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Utilization of Moringa Leaves Extract in The Production of Functional White Soft Cheese

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ABSTRACT

The present study was carried out to determine the vital compounds in the *Moringa peregrina* leaves ethanolic extract (MPLEE) and estimate the effect of that extract on the oxidative stability, chemical composition, microbiological and sensory properties of UF-soft cheese made with MPLEE at different concentrations (10, 20 and 50 mg/ml) compared to control cheese and cheese contained 0.02% of butylated hydroxy anisole. The results showed that the yield of extract was 35% of dry weight of leaves, Total phenolics (116.6 mg chlorogenic acid equivalents /g extract) and total flavonoids (45.2 quercetin equivalents/g extract). The main components for MPLEE were rutin, narengin, rosmarinic and hisperdin (17412, 1328, 1047 and 984.90 mg/L, respectively). The concentration of the sample that scavenges 50% of the DPPH radicals (SC₅₀) of MPLEE was recorded 500 µg mL⁻¹. Results showed that, addition of MPLEE had increasing significant effect (P≤0.05) on fat%, Nitrogen fractions%, salt% and total volatile fatty acids. On the other hand, titratable acidity was decreased with addition of the ethanolic extract compared to control. Cheese containing MPLEE showed the highest oxidative stability. Also, the extract reduced the total bacterial and coliform count in all samples. However, fungi counts showed opposite trend and they were not detected in all treated samples up to 60 days of storage. Results also showed that cheese samples containing MPLEE showed better organoleptic characteristics than other treatments. It could be recommended to use this extract at level of 20 mg/ml as a useful additive to improve the microbiological quality and shelf life of the product

Keywords: *Moringa peregrina*, Antimicrobial activity, Natural antioxidants, UF-white soft cheese

INTRODUCTION

Moringa trees are an important food commodity as almost all plant parts are edible and consumed as nutritive vegetable in many countries (Oluduro, 2012).

All parts of moringa trees possess high nutritional properties due to its content of numerous essential phytochemical compounds. For example, leaves of moringa had protein content more than Zabady (as fermented milk) by 9 times, also, have vitamin A more than carrots by 10 times, and have more than 15 times of banana in potassium content, in addition it has more than 17 times of milk for calcium content. Moreover, moringa leaves have more than 7 and 25 times of orange and spinach for vitamin C and iron respectively. (Said-Al-Ahl et al., 2017 and Rockwood et al., 2013).

Family Moringaceae have many medicinal plants, which are used globally in traditional medicine for various illnesses treatment. *Moringa peregrina* is commonly known by many names such as horseradish tree or drumstick tree in English and Habb Elyasar or Yen in Arabic, which grows in many areas of the world like Northeastern tropical Africa, Madagascar and Arabia (Miller et al., 1988) and in the eastern desert mountains in Egypt (Batanouny et al., 1999). In Egypt, it is used locally to treat slimness, constipation, headache, fever, burns, back and muscle pains (Abdel-Rahman et al., 2010).

Antimicrobial, anticancer, antioxidant, analgesic and anti-inflammatory activities for *M. peregrina* leaves were revealed by many literatures for its pharmacological actions (Abdel-Rahman et al., 2010; El-Batran et al., 2005; El-Alfy et al., 2011 and Dehshahri et al., 2012). Moreover, its ethanolic extracts showed significant antibacterial effect against Gram -ve bacteria (*Escherichia coli* ATCC25922 and *Klebsiella pneumoniae* ATCC 13883) and Gram +ve bacteria (*Staphylococcus aureus* ATCC 43300) (Majali et al., 2015). It has antimicrobial effect against *S. aureus* due to the presence of terpenoids and steroids. In addition, the presence of alkaloids in moringa leaves makes a disrupt microorganism's DNA and that makes it have antimicrobial effect (Bennett et al., 2003). Also, the presence of flavonoids in moringa leaves inhibits the enzyme bound membrane activity for many types of microorganisms (Li-Weber, 2009).

