Manufacture of Low Fat UF-Soft Cheese Supplemented with Rosemary Extract (as Natural Antioxidant)

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Abstract: UF- soft cheese was prepared from UF milk retentate (1.5% fat) and supplemented with 1-5% rosemary extract (RE) and cold stored for 30 days. The total phenolic content (TPC) and antioxidant capacity were evaluated using DPPH and FRAP methods in retentate before and after pasteurization and salting and in the resultant cheese. Also, the chemical composition and sensory properties were evaluated. Fortification of retentate with rosemary extract increased its content of phenolic content and consequently, its antioxidant activity. Pasteurization increased the TPC and antioxidant activity. Addition of 3% Nacl reduced slightly the TPC, RSA% and FRAP values. Moreover, it was noticed that UF- soft cheese fortified with 1% rosemary extract retained more TPC and antioxidant activity; also, increasing the concentration of rosemary extract to 5% had more acceptable flavor, body and texture, and antioxidant activity until 30 days. Furthermore, the rate of decrease in TPC , RSA% and FRAP values in cheese samples with rosemary extract after 30 days of storage were less comparable to control cheese (without RE). [Journal of American Science 2010;6(10):570-579]. (ISSN: 1545-1003).

Keywords: UF milk retentate, UF-Soft Cheese, Rosemary Extract, Antioxidant.

1. Introduction:

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 (Sukkar and Bounous 2004). Also, the focus of nutrition research has shifted towards specific food ingredients contributing to nutrition and health.

Rosemary (*Rosemarinus offcinalis*) has always been known a versatile, aromatic herb and addition to being used as a food flavoring is also known medicinally for its powerful antioxidant activity, antibacterial and antimutagenic properties (Oluwatuyi et al 2004). Moreover, its extract contains several beneficial substances with antioxidant properties. It contains chemicals called quinines which can inhibit carcinogens. Therefore, rosemary antioxidant extract is ranked high on the list of cancer-prevention and reduction foods (Mahmoud et al 2005).

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

2. Materials and Methods:

Materials

Buffalo's Skim Milk Retentate was obtained

from Dairy Industry Units, Animal Production Research Institute, Ministry of agriculture, Giza, Egypt. Rosemary herb was purchased from the commercial market for medicinal and herbal plants in Cairo, Egypt. Commercial rennet powder: Valirren 150-microbial cheese rennet was obtained from valley Reasearch, Inc.,USA.(1:150000Mcu/gr).

Methods:

1- Total phenolic contents (TPC)

Total phenolic compounds were determined According to Zheng & Wang (2001) by using Folin-Ciocalteu reagent and expressed as milligrams of Gallic acid equivalents (GAE) per 100 gm.

Antioxidant Capacity

A-Radical Scavenging Activity (RSA %) assay

Free radical Scavenging activity (RSA) of the samples was measured using the method of Brand-Williams et al (1995) and expressed as percentage inhibition of the DPPH radical and was determined by the following equation:

$$RSA\% = \frac{Abs \text{ control} - Abs \text{ sample x } 100}{Abs \text{ control}}$$

B - The Ferric Reducing Antioxidant Power (FRAP) assay

Antioxidant activity was measured using the ferric antioxidant power (FRAP) assay of Benzie and Strain (1996).

Preparation of rosemary extract:

The ground sample (10g) was infused in 90 ml hot water then stirred for 2h and allowed to stand in the refrigerator overnight. Then it was filtered through a four-layer of cloth cheese.

Cheese manufacture (supplementation with rosemary extract)

Retentate 1.5% milk fat was supplemented with rosemary extract at the rate of 1,2,3,4 and 5% and well mixed with a blender with high speed. All treatments pasteurized 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°C±2°C until a proper coagulum was formed after about one hour and then transferred to refrigerator at 5°C±2°C. Total phenolic, and antioxidant capacity (RSA% and FRAP) were determined in both retentates before and after pasteurization and after salting, as well as, the resultant cheeses when fresh and after 7, 15 and 30 days. In addition, the chemical composition 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 composition

Cheese samples were analyzed for its total solids and titeratable acidity (AOAC 2000), total nitrogen, soluble nitrogen using micro-kjeldahl method and calcium (IDF 2000) while pH was determined using pH meter (Knic Digital pH meter 646)

Sensory Evaluation

Ten trained panelists from the staff members of the Dairy Science Department, National Research Center, Egypt used a quality rating score card for evaluation of flavor (30 points) and body and texture (60 points) and appearance (10 points) (Body felt et al 1998).

