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Utilization of Portulaca Oleracea L. to Improve Quality of Yoghurt

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ABSTRACT

The present investigation was conducted to study the possibility of using Portulaca Oleracea L. as a source of Omega-3 and Omega-6 fatty acids as well as high vitamins and minerals, to improve the quality of yoghurt. Also, the microbial characteristics the treated yoghurt were evaluated. The obtained results showed that the replacement of milk fat by dry leaves of P. Oleracea had no effect on the chemical composition and the sensory properties of the treated yoghurt with 50 and 100% P. Oleracea L. leaves oil as milk fat substitute compared to the untreated one. In conclusion, manufacturing yoghurt is suitable as a rich nutrient food stuff for people suffering from blood hypertension, high blood cholesterol, liver and heart diseases.

Key words: - Portulaca. oleracea L. leaves oil - omega-3 (ω-3) - yoghurt – macro and micro elements

INTRODUCTION

Portulaca oleracea L., a member of family Portulacaceae, is a warm climate, annual, green herb, with branched and succulent stems which are decumbent near the base and ascending near the top to a height of 15-30 cm. The plant is fleshy, stout and succulent with obovate to spathulate, obtuse opposite leaves tapering towards the base. The flowers are small, yellow, and sessile in clusters of 3-5 on the forks and tips of the branches. The fruit is oblong and transversely dehiscent. The seeds are orbicular and 0.5 mm in diameter ***(Hussein, 1985; Mossa et al., 1987 ; Feinbrun-Dothan & Darin .(1991*) *and Mitich, 1997)*** It has a cosmopolitan distribution in Africa, China, India, Australia, Middle East, Europe and United States ***(Mitich, 1997; Oran & Al-Eisawi, 1998 and Chan et al., 200).***

P. oleracea reported to contain also other chemical constituents, including urea, calcium, iron, phosphorous, manganese, copper and fatty acids, especially omega-3-acids whose concentration in P. oleracea is the highest found in leafy vegetables ***(Hussein, 1985; Simopoulos et al., 1992; Mohamed & Hussein, 1994; Ezekwe et al., 1999 and; Garti et al., 1999).*** Furthermore, the occurrence of glutathione; glutamic acid; and aspartic acid has been published by ***Simopoulos et al. (1992).***

Recent research has shown that P. oleracea (purslane) is a rich source of omega-3 (ω-3) fatty acids, which is important in preventing heart attacks and strengthening the immune system ***(Simopoulos, 2004)***.

In addition, purslane is reported to be rich in linolenic acid and ß-carotene and its used as a health food for patients with cardiovascular diseases ***(Liu et al., 2000).*** It contain several types of vitamins and minerals especially calcium and potassium.

Although purslane (Portulaca oleracea) has long been known as a nuisance weed ***(Vengris et al., 1972),*** more recently strong interest has developed in this species as a highly nutritious salad green or potherb. This interest is encouraged by the perceived health benefits associated with α-linolenic acid (LNA), an omega-3 fatty acid (ω3FA) often referred to as fish oil, and the antioxidant α-tocopherol. In fact, the health benefits of ω3FAs, as part of a balanced diet, have been well-established ***(Simopoulos et al., 1985; Simopoulos, 1991;and British Nutrition Foundation1993).*** Purslane leaves are a rich source of both the 18:3 fatty acid (LNA) and α-tocopherol ***(Simopoulos et al., 1992)***.

Recently the use of vegetable fats and oils in dairy industry is one of the most important and controversial topics. Among the major reasons for the growing acceptance of these new foods that is could be kept for several weeks under normal refrigeration condition. Besides that, the primary risk factor associated with cardiovascular disease is the highly consumption of animal fat and cholesterol ***Durkee, (1968).***

In addition, diets containing a large amount of polyunsaturated fatty acids tend to decrease blood cholesterol concentration, whereas diets high in saturated fatty acids tend to increase blood cholesterol. Thus an individual should substantially reduce dietary intake containing lower saturated fatty acids and cholesterol content. This can be accomplished by modified dairy products containing lower saturated fatty acids and cholesterol content ***Hurt, (1972)***.

Therefore, P. oleracea L. leaves oil is considered a source of significant nutritional value in human health and a preventive factor in many diet related illness like coronary heart disease and cardiovascular disease .

Therefore, this investigation aims to study the possibility of using P. oleracea L. leaves oil as fat milk substitute in Yoghurt.

MATERIAL AND METHODS

Plant Material

P. oleracea L. was collected from Minufiya province-Egypt in September 2014, and identified by Corps unit, Plant Research Department, Nuclear Research Center, Atomic Energy Authority.

Sample Treatment

The fresh samples were manually washed with distilled water and residual moisture evaporated at room temperature .Plant roots were discarded and residues of plant were cut into species then dried in oven at 45ºC for 72 hr then ground to a fine powder in a coffee grinder for 2 min., at 15 sec. intervals, the process was stopped for 15 sec. to avoid heating of sample.

Nutritional Analysis

Using the standard methods of the Association of the Analytical Chemists (AOAC) determination of moisture, ash, and crude fibers (on dry basis) was carried out ***(Anon, 2000).*** The determination of proteins in terms of nitrogen was done by micro Kjeldahl method **(Kjeldahl, 1983)**. The nitrogen value was converted to protein by multiplying to a factor of 6.25 . The lipid content of the samples was done using Soxhlet type of the direct solvent extraction method. The solvent used was petroleum ether (boiling range 40-60ºC) **(Anon2007)** .The crude fiber was also determined by the method described by ***(Boussama, 1999).*** The total carbohydrates were determined by difference method [100 - (proteins + fats + moisture + ash in percentage)] ***(Muller & Tobin, 1980; Hussain et al ,.(2009)*** All the proximate values were reported in percentage.

Fatty Acids

1-The fatty acid methyesters were prepared using sulfuric acid in methanol (2.5%) as a reagent and the methylation was carried out by refluxing the oil for 2.5 hours on water bath, according to the method reported by ***Anon (1966)***.

2-Separation and identification of fatty acid methyl esters: The fatty acid methyl esters were analyzed by gas Chromatephic technique using a model HP 6890 instrument equipped with aflame ionization detector, The fractionation of fatty Acid methyl ester was conducted by using a coiled glass column (30m x 320 μm x 0.5μm) and coated with in nowax polyethrles glycol.

The oven temperature was 150 ºC hile the temperature of injector and detectors were 260 ºC nd 275 ºC espectively. the Nitrogen flow was 1.5 ml/min and split ration was 30: 1. The peak areas and retention times were measured using enhanced integrator. The identification of fatty acid methyl esters were performed by comparing their relative retention times. With those of authentic fatty acid methyl esters.

Elemental Analysis

Accurately weighed sample (3 g) in a crucible was subjected to ashing in furnace for 4 hour at 550 ºC. After cooling in desiccator, 2.5 mL of 6N HNO3 was added to the crucible. The solution was filtered and diluted up to 100 mL with distilled water. The solution was analyzed for Ca, Mg, Na, K, P, Fe, Cu, Zn, Ni, and Mn by using Atomic Absorption Spectrophotometer.The results were obtained while using a working standard of 1000 ppm for each of the species ***(Khan & Hidayat, 2008; Hussain et al., 2009 and Hussain et al.(2010)) .***

Source of Milk

Fresh bulk whole cows' and buffaloes' milk (morning milking) were obtained from the herds belong to the Agriculture Faculty Minufiya University

Yoghurt Starter Culture

An active Yoghurt starter culture containing pure strains of *Streptococcus thermohpiles (11486) and Lactobacillus bulgaricus (11842),* 1: 1, was obtained from Christian Hansen (Denmark) and was used in yoghurt manufacture.

Yoghurt Manufacture

Experimental procedure: Fresh mixture of cows' and buffaloes' milk (9 kgm with 4% fat) were divided into tree equal portions: one portion was used as a control sample, the fat of the other portions were replaced with P. oleracea L leaf oil by ratio of 50% and 100%. All milk batches were heated to the boiling point for 10 min. then, cooled to 40ºC, then inculated with 2% starter (a mixed culture of S. thermophilus and L. bulgaricus) and mixed well. The inoculated batches were packed into plastic cups (capacity of 80 gm) and incubated at 42 ºCfor about 3 hr. until coagulation occurs. The result yoghurt samples were stored at 5± 1 ºC)in the refrigerator.

These samples were analyzed periodically every 4 days for acidity and pH and were analyzed as fresh and after 12 days for total solid and fat content.

The samples were judged every 4 days by The pannel consisted of 10 judges from the staff members of the Food unit; Plant Research Department, Nuclear Research center , Atomic Energy Authority were evaluated yoghurt samples according to score sheet suggested by ***Younis (1983)***. Experiments were repeated and analyzed in duplicated and average results were recorded.

Chemical Analysis

The total solid, fat content and total nitrogen contents, titratable acidity and pH values of yoghurt were determined according to ***Ling (1963).***

Microbiological Analysis

Total viable bacterial counts were determined using plate count agar media ***(APHA, 1992).*** Mould and yeast counts were counted on Oxytetracycline glucose yeast extract agar medium according to ***Oxoid (1982).***

RESULTS AND DISCUSSIONS

Nutritional Composition

Nutritional analysis of P. oleracea except moisture (wet and dry basis) was carried out on dry basis and has been reported in the Table 1. The moisture content of P. oleracea on wet basis was (87.00%), while moisture content on dry basis was 5.00% in P. oleracea (23.2%),. Ash content determined in P. oleracea was 23.2% these results trend with (Srivastava et al., 2006). Lockett had also reported high ash content in some greens used by the lactating mother such as bitter leaves, (Lockeett et al., 2000). This indicates P. oleracea could be good sources of mineral elements. Total proteins T.N x (6.25),crud fiber and total carbohydrates were 13.50 ,15.00,and 36.8 respectively

Table (1): Nutritional composition: of P. oleracea.(dry weight).

Moisture content %	87.00 %
Moisture content (dry) %	5.00%
Crude protein (T.N x 6.25)%	13.50%
Total lipids %	6.50 %
Crude fiber %	15.00%
Ash %	23.2%
Total carbohydrate %	36.80 %

Fatty Acids in Pruslane

Fatty acids are given in table (2) showed that P. oleracea leaves oil is rich in linolenic acid ((ω-3) C18:3) and lenolice acid ((ω-6) C18:2) which reached content ratio to 45.65 % and 12.37 % respectively . This percentage is much higher than that found in some leafy vegetables and even higher than that found in some commonly fish species . So that used P. oleracea leaves oil as fat substitute in manufacture yoghurt as a health food for patients with cardiovascular diseases.

Table (2): Fatty Acid composition of P. oleracea.

Fatty acids (%)	
Myristic C14:0	2.00%
Palmitic C16:0	9.31 %
Citric C18:0	2.15 %
Oleic C18:1	5.356 %
Lenolice((ω-6) C18:2	12.37 %
Lenolenice((ω-3) C18:3	45.65 %
Eicosapentaenoic C20:5	0.13 %
Other acids	22.8

Elemental Composition

Elemental composition of the dry samples, reported on dry weight basis, is given in Tables(3). The P. oleracea are found to be good sources of Ca, Mg, P, Na, K, Ni, Cu, and Mn. These P. oleracea considered as sole source of macro and micro elements and can be used as one of the potential sources of the elements in the diet.

The positive impact of zinc supplementation on the growth of some stunted children, and on the prevalence of selected childhood diseases such as diarrhoea, suggests that zinc deficiency is likely to

be a significant public health problem, especially in developing countries (***Osendarp et al., 2003)***. According to FAO's food balance data, it has been calculated that about 20% of the world's population could be at risk of zinc deficiency. The average daily intake is less than 70 µg per day (***Holt & Brown, 2004)***.

Biologically, nickel also plays a key role in plants. As a matter of fact urease (an enzyme whish assist in the hydrolysis of urea) contains nickel. Other nickel containing enzymes include a class of superoxide dismutase ***(Sezilagyi et al., 2004)***. These nickel containing enzymes play integral role in human biological system.

Dairy products supply 50-80% of dietary calcium in most industrialized countries, while foods of plant origin supply about 25%. The calcium concentration.

Table (3): Composition of micro elements and Macro elements of the P. oleracea (ppm).

Micro elements		Macro elements	
Fe	9.19	Ca	157
Zn	0.257	Mg	100
Mn	2.31	K	1102
Cu	0.138	Na	63
Ni	0.10	P	317

Sensory Evaluation in Yoghurt

Sensory evaluation scores given in Table (4) showed that for the control yoghurt and other treatments containing P. oleracea leaves oil by percentage 50% and 100% as fat substitute decreased with advancing storage time .As seen from Table (4) remarkable difference was observed in the appearance of the control (yoghurt containing the milk fat) and other treatments that contained P. oleracea leaves oil as fat substitute .A white colour was observed in the yoghurt containing P. oleracea leaves oil while a yellow colour was revealed in the yoghurt containing milk fat . From the same table it can be seen that the body and texture of yoghurt containing P. oleracea leaves oil obtained lower scoring compared with the control.

Dealing the flavour item the control yoghurt showed higher scoring points for flavour than the yoghurt of the treated samples due to the oily flavour of the latter ones.

The control yoghurt was ranked higher score as fresh compared with other treatments containing P. oleracea leaves oil as fat substitute specially that contained to 100% P. oleracea leaves oil . This may be due to the replacement of milk fat with P. oleracea leaves oil induced an oily flavour which effected the acceptability of these yoghurt by the score panel testers.

The yoghurt samples in the control or that containing P. oleracea leaves oil showed rapid decrease in their quality after 10 days of cold storage showing slimy surface and yeasty flavour and became unacceptable with a slight bitter taste and were rejected after 12 days for the control cheese and that containing P. oleracea leaves oil. This is obviously due to the growth of the microorganisms.

From the previously mentioned results it can be concluded that a safe and good quality yoghurt can be made from homogenized milk containing 4 % P. oleracea leaves oil as milk fat substitute (containing a higher percentage of omega 3 (ω-3) C18:3).

This yoghurt is more suitable as a higher nutrient food stuff for people suffering from blood hypertension , high blood cholesterol ,liver and heart diseases, as P. oleracea leaves oil is a source of significant nutritional value in human health and a preventive factor in many diet related illness.

Table (4): Effect of different treatments and storage on Sensory evaluation of yoghurt made by using P. oleracea L. leaves oil as fat substitute.

Treatments / Storage period		control	Percentage of P. oleracea L. leaves oil replacement fat	
			replaced fat % 50	replaced fat % 100
Fresh	Appearance	10	9	9
	Body &Texture	60	57	87
	Flavour	30	26	23
	Total	100	92	89
4	Appearance	9	8	7
	Body &Texture	55	53	51
	Flavour	28	23	22
	Total	92	84	80
8	Appearance	7	6	5
	Body &Texture	50	44	40
	Flavour	21	20	18
	Total	79	70	63
12	Appearance	6	5	5
	Body &Texture	38	35	32
	Flavour	17	15	13
	Total	61	55	50

Table (5) clears that the total solid in control and other yoghurt treatment samples (50% and 100% as fat substitute) showed about the same percentage with slight differences. Total solid of all samples was 13.50.

During storage it was noticed that total solid of control samples and other samples decreased by proceeding the storage period up to the end of storage period.

The slight decrease in total solid of all treatments can be attributed to lactose fermentation, protein and fat hydrolysis producing and volatile substances, in additions to lactic acid, acetaldehyde and acetone. Similar results were reported by***, El-Shibiny et al. (1979), Ibrahim (1984),Tamime, and Robinsin (1985):, Mehanna et al (1988) Abd El-salam et al (1996), Kebary and Hussen (1999) Sallam (2003) and Anwar et al. (2009)*** on yoghurt. who found that the average total solids% of yoghurt gradually decreased during storage period.

Total nitrogen content in dry matter of all yoghurt treatments did not change as storage period proceeded (Table 5). Similar results were reported by ***El-Shibiny et al. (1979) Ibrahim (1984), Mehanna et al (1988); Abd El-salam et al (1996), Kebary and Hussen (1999) Sallam (2003) and Anwar et al. (2009).*** Total nitrogen content of all yoghurt treatments did not differ from each other, this means that the replacement of fat treatments did not have any effect on total nitrogen content of yoghurt. The same trend was noticed by ***Sallam (2003) and Anwar et al. (2009)*** on yoghurt.

The fat content in control and other yoghurt treatments showed about the same percentages. Fat contents of all samples were 4%. (Table 5).During storage, it can be noticed that the average fat content decreased during storage periods to 3.8, 3.7 and 3.7 for the control and other samples.The slight decrease in the fat content of Yoghurt during storage may be due to the slight hydrolysis of fat. These results are in accordance with those reported by ***El-Shibiny et al. (1979), Ibrahim (1984), Tamime, and Robinsin (1985):, Mehanna et al (1988) , Tamime,(1994), Abd El-salam et al (1996), Abd-El-Aty et al (1998),Kebary and Hussen (1999) Sallam (2003) and Anwar et al. (2009).***

Table (5): Effect of Substitute milk fat by P. oleracea L. leaves oil and storage on the total solid, total nitrogen and fat contents of yoghurt during storage in at 5 ºC.

Treatments / Determination	Control		Percentage of P. oleracea L. leaves oil replacement fat			
			%50		100%	
	Fresh	12	Fresh	12	Fresh	12
Total Solid%	13.50	12.90	13.50	12.81	13.50	12.82
Total nitrogen%	0.701	0.700	0.710	0.715	0.691	0.691
Fat %	4	3.8	4	3.7	4	3.7

Titratable acidity % of all fresh yoghurt samples was nearly the same. Titratable acidity of fresh control, 50% and 100 % P. oleracea leaves oil as fat substitute yoghurt samples ranged from 0.83 to 0.90 %. Titratable acidity of all yoghurt samples increased significantly ($p \leq 0.05$) as the storage period advanced (Tables 6).The increase of titratable acidity is most likely due to the fermentation of residual lactose. Similar results were reported by ***El-Shibiny et al. (1979), Ibrahim (1984), Mehanna et al (1988); Al Salah (1992); Abd El-salam et al (1996), Abd-El-Aty et al (1998), Kebary and Hussen (1999) Sallam (2003) and Anwar et al. (2009).***

It is obvious that the pH values of all fresh yoghurt samples were almost similar and ranged from pH 4.70 to 4.73. The pH values of the control yoghurt samples decreased significantly ($p \leq 0.05$) and reached 4.25 after 12 days of cold storage only, These results are in agreement with those reported ***Ibrahim (1984)*** and ***Anwar et al. (2009)*** on yoghurt.

Table (6): Effect of different treatments and storage on the acidity and pH values of yoghurt made by using P. oleracea L. leaves oil as fat substitute.

Storage period (days)	Acidity			PH		
	Control	Percentage of P. oleracea L. leaves oil replacement fat		Control	Percentage of P. oleracea L. leaves oil replacement fat	
		50%	100%		50%	100%
Fresh	0.90	0.83	0.84	4.70	4.72	4.73
4	0.95	0.90	0.89	4.60	4.65	4.65
8	1.10	0.95	0.94	4.49	4.55	4.58
12	1.15	1.11	1.10	4.25	4.36	4.39

® = Organoleptically unaccepted and rejected.

Total viable bacterial count of fresh yoghurt samples were 91×10^6, 101×10^6, and 99×10^6cfu /g^{-1} for fresh control, 50% and 100 % P. oleracea leaves oil as fat substitute yoghurt samples respectively (Table 7). Total viable bacterial counts of all yoghurt treatments increased gradually as storage period progressed and reaching $107x10^6$,$105x10^6$ and 103×10^6 cfu /g^{-1} at the end of the storage period (12 days). These finding are in agreement with those obtained by ***Anwar et al. (2009)*** on yoghurt.

Counts of moulds and yeasts in fresh samples were 10×10^2 , 12×10^2 and 11×10^2 for fresh control, 50% and 100 % P. oleracea leaves oil as fat substitute yoghurt samples respectively table (7) . The same trend was stated by ***sallam et al. (2003) and Anwar et al. (2009)*** on yoghurt. Control yoghurt samples showed a gradual increase in mould and yeast counts during the cold storage period and reaching 60×10^5 , 59×10^5 cfu /g^{-1} and 62×10^5 after 12 days for control, 50% and 100 %P. oleracea leaves oil as fat substitute yoghurt samples respectively, when it was rejected.

Table (7): Effect of different treatments and storage on the Total bacterial count and total mold and yeast of yoghurt made by using P. oleracea L. leaves oil as fat substitute.

Storage period (days)	Total bacterial count			Total Mold and yeast		
	Control	Percentage of P. oleracea L. leaves oil replacement fat		Control	Percentage of P. oleracea L. leaves oil replacement fat	
		50%	100%		50%	100%
Fresh	91×10^6	101×10^6	99×10^6	10×10^2	12×10^2	11×10^2
4	125×10^6	111×10^6	112×10^6	39×10^3	39×10^3	44×10^3
8	136×10^6	133×10^6	132×10^6	48×10^4	52×10^4	55×10^4
12	$107\ x10^6$	$105x10^6$	$103x10^6$	$60x10^5$	$59x\ 10^5$	$62x10^5$

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