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IMPACT OF USING CHITOSAN AND JOJOBA OIL AS AN EDIBLE COATING ON RAS CHEESE QUALITY

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ABSTRACT: *Six cheese treatments were made to study the effect of coating cheese with chitosan and jojoba oil on cheese quality. One cheese treatment was uncoated, while another cheese treatment was coated with 2.0% chitosan solution. The other four treatments were coated by replacing 25, 50, 75 and 100% of chitosan with jojoba oil. Replacement of chitosan with jojoba oil caused a significant increase in moisture, soluble nitrogen, total volatile fatty acids, total proteolytic and lipolytic bacterial counts and scores of organoleptic properties, while caused a significant reduction of cheese loss and mould and yeast counts. Fat, total nitrogen, soluble nitrogen and total volatile fatty acids contents and organoleptic scores of Ras cheese treatments increased as ripening period proceeded, while moisture decreased.*

Key words: *Ras cheese, chitosan, jojoba oil, cheese loss, coating*

INTRODUCTION

During ripening of Ras cheese, moulds might grow on cheese surface and within cracks in cheese, which is one of the most problems facing Ras cheese manufacture in Egypt. Common genera of molds, which may grow on cheese are *Penicillium* and *Aspesgillus* (Bullerman and olivigni 1974; Bullerman, 1976). These moulds have considerable proteolytic and/or lipolytic activities. The growth of moulds on cheese surface and in the cracks not only detracts form the appearance but also may affect of cheese, which make the cheese unsalable. In addition moulds growth on cheese may produce toxigenic substance, which called mycotoxins that exhibit properties of acute, sub acute and chronic toxicities. Some of mycotoxins are carcinogenic, mutagenic and teratogenic. To overcome the production of mycotoxins, about 1.3cm of cheese around and beneath the mould should be removed (Bullerman, 1986), which results in a considerable loss in the cheese yield.

To prevent moulds growth on cheese, several attempts have been tried such as using coating with special materials, waxing cheese, treating with preservatives such as natamycin, potassium sorbate and irradiation (Kebary *et al*., 2001 a, b; Kebary *et al*., 2010, El-Sisi *et al*., 2015).

Recently, efforts have been devoted to develop and use an edible coating during cheese making because of its biodegradability, biocompatibility, non-toxicity and food preservation. Chitosan coating was tested on Ras cheese (El-sisi, 2015) because it has exhibited high antimicrobial activity against fungi and Gram-positive and Gram-negative bacteria. Jojoba oil is prepared by pressing the jojoba seeds to extract the oil, followed by filtration. It is then pasteurized to ensure product safety. Its chemical classification is wax. It has both plant and synthetic sources (Gottschalck and Bailey 2008). According to the International Cosmetic Ingredient Dictionary and Handbook, a synthetic source is assigned to an ingredient that is prepared (synthesized) by the reaction of a substance with one or more other substances to form a new chemical entity. In cases possesses an anti-inflammatory activity therefore it was used in folk

remedies for renal colic, sunburn, chafed skin wounds and sore throat (Guirguis *et al*., 2013) Chitosan and jojoba oil have been used in coating fruits and vegetables crops to extend their shelf life (Ahmed *et al*., 2007; Cerqueira *et al*., 2010 and Abld El-Motty & El-Faham, 2013).

In view of the aforementioned, the objectives of this study were to evaluate the effects of treating Ras cheese with different combinations from chitosan and Jojoba oil on cheese loss, cheese quality and growth of moulds & yeasts on cheese and to monitor changes in cheese quality during ripening period.

MATERIALS AND METHODS

Bacterial strains:

Lactococus lactis CH, was gratefully obtained from Chr. Hansen's laboratory (Horsholm, Denmark) and used as a starter. It was activated by three successive transfers in sterile 10% reconstituted non-fat dry milk.