Statistical analysis

All data are presented as the mean \pm SD. Two-ways analysis of variance (ANOVA) using PC-STAT (P 0.05) was the level of significance.

3. Results and Discussion:

Retentate - rosemary extract mixture Total Phenolic Content (TPC):

The total phenolic content (TPC) of retentate supplemented with rosemary extract before and after pasteurizing and salting the mixture was presented in table (1) and fig (1). It was obvious that, as the

concentration of rosemary extract increased, the TPC of the mixture was considerably increased as a result of the high TPC of rosemary extract (965 mg Gallic acid/100g) which ascribed to the phenolic diterpenes of extract. In this respect Schwarz et al 1992, Debersac et al 2001 and Ibanez et al 2003 reported that the main compounds responsible for rosemary's antioxidant properties have been identified as phenolic diterpenes, such as carnosic acid, carnosol, rosmanol, epi and iso-rosmanol, rosmadial and methyl carnosate. Other compounds, such as rosmarinic acid, caffeic acid and flavonoids, have also been associated with the antioxidant activity of rosemary (Del Bano et al 2003, Stefanovits-Banjai et al 2003 and Suhaj 2006). Wojdylo et al (2007) demonstrated that rosemary posed higher amount in total phenolic content and this confirmed the previous results by Cosio et al (2006) and Parejo et al (2002).

The obtained data clearly showed that adding 1% rosemary extract highly increased the TPC of the mixture by 131.553%, then this rate gradually increased to reach 210.679% by adding 5% rosemary extract.

It was noticed that pasteurizing the mixture increased its content of phenolic compounds for all treatments. Stewart et al (2000) found that heat treatment (cooking) increased the level of free flavonols and this was confirmed by Turkmen et al (2005). This ascribed to the effect of pasteurization on the hydrogen bonds between the phenolic hydroxyl group and the receptor group of the proteins such as NH and CO Bartolome et al (2000).

Salting the previous pasteurized samples slightly decreased its content of phenolics, but it was still higher than the corresponding values before heat treatment (pasteurization). This was presumably caused by the effect of salting on the ionic strength of the media, hence, the hydrophilic capacity of it was increased, consequently, the association partially increased, hiding again the approach of PC to react with Folin reagent.

Antioxidant Capacity

Preliminary studies confirm that a multi method approach is necessary in the antioxidant activity assessment. A rapid and reproducible methods should be used whenever an antioxidant activity is designed (Frankel and Meyer 2000, Parjo et al 2002 and Deepa et al 2007). Two spectrophotometric methods were used, DPPH assay to determine the free radical scavenging ability and the FRAP assay to measure ferric reducing ability were used.

Radical Scavenging Activity (RSA):

Many investigators reported that milk and its

components had different antioxidant properties (Chen et al 2003, Kitts 2005 and El-Din and El-Messery 2007). Supplementing retentate with rosemary extract increased its RSA [table (2) and fig (2)]. The rate of increase in RSA by adding 1% and 5% rosemary extract were 45.373% and 97.611% respectively. Perez et al (2007) reported that phenolic compounds appear to be not the only compounds responsible for antioxidant activity of the rosemary extracts (water, methanol and ethanol). Also, they revealed that rosemary extracts could act as hydrogen and/or electron donors and react with free radicals converting than into more stable products and in this way terminating radical chain reactions.

Also, Wojdylo et al (2007) found that rosemary exhibited twice as good activity in scavenging the DPPH radical, this was in agreement with that reported by Houlihan et al (1984) and Cosio et al (2006).

