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Manufacture of Healthy Kariesh Cheese Using Pomegranate Peel Powder

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POMEGRANATE peels powder (PPP) contain total phenolic compounds, antioxidation activity, which can provide numerous health benefits. Kareish cheese was prepared from buffalo's milk supplemented with different levels 0.5, 1.0, 1.5 and 2.0% of PPP. Adding PPP increased significantly ($p < 0.05$) cheese yield, total protein, soluble nitrogen (SN), ash contents, the counts of lactic acid bacteria, the scores of organoleptic properties but decreased significantly ($p < 0.05$) the moisture content, pH values and The counts of mould and yeast of kareish cheese ($p < 0.05$). Cheese yield, moisture, total protein content, and score of organoleptic properties decreased throughout storage period, while ash and soluble nitrogen contents increased. The counts of lactic acid bacteria increased up to the seventh day of storage and then decreased up to the end of storage period. Cheese sample supplemented with 2% PPP gained the highest of total antioxidant activity and phenolic compounds. The PPP contained high levels of ephenolic compounds, consequently it exhibited effective antimicrobial properties and high antioxidant activity.

Keywords: Kareish cheese, Pomegranate peel powder, Antioxidation activity.

Introdiction

Kariesh cheese is one of the most popular and oldest cheese varieties consumed in Egypt (Abou Donia, 2008). Kariesh cheese is considered one of the most food production rich in calcium and phosphorus. These elements essential for bones and teeth formation. It is rich in sodium and potassium, which play an important role in the formation of body liquids and muscles (Francois et al., 2004).

Pomegranate peels have attracted a great attention for their nutritional and health benefits in the last years (Mehder, 2013). The PPP contain proteins much higher leucine, lysine, aromatic amino acids (phenylalanine and tyrosine), valine and threonine contents and slightly lower sulpher containing amino acids (methionine and cysteine) and isoleucine that the reference protein pattern (Rowayshed et al., 2013). Pomegranate peels is good source of bioactive compounds such as polyphends, carotenoids, vitamins, enzymes and dietary fibers (Cai et al., 2004 and Ashoush et al., 2013).

The PPP is a good source of total Phenolic compounds and has a great free radical scavenging activity (Ashoush et al., 2013). Moreover, PPP can be used in the food industry as a potential ingredient to develop functional foods and to promote health benefits (Gullon et al., 2015). Also, PPP can exhibit antibacterial activity (Khan and Hanee, 2011) against *E. coli* and *P.aeruglosa Staph. aureus*, which can play an important role in prevention of diseases.

The objective of this work was to prepare functional kariesh cheese by using PPP that improving the quality attributes of kareish cheese.

Materials and Methods

Materials

Fresh bulk buffalo's milk was obtained from the Faculty herd and separated at the pilot plant at Department of Dairy Science and Technology. Faculty of Agriculture, Menoufia University, Shibin El-Kom, Egypt.

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Starter culture

Active *Streptococcus thermophiles* EMCC 1043, *lactobacillus delbruekil* subsp. *bulgaricus* EMCC 1102 were obtained from Egyptian Microbial Culture Collection (EMCC) at Microbiological Resources Center, Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

Methods

Preparation of pomegranate peels powder (PPP)

Pomegranate fruits were washed by distilled water then peeled. The peels were air dried in a ventilated oven at 40°C for 48 h and ground and passed through a 24 mesh sieve to get a fine powder as described by Shan et al. (2009). The chemical composition of pomegranate peels powder used in making Kareish cheese was TS 89.31%, TP 4.42%, fiber 11.5%, fat 2.75%, ash 3.60% and total phenolic compounds 26.54 mg/100 g (Sakr et al., 2018).

Kareish cheese making

Skimmed buffalo's milk was heated at 63°C for 30 min and then cooled to 42°C according to Abd–Elhamid (2012). Pomegranate peels powder (PPP) was added to warm milk at the rate of 0, 0.5, 1.0, 1.5, and 2.0% (C, T_1, T_2, T_3 and T_4, respectively). Yoghurt starter (2%) was added to prepared milk containing PPP. The inoculated milks were incubated at 42°C until complete coagulation. The curd was ladled into plastic frames lined with muslin cloth and left until complete whey drainage. The cheese treatments were packed in plastic bags contained salted whey (5%) and stored in refrigerator at 6 $\pm$ 2 °C. Samples from each cheese treatment was taken when fresh and at 7, 14, 21 and 28 days for chemical, microbiological and sensory evaluation. The whole experiment was triplicate.

Cheese yield

Cheese yield was calculated as kg of fresh cheese per 100 kg of skim milk used.

Chemical analysis

The moisture, ash content, total nitrogen and soluble nitrogen content were determined according to the method described by AOAC (2007). pH was measured using laboratory pH meter [Jen way electric pH meter] with a combined glass electrode.

Determination of total phenols, and antioxidant activity

Five grams of sample were mixed with 50 ml of 50% ethanol and stirred at room temperature for 1 hr and filtered through Whatman No.1 filter paper. The total phenols and antioxidant activity were determined in the ethanolic extract. The total phenolic compounds were determined as described by Singleton and Rossi (1965). Briefly, 1.5 ml of 10-fold diluted Folin-Ciocalteau reagent and 1.2 ml of sodium carbonate (7.5% w/v) were added to 0.3 ml of the ethanolic extract, mixed well and allowed to stand for 30 min. Absorbance of the reaction mixture was measured using a spectrophotometer (6505 UV/Vis, Jenway LTD., Felsted, Dunmow, UK) at 765 nm using gallic acid as a standared. Results were expressed as mg of gallic acid equivalents per g of the sample.

The antioxidant activity was evaluated by using 2, 3-diphenyl-1-pricrl-hydrazyl (HPPH) assay (Cuendet & Potterat, 1997 and Burits & Bucar, 2000). One hundred microliters of the ethanolic extract were added to 5 ml of a 0.004% (w/v) of DPPH in methanol. The mixture was vortexed for 15 s and then left to stand at room temperature for 30 min. Absorbance was checked at 517 nm against a blank (ethanol) using the spectrophotometer and the decreased in the solution absorbance was due to proton-donating activity. The control was prepared as above whithout extract. The DPPH radical-scavenging activity was calculated using the following formula:

DPPH radical - scavenging activity (%) = $[(1- A_1/A_0)\times 100]$

Where A_0 is the absorbance of the sample.

Microbiological analysis

Total bacterial count and mould & yeast were determined as suggested by American Public Health Association (APHA, 2005).

Sensory evaluation

The organoleptic properties of Kareish cheese were assessed by 7 panelists from the staff members of Dairy Science Department, Faculty of Agriculture, Menoufiya University, according to Kebary et al. (2018).

Statistical analysis

Data were analyzed using 2 × 3 factorial design. New Man Keuls Test was used to make the multiple comparisons (Steel and Torrie, 1980) using Costat program. Significant differences were determined at $p \leq 0.05$.

Results and Discussion

The yield of kareish cheese is one of the

most economically important aspects of cheese manufacturing. Cheese yield content of kareish cheese made with pomegranate peels powder increased significantly ($p \leq 0.05$) and this increase was proportional to the rate of adding PPP (Table 1). The moisture content was in adverse trend to cheese yield and increased with the low concentrates of PPP. Treatment made with adding 2% PPP (T_4) had the highest cheese yield and the lowest moisture content. This increased might be due to the amount of PPP added which contained high total solids (4.42% proteins, 11.5% fiber and 2.75% fat). Cheese yield and moisture content decreased in all treatments with the progression of storage period. This might be due to the development of acidity that led to shrink contract the curd that helps to expel the whey from the curd (Effat et al. 2001 and Kebary et al. 2018).

Kareish cheeses treatments were significantly ($p < 0.05$) different in ash content, which means that adding PPP caused a significant ($p < 0.05$) increase in ash content (Table 1) .This increase may be due to added PPP which contained 3.60% ash. Also, ash content of all kareish cheese treatments increased slightly throughout storage period, this increased might be due to the loss of moisture content. These results are in agreement with those reported by Alnemr et al. (2013) and Kebary et al. (2018).

Significant differences in the total protein content among all kareish cheese treatments, which means incorporating of PPP affected significantly ($p \leq 0.05$) total protein content of kareish cheese treatments (Table 2). Total protein content of kareish cheese made with pomegranate peels powder increased significantly ($p \leq 0.05$) and this increase was proportional to the rate of adding PPP. Cheese treatment T_4 which made with adding 2% PPP contained the highest total protein content (Sakr et al., 2018). Total protein content of all treatments decreased significantly ($p \leq 0.05$) as storage period proceeded. This decreased might be due to protein and the loss of water soluble nitrogen (WSN) during storage period in the pickling medium (Kebary et al., 2018).

There were significant ($p \leq 0.05$) differences among all kareish cheese treatments in soluble nitrogen content , which means addition PPP has significantly ($p \leq 0.05$) the soluble nitrogen content of kareish cheese treatments (Table 2). Cheese treatment T_4 which made with adding 2% PPP contained the highest total protein content soluble nitrogen content. These results could be attributed the prebiotic effect of PPP that enhance the growth of lactic acid bacteria which subsequently increases the proteolysis ability and increased the soluble nitrogen content of the resultant kareish cheese treatments. Soluble nitrogen content of all cheese treatments increased significantly ($p \leq 0.05$) during storage period. These results are in agreement with those reported by Alnemr et al. (2013), Kebary et al. (2018) and Sakr et al. (2018).

There were significant ($p \leq 0.05$) differences among all kareish cheese treatments (Table 2) in pH values, which means addition PPP has significant ($p \leq 0.05$) effect on pH values. Cheese treatment T_4 that made with adding 2% PPP exhibited the lowest pH values, which might be due to the prebiotic effect of PPP that stimulate the growth of lactic acid bacteria and increases the ability to ferment lactose and consequently decrease pH of cheese. On the other hand the pH values of all kareish cheese treatments decreased significantly ($p \leq 0.05$) as storage period progressed. These results are in agreement with those of Mahmoud et al. (2013), Kebary et al. (2018) and Sakr et al. (2018).

Data in Table 3 indicated that the PPP was a good source of total phenolic compounds (TPC) and exhibited pronounced free radical scavenging activity. The TPC of all treatments was higher than the control cheese. The TPC generally decreased as storage period advanced. From this results, it can be concluded that the level of phenolic compounds and free radical scavenging capacities of resultant cheese increased with the increase PPP concentrate added. These results are in harmony with those of Ashoush et al. (2013) and Skar et al. (2018). Antioxidant activity had the similar trend with TPC. Cheese containing 2% PPP was higher antioxidant activities than other treatments and control. El-Batway et al. (2014) and Sakr et al. (2018) reported that the use of PPP increased the antioxidants and enhanced the functional and nutritional values of the product.

The results show that lactic acid bacterial counts increased till the seventh day of storage, then decreased pronouncely as the storage period proceeded. The highest numbers of bacteria were observed at the seventh day of storage period, which was 21×10^6 cfu/g. The lowest number of bacteria was observed at 28 days, it was 81×10^4 cfu/g (Table 4). These results are in agreement with those reported by Badawi and Hassan (1999) and Mahmoud et al. (2013). Adding PPP caused a significant ($p < 0.05$) increased in lactic acid bacterial count, which might be due to prebiotic effect of PPP.

TABLE 1. Cheese yield, ash content and moisture content (%) of kareish cheese as affected by added PPP percentage.

Cheese treatments	Cheese yield (%)					Ash content (%)					Moisture content (%)				
	0	7	14	21	28	0	7	14	21	28	0	7	14	21	28
C	18.7^{Ae}	18.5^{Be}	18.3^{Ce}	18.2^{De}	18.1^{Ee}	1.97^{Dc}	1.97^{Dc}	1.98^{Cc}	2.01^{Bc}	2.06^{Ac}	74.85^{Ad}	73.27^{Bd}	72.64^{Cd}	71.84^{Dd}	71.21^{Ed}
T_1	21.3^{Ad}	19.9^{Bd}	19.6^{C}d	19.4^{Dd}	19.3^{Ed}	1.99^{Dbc}	2.00^{Dbc}	2.03^{Cbc}	2.07^{Bbc}	2.11^{Abc}	74.20^{Aa}	73.77^{Ba}	73.39^{Ca}	73.10^{Da}	72.86^{Ea}
T_2	23.2^{Ac}	22.9^{Bc}	22.7^{Cc}	22.5^{Dc}	22.4^{Ec}	2.00^{Db}	2.01^{Db}	2.04^{Cb}	2.10^{Bb}	2.15^{Ab}	73.81^{Ab}	73.28^{Bb}	72.87^{Cb}	72.52^{Db}	72.15^{Eb}
T_3	25.4^{Ab}	25.1^{Bb}	24.8^{Cb}	24.6^{Db}	24.4^{Eb}	2.00^{Dab}	2.03^{Dab}	2.07^{Cab}	2.14^{Bab}	2.20^{Aab}	73.68^{Abc}	73.11^{Bbc}	72.73^{Cbc}	72.46^{Dbc}	72.15^{Ebc}
T_4	27.2^{Aa}	26.8^{Ba}	26.4^{Ca}	26.2^{Da}	26.1^{Ea}	2.02^{Da}	2.06^{Da}	2.11^{Ca}	2.18^{Ba}	2.24^{Aa}	73.17^{Ac}	72.72^{Bc}	72.34^{Cc}	72.06^{Dc}	71.72^{Ec}

Different capital letters in the same column are different significantly at $p \leq 0.05$.
Different small letters in the same row are different significantly at $p \leq 0.05$.
C: Control cheese.
T_1: Kareish cheese made with 0.5% PPP.
T_2: Kareish cheese made with 1% PPP.
T_3: Kareish cheese made with 1.5% PPP.
T_4: Kareish cheese made with 2% PPP.

TABLE 2. Total protein, soluble nitrogen content (%) and pH value of kareish cheese as affected by added PPP percentage.

Cheese treatments	Total protein content (%)					Soluble nitrogen content (%)					pH value				
	7	14	21	28	0	7	14	21	28	0	7	14	21	28	
C	16.63^{Ae}	16.15^{Be}	15.65^{Ce}	15.30^{De}	15.10^{Ee}	0.32^{Ee}	0.45^{De}	0.61^{Ce}	0.67^{Be}	0.80^{Ae}	5.40^{Aa}	5.29^{Ba}	5.15^{Ca}	5.07^{Da}	5.04^{Ea}
T_1	16.85^{Ad}	16.34^{Bd}	15.83^{Cd}	15.56^{Dd}	15.40^{Ed}	0.35^{Ed}	0.51^{Dd}	0.69^{Cd}	0.78^{Bd}	0.92^{Ad}	5.35^{Ab}	5.20^{Bb}	5.07^{Cb}	5.00^{Db}	4.97^{Eb}
T_2	17.27^{Ac}	16.69^{Bc}	16.29^{Cc}	15.89^{Dc}	15.71^{Ec}	0.40^{Ec}	0.64^{Dc}	0.81^{Cc}	0.94^{Bc}	1.09^{Ac}	5.27^{Ac}	5.15^{Bc}	5.05^{Cc}	4.93^{Dc}	4.89^{Ec}
T_3	17.55^{Ab}	16.95^{Bb}	16.55^{Cb}	16.07^{Db}	16.00^{Eb}	0.46^{Eb}	0.77^{Db}	0.90^{Cb}	1.05^{Bb}	1.18^{Ab}	5.21^{Ad}	5.12^{Bd}	4.99^{Cd}	4.87^{Dd}	4.83^{Ed}
T_4	17.80^{Aa}	17.31^{Ba}	16.58^{Ca}	16.12^{Da}	16.23^{Ea}	0.51^{Ea}	0.81^{Da}	0.99^{Ca}	1.10^{Ba}	1.15^{Aa}	5.14^{Ae}	5.04^{Be}	4.92^{Ce}	4.79^{De}	4.76^{Ee}

*See legend in Table 1

TABLE 3. Total phenolic compounds and Total antioxidant activity of cheese as affected by added PPP percentage.

Cheese treatments	Phenolic compounds (mg/100 g)					Total antioxidant activity(%)				
	0	7	14	21	28	0	7	14	21	28
C	0.0	0.0	0.0	0.0	0.0	24.40^{Ae}	15.00^{Be}	11.86^{Ce}	10.06^{De}	8.96^{Ee}
T_1	11.15^{Ad}	9.51^{Bd}	8.85^{Cd}	7.94^{Dd}	7.11^{Ed}	68.63^{Ad}	47.76^{Bd}	39.24^{Cd}	35.18^{Dd}	32.74^{Ed}
T_2	11.90^{Ac}	10.64^{Bc}	9.95^{Cc}	9.10^{Dc}	8.27^{Ec}	75.82^{Ac}	51.74^{Bc}	46.09^{Cc}	43.20^{Dc}	41.08^{Ec}
T_3	$13.19^{A}b$	12.08^{Bb}	11.82^{Cb}	10.56^{Db}	9.78^{Eb}	80.51^{Ab}	57.43^{Bb}	48.76^{bC}	$46,57^{Db}$	45.02^{Eb}
T_4	14.80^{Aa}	13.47^{Ba}	12.85^{Ca}	12.10^{Da}	11.18^{Ea}	85.4^{2Aa}	65.97^{Ba}	60.94^{Ca}	57.24^{Da}	55.84^{Ea}

*See legend in Table 1

TABLE 4. Total bacterial count and mould& yeast of kareish cheese as affected by added PPP percentage.

Cheese treatments	Storage period (days)				
	0	7	14	21	28
	LAB count (cfu/ g)				
C	35×10^{5Ec}	80×10^{5Ea}	56×10^{5Eb}	28×10^{5Ed}	81×10^{4Ee}
T_1	35×10^{5Dc}	92×10^{5Da}	69×10^{5Db}	30×10^{5Dd}	90×10^{4De}
T_2	39×10^{5Cc}	98×10^{5Ca}	72×10^{5Cb}	42×10^{5Cd}	12×10^{5Ce}
T_3	50×10^{5Bc}	16×10^{6Ba}	82×10^{5Bb}	50×10^{5Bd}	38×10^{5Be}
T_4	67×10^{5Ac}	21×10^{6Aa}	98×10^{5Ab}	60×10^{5Ad}	55×10^{5Ae}
	Mould & Yeast (cfu/g)				
C	20×10^{2Ae}	31×10^{2Ad}	35×10^{2Ac}	40×10^{2Ab}	55×10^{2Aa}
T_1	10×10^{2Be}	14×10^{2Bd}	17×10^{2Bc}	18×10^{2Bb}	22×10^{2Ba}
T_2	6×10^{2Ce}	9×10^{2Cd}	12×10^{2Cc}	14×10^{2Cb}	18×10^{2Ca}
T_3	ND	ND	ND	2×10^{2Db}	6×10^{2Da}
T_4	ND	ND	ND	ND	ND

N D = Not Detected
*See legend in Table 1

On the other hand, the counts of mould and yeast increased gradually during storage period until reached its maximum at the end of storage period for all cheese treatments (Table 4). Kareish cheese treatments made with adding PPP contained lower counts of mould and yeast than control treatment. Adding PPP caused a significant ($p < 0.05$) decreased in counts of mould and yeast due to the antimicrobial phenolic compounds in PPP. These compounds can degrade the cell wall, disrupt the cytoplasmic membrane, damage membrane proteins and interfere with membrane-intergrated enzymes, which may eventually lead to cell death (Shan et al., 2007).

