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The Chemical and Microbiological Properties of Ricotta Cheese Supplemented with Curcumin and Bifidobacteria

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PRODUCTION of Ricotta cheese has been considered to be one of the economic ways for the utilization of whey. The aim of this study was investigating the possibility of improving the quality attributes of Ricotta cheese by using curcumin, and incorporating Bifidobacteria, with monitoring the changes of cheese quality and the survival of bifidobacteria during storage period. Four treatments were made to determine the effects of curcumin on the chemical properties of Ricotta cheese stored for 14 day refrigerated condition. Addition of 2% curcumin to Ricotta cheese affected significantly ($p \leq 0.05$) total protein, titratable acidity, lactose, fat and ash contents. These parameters were increased while cheese yield decreased gradually during storage period. Ricotta supplemented with curcumin was characterized by increasing phenol compounds (PC), antioxidant activity % (AA) and scores of organoleptic properties. Curcumin-treated probiotic Ricotta gained the highest PC, TAA. Curcumin could be considered as a good source of total phenolics. Also, high retention of antioxidant activity in curcumin-treated Ricotta could be related to the stability of the curcumin. Organoleptic properties of Ricotta cheese revealed that all curcumin-treated samples gained higher scores as compared to untreated curcumin treatment (control). Also, it was characterized by high counts of Bifidobacteria during storage period. It could be concluded that functional Ricotta of acceptable composition and acceptable quality can be made by the addition of *Bif. infants* and curcumin.

Keywords: Ricotta, Curcumin, Bifidobacteria.

Introduction

Ricotta cheese is a high moisture soft cheese (Modler and Emmons, 2001). It can be made using cheese whey or milk, or a mixture of both (Pizzillo et al., 2005). Ricotta cheese is very mild and it is used in many Italian dishes, fresh Ricotta had a mild and nutty flavour. Production of Ricotta cheese has been considered to be one of the economic ways for the utilization of whey. So, it can be ussed to recreate an additional source of income (Shukla et al., 1986).

Curcumin (CUR) is a phenolic compound obtained from the rhizome of plant turmeric (Curcuma longa). CUR has many health promoting benefits such as antioxidant, anticancer, anti-inflammatory (Aggarwal et al., 2007; Anand et al., 2007 and Anand et al., 2008) and anticarcinogenic activities (Miller et al., 2008). Hosney et al. (2011) reported that Karish cheese with added curcumin showed better keeping quality and longer shelf-life than plane Karish cheese (control).

Bifidobacteria as a probiotic show antagonistic effects towards some pathogenic bacteria, reduce the risk of diarrhea, normalize the bowel movement, enhance immune functions, reduce cholesterol level, reduce the risk of eczema, synthesize several vitamins, protect from cancer and relieve of lactose intolerance symptoms (Burgain et al., 2011; Prasanna et al., 2014 and Martin

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et al., 2015). To achieve these health benefits of bifidobacteria, their numbers in any product should be about $10^6 – 10^7$ cfu / gm.

The objectives of this study were to investigate the possibility of improving the quality attributes of Ricotta cheese by using curcumin, study the effect of incorporating Bifidobacteria on Ricotta cheese quality and to monitor the changes of cheese quality and the survival of bifidobacteria during storage period.

Materials and Methods

Curcumin

Curcumin (CUR) from *Curcum longa* (> 65% pure), was purchased from sigma (St. Louis, Mo, USA).

Sweet whey

Sweet whey of Mozzarella cheese processing was obtained from own Lab, Kafr El-Sheikh Governorate, Egypt.

Cultures

Active *Bifidobacterium infantis* ATCC 15697 was obtained from Egyptian Microbial Culture Collection (EMCC) at Microbiological Resources Center, Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

Ricotta Cheese making

Sweet whey obtained after processing of Mozzarella cheese were divided to four portions. The whey samples were kept in four separate tanks according to El-Sheikh et al. (2010). The first portion was control treatment without addition of neither curcumin nor bifidobacteria, the second portion was curcumin – treated cheese (2%), the third portion was probiotic-treated ($1.3 – 1.5 \times 10^8$ cfu/g), the fourth portion was curcumin – treated probiotic cheese ($1.3 – 1.5 \times 10^8$ cfu/g). The pH was adjusted to 4.5,the sweet whey heated at 90°C for 15 – 30 min, the curd was left in the whey for 10 min and then scooped in a plastic frame lined with cheesecloth, then added (bifidobacteria and/ or curcumin) and placed over a drainage table and allows to achieve complete drainage in 24 h. The curd was removed from the molds and cut into small cubes, cheese samples was packed in polyethylene bags, and then warm water containing 10% salt was added and stored in refrigerator at 6 ± 2°C. Samples from each cheese treatment was taken when fresh and at 3, 7, 10, 14 day for chemical, microbiological and sensory evaluation. The whole experiment was triplicate.

Chemical analysis

The total solids, titratable acidity, protein, fat and ash contents were determined according to AOAC (2007). Lactose was determined according to Barnett and Abdel-Tawab (1957).

Antioxidant activity was determined by using 2, 3-diphenyl-1-pricrl-hydrazyl (HPPH) assay (Cuendet & Potterat, 1997 and Burits & Bucar, 2000). Phenolic compound was determined in the ethanolic extract of cheese according to Singleton and Rossi (1965).

Cheese yield

Cheese yield was calculated as kg of fresh cheese per 100 kg of sweet whey used.

Microbiological analysis

Count of Bifidobacteria was determined using modified MRS agar containing solution of antibiotics and lithium chloride (Wijsman et al., 1989). Plates were incubated at 37°C for 2 – 3 days under anaerobic conditions.

Organoleptic properties

Ricotta cheese samples were scored for organoleptic properties by a taste panel of 13 staff members (trained) of Dairy Department, Food Technology Institute, Agricultural Research Center according to Mahran et al. (1999). The panelists scored the cheese flavor (out of 40 points); body & texture (out of 50 points) and appearance (10 points).

Statistical analysis:

Data were analyzed using factorial design, and the Student NewMan Keuls test was followed to make the multiple comparisons (Steel and Torrie, 1980) using Costat program. Significant differences were calculated at $p < 0.05$.

Results and Discussion

The cheese yield and total solids content of Ricotta cheese made with curcumin increase significantly (P 0.05 (Table 1,6). This yield increase might be due to the formation of complexs between curcumin and immunoglobulin (Liu et al., 2008) and -lactoglobulin (Sneharani et al., 2010). Curcumin-treated probiotic Ricotta had the highest yield and total solids. The cheese yield decreased and total solid increased in all treatments with the progression of storage period. This may be due to expel the whey from the curd (Effat et al., 2001 and Kebary et al., 2018).

TABLE 1. Effect of curcumin and *Bif. infantis* on cheese yield, total solids, ash and fat contents of Ricotta cheese during cold storage (6 ± 2°C / 14 day).

Cheese treatments	Cheese yield (%)					Total solids content (%)					Ash content (%)					Fat content (%)				
	Storage period (days)																			
	0	3	6	10	14	0	3	6	10	14	0	3	6	10	14	0	3	6	10	14
Control	4.41	4.23	4.00	3.78	3.35	23.39	27.16	30.07	32.96	34.00	2.03	2.30	2.46	2.57	2.70	6.17	6.20	6.36	6.52	6.80
Curcumin-treated cheese	4.55	4.38	4.10	3.90	3.64	23.65	27.20	30.12	33.11	34.27	2.09	2.50	2.68	2.78	2.92	6.22	6.38	6.68	6.98	7.07
Probiotic-treated cheese	4.43	4.24	4.03	3.80	3.40	23.71	27.23	30.17	33.15	34.31	2.05	2.33	2.50	2.61	2.77	6.17	6.24	6.35	6.51	7.81
Curcumin-treated probiotic cheese	4.64	4.47	4.15	4.09	4.00	23.88	27.34	30.29	33.26	34.45	2.09	2.51	2.71	2.80	2.97	6.23	6.40	6.68	6.95	7.10

Data in Tables 1& 6 show that the ash content of curcumin-treated Ricotta was comparable (p □ 0.05) with control. It seems that binding of curcumin with transition metals like Fe^{3+}, Mn^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Pb^{2+}, Cd^{2+}, Ru^{3+} and Re^{3+} render curcumin stable and functionl (Zebib et al., 2010). Also, ash content increased in all treatments with the progression of storage period. There were no significant differences ($p > 0.05$) in fat content among different treatments. While, fat content increased in all treatments with the progression of storage period. This may be due to expel the whey from the curd (Kebary et al., 2018).

Total protein and lactose content of all treatments increased significantly as storage period proceeded (Table 2, 6). This may be due to expel the whey from the curd. There were significant (p □ 0.05) differences among cheese treatments, which means that supplementation of curcumin increased significantly (p □ 0.05) the total protein of Ricotta cheese. This increase might be due to the formation of complexs between curcumin and immunoglobulin (Liu et al., 2008) and □-lactoglobulin (Sneharani et al., 2010).

The titratable acidity of Ricotta from different treatment and control increased significantly ($p \leq 0.05$) as storage period progressed (Table 2, 6). Supplementation of Ricotta with curcumin did not affect significantly in titratable acidity as compared to control cheese. After 14 day of storage, the acidity of curcumin-treated probiotic Ricotta was the lowest (1.32 %) with curcumin-untreated Ricotta (Table 2). These results might be due to the high buffering capacity of milk constituents (Salaun et al., 2005).

Tables 3&6 show the changes in the antioxidant activity of curcumin-treated Ricotta from different treatments during storage . The initial levels of antioxidant activity were high in curcumin-treated Ricotta as compared in control Ricotta and probiotic-treated Ricotta. After 14 day of storage, Ricotta from all treatment showed a decline in their antioxidant activity. The antioxidant activity recorded the lowest value in control Ricotta and the highest level in curcumin-treated probiotic Ricotta. High retention of antioxidant activity in curcumin-treated Ricotta could be related to the stability of the curcumin (Anand et al., 2008).

Significant differences in the antioxidant activity and total phenolics content among all Ricotta cheese treatments (Tables 3&6). Curcumin-treated probiotic Ricotta contained the

TABLE 2. Effect of curcumin and Bif. infantis on total protein, lactose and titratable acidity contents of Ricotta cheese during cold storage (6 + 2°C / 14 day).

Cheese treatments	Total protein content (%)					Lactose content (%)					Titratable acidity content (%)				
	0	3	6	10	14	0	3	6	10	14	0	3	6	10	14
Control	15.74	15.97	17.13	17.60	18.00	3.56	4.13	4.35	4.57	4.86	1.09	1.30	1.31	1.36	1.40
Curcumin-treated cheese	16.10	16.50	17.90	18.25	18.44	3.76	4.33	4.51	4.84	4.92	1.06	1.23	1.26	1.30	1.34
Probiotic-treated cheese	15.78	16.02	17.17	17.63	18.05	3.60	4.27	4.43	4.62	4.87	1.07	1.26	1.28	1.32	1.35
Curcumin-treated probiotic cheese	16.18	16.54	17.96	18.30	18.49	3.85	4.45	4.57	4.86	4.98	1.04	1.23	1.27	1.30	1.32

TABLE 3. Total phenol (mg/g) and antioxidant activity (%) of Ricotta cheese treated with curcumin and Bif. infantis during cold storage (6 + 2°C / 14 day).

Cheese treatments	Total phenol (mg/g)					Antioxidant activity (%)				
	0	3	6	10	14	0	3	6	10	14
Control	_	_	_	_	_	22.02	20.91	19.30	17.85	16.20
Curcumin-treated cheese	20.50	17.87	16.57	15.03	13.81	47.42	43.83	40.17	37.99	35.37
Probiotic-treated Kishk	_	_	_	_	_	25.71	22.54	20.58	18.98	16.77
Curcumin-treated probiotic cheese	52.46	50.90	49.00	47.73	46.82	58.24	54.03	52.66	50.60	49.52

TABLE 4. *Bif. infantis* counts of Ricotta cheese treated with curcumin and *Bif. infantis* during cold storage (6 ± 2°C / 14 day).

Viable counts of *Bif. infantis* (cfu/g)					Cheese treatments
Storage period (days)					
14	10	6	3	0	
ND	ND	ND	ND	ND	Control
ND	ND	ND	ND	ND	Curcumin-treated cheese
44×10^6	85×10^6	23×10^7	63×10^7	79×10^6	Probiotic-treated cheese
70×10^6	20×10^7	52×10^7	87×10^7	85×10^6	Curcumin-treated probiotic cheese

* cfu: Colony forming unit. ND: Not detected.

highest antioxidant activity and total phenolics content. The variation might be attributed to the high antioxidant capacity of curcumin. Also, curcumin could be considered as a good source of total phenolics. These results were in line with those reported that curcumin has exhibited antioxidant activity (Sharma, 1976).

The viable counts of *Bif. infantis* of probiotic-treated cheese and curcumin-treated probiotic cheese are presented in Tables 4& 6). The populations of *Bif. infantis* showed a gradual decrease during storage period. After 14 days of storage the counts of *Bif. infantis* of probiotic-treated cheese and curcumin-treated probiotic cheese were reduced to 44×10^6 and

70 × 10^6, respectively. It is evident that the growth of *Bif. Infantis* retained high count by the presence of curcumin which fall within the recommended count (10^6 – 10^7) claimed for probiotic foods to accomplish their health benefits (IDF, 1992).

Changes in total scores of organoleptic properties of Ricotta cheese treatments during storage period are presented in Tables (5, 6). The results showed that properties qualities were affected significantly by adding curcumin and storage period. Curcumin–treated probiotic cheese gained the highest scores of organoleptic properties. Organoleptic properties of Ricotta cheese revealed that all curcumin-treated samples gained higher scores as compared to untreated curcumin treatment (control).

Conclusions

Production of Ricotta cheese has been considered to be one of the economic ways for the utilization of whey. Results showed that functional Ricotta of acceptable composition and acceptable quality can be made by the addition of *Bif. infants* and curcumin.

TABLE 5. Sensory evaluation of Ricotta cheese treated with curcumin and *Bif. infantis* during cold storage (6 ± 2°C / 14 day).

Storage period (days)	Cheese treatments			
	Control	Curcumin-treated cheese	Probiotic-treated Kishk	Curcumin-treated probiotic cheese
			Body & Texture (35)	
Fresh (0)	33.0	34.0	34.0	34.2
3	32.5	33.5	33.5	34.0
6	29.5	32.7	33.0	33.5
10	29.0	32.5	32.5	33.0
14	27.5	32.5	32.0	33.0
			Color and Appearance (15)	
Fresh (0)	13.05	13.0	13.5	13.0
3	12.0	13.0	13.5	13.0
6	11.5	12.5	13.0	12.5
10	11.0	12.0	13.0	12.0
14	10.0	11.7	12.0	11.5
			Flavor (50)	
Fresh (0)	43.5	45.3	43.5	45.8
3	42.0	44.5	43.0	45.0
6	40.0	44.3	42.6	44.5
10	39.0	44.0	42.0	44.0
14	37.8	43.5	42.0	44.0
			Overall acceptability (100)	
Fresh (0)	90.0	92.3	91.0	93.0
3	86.5	91.0	89.0	92.0
6	81.0	89.5	88.6	90.5
10	79.0	88.5	87.5	89.0
14	75.3	87.7	86.0	88.5

TABLE 6. Statistical analysis of the chemical composition and microbiological counts of Ricotta cheese with or without curcumin as affected by and storage period.

Ricotta properties	Effect of treatments					Effect of storage period (days)					
	Multiple comparisons●					Multiple comparisons●					
	Mean squares	Control	Curcumin treated cheese	Probiotic treated cheese	Curcumin treated probiotic cheese	Mean squares	Fresh (0)	3	6	10	14
Cheese yield	0.205*	C	B	C	A	0.119*	A	B	C	D	E
Total soild (%)	0.325*	C	B	C	A	0.166*	D	C	BC	B	A
Ash (%)	1.320*	B	A	B	A	4.714*	D	C	BC	B	A
Fat (%)	0.963*	B	A	B	A	0.26*	C	CB	B	AB	A
Total protein (%)	0.308*	B	A	B	A	0.217*	C	BC	B	A	A
Lactose (%)	0.057*	C	B	BC	A	0.941*	C	BC	B	AB	A
Titratable acidity (%)	0.068	C	BC	B	A	0.281*	D	C	BC	B	A
Total phenol (mg/g)	67.264*	-	C	-	A	2.814*	A	B	C	D	E
Antioxidatio activity (%)	21.495*	D	B	C	A	1.941*	A	B	C	D	E
Sensory evaluation	0.184*	D	B	C	A	3.318*	A	B	C	D	E
Bifidobacterial counts	14.159*	-	B	-	A	0.033*	D	A	B	C	E

● For each effect the different letters in the same row means the multiple comparisons are different from each other, letter A is the highest mean followed by B, C, … etc.

* Significant at 0.05 level ($p \leq 0.05$).

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الخواص الكيميائية والميكروبيولوجية لجبن الريكوتا المدعم بالكركمين والبيفيدوبكتريا

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أجريت الدراسة لتصنيع جبن ريكوتا وذلك للاستفادة من كميات الشرش الكبيرة التى تعد منتج ثانوي في مصانع الألبان وتم تدعيمها بالكركمين وبكتريا البروبيوتك (نظراً لما له من فوائد صحية عديدة). تم تتبع نشاط بكتريا البروبيوتك, التركيب الكيماوي خلال فترة التخزين (١٤ يوم على درجة حرارة ٦ ± ٢°م).

ارتفعت نسبة كلا من التصافي والجوامد الصلبة في المعاملات المحتوية على الكركمين. كما زادت نسبة الجوامد الصلبة وانخفضت نسبة التصافي بتقدم فترة التخزين. ارتفعت الحموضة تدريجياً خلال فترة التخزين بينما لم تظهر اختلافات معنوية بين المعاملات. المُعاملات المُحتوية على الكركميين كانت أعلى في مُحتواها من اللاكتوز والبروتين مُقارنةً بباقي المُعاملات, كما زادت نسبة كلا من اللاكتوز والبروتين بتقدم فترة التخزين. المُعاملات المُحتوية على الكركميين كانت أعلى في مُحتواها من الدهن والرماد مُقارنةً بباقي المُعاملات, كما ازدادت نسبة الدهن والرماد بتقدم فترة التخزين. إضافة الكركميين أدت إلى زيادة أعداد البيفيدوبكتريا. المعاملات المحتوية علي الكركمين كانت اعلي بشكل كبير في محتواها من مضادات الاكسدة عن باقي المعاملات. كما احتوت المُعاملات المُحتوية على الكركميين مركبات فينولية. حصلت المُعاملات المُحتوية على الكركميين على أعلى درجات تقييم حسّي مُقارنةً بباقي المُعاملات.