Cheese making:

Six Batches of Ras cheese were made from bulk fresh cow's milk (obtained from the herd of Tokh Tanbisha farm, Menoufia University, Shibin El-Kom, Egypt) Ras cheese treatments were made according to Abdel Tawab (1963). Two wheels were made for each of the six treatments, one was left to study the effect of chitosan and Jojoba oil on the moulds growth on cheese and calculate the cheese loss and the second was used to sample cheese for chemical, microbiological, and organoleptic analysis. All cheese wheels were coated with different solutions from chitosan (was provided by ROTH Bestellen Sie Zum Nulltarif, Germany) and Jojoba oil (was obtained from El-Masry Ever line Company, El-Gharbiya Governorate, Egypt) prepared according Gurisguis *et al* (2013) as showers in Table (1). All cheese treatments were sampled when fresh and at monthly intervals up to six months. The whole experiment was duplicated.

Chemical analysis

Moisture, fat, titratable acidity, total and soluble nitrogen were measured according to Ling (1963). Total volatile fatty acids were determined by the method described by Kosikowski (1986).

Weight loss:

At the end of ripening period every cheese wheel was weighted then scraped and cleaned, then weighted again. The loss was calculated by the difference in the weight and recorded as a percentage.

Table (1): Chitosan and Jojoba oil concentration for cheese coating

Cheese treatments	Concentration of Chitosan and Jojoba oil
C	Control cheese (uncoated)
C_1	Chitosan solution (2.0%
T_1	Chitosan solution + Jojoba oil (3:1)
T_2	chitosan solution + Jojoba oil (1:1)
T_3	Chitosan solution + Jojoba oil (1:3)
T_4	Jojoba oil (only)

Microbiological analysis:

Total viable bacterial counts were enumerated on nutrient agar as recommended by APHA (1992). Proteolytic bacterial count was determined according to Sharf (1970), while count of lipolytic bacteria was determined according to Salle (1961). Moulds and yeasts counts were enumerated on potato glucose agar (acidified) medium (Difico, 1984).

Sensory evaluation:

Ras cheese samples were evaluated by 5 judges from the staff members of Dairy Department. Food Technology Institute, Agriculture Research Center, Giza, Egypt using the score sheet suggested by Gaber (2000).

Statistical analysis:

Factorial experiment was used to analyze all data and the Student Newman Keuls was followed to make the multiple comparisons (Steel and Torrie, 1980) using Costat Program. Significant differences were calculated at $p \leq 0.05$.

RESULTS AND DISCUSSION

Weight loss:

Coating of cheese with chitosan and Jojoba oil caused a pronounced ($P \leq 0.05$) reduction in cheese loss compared with control cheese (uncoated cheese) as shown in Tables 2&7. Although cheese treatments were made and ripened under good hygienic condition, the loss in control cheese was almost 10%. There were negative correlation between the amount of cheese loss and concentration of jojoba oil Cheese treatment that coated with Jojoba oil only exhibited the lowest cheese loss These results might be due to the ability of jojoba oil and chitosan to inhibit the mould and yeast growth on the surface of cheese and/or decrease the loss of moisture content (El-Sisi *et al*., 2015 and Guirguis *et al*., 2013).

Moisture content of all cheese treatments decreased significantly ($P \leq 0.05$) throughout the ripening period (Tables 3&7), which might be due to the loss of moisture during ripening. Similar results have been reported by Mehanna *et al*., (2009) Kebary *et al.*, (2010b) and El-Sisi *et al*., (2015). Coating of cheese with chitosan and jojoba oil caused a significant effect ($P \leq 0.05$) on moisture content of the cheese treatments. There were positive correlation between the moisture content and the rate of replacing chitosan with jojoba oil, which means increasing the concentration of jojoba oil caused a significant ($P \leq 0.05$) reduction of moisture content during cheese ripening consequently (Tables 3&7). These results are in agreement with those reported by El-Sisi *et al.,* 2015.

Table (2): Effect of coating Ras cheese with chitosan and jojoba oil on cheese loss after six months

Cheese Treatment	Cheese loss (%)
C1	10.7
C2	9.1
T1	8.56
T2	4.15
T3	1.70
T4	1.15

See table (1)

Total nitrogen, fat, salt and ash contents of all cheese treatments followed similar trend, where they increased with different rates during cheese ripening, which might be due to the loss in moisture content during cheese ripening (Tables 3&7). There were slight differences (P ≤ 0.05) among cheese treatments in total nitrogen, fat, ash and salt content, which might be due to the differences in moisture content of these cheese treatments (Table 3). These results are in accordance with those reported by Kebary *et al.* (2001 a, b) and El-Sisi *et al*. (2015).