Regarding to the effect of heat treatment it was noticed that pasteurizing the previous mixtures increased its RSA as a result of the intermediate oxidation state of the polyphenols which increased its ability to donate hydrogen atom from the aromatic hydroxyl group to a free radical Kaur and Kappor (2001). While salting the previous mixtures slightly reduced its radical scavenging activity but still higher than before pasteurization for all treatments.

Ferric Reducing Antioxidant Power (FRAP):

The ability of the sample to reduce Fe³⁺-TPTZ to Fe²⁺ -TPTZ was used as a criterion on antioxidant capacity. Results illustrated in table (3) and fig (3) showed that supplementing retentate with rosemary extract greatly increased its FRAP values as a result of the high value of FRAP rosemary extract (90 mmol/l). Since the FRAP assay treats the antioxidants contained in the samples as reduction in a red-linked colorimetric reactor and the value reflects the reducing power of the antioxidants (Li et al 2006). The rate of increase in FRAP values by adding 1% and 5% rosemary extract were 90.697% and 234.883% respectively. Pasteurizing the previous samples increased it's FRAP values for all treatments. This ascribed to the increment of total phenolic content of the samples as mentioned before and presumably to some reductants compounds as a result of the heat treatment indicating that pasteurized retentate- rosemary extract mixture has more potential antioxidant activity.

Adding 3% NaCl to the previous pasteurized samples slightly decreased its ability to reduce ferric-TPTZ complex, but it was still more than the corresponding values of the mixture before heat treatment. The Resultant UF-Soft Cheese Chemical Composition:

The moisture content of the resultant UF-soft cheese supplemented with different concentration of rosemary extract during cold storage was shown in fig (4). It was obvious that increasing the conc. of extract significantly increased the moisture content for all cheese samples and this trend was observed throughout storage periods as a result of retaining the whey proteins in the texture of the cheese which had water binding capacity much higher than casein (Galal et al 2003 and Romeih et al 2004).

As shown in fig (5), the soluble nitrogen (SN) of UF-soft cheese slightly decreased with increasing the concentration of rosemary extract added, while it had slight effect on the SN/TN% fig (6) and SN/DM% fig (7). The later increased with prolonged storage of cheese samples until 30 days as a result of the proteolytic activity of the residues microbial rennet in cheese curd and the proteinases enzymes in milk .This finding was in agreement with that reported by Galal et al (2003) and Foda et al (2008).

Lactose content and lactose/DM% of the resultant UF-soft cheese supplemented with rosemary extract were shown in fig (8, 9). It was evident that prolonged the cold storage until 30 days gradually reduced the lactose content for all samples, this may be caused by the easy hydrolysis of lactose with increasing the moisture content of cheese samples.

Regarding to the total calcium content of UF-soft cheese samples as illustrated in fig (10). It was clear that the calcium content slightly decreased as a function of rosemary extract addition in fresh cheese samples. Prolonged the storage until 30 days gradually decreased total calcium content for all treatments, this may be ascribed to the lowness of pH values during storage. These results agreed with that reported by McMahon et al (2005) and Choi et al (2008).

Fig (11, 12) showed a slight decrease in pH and increase in acidity values of fresh cheese samples with increasing the concentration of extract added. While during storage, a continues decrease and increase in pH and acidity values were clearly observed for all cheese samples. Also, it was noticed that after 30 days of storage, the acidity of control and experimental cheese samples were nearly the same.

Total Phenolic Content (TPC):

Table (4) and fig (13) demonstrated that, there was highly increase in TPC in cheese samples supplemented with rosemary extract and was proportional to the concentration of extract added. This ascribed to the TPC of rosemary extract as mentioned before which remained in cheese curd.

The rate of increase in TPC in fresh cheese samples supplemented with 1%, 5% rosemary extract was 120.627% and 196.412% respectively. During cold storage, the TPC gradually decreased for all treated samples, this may attributed to the transformation of PC which highly unstable compounds and undergo numerous enzymatic and chemical reactions during food storage as stated by Legrand et al (2005). After 30 days, cheese had more TPC than control by 166.66% and 272.73% for cheese samples supplemented with 1% and 5% rosemary extract respectively.

Antioxidant Capacity

Radical Scavenging Activity (RSA)

Data presented in table (5) and fig (14) revealed that, supplementing retentate with rosemary extract increased the RSA in the resultant UF-soft cheese by increasing the percentage of added rosemary extract. It was noticed that, RSA% of fresh cheese supplemented with 1% and 5% extract increased by 38.554% and 83.015% respectively, while the corresponding rates after 30 days of storage were 63.883% and 119.401% in the same order. However, the percentage of RSA gradually decreased during cold storage for all cheese treatments.

Ferric Reducing Antioxidant Power (FRAP)

Table (6) and fig (15) Showed that supplementing retentate with rosemary extract proportionally increased the FRAP values of the resultant fresh cheese by increasing the additive concentration. This ascribed to the FRAP assay which permits the overall estimation of water - soluble compounds influencing the total antioxidant. The rate of increase in FRAP values for fresh UF cheese samples supplemented with 1, 5% extract was 86.956% and 221.739% respectively.

Also, it is noteworthy that the reducing power of control cheese samples was more affected by storage rather than the fortified samples. After 30 days, the FRAP values of cheese samples supplemented with 1% and 5% extract were more by 175% and 416.66% than the corresponding value of control cheese.

Sensory evaluation of the resultant cheese

Organoleptic evaluation of UF-soft cheese supplemented with rosemary extract during cold storage ($6^{\circ}C\pm 2^{\circ}C$) were demonstrated in table (7). Data revealed that cheese samples with rosemary extract until 3% concentration gained similar scores as control for their appearance when fresh and after 30 days of storage, while, cheese samples with more than 3% rosemary extract were highly acceptable by most panelists and had significantly (p 0.05) higher scores for flavor than control cheese (without rosemary extract) when fresh and throughout storage periods. This ascribed to the aroma and intensity flavor of rosemary extract which were accepted particularly at the end of storage (30 days). The rate of increasing flavors scores in cheese samples was parallel to the concentration of extract added. Also, it was noticed that cheese body and texture did not highly changed significantly (p 0.05) with rosemary extract added. In general, UF- soft cheese supplemented with rosemary extract had higher total scores than control cheese (without extract) when fresh and allover the storage periods.

From the foregoing results one can conclude that, supplementing retentate with rosemary extract increased its antioxidant activity (TPC, RSA, reducing power). Pasteurization increased these parameters; the resultant UF-soft cheese fortified with 1% rosemary extract had TPC, RSA and FRAP values (antioxidant activity) greater than control cheese (without extract).

Moreover, the rate of decrease in TPC after 30 days of storage comparable to the content of the mixture of retentate and rosemary extract before pasteurization was 8% with adding 1,5% extract, while it was 20% for control cheese (fig 16).

The RSA% was decreased by 15% with the same concentration (1, 5%) where as the corresponding decrease for control cheese was 25% g fig (17). Also, it was noticed from fig (18) that , the FRAP values of control cheese at 30 days storage decreased by 44%, while this reduction of FRAP values were 20% and 14% for cheese samples supplemented with 1,5% extract this means that UF-soft cheese fortified with 1% rosemary extract retained more TPC and antioxidant activity . Also, increasing the concentration of extract to 5% had more acceptable flavor, body and texture and antioxidant activity until 30 days and close to ideal recommended for human diet.

Table (1): Total Phenolic content (equivalent mg
Gallic acid/ 100 gm) of retentates
supplemented with rosemary extract
(Average of 3 replicates).

Conc. of extract	Before pasteurization	After pasteurization	After salting		
0	206	249	229		
1%	477	530	500		
2%	521	571	550		
3%	560	600	585		
4%	600	645	630		
5%	640	689	670		

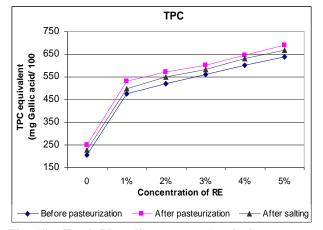


Fig (1): Total Phenolic content (equivalent mg Gallic acid/ 100 gm) of retentates supplemented with rosemary extract (Average of 3 replicates).

 Table (2): RSA% of retentates supplemented with rosemary extract (Average of 3 replicates).

Conc. of extract	Before pasteurization	After pasteurization	After salting		
0	33.5	40.747	38.1		
1%	48.7	54.479	52.25		
2%	53.555	58.365	56.149		
3%	57.704	62.875	60.9		
4%	61.5	66.148	64.048		
5% 66.2		71.006	69		

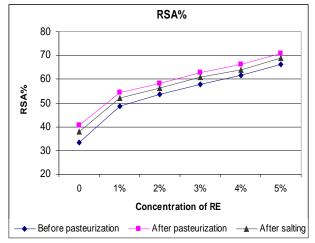


Fig (2): RSA% of retentates supplemented with rosemary extract (Average of 3 replicates).

Table (3): FRAP (mmol/l) of retentatessupplemented with rosemary extract(Average of 3 replicates).

Conc. of extract	Before pasteurization	After pasteurization	After salting
0	21.5	26	24.6
1%	41	48	45
2%	49.8	56	53.5
3%	56	62	60
4%	63	68	66
5%	72	79	76

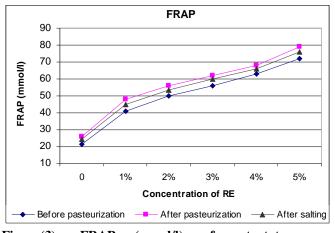


Fig (3): FRAP (mmol/l) of retentates supplemented with rosemary extract (Average of 3 replicates).

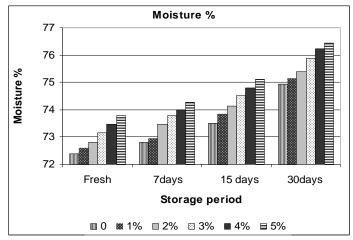


Fig (4): Moisture of low fat UF soft cheese with rosemary extract during storage (5±2 °C).

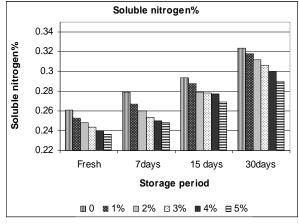


Fig (5): Soluble nitrogen% of low fat UF soft cheese with rosemary extract during storage $(5\pm2$ °C).

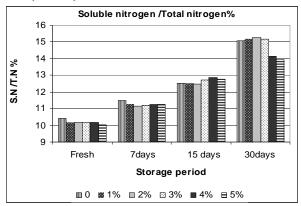


Fig (6): Soluble nitrogen /Total nitrogen (S.N /T.N %) of low fat UF soft cheese with rosemary extract during storage (5±2 °C).

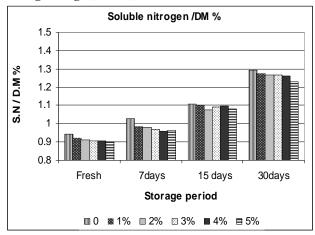


Fig (7): Soluble nitrogen /DM % of low fat UF soft cheese with rosemary extract during storage (5±2 °C).

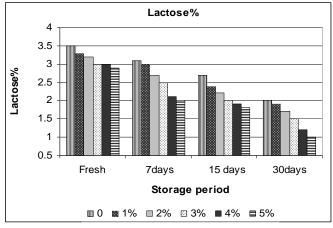
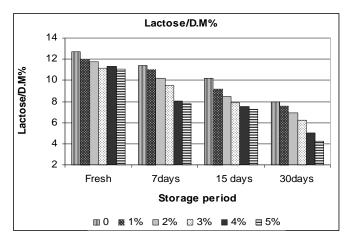
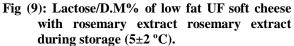


Fig (8): Lactose% of low fat UF soft cheese with rosemary extract during storage (5±2 °C).





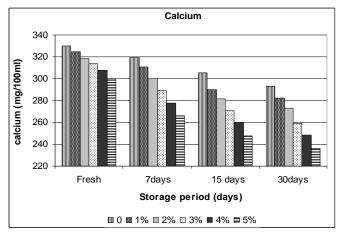


Fig (10): Calcium (mg/100ml) of low fat UF soft cheese with rosemary extract during storage (5 \pm 2 °C).

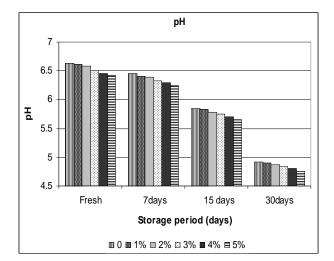


Fig (11): pH of low fat UF soft cheese with rosemary extract during storage (5±2 °C).

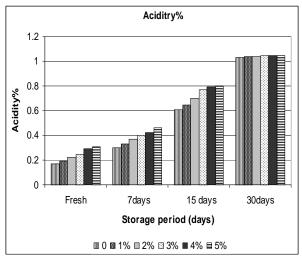


Fig (12): Acidity% of low fat UF soft cheese with rosemary extract during storage (5±2 °C).

Table (4): Total Phenolic content (equivalent mg Gallic acid/ 100 gm) of low fat UF soft cheese with rosemary extract during storage $(5\pm2$ °C).

Conc. of	Fresh	7 days	15	30day		
extract			days	S		
0	223	201	182	165		
1%	492	480	465	440		
2%	541	530	510	488		
3%	580	563	545	522		
4%	620	608	590	569		
5%	661	647	630	615		

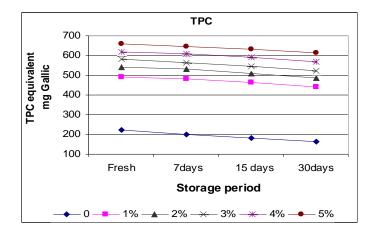


Fig (13): Total Phenolic content (equivalent mg Gallic acid/ 100 gm) of low fat UF soft cheese with green bell pepper juice during storage (5±2 °C).

Conc. of Fresh extract		7 days	15 days	30days		
0	36.128	33	30.047	25.21		
1%	50.057	48	45.157	41.315		
2%	2% 53.007		47.06	42.213		
3% 57.31		53.361	50	45.069		
4%	4% 62.148		55.075	51.048		
5%	5% 66.12		60.147	55.311		

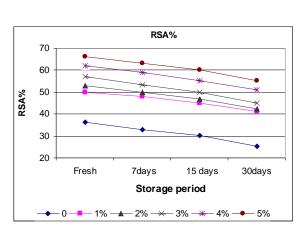


Fig (14): RSA% of low fat UF soft cheese with green bell pepper juice during storage (5±2 °C).

Table (5): RSA% of low fat UF soft cheese with rosemary extract during storage (5 ± 2 °C).

with roseniary extract during storage (5±2 °C).											
Conc. of extract	Fresh	7 days	15 days	30days							
0	23	18	16	12							
1%	43	40	37	33							
2%	51	49	47	42							
3%	57	55	53	46							
4%	63	61	58	53							
5% 74		71	68	62							

Table (6): FRAP (mmol/l) of low fat UF soft cheese with rosemary extract during storage (5±2 °C).

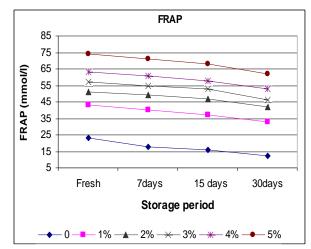


Fig (15): FRAP (mmol/l) of low fat UF soft cheese with rosemary extract during storage (5±2 °C).

Table (7): Sensory evaluation	n of low fat UF soft cheese wit	h rosemary extract during storage.

	Appearance (10)			Flavor (30)		Body and Texture (60)			Total (100)							
	Fresh	7 d.	15 d.	30 d.	Fresh	7 d.	15 d.	30 d.	Fresh	7 d.	15 d.	30 d.	Fresh	7 d.	15 d.	30 d.
0	10 ^{Aa}	9 ^{Ab}	7.4 ^{Bc}	6 ^{Ad}	27.3 ^{Ca}	26.3 ^{Cb}	26.1 ^{Cb}	25 ^{Bc}	58.2 ^{Ba}	56.5 ^{Cb}	56.9 ^{Bb}	55.8 ^{Bc}	95.5 ^{Ca}	91.8 ^{Cb}	90.4 ^{Cc}	86.8 ^{Cd}
1%	10 ^{Aa}	9.5 ^{Ab}	7.7 ^{Bc}	6.2 ^{Ad}	27.8 ^{Ca}	26.8 ^{Cb}	26.4 ^{Cb}	25.3 ^{Bc}	58.5 ^{Ba}	58.1 ^{Aa}	57.6 ^{Ab}	56.1 ^{Ac}	96.3 ^{Ba}	94.4 ^{Ab}	91.7 ^{Bc}	87.6 ^{Bd}
2%	10 ^{Aa}	8.7 ^{Bb}	8 ^{Ab}	6.6 ^{Ac}	28.1 ^{Ba}	27.2 ^{Bb}	26.7 ^{Cc}	25.7 ^{Bd}	58.8 ^{Ba}	58.4 ^{Aa}	57.4 ^{Ab}	56.3 ^{Ac}	96.9 ^{Ba}	94.3 ^{Ab}	92.1 ^{Ac}	88.6 ^{Ad}
3%	9.5 ^{Ba}	8.5 ^{Bb}	7.2 ^{Bc}	6.9 Ad	28.5 ^{Ba}	27.5 ^{Bb}	27.2 ^{Bb}	26.1 ^{Ac}	59.3 ^{Aa}	57.6 ^{Bb}	57.1 ^{Ab}	55.4 ^{Bc}	97.3 ^{Aa}	93.6 ^{Bb}	91.5 ^{Bc}	88.4 ^{Ad}
4%	9.1 ^{Ba}	7.9 ^{Cb}	6.8 ^{C c}	5.8 ^{Bd}	28.7 ^{Ba}	28 ^{Aa}	27.6 ^{Bb}	26.5 ^{Ac}	58 ^{Ba}	57.3 ^{Bb}	56.6 ^{Bc}	55.2 ^{Bd}	95.8 ^{Ca}	93.2 ^{Bb}	91 ^{Bc}	87.5 ^{Bd}
5%	9 ^{Ba}	7.7 ^{Cb}	6.5 ^{C c}	5.5 ^{Bd}	29 ^{Aa}	28.5 ^{Ab}	28 ^{Ab}	26.9 ^{Ac}	57.7 ^{Ca}	57 ^{Ba}	56.5 ^{Bb}	55 ^{Bc}	95.7 ^{Ca}	93.2 ^{Bb}	91 ^{Bc}	87.4 ^{Bd}

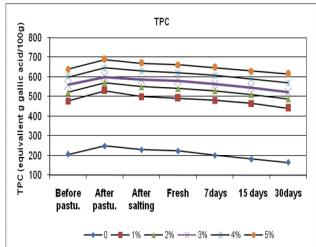


Fig (16) Phenolic compound (equivalent mg Gallic acid/100mg) of the retentate-rosemary extract and the resultant UF-soft cheese during cold storage.

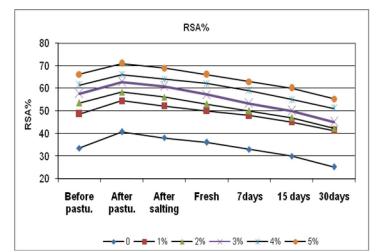


Fig (17) RSA% of the retentate-rosemary extract and the resultant UF-soft cheese during cold storage.

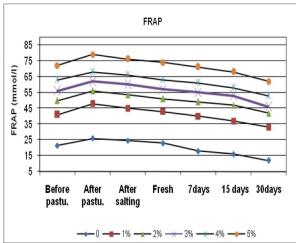


Fig (18) FRAP (mmol/l) of the retentate-rosemary extract and the resultant UF-soft cheese during cold storage.

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