Score for the organoleptic properties of cheese fortified by PPP are cleared in Table 5. Treatments T_2 & T_3 gained the highest total scores. There were obvious differences among all treatments during storage period. Some studies demonstrated that addition of PPP had no significant effect on the organoleptic properties as compared to the control treatment (El-Said, 2014). While other studies demonstrated that addition of PPP had significant effect on the organoleptic properties, this might be due to the inhibitory effect of the phenolic compound present pomegranate on the metabolic activity of starter bacteria, which decreased changes of milk compounds.

TABLE 5. Change in organoleptic scores of kareish cheese as affected by adding PPP percentage during storage (6 ± 2°C /28 day).

Storage period (days)	Cheese treatments				
	T_0	T_1	T_2	T_3	T_4
			Body & Texture (35)		
Fresh (0)	28.5	32.0	32.5	33.5	32.5
7	28.0	31.0	31.0	32.5	32.2
14	27.0	30.4	32.0	32.0	31.2
21	25.5	30.0	31.6	31.2	31.0
28	25.0	29.5	31.2	31.0	30.5
			Color and Appearance (15)		
Fresh (0)	13.5	13.6	13.8	13.8	13.2
7	12.6	13.4	13.6	13.6	13.0
14	12.2	13.2	13.8	13.5	13.0
21	12.0	13.0	13.0	13.2	12.7
28	11.5	12.4	12.6	13.0	12.2
			Flavor (50)		
Fresh (0)	42.5	45.2	46.5	46.8	45.6
7	41.2	43.0	45.2	45.4	44.8
14	41.0	43.2	44.8	45.0	44.6
21	40.0	43.0	44.6	45.4	45.0
28	39.4	43.0	45.0	45.6	45.0
			Overall acceptability (100)		
Fresh (0)	84.5^{Ea}	90.8^{Da}	92.8^{Ba}	94.1^{Aa}	91.1^{Ca}
7	81.8^{Eb}	77.4^{Db}	89.8^{Bb}	91.5^{Ab}	87.0^{Cb}
14	80.2^{Ec}	86.8^{Dc}	90.6^{Bc}	90.5^{Ac}	88.8^{Cc}
21	77.5^{Ed}	86.0^{Dd}	89.2^{Bd}	89.8^{Ad}	88.7^{Cd}
28	75.9^{Ee}	85.9^{De}	88.8^{Be}	88.6^{Ae}	87.7^{Ce}

*See legend in Table 1

Conclusion

It could be concluded that, PPP contained high levels of ephenolic compounds, consequently it exhibited effective antimicrobial properties and high antioxidant activity. Therefore the PPP might be used to production of functional kareish cheese. This study indicates that peel of pomegranate can be used commercially in the dairy manufacture as potential natural preservative.

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إنتاج جبن قريش صحي بإضافة مسحوق الرمان كمكمل غذائى

إلهام الدن
قسم علوم وتكنولوجيا الألبان - كلية الزراعة - جامعة المنوفية - شبين الكوم - مصر

يهدف هذا البحث إلى رفع القيمة الغذائية و الصحية للجبن القريش وإطالة فترة حفظها، وذلك من خلال تدعيمها بإضافة ٠,٥ ، ١,٠ ، ١,٥ ، ٢,٠٪ من مسحوق قشر الرمان الغني بمضادات الأكسدة والمضادات البكتيرية ، خزن الجبن الناتج لمدة ٢٨ يوم على ٦ ± ٢° م ، و تم قياس مضادات الأكسدة وكذا تم تقدير الخواص الكيميائية والحسية والميكروبيولوجية للجبن.

وجد اختلافات معنوية في التركيب الكيماوي ، رقم الـ pH في جميع المعاملات. أدى تدعيم الجبن القريش بمسحوق قشر الرمان لزيادة محتوى المنتج من المواد الفينولية وما يرتبط بها من نشاط مضادات الأكسدة بالمقارنة بالعينة الكنترول. أدى هذا التدعيم إلى تحسين الخواص في القيمة الحسية للجبن الناتج ، كما أدى إلى زيادة أعداد بكتريا حمض اللاكتيك LAB خاصةً المعاملة المدعمة بإضافة ١,٥٪ مسحوق قشر الرمان ، كما ظهر انخفاض معنوي في أعداد الخمائر والفطريات.