Titratable acidity of all cheese treatments increased significantly (P ≤ 0.05) as ripening period proceeded (Tables 4&7). These results are in agreements with those reported by Kebary *et al.* (2001 a,b), and Kebary *et al.*, (2010). Coating of cheese with chitosan and Jojoba oil caused a significant (P ≤ 0.05) increase in titrabale acidity (El-Sisi *et al*., 2015). These results might be due to the increase of moisture content that increase the water activity, which consequently enhance the growth of microflora especially lactic acid bacteria and or the decrease of oxygen penetration, which enhance the lactic acid bacteria and consequently develop the acidity.

Ripening indices (soluble nitrogen and total volatile fatty acids) followed almost similar trends (Tables 4&7). They increased significantly (P ≤ 0.05) in all cheese treatments as ripening period progressed. These results are in accordance with those reported by Kebary *et al.* (2001 a,b) and El-Sisi *et al*., (2015) Coating of cheese with chitosan and Jojoba oil increased significantly (P ≤ 0.05) the ripening indices. There were a positive correlation between the concentration of Jojoba oil and the values ripening indices. These results might be due to the higher moisture content which enhanced the growth of microflora in cheese especially the proteolytic and lipolytic bacteria and consequently increased lipolysis and proteolysis activities.

Microbiological quality of Ras cheese :

Total bacterial count, proteolytic and lipolytic counts followed almost similar trends (table 5). They increased during the first three months of ripening period then decreased up to the end of ripening period (savijoki, 2006).

Total, proteolytic and lipolytic bacterial counts increased by increasing the rate of replacting chitosan with jojoba oil. These results might be due to the increase of moisture content and consequently increase the water activity that enhance the microbial growth and/or decreasing of oxygen penetration that enhance the growth of lactic acid bacterial (El-Sisi *et al*., 2015). On the other hand mould and yeast were not detected in fresh cheese treatments, while they appeared on the third month of ripening period and increased up to the end of ripening period (table 5). Coating of cheese with chitosan and Jojoba oil caused a marked reduction of mould and yeast counts (Table 5). Similar results are reported by Aider, 2010; Fajardo *et. al*., 2010 and El-Sisi *et al*., 2015.

Organoleptic evaluation of Ras cheese:

Scores of organoleptic properties are presented in table (6). Organoleptic properties of all cheese treatments improved and their scores increased as ripening period proceeded (Tables 6&7). These results are in agreements with those reported by Gabr *et al.* (2000) ; Kebary *et al.* (2001 a, b); Kebary *et al*., (2010); and El-Sisi *et al.,* (2015).

Table 3

Table 4

Table (5): Microbiological quality of Ras cheese coated with Jojoba oil during ripening

Storage period (months)			
Total bacterial count (cfu/g)			
Cheese Treatment	**0**	**3**	**6 months**
C1	6×10^{4}	7×10^{6}	2×10^{5}
C2	4×10^{4}	6×10^{6}	3×10^{4}
T1	3×10^{4}	5×10^{6}	9×10^{3}
T2	2×10^{4}	3×10^{6}	5×10^{3}
T3	2×10^{4}	8×10^{5}	3×10^{3}
T4	6×10^{3}	1×10^{5}	1×10^{3}
Yest & mould			
C1	N.D	4×10^{2}	1×10^{4}
C2	N.D	2×10^{2}	3×10^{3}
T1	N.D	8×10^{1}	1×10^{3}
T2	N.D	8×10^{1}	9×10^{2}
T3	N.D	5×10^{1}	6×10^{1}
T4	N.D	2×10^{1}	3×10^{1}
Lypolytic bacteria			
C1	6×10^{2}	2×10^{3}	1×10^{3}
C2	7×10^{2}	4×10^{3}	2×10^{3}
T1	8×10^{2}	4×10^{3}	7×10^{3}
T2	9×10^{2}	12×10^{3}	2×10^{3}
T3	11×10^{2}	16×10^{3}	4×10^{3}
T4	13×10^{2}	26×10^{3}	7×10^{3}
Proteolytic bacteria			
C1	1×10^{2}	3×10^{4}	2×10^{3}
C2	3×10^{2}	8×10^{4}	1×10^{3}
T1	11×10^{2}	17×10^{4}	4×10^{3}
T2	14×10^{2}	25×10^{4}	12×10^{3}
T3	18×10^{2}	29×10^{4}	13×10^{3}
T4	21×10^{2}	33×10^{4}	14×10^{3}

