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Research Article
Physicochemical Characteristics and Antioxidant Capacity of Bio Drinking Yoghurt Fortified with *Salvia officinalis* Extract

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Abstract

Background and Objective: Fermented dairy products are widely consumed as functional foods. *Salvia officinalis* has many health benefits. Bio-products are usually standardized based on the presumption that culture viability is a reasonable measure of probiotic activity, thus the ability of the strain to attain high cell population is of primary importance. **Materials and Methods:** In this study, sage extracts and probiotic culture were utilized in drinking yoghurt of buffalos' milk. The experiment was carried out at Faculty of Agriculture, Damanhour University and Faculty of Agriculture (El-Shatby), Alexandria University, Egypt during October-December, 2016. Three replicates of each treatment were manufactured and the products were analyzed during storage for physicochemical, microbiological, viscosity and sensory properties. **Results:** The results showed that the sage extract and probiotic culture have no any influence on physicochemical properties. While, sage extract stimulates the growth of probiotic culture and increased the antioxidant capacity of drinking yoghurt. The drinking yoghurt made with sage extract and probiotic culture was highly accepted in flavor by panelists. **Conclusion:** This study recommended that sage extract could be a good source of anti-oxidant compounds as well as increasing the viable probiotic culture to increase the health benefits of bio-drinking yoghurt.

Key words: Drinking yoghurt, probiotic, sage

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

There has been an increasing demand of consumer interest in functional foods containing live probiotic cultures and high level of anti-oxidant compounds[1]. Daily consumption of fermented milk enhances immune system, improve lactose digestibility, mange blood glucose level, eliminate some diseases such as colon cancer, inflammatory bowel disease, diarrhea and allergies[2]. Drinking yoghurt is categorized as stirred yoghurt with low viscosity and this product is consumed as a refreshing drink in many countries[3]. Drinking yoghurt, also known as drinkable yoghurt is one of the fastest growing fermented products in the world[1,4]. Supplementation of drinking yoghurts with probiotic culture and prebiotic represents a new option to add further value to dairy beverages[5].

Sage, garden sage or common sage (*Salvia officinalis*, Lamiaceae) is a perennial, evergreen sub-shrub, with woody stems, grayish leaves and blue to purplish flowers. It is native to the Mediterranean region, being currently cultivated in many countries[6,7]. Sage is mostly diffuse in the Mediterranean Basin, in South East Africa and in Central and South America[8]. Sage and some of its constituents were good sources of anti-oxidant components, mainly phenolic compounds such as carnosic, caffeic, rosmarinic and salvianolic acids as well as other phenolic structure-based compounds[9,10]. Rosmarinic acid (RA-1) is the major phenol compound of sage[11]. Also, carnosol, ursolic acid, oleanolic acid and rosmarinic acid were presence in the leaves of *S. officinalis*[12]. Sage has one of the longest histories and widely used in medicine against fever, rheumatism, perspiration, sexual debility and in the treatment of chronic bronchitis, as well as mental and nervous diseases[13]. The potential health benefits of sage phenolic compounds, especially flavonoids, which is efficient in trapping superoxide anion, hydroxyl, peroxyl and alcohoxyl radicals[14,15]. It has a potential in treating cancer as it shows strong antitumor genic activities as well as in the cosmetics, perfumery and pharmaceutical industries[4,16]. Moreover, sage is used as a traditional herbal medicine against gastric disturbances and inflammatory processes[17]. Sage leaves and its essential oil possess carminative, anti-spasmodic, anti-septic, astringent and anti-hidrotic properties, as well various extracts of different *Salvia* species have been examined for anti-microbial, anti-inflammatory, anti-oxidant, spasmolytic, cholinergic and cytoprotection binding properties and the involved mechanisms have been partially described[17,18]. Sage is also used traditionally in some food preparations and in some cheeses[10,19]. Sage is largely used as a savory food flavoring either as dried leaves or essential oil. It was one of the favorite candidate species as a source of natural anti-oxidants in health care products[20]. Its essential oil inhibits fungal growth, which is associated with its camphor content. In addition, sage volatile oil can also act as anti-oxidants and preservatives in some foods[21].

Modern food science and nutrition have suggested the involvement of probiotic cultures and prebiotic ingredients in order to increase the nutritional value of some food products. Since the sage "*Salvia officinalis*" is used for a long time as medicine, the aim of this work was to increase the functional properties of bio-drinking yoghurt by sage extract and to evaluate the chemical, microbial, rheological characteristics, anti-oxidant capacity and sensory properties of final product.

MATERIALS AND METHODS

Milk: Fresh buffalo milk was obtained from the herds of Nasser Secondary School of Agriculture, Damanhour, Behera Governorate, Egypt. *Salvia officinalis* (Sage) plant was obtained from local market, Shoubrikhit, Behera Governorate, Egypt. Yoghurt commercial culture (DVS Express 0.1) and Probiotic commercial culture (FD-DVS ABY-3 Probio-Tec) were obtained from Chr. Hansen's Laboratory, Denmark.

Preparation of sage extract solutions: Sage plant was washed with tap water after that with distilled water and dried in shade drying for few days and then powdered with electric blender. The dried leaves powder was passed through sieve no. 20. About 100 g of leaves powder were extracted by boiling in 1000 mL of distilled water and stirring for 5 min and then cooled to room temperature, adjusting the volume to 1000 mL and then filtrated through Whitman filter paper No. 1. The filtrate extract was stored at -20°C until use.

Preparation of drinking yoghurt: Egyptian buffalo milk was analyzed for physicochemical properties. Sugar (8.5%) was dissolved in milk at 50°C and then heated at 90°C for 5 min. The milk was cooled to 45°C and then it was divided to 2 potions. The first potion was inoculated with starter culture (DVS Express 1) and the second potion was inoculated with probiotic starter culture. Inoculated milk of each treatment was then divided to 4 potions. Six treatments were carried out as following C1; yoghurt culture with add 10% of boiled water as a control, T1; yoghurt culture with adding 5% extract solution and 5% of boiled water, T2; yoghurt culture with adding 7% extract solution and 3% of boiled water, T3; yoghurt culture with adding 10% extract solution; C2; probiotic culture with add 10% of boiled water as a control,

T4; probiotic culture with adding 5% extract solution and 5% of boiled water, T5; probiotic culture with adding 7% extract solution and 3% of boiled water, T6; probiotic culture with adding 10% extract solution. Treatments were incubated at 42°C until pH drops to 4.7 and then kept at 5°C for overnight. Coagulated yoghurt was stirred by mechanical mixer, filled in plastic bottles and stored at 5°C for 15 days.

Chemical analysis of the product: Moisture, ash, fat, protein contents, pH and Titratable acidity were determined by the methods of AOAC[22].

Phenolic content: Twenty grams of drinking yoghurt were mixed with 20 mL of distilled water; the mixture was vortexed for 1 min then filtrate through Whitman filter paper No. 42. Total extractable phenolic contents of sample were evaluated with Folin-Ciocalteu (FC) reagent using Gallic acid as standard materials[23]. A 100 µL aliquot of sample extract or a standard dilution was mixed with 2 mL water followed by 200 µL of FC reagent (2N). Tubes were vortexed and incubated at room temperature for 5 min and then 1 mL of aqueous sodium carbonate solution (10%) was added. Samples were vortexed and kept at room temperature for 1 h. Absorbance was measured at 765 nm in a Beckman DU 640-spectrophotometer.

Microbiological tests of extracts (sage) sample and drinking yoghurt: Standard plate count agar was used for total bacterial count (ISO)[24] 4833:1:2013). MRS agar was used for total lactic acid bacterial count[25]. The enumerations of yeasts and molds were carried out according to (ISO)[26]. Violet red bile agar (VRBA) was used for Coliform bacterial count (ISO)[27].

Sensory evaluation: Sensory test was carried out at Department of Food and Dairy Science and Technology, Faculty of Agriculture, Damanhour University. Ten of well-trained panelists with no known allergies to milk products were recruited. Panelists scored their responses onto a print-out paper sheet with a scale of 10 points for texture, 10 points for color, 20 points for flavor and 40 points for overall acceptability, 1 was the lowest evaluation[28].

Determination of viscosity: The viscosity of drinking yoghurt samples that stored at 10°C overnight were measured using oscillatory viscometer (VR 3000M YR Viscometers, Spain).

Statistical analysis: The data were analyzed by a general linear model procedure of the Fisher's protected least-significant difference (PLSD) test using SAS[29], (SAS Institute, Inc., Cary, NC). This test combines one-way ANOVA with comparison of differences between the means of the treatments at the significance level of $p \leq 0.05$. Correlations were calculated using Pearson's correlation coefficient.

RESULTS

Physicochemical properties of drinkable yoghurt: Statistical analysis has shown that there was no significant difference ($p > 0.05$) in moisture content among all treatments during the storage period (Table 1). The obtained results showed that there is no significant difference in fat and protein contents among all treatments (Table 2). The drinking yoghurt samples have pH values between 4.63 and 4.77 at 1st day of manufacturing, but the pH decreased at the end of storage periods in all treatments, but there was no significant difference ($p > 0.05$) among all samples.

Table 3 shows the mean total phenolic contents in all samples of drinkable yoghurt. The total phenolic content was increased with the increasing aqueous extract of the herb sage. The total phenolic contents of drinkable yoghurt samples were significantly higher in treatments of adding 10% extract when compared to control.

Ash content of the experimental drinking yoghurt samples has significant increased ($p \leq 0.05$) with increasing the levels of sage extract. The ash content was at highest level in treatments of adding 10% extract and lowest level was in control samples.

Microbiological analysis: The results show that the all samples of drinking yoghurt were free from coliform bacteria, yeasts and molds. The data in Table 3 also show that the level of colony forming units of on the MRS agar were in range of $\log_{10}$ 7.3-8.7 at 1st day of manufacturing. Drinking yoghurt samples made with probiotic culture (Table 4-6) had high count of colonies when compared with samples made with yoghurt culture and adding sage extract increased the count of colonies.

Table 1: Physicochemical analysis *Salvia officinalis* extract

Properties	Samples (sage)
Moisture (%)	98.63
Ash (%)	0.345
Acidity (%)	0.29
pH	6.254
Brix (%)	0.70
Viscosity (cp)	7.92
Total phenol mg/100 g	95.25
Estimate the color at wavelength 500 nm	2.774

Table 2: Physicochemical analysis of drinking yoghurt produced using *Salvia officinalis* extract

Composition (%)	Storage period (day)	Treatments							
		C1	T1	T2	T3	C2	T4	T5	T6
Moisture (%)	0	78.32^{a}	78.25^{a}	78.21^{a}	78.16^{a}	78.45^{a}	78.37^{a}	78.31^{a}	78.28^{a}
	7	77.61^{a}	77.52^{a}	77.47^{a}	77.39^{a}	77.56^{a}	77.49^{a}	77.45^{a}	77.41^{a}
	15	76.22^{a}	76.15^{a}	76.12^{a}	76.05^{a}	76.37^{a}	76.34^{a}	76.29^{a}	76.24^{a}
Ash (%)	0	0.416^{g}	0.438^{efg}	0.467^{abcdef}	0.475^{abcde}	0.415^{g}	0.436^{efg}	0.463^{abcdef}	0.478^{abcde}
	7	0.427^{fg}	0.448^{cdefg}	0.477^{abcde}	0.486^{abc}	0.427^{fg}	0.443^{defg}	0.472^{abcde}	0.487^{abc}
	15	0.436^{efg}	0.459^{abcdef}	0.486^{abc}	0.495^{ab}	0.440^{defg}	0.455^{bcdefg}	0.481^{abcd}	0.498^{a}
Fat (%)	0	6.20^{a}	6.30^{a}	6.40^{a}	6.50^{a}	6.10^{a}	6.30^{a}	6.40^{a}	6.50^{a}
	7	6.30^{a}	6.50^{a}	6.60^{a}	6.70^{a}	6.30^{a}	6.43^{a}	6.60^{a}	6.70^{a}
	15	6.40^{a}	6.60^{a}	6.70^{a}	6.80^{a}	6.40^{a}	6.60^{a}	6.70^{a}	6.80^{a}
Protein (%)	0	3.703^{a}	3.550^{a}	3.580^{a}	3.603^{a}	3.520^{a}	3.560^{a}	3.580^{a}	3.610^{a}
	7	3.540^{a}	3.580^{a}	3.620^{a}	3.670^{a}	3.560^{a}	3.590^{a}	3.630^{a}	3.660^{a}
	15	3.580^{a}	3.620^{a}	3.660^{a}	3.710^{a}	3.570^{a}	3.630^{a}	3.670^{a}	3.703^{a}
Total phenol	0	7.626^{i}	8.033^{ghi}	8.360^{efgh}	8.806^{def}	7.540^{i}	7.920^{hi}	8.120^{fghi}	8.240^{efghi}
	7	7.940^{ghi}	8.360^{efgh}	8.620^{defgh}	10.106^{ab}	8.100^{fghi}	8.386^{efgh}	8.640^{defg}	8.860^{de}
	15	8.800^{def}	9.166^{cd}	9.720^{bc}	10.600^{a}	8.940^{de}	9.740^{bc}	10.280^{ab}	10.706^{a}
pH	0	4.760^{a}	4.670^{a}	4.660^{a}	4.640^{a}	4.770^{a}	4.703^{a}	4.650^{a}	4.630^{a}
	7	4.630^{b}	4.610^{b}	4.603^{b}	4.580^{b}	4.683^{b}	4.640^{b}	4.620^{b}	4.593^{b}
	15	4.580^{c}	4.570^{c}	4.550^{c}	4.503^{c}	4.603^{c}	4.590^{c}	4.560^{c}	4.520^{c}

$^{a-i}$Means at the row with different superscripts are different ($p<0.05$). C1: Yoghurt culture with add 10% of boiled water as a control, T1: Yoghurt culture with adding 5% extract solution and 5% of boiled water, T2: Yoghurt culture with adding 7% extract solution and 3% of boiled water, T3: Yoghurt culture with adding 10% extract solution, C2: Probiotic culture with add 10% of boiled water as a control, T4: Probiotic culture with adding 5% extract solution and 5% of boiled water, T5: Probiotic culture with adding 7% extract solution and 3% of boiled water, T6: Probiotic culture with adding 10% extract solution

Table 3: Microbiological analysis of drinking yoghurt produced using *Salvia officinalis* extract

Microbial test	Storage period (days)	Treatments							
		C1	T1	T2	T3	C2	T4	T5	T6
Lactobacillus (Log_{10})	0	7.39	7.95	7.83	7.54	7.66	8.01	8.03	8.04
	7	6.56	7.32	7.49	6.73	7.65	7.67	7.81	7.83
	15	6.81	6.54	6.38	6.56	7.54	7.55	7.63	7.67

*ND: Non detected, C1: Yoghurt culture with add 10% of boiled water as a control, T1: Yoghurt culture with adding 5% extract solution and 5% of boiled water, T2: Yoghurt culture with adding 7% extract solution and 3% of boiled water, T3: Yoghurt culture with adding 10% extract solution, C2: Probiotic culture with add 10% of boiled water as a control, T4: Probiotic culture with adding 5% extract solution and 5% of boiled water, T5: Probiotic culture with adding 7% extract solution and 3% of boiled water and T6: Probiotic culture with adding 10% extract solution

Table 4: Viscosity (cp) of drinking yoghurt produced using *Salvia officinalis* extract

Treatments	Storage period (day)		
	0	7	15
C1	410^{p}	434^{op}	450^{mno}
T1	430^{op}	455^{lmno}	510^{ghij}
T2	459^{lmno}	478^{jklm}	542^{efg}
T3	502^{hijk}	550^{def}	583^{bcd}
C2	442^{nop}	510^{ghij}	572^{cde}
T4	470^{klmn}	530^{fgh}	610^{ab}
T5	490^{ijkl}	520^{fghi}	590^{bc}
T6	510^{ghij}	550^{def}	630^{a}

$^{a-i}$Means with different superscripts are different ($p<0.05$). C1: Yoghurt culture with add 10% of boiled water as a control, T1: Yoghurt culture with adding 5% extract solution and 5% of boiled water, T2: Yoghurt culture with adding 7% extract solution and 3% of boiled water, T3: Yoghurt culture with adding 10% extract solution, C2: Probiotic culture with add 10% of boiled water as a control, T4: Probiotic culture with adding 5% extract solution and 5% of boiled water, T5: Probiotic culture with adding 7% extract solution and 3% of boiled water, T6: Probiotic culture with adding 10% extract solution

Viscosity of drinking yoghurt: The viscosity of drinking yoghurt at 1st day of manufacturing was in range of 410-510 cp at 10°C (Table 4). The viscosity is increased with increasing the level of sage extract as well the viscosity was higher in probiotic drinking yoghurt when compared to normal drinking yoghurt.

Sensory evaluation: The results of sensory evaluation are the values of texture, color, flavor and overall acceptability (Table 5). It is evident from the results of sensory evaluation that the addition of the aqueous extract solution of sage (*Saliva officinalis*) at all levels did not have any significant effect ($p>0.05$) on all parameters, texture, color, flavor and overall acceptability scores at 1st day of manufacturing. At the end of storage period, there was a significantly decrease ($p<0.05$) in all parameters and overall acceptability scores.

Table 5: Effect of salvia officinalis extract on sensory evaluation of drinking yoghurt

Properties	Storage period (day)	Treatments C1	T1	T2	T3	C2	T4	T5	T6
Texture-10	1	8.83^{ab}	8.91^{a}	8.50^{abc}	8.91^{a}	8.66^{abc}	8.75^{abc}	8.66^{abc}	8.50^{abc}
	15	8.00^{abc}	8.75^{abc}	7.83^{bc}	8.66^{abc}	8.83^{ab}	8.25^{abc}	8.58^{abc}	7.75^{c}
Color-10	0	9.33^{a}	9.00^{ab}	8.41^{abc}	8.50^{abc}	9.00^{ab}	8.75^{ab}	8.16^{abc}	8.00^{bc}
	15	8.33^{abc}	7.91^{bc}	7.91^{bc}	7.50^{bc}	7.91^{bc}	8.00^{bc}	7.91^{bc}	7.50^{c}
Flavor (20)	1	16.41^{abcd}	17.58^{a}	17.58^{a}	16.16^{abcd}	16.25^{abcd}	16.91^{ab}	16.75^{abc}	15.91^{abcde}
	15	15.66^{bcde}	16.25^{abcd}	15.75^{bcde}	15.33^{bcde}	15.08^{cde}	16.08^{abcde}	14.91^{de}	14.41^{e}
Appearance-40	1	34.58^{ab}	35.50^{a}	33.50^{abc}	33.58^{abcd}	33.91^{abcd}	34.41^{abc}	33.58^{abcd}	32.41^{bcd}
	15	32.00^{cde}	32.91^{bcd}	31.50^{de}	31.50^{de}	31.83^{de}	32.33^{bcd}	31.41^{de}	29.66^{e}

$^{a-j}$Means at the row with different superscripts are different ($p<0.05$). C1: Yoghurt culture with add 10% of boiled water as a control, T1: Yoghurt culture with adding 5% extract solution and 5% of boiled water, T2: Yoghurt culture with adding 7% extract solution and 3% of boiled water, T3: Yoghurt culture with adding 10% extract solution, C2: Probiotic culture with add 10% of boiled water as a control, T4: Probiotic culture with adding 5% extract solution and 5% of boiled water, T5: Probiotic culture with adding 7% extract solution and 3% of boiled water, T6: Probiotic culture with adding 10% extract solution

DISCUSSION

The obtained data showed that the adding sage extract does not have significant effect on pH, fat and protein of drinking yoghurt during storage, this is related to standardization process of milk. These data also recommended that the starter cultures (yoghurt culture or probiotic culture) have no any effect on the pH and chemical analysis of drinking yoghurt. It was suggested that the prebiotic or probiotic not a significant factor for determining fermentation time of drinking yoghurt[30]. The moisture decreased during storage, this is related to natural evaporation[31]. The ash content was higher in treatments made with high level of extract when compared to control samples. This is expected since the ash content is higher in sage leaves (Table 1).

The total phenolic is higher in treatments made with sage extract, this is expected since the sage is good source of phenolic components[32].

All samples of drinking yoghurt were free from undesirable micro-organisms (coliform bacteria, yeasts and molds), this is due to using boiling sage extract and no microbial contaminations during processing. The obtained data recommended that sage extract stimulate the growth of lactobacilli as the level of lactobacilli was much higher in samples that contain sage extract when compared with control. As well as, the level of lactobacilli was higher in probiotic culture when compared to yoghurt culture. The ability of the probiotic strain to attain at high population is of primary importance, approximately 10^7 cells mL^{-1} at the time of consumption is considered functional[33]. Probiotic bacteria lost viability throughout storage due to low pH of fermented foods and some anti-microbial substances which produced by some strains[34].

The viscosity of drinking yoghurt is affected by supplementation with sage extract and its level. Rheology is the study of the deformation and flow of materials[35]. Yoghurt can be classified as pseudo-plastic material (contains a yield stress that has to be exceeded for flow to be initiated) that can be either a viscoelastic fluid if in stirred or drinking yoghurt or a viscoelastic solid in set yoghurt. Viscoelastic indicated the material has some of the elastic properties of an ideal solid and some of the flow properties of an ideal (viscous) liquid. Yoghurt also exhibits time-dependent shear thinning behavior, but yoghurt is not a true thixotropic material since structural breakdown due to shear is not completely reversible once the shear stops[36]. Structural recovery also affects the apparent viscosity of yoghurts. Lee and Lucey[37] found that the rheological properties of stirred yoghurts were greatly influenced by the physical properties of the original intact (set) yoghurt gels. The increasing of viscosity during the cold storage is related to the structural recovery after stirring.

Samples of drinking yoghurt made with sage extract were accepted by panelists. Sensory evaluation is the ultimate measure of product success. Sensory analysis comprises a variety of powerful and sensitive tools to measure human responses to foods and other products. Since the high health benefits of sage extract and there is no any application in dairy products, the adding sage extract in drinking yoghurt was accepted by panelists and could be a good source of anti-oxidant and other benefits in drinking yoghurt.

The results recemented that the sage extract has high level of phenolic component which is good source of antioxidant capacity, as well as increase the health benefits of drinking yoghurt. As it well established that the phenolic component has strong antimicrobial effect, against pathogenic bacteria[38], but in this research the sage extract

stimulates the growth of probiotic bacteria. Future research should be carried out to isolate the component from sage extract that activate the probiotic bacteria.

CONCLUSION

Drinking yoghurt is widely consumed as freshly drinking and good source of nutrients elements. The results of this study concluded that the sage extract stimulated the growth of probiotic culture with no affecting on the chemical and physical properties of final products. As well as drinking yoghurt made with probiotic culture and sage extract was accepted in flavor by panelists. So, our recommendation is the bio-drinking yoghurt fortified with sage extract has good health benefits.

SIGNIFICANCE STATEMENT

This study discovered the affecting of sage extract on the growth of probiotic culture that can be beneficial for human health and overall acceptability of drinking yoghurt. This study will help the researchers to uncover the critical areas of probiotic fermented dairy products that many researchers were not able to explore. Thus, a new theory on health benefit of bio-fermented milk products fortified with sage extract is arrived.

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