UF-white soft cheese is a soft, spreadable cheese produced from concentrated milk by ultrafiltration, to achieve a total solids ratio of 35%, and then enzymatically coagulation of retentate. This type of cheese contains 45-60% fat (on a dry basis), 28% protein (on a dry basis) and maximum 3% salt and the final pH after 72 hours are 4.8. Soft white cheese UF is fresh cheese that can be consumed directly after production. The shelf life of this type of cheese is a maximum two months. (Abd El-Salam et al., 1993 and

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Ramadan et al., 2014).

Mukunzi et al. (2001), decided that new products with low costs and characterize with an improvement in its nutritional and sensory evaluation can be produce from the incorporation of *Moringa oleifera* extracts during the processing of that products.

Some dairy products as yoghurt (Hassan et al., 2016 and Akajiaku et al., 2018), labneh (Salem et al., 2013), cottage cheese (Mahami et al., 2012) and soft cheese (Belewu et al., 2012 and Hassan et al., 2017) were produced by adding dry leaves of moringa and had an acceptable characteristics.

The aim of this study is to examine and utilizing the leave alcoholic extract of *Moringa peregrina* in enhancements some characteristics (nutritional, sensory evaluation, oxidative stability and microbiological quality) of soft white cheese made by Ultrafiltration technique.

MATERIALS AND METHODS

Materials

Chemicals

Some chemicals were purchased from Sigma–Aldrich (St. Louis, MO, USA) such as Radical ,chlorogenic acid and 1,1- diphenyl-2-picrylhydrazyl (DPPH). In addition other was obtained from Fluka Biochemika (Steinheim, Germany) such as Folin-Ciocalteu reagent. other chemicals such as Ethanol obtained from Egypt company (El-Nasr Company for Chemical Industries).

Plant materials

Leaves of *Moringa peregrina* were collected from Al-Orman garden, ministry of agriculture, Giza, Egypt at April 2020.

Rennet

Microbial rennet powder (Marzyme – protease Rhizomucor miehei) has obtained from Danisco France-2, Avenue Brun- Fauuiier- 38470 VINAY (Franc) by Misr Food Additives (MIFAD), Egypt, and used in cheese making after dilution (0.5 g / 20 ml water/10 kg retentate).

Retentate

Retentate of cow's milk (40% solids), was obtained from Obour Land Company for food industries (Obour City, Egypt).

Methods

Preparation of defatted moringa leaves flour

Moringa leaves were washed with tap water, then it was dried in air circulated oven at 50° C for 3 hrs., ground to fine powder for 3 min using a Moulinex mixer (Type 716, France) at the maximum speed setting and the meals were ground to pass through a 1 mm2 sieve. The powder was then defatted using n-hexane. Solvent was evaporated by rotary-evaporator and dried-defatted meal was stored at 4 °C until sample extraction.

Preparation of the extracts

Twenty grams of defatted flour were extracted with ethanol 70% (200 ml) using magnetic stirrer at room temperature , filtration through Whatman No.1 filter paper, re-extracted of the residues under the same conditions, evaporated by rotary-evaporator and then freeze- dried (Thermo- electron Corporation – Heto

power dry LL 300 Freeze dryer). The dried extracts were weighed after lyophilization to determine the yield and stored at -20°C until analysis was carried out. The yield of lyophilized extracts based on dry weight basis was calculated from the following equation:

$$\text{Yield (g/100 g of dry plant material)} = (\text{W1} \times 100) / \text{W2}$$

Where: W1 was the weight of the extract after the solvent evaporation and W2 was the weight of the dry plant material.

Cheese manufacturing

Cow's milk retentate containing 0.02% CaCl₂ and 4% NaCl was divided into five equal portions. The first portion was without any additives (control). The Butylated hydroxyl anisole (BHA) as synthetic antioxidant was added to the second portion at ratio of 0.02%. Ethanolic extract of *Moringa peregrina* leaves were added to the third, fourth and fifth portions at the rate of (10, 20, 50 mg/mL of retentate) respectively. Microbial rennet powder was used in cheese making after dilution (0.5 g / 20 ml water/10 kg retentate). White soft cheese was made by the method of Renner and Abd El-Salam (1991). The resultant cheese of all treatments was packaged into a plastic container (1 Kg), sealed and incubated at 45°C for one hour. The resultant cheese treatments were stored in pasteurized brine solution containing 6% NaCl at 5±2°C for 90 days. Samples were taken for organoleptic properties, chemical, and microbiological analysis at zero time (just after manufacture), 30, 60 and 90 days.

Determination of total phenolic compounds content

The content of total phenolic compounds was determined using Folin-Ciocalteu procedure (Pothitirat et al., 2009). Lyophilized *Moringa peregrina* leaves ethanolic extract (1000 µg/mL), 200 µL was mixed with 500 µL of the Folin-Ciocalteu reagent (diluted 1:10 with deionized water) and 800 µL of sodium bicarbonate solution (7.5%, w/v). The mixture was allowed to stand at room temperature for 30 min with intermittent shaking. The absorbance was measured at 765 nm using JENWAY 6405 UV/visible spectrophotometer (UK). The content of total phenolic compounds was calculated as mean ± SD and expressed as grams of chlorogenic acid equivalents (CAE) in 100 g of the extract.

Determination of total flavonoid compounds content

Total flavonoids were analyzed using aluminum chloride colorimetric method (Pothitirat *et al.*, 2009). Sample (1000 µg/mL) of 500 µL was mixed with 500 µL of 2% aluminum chloride solution. The mixture was allowed to stand at room temperature for 10 min with intermittent shaking. The absorbance of the mixture was measured at 415 nm against a blank sample without aluminum chloride using JENWAY 6405 UV/visible spectrophotometer (UK). The content of total flavonoids was calculated as mean ±SD (n = 3) and expressed as grams of quercetin equivalents (QE) in 100 g of the extract.

Quantitative analysis of major active compounds by HPLC

HPLC analysis of flavonoid compounds

For the HPLC finger print analysis of flavonoid compounds present in extracts, a Shimadzu system (Shimadzu Corp., Kyoto, Japan) consisting of two LC-10AD pumps, SCL-10A system controller, SPD-M10A

photo-diode array detector, and a prepacked LUNA C18 (4 × 250 mm, 5 µm, Phenomenex) was used. A flow rate of 1 mL/min, and gradient elution of acetonitrile- water-acetic acid (5:93:2, v/v/v) [solvent A] and of acetonitrile-water-acetic acid (40:58:2, v/v/v) [solvent B], 0– 50 min solvent B from 0 to 100%; and injection volume of 20 µL were applied; whereas the separation of compounds was monitored at 280 and 320 nm.

Antioxidant activity evaluation

DPPH radical-scavenging activity

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 sample (10 mg extract / 10 ml ethanol 70%) was added to 3 ml of 0.1 mM DPPH dissolved in ethanol. After incubation period of 30, 60 and 120 min at room temperature, the absorbance was determined against a control at 517 nm (Gulcin *et al.*, 2004). Percentage of antioxidant activity of free radical DPPH was calculated as follow:

$$\text{Antioxidant activity (\% Inhibition)} = [(A \text{ control} - A \text{ sample})/A \text{ control}] \times 100$$

Where A control is the absorbance of the control reaction and A sample is the absorbance in the presence of plant extract. Samples were analyzed in triplicate. The concentration of the sample that scavenges 50% of the DPPH radicals (SC₅₀) was calculated by linear regression of curves showing percentage scavenging versus sample concentration.

Examination of UF-white soft cheese:

Chemical analysis

Total solids (T.S%), Fat/DM content, total nitrogen (TN/DM), soluble nitrogen (SN/TN), non-casein nitrogen (NCN/TN) and non-protein nitrogen (NPN/TN) fractions were determined as described in (Fox, 1997). Titratable acidity (T.A) was determined by the method developed by (Lau *et al.*, 1991). Total volatile fatty acid (TVFA) contents were determined by the method described by Kosikowski (1982).

Oxidative stability tests:

Cheese fat was extracted from the samples according to Abd El-Fattah (2006). Peroxide and acid values of white soft cheese fat were determined according to AOAC (2007). Thiobarbituric acid test (TBA) of cheese fat was determined according to Lee *et al.* (2003).

Sensory evaluation

According to Meilgaard *et al.* (2006) the sensory evaluation was carried out by using nine points structured hedonic scale, for the attributes of Flavor, Body & Texture and Appearance. Based on 9-point hedonic scale; like extremely=9, like very much =8, like moderately =7, like slightly =6, neither like nor dislike =5, dislike slightly =4, dislike moderately =3, dislike very much =2, dislike extremely =1. About 20 g of each sample were served, at approximately 7°C, in white 50 ml plastic cups coded with random three-digit numbers.

Microbiological examination

One gram of cheese was accurately weighed and transferred to a sterile mortar. Cheese was then thoroughly ground in 10 ml of a sterile aqueous sodium citrate solution (2%) to a homogenous mass. The mortar contents were transferred quantitatively to a sterile volumetric flask 100 ml using a sterile saline solution and the volume was made

up to the mark to get the final 1/100 dilution of the cheese which was used in final making further dilutions (APHA, 2005) for the determination of the different microbial groups as follow:

The total bacterial count (TBC) was determined according to (Oxoid, 2006) using Tryptone Glucose Extract Agar (T.G.E.A) medium. Plates were incubated at 37 °C for 2 to 3 days. The total coliform count was estimated by plating suitable dilutions on MacConkey agar medium as described by APHA (2005). The plates were incubated for 24 hr., at 35±1°C, and the small non-mucoid red colonies were counted. The total mould and yeast counts were determined according to APHA (2005) by plating suitable dilutions in duplicates on Sabouraud Dextrose Agar medium (Oxoid, 2006). Plates were incubated at 28°C for 3 days then the counts were recorded.

Statistical analysis

Results were statistically analyzed using a computer program “SAS system for windows version 9.00 TS M0” (Guide, 2008) for analysis of variance by two ways (ANOVA) and comparison of means by Duncan’s multiple comparison test where P< 0.05 was considered for significant difference.

RESULTS AND DISCUSSION

The yield, total phenolics content, total flavonoids content and DPPH radical scavenging activity of MPLEE are presented in Table 1. *Moringa peregrina* gave yield extract (35%), total phenolic content (116.6 mg CAE/ g extract) and total flavonoids content (45.2 mg QE/g extract). The antioxidant activity of MPLEE is also presented in Table 1. The antioxidant activity expressed as SC₅₀ (µg mL⁻¹). Low SC₅₀ values mean high antioxidant activity. SC₅₀ of MPLEE was recorded 500 µg mL⁻¹.

Table 1. Yield of extract, total phenolic, total flavonoids and DPPH activity (SC₅₀; µg mL⁻¹) of *Moringa peregrina* using ethanol 70%

Item	<i>Moringa peregrina</i>
Yield of extract (% dry weight)	35
Total phenolics (mg GAE/ g extract)	116.6
Total flavonoids (mg QE/ g extract)	45.2
DPPH activity (SC ₅₀ ; µg mL ⁻¹)	500

The flavonoid compounds identified in the MPLEE are listed in Table 2. Ten components were represented in the MPLEE. The main components were rutin, narengin, rosmarinic and hisperdin (17412, 1328, 1047and 984.90 mg/L, respectively).

Table 2. Bioactive flavonoid compounds in *Moringa peregrina* leaves ethanolic 70% extract evaluated by HPLC.

Compound	Concentration (mg/L)
Narengin	1328.00
Hisperdin	984.90
Rosmarinic	1047.00
Rutin	17412.00
Quercitrin	59.65
Narenginin	84.72
Hispertin	7.038
Kampferol	64.21
Apignin	95.90
7-Hydroxyflavon	16.03

Illustrated data in Table 3 show the total solids content of resultant cheese (control and treatments) through all storage periods. These data indicate that the control cheese have the lowest T.S% content when compared with other treatments either in fresh or other storage periods. The increasing of T.S% in cheese treatments might be due to the total solids content of moringa's ethanolic extract. Also, the total solids content showed gradual increasing in all cheese treatment with the progress of storage period until 90 days. These data came in harmony with those reported by Salem *et al.*, (2010); Khalifa and Wahdan, (2015) & El-Zawahry and Abd El-Wahed (2018)

Data in the same Table revealed that the fat/ dray matter and protein/ dry matter gained the same behavior which occurred in the total solids content either in fresh or throughout all storage periods and that might be as a

result to the decreasing of moisture content of stored control and treated cheese. that result came in compatible with those reported by Hassan *et al.*, (2017) & El-Zawahry and Abd El-Wahed, (2018)

Also, Table (3) shows that, salt in moisture of all cheese treatments was increased with the progress of the storage period. This could be attributed to the loss in water as a result of water exudation during storage period which in turn lead to a more salt concentration.

The same Table shows that, titratable acidity increased gradually until the end of the storage period for all treatments. The control cheese samples had higher titratable acidity than all other cheese treatments during storage period. Cheese containing extracts showed lower titratable acidity. This may be due to antimicrobial activity of these extracts Majali *et al.*, (2015)

Table 3. Chemical composition of UF-soft cheese affected by addition of Moringa leaves ethanolic extract.

Property	Storage period (day)	Control	Moringa leaves ethanolic extracts (mg/ml)		
			10	20	50
TS (%)	Fresh	35.45 ^j	35.40 ^j	35.26 ^j	37.10 ^{hi}
	30	37.60 ^{gh}	37.45 ^{gh}	36.64 ⁱ	39.47 ^c
	60	38.48 ^{ef}	38.35 ^{ef}	37.90 ^{fg}	41.30 ^b
	90	39.44 ^c	39.19 ^{cd}	38.77 ^{de}	42.74 ^a
Fat/DM (%)	Fresh	37.11 ^h	37.88 ^{fg}	38.65 ^{bcd}	39.42 ^a
	30	37.23 ^h	37.90 ^{fg}	38.57 ^{cde}	39.24 ^{ab}
	60	37.44 ^{gh}	37.93 ^{fg}	38.42 ^{cdef}	38.91 ^{abc}
	90	37.94 ^{efg}	38.03 ^{def}	38.12 ^{def}	38.21 ^{def}
Total Protein/DM (%)	Fresh	31.02 ^k	31.33 ^k	31.64 ^{ij}	31.95 ^{hi}
	30	32.30 ^{gh}	32.36 ^{gh}	32.42 ^{fgh}	32.48 ^{fgh}
	60	32.54 ^{efg}	33.09 ^{de}	33.64 ^{cd}	34.19 ^{bc}
	90	32.98 ^{ef}	33.96 ^{bc}	34.51 ^b	35.49 ^a
Salt/moisture (%)	Fresh	8.74 ^g	8.79 ^g	8.84 ^g	9.19 ^f
	30	9.92 ^e	9.96 ^e	10.04 ^e	10.30 ^d
	60	10.35 ^d	10.38 ^d	10.46 ^d	10.66 ^c
	90	10.84 ^b	10.87 ^b	10.95 ^{ab}	11.09 ^a
Titratable acidity (as lactic acid %)	Fresh	0.22 ^j	0.22 ^j	0.21 ^j	0.20 ^j
	30	0.80 ^g	0.79 ^{gh}	0.75 ^{hi}	0.71 ⁱ
	60	1.39 ^d	1.38 ^d	1.32 ^e	1.25 ^f
	90	1.90 ^a	1.88 ^a	1.75 ^b	1.63 ^c

Values in the same raw having different letters are significantly differed $p > 0.05$
 Values are presented as means \pm SD.

Mentioned data in Table 4 show the changes of protein fraction in correlation with the total protein of resultant cheese. These data revealed a progress in the soluble and non-protein nitrogen of all cheese treatments and control with the increasing of storage periods and that came in harmony with the clarification of Ismail *et al.*, (2006) and Khalifa and Wahdan (2015), which explain that by the protein breakdown by microflora and or the action of the enzymes of proteolysis.

Illustrated data in the same Table show the changes and the progress of Total volatile fatty acids content of resultant cheese. These data clarified that there were an increase in the TVFA in all cheese treatments and the control one with the progress in the storage period. These result came in harmony with those reported by Abd El-Gawad, (2009) and Hassan *et al.*, (2017).

Table 4. Ripening indices of UF-soft cheese affected by addition of Moringa leaves ethanolic extract.

Property	Storage period (day)	Control	Moringa leaves ethanolic extracts (mg/ml)		
			10	20	50
SN/TN %	Fresh	7.31 ^j	8.7 ^h	8.18 ^{hi}	8.49 ^h
	30	7.56 ^{ji}	8.87 ^h	10.09 ^g	11.48 ^f
	60	13.67 ^e	14.98 ^d	16.29 ^c	17.44 ^a
	90	16.57 ^{bc}	16.86 ^{abc}	17.15 ^{ab}	17.6 ^a
NPN/TN %	Fresh	3.95 ^g	4.03 ^g	4.23 ^g	4.16 ^g
	30	7.70 ^f	7.72 ^f	8.15 ^e	7.95 ^{ef}
	60	12.00 ^d	12.10 ^{cd}	12.55 ^{ab}	12.37 ^{bcd}
	90	12.40 ^{abcd}	12.43 ^{abc}	12.81 ^a	12.60 ^{ab}
TVFA ml (NaOH 0.1N /100 g)	Fresh	7.69 ^j	8.09 ^k	8.49 ^j	8.89 ⁱ
	30	10.07 ^h	10.87 ^g	11.67 ^f	12.47 ^e
	60	12.45 ^e	13.06 ^d	13.67 ^c	14.28 ^b
	90	13.07 ^d	13.74 ^c	14.41 ^b	15.08 ^a

Values in the same raw having different letters are significantly differed $p > 0.05$
 Values are presented as means \pm SD.

Results presented in Table (5) shows that, cheese made with Moringa leaves ethanolic extracts had

significant ($p \leq 0.05$) lower peroxide values compared with control and BHA cheeses at the end of the storage period. The lower peroxide values of cheeses fortified with the extracts may be due to antioxidant activity of Moringa leaves ethanolic extract Kumar and Pari (2003) & Al-Owaisi *et al.* (2014).

The peroxide values increased in all cheeses treatments as storage period progressed up to the end of the storage period. The The obtained results are similar to those obtained by Omido *et al.* (2013); Singh *et al.*, (2013) & El-Zawahry and Abd El-Wahed, (2018).

As storage period progress, the acid value increase gradually in all treatments as shown in table (3). That might be due to the set free of short chain fatty acids and this gradual increase continued through the progress of storage periods. The lowest change in the acid value was

observed in cheese which treated with ethanolic extracts followed by BHA treated cheese finally the control cheese gained the highest acid value content than other treatments either in the beginning or the end of storage period. These results came in compatible with the mentioned results by Abd El-Aziz *et al.*, (2012); Omido *et al.* (2013) & El-Zawahry and Abd El-Wahed, (2018).

The trend of the changes in Thiobarbituric acid values of all treatments was increased in all treatments with the progress of the storage period. The obtained results are similar to those obtained by Azzam, 2007. The Moringa leaves ethanolic extract treatments were lower significantly ($p \leq 0.05$) in thiobarbituric acid compared to cheese treatments with synthetic antioxidant (BHA) and control cheese. Similar results were obtained by Bandyopadhyay *et al.* (2007) & El-Zawahry and Abd El-Wahed, (2018).

Table 5. Oxidative stability of UF-soft cheese affected by addition of Moringa leaves ethanolic extracts.

Property	Storage period (day)	Control	BHA (0.02%)	Moringa leaves ethanolic extracts (mg/ml)		
				10	20	50
Acid value (mg KoH / g fat)	Fresh	0.76 ^k	0.72 ^{kl}	0.70 ^l	0.67 ^{lm}	0.63 ^m
	30	1.05 ^g	0.99 ^g	0.94 ^{gh}	0.86 ⁱ	0.81 ^{ij}
	60	1.21 ^d	1.13 ^e	1.06 ^f	0.98 ^g	0.92 ^h
	90	1.43 ^a	1.34 ^b	1.27 ^c	1.20 ^d	1.14 ^e
Peroxide value (meq/ kg)	Fresh	4.74 ^j	4.50 ^k	4.28 ^k	4.21 ^k	4.20 ^k
	30	9.13 ^g	8.74 ^h	8.36 ⁱ	8.30 ⁱ	8.23 ⁱ
	60	13.44 ^d	13.04 ^e	12.65 ^f	12.51 ^f	12.40 ^f
	90	19.00 ^a	16.47 ^b	13.95 ^c	13.68 ^{cd}	13.51 ^d
TBA test	Fresh	0.158 ^j	0.144 ^{mm}	0.142 ^{no}	0.141 ^o	0.137 ^p
	30	0.165 ⁱ	0.157 ^j	0.151 ^k	0.148 ^l	0.145 ^m
	60	0.220 ^e	0.203 ^f	0.202 ^f	0.192 ^g	0.184 ^h
	90	0.280 ^a	0.265 ^b	0.263 ^b	0.250 ^c	0.243 ^d

Values in the same raw having different letters are significantly differed $p > 0.05$

Values are presented as means \pm SD.

Data presented in Table (6) shows the total bacterial, coliform, yeast and mould counts of white soft cheese during storage period. The total bacterial counts decreased gradually in all treatments till the end of the storage period. Addition of Moringa leaves ethanolic extracts reduced the total bacterial count than the control along the storage period. This reduction may be attributed to the inhibitory effect of the extract as antimicrobial agent. These results agree with Majali *et al.* (2015)

Coliform bacteria were observed with slightly numbers at the beginning of the storage period in cheese samples, then not detected with the progress of storage period. These results are in agreement with Majali *et al.* (2015). The absence of coliform bacteria could due to the efficient heat treatment and good sanitation conditions applied during manufacture and storage of cheese samples and the development of acidity in cheese. These results agree with the results of Monzano *et al.* (1992). Yeast and mould counts were not detected in all samples up to 60 days of storage except the control treatment which observed after 30 days of storage period. This could be attributed to the inhibitory effect of Moringa leaves ethanolic extracts. Also, it could be noticed that the yeasts and moulds were higher in control cheese compared with other treatments. These illustrated data came in harmony with those mentioned by Singh *et al.* (2005). Meanwhile counts of yeast, mould and coliform in all samples were in accordance to the legal Egyptian standards.

Table 6. Microbiological properties of UF-soft cheese affected by addition of Moringa leaves ethanolic extract (log cfu/g)

Property	Storage period (day)	Control	Moringa leaves ethanolic extracts (mg/ml)		
			10	20	50
Total viable count	Fresh	7.897	7.681	7.255	7.146
	30	8.012	7.544	7.00	6.301
	60	8.193	7.447	6.477	N.D
	90	8.328	7.204	N.D	N.D
Coliform group	Fresh	2.301	2.301	2.000	N.D
	30	2.301	2.000	N.D	N.D
	60	2.000	N.D	N.D	N.D
	90	N.D	N.D	N.D	N.D
Yeasts	Fresh	4.919	N.D	N.D	N.D
	30	4.982	4.361	N.D	N.D
	60	5.152	4.612	N.D	N.D
	90	5.281	4.732	4.230	3.301
Mould	Fresh	N.D	N.D	N.D	N.D
	30	4.342	3.301	N.D	N.D
	60	4.397	3.698	3.301	N.D
	90	4.278	3.845	3.903	3.477

N.D: Not Detected

Data presented in table (7) showed the average score points given for flavour, body and texture, and appearance of UF-white soft cheese as affected by adding natural antioxidants. The results showed that there were significant differences between the control cheese and all experimental cheeses when fresh and during the storage period up to 90 days. Cheese made with Moringa leave extracts at different ratios showed

the highest score for organoleptic properties than other cheese. Control cheese showed the lowest scores for organoleptic properties. All cheese treatments had been acceptable by panelists it is also that all additives improved cheese properties and overall acceptability.

Also, organoleptic properties of all cheese treatments improved by progressed of the storage period until the end of storage. The obtained results are similar to those reported by Osman and Abd El-Wahed, (2019).

Table 7. Organoleptic properties of UF-soft cheese affected by addition of Moringa leaves ethanolic extract.