* See table (1)
* N.D = not detected

Table 6

Table 7

Although many cheese treatments were accepted by panelists, cheese treatment that was coated with 2.0% jojoba oil was the most acceptable cheese and gained the highest scores of organoleptic properties (Tables 6&7). Increasing the rate of replacing chitosan with jojoba oil improved the organoleptic properties of Ras cheese.

It could be concluded that increasing the rate of replacing chitosan with jojoba oil increased moisture content, soluble nitrogen, total volatile fatty acids, total bacterial counts, proteolytic and lipolytic bacterial counts and improved the organoleptic properties, while decreased cheese loss and mould and yeast counts. Therefore, it could be recommended that coating Ras cheese with Jojoba cheese will improve the acceptability of cheese and decrease the cheese loss.

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تأثير استخدام الشيتوزان وزيت الجوجوبا فى تغليف الجبن على جودة الجبن الراس

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الملخص العربى

يهدف هذا البحث لدراسة تأثير إستخدام الشيتوزان وزيت الجوجوبا فى تغليف على جودة الجبن الراس ولذلك فقد تم تصنيع ستة معاملات بإستخدام تركيزات مختلفة من الشيتوزان وزيت الجوجوبا المعاملة الأولى غير مغلفة (كنترول) بينما المعاملة الثانية غلفت بشيتوزان تركيزه ٢% . الأربع معاملات الأخرى غلفت بنسب استبدال الشيتوزان بواسطة زيت الجوجوبا بنسب ٢٥% ، ٥٠% ، ٧٥% ، ١٠٠% زيت الجوجوبا فقط أدي استبدال الشيتوزان بزيت الجوجوبا الى زيادة كل من الرطوبة والنيتروجين الذائب والاحماض الدهنية الطيارة وكذلك البكتيريا المحللة للبروتين والمحللة للدهون وكذلك قيم التحكيم الحسى بالمقارنة بالكونترول كما أدت أيضاً الى قلة الفاقد من الجبن وكذلك انخفاض الفطريات والخمائر مقارنة بالكونترول ولقد أوضحت النتائج أن إستخدام زيت الجوجوبا أدى الى تحسين الصفات الحسية للجبن وكذلك تقليل الفاقد من الجبن .

Table (3): Gross composition of Ras cheese coated with chitosan and jojoba oil during the ripening period

Cheese Treatment	Ripening Period (months														
	Moisture (%)			Fat (%)			Salt (%)			Ash (%)			Total Nitrogen (%)		
	0	3	6	0	3	6	0	3	6	0	3	6	0	3	6
C1	38.22	36.12	30.25	40.56	39.52	41 .22	3.30	4.30	4.80	4.28	5.21	5.52	3 .35	3 .39	3.60
C2	38.42	36.46	3 1.76	40.50	40.50	41.09	3.26	4.00	4.30	4.35	5.30	5.32	3.32	3 .38	3.42
T1	38.52	37.00	32 .46	40.37	41.00	41.70	3.50	3.60	3.80	4.80	5.20	5.53	3.36	3 .38	3.45
T2	38.53	37.20	32 .46	40.75	41.20	41.72	3.51	3.62	3.82	4.87	5.30	5.63	3.38	3 .40	3. 46
T3	38.57	37.40	33.16	40.95	41.30	41.80	3.56	3.70	3.91	4.90	5.40	5.92	3.38	3 .43	3.52
T4	38.86	37.62	3 4.30	40.99	41.40	41.97	3.70	3.82	3.92	4.93	5.50	5.94	3.39	3 .44	3. 61

C1 : