فترة التخزين عن عينات جبن المقارنة، ومن حيث الاختبارات الحسية فإن عينات الجبن المحتوية على مستخلصات قشور الليمون أعطت أقل معدل تحكيم حسي عن باقي المعاملات وذلك خلال فترة التخزين، ومن خلال النتائج السابقة فإنه يمكن استخدام بعض المستخلصات الطبيعية في صناعة جبن شبيه الفيتا مثل مستخلص قشور الرمان والليمون بمعدل ٠,٥% حيث أنها حسنت من الخواص البكتيريولوجية لعينات الجبن وزادت من ثباتها ضد الأكسدة.

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