Storage period (day)	Property	Control	Moringa leaves ethanolic extracts (mg/ml)		
			10	20	50
Fresh	Flavour	7.04 ^e	7.09 ^d	7.17 ^c	7.33 ^a
	Body & Texture	6.39 ⁱ	6.43 ^j	6.49 ⁱ	6.98 ^f
	Appearance	6.48 ⁱ	6.55 ^h	6.88 ^g	7.28 ^b
30	Flavour	7.45 ^e	7.50 ^d	7.61 ^b	8.25 ^a
	Body & Texture	6.49 ^j	6.58 ^k	6.88 ^h	7.35 ^f
	Appearance	6.65 ^j	6.73 ⁱ	6.95 ^g	7.55 ^c
60	Flavour	8.06 ^e	8.15 ^e	8.16 ^c	9.09 ^a
	Body & Texture	7.38 ^e	7.48 ^{de}	7.57 ^d	8.41 ^b
	Appearance	7.40 ^{de}	7.55 ^{de}	7.55 ^{de}	8.00 ^{de}
90	Flavour	8.1 ^f	8.15 ^e	8.30 ^c	9.25 ^a
	Body & Texture	7.49 ^j	7.60 ⁱ	7.78 ^g	8.52 ^b
	Appearance	7.40 ^l	7.45 ^k	7.65 ^h	8.27 ^d

Values in the same raw having different letters are significantly differed $p > 0.05$

Values are presented as means \pm SD.

CONCLUSION

Moringa leaf extracts can be added to dairy products such as (UF-soft cheese) as a new source of natural antioxidants and antibacterial agents. This treatment was improved the oxidative stability, organoleptic properties and reduce the total bacterial, coliform, mold and yeast counts of the treated cheese compared with control. It can be recommended to use this extract at a rate of 20 mg/ml which improves the microbiological quality and shelf life of the resulting cheese.

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الاستفادة من مستخلص أوراق المورينجا لإنتاج الجبن الأبيض الطري الوظيفي السيد محمد عبدالواحد¹ و علي عثمان محمد خليل² اقسم علوم الاغذية – كلية الزراعة- جامعة الزقازيق اقسم الكيمياء الحيوية الزراعية- كلية الزراعة- جامعة الزقازيق

أجريت الدراسة الحالية لتحضير لتقدير المركبات الحيوية بالمستخلص الإيثانولي لأوراق نبات المورينجا بيريجرينا وتقدير تأثير هذا المستخلص على الثبات التأكسدي والتركيبي الكيميائي والخصائص الميكروبيولوجية والحسية للجبن الأبيض الطري المصنع بطريقة الترشيح الفائق وذلك بإضافة المستخلص الإيثانولي بتركيزات مختلفة (10، 20 و 50 ملجم/مل) مقارنة بالجبن الكنترول والجبن المحتوي على 0.02% من بيوتيلينيد هيدروكسي أنيسول. أوضحت النتائج أن محصول المستخلص كان 35% من الوزن الجاف لأوراق المورينجا، الفينولات الكلية 116.6 مجم مكافئ حمض الكلوروجينيك/جم مستخلص والفلافونويدات الكلية 54.2 مكافئ الكيرسيتين/جم من المستخلص. المكونات الرئيسية للمستخلص الإيثانولي كانت روتين ، نارينجين، روزمارينيك وهيسبردين (17412 ، 1328 ، 1047 و 984.90 ملجم/لتر، على التوالي). تم تسجيل تركيز عينة المستخلص الكحولي التي تزيل 50% من الشقوق الحرة DPPH (SC₅₀) لـ 500 ميكروجرام/مل. أظهرت النتائج أن إضافة MPLEE كان لها تأثير معنوي متزايد ($P \leq 0.05$) على نسب كل من الدهن، شقوق النبتروجين، والملح، والأحماض الدهنية الكلية الطيارة. من ناحية أخرى، تم انخفاض الحموضة بإضافة مستخلص الإيثانول مقارنة بالمجموعة الكنترول. أظهر الجبن المحتوي على MPLEE أعلى ثباتاً للأكسدة. أيضاً، عمل المستخلص على تقليل إجمالي عدد البكتيريا الكلي ويكتريا القولون في جميع العينات. ومع ذلك ، أظهر تعداد الفطريات اتجاهًا معاكسًا ولم يتم اكتشافها في جميع العينات المعالجة حتى 60 يوماً من التخزين. أظهرت النتائج أيضاً أن عينات الجبن المحتوية على المستخلص الإيثانولي أظهرت خصائص حسية أفضل من المعاملات الأخرى. لذلك يمكن التوصية باستخدام هذا المستخلص عند مستوى 20 مجم/مل لتحسين الجودة الميكروبيولوجية ومدة صلاحية الجبن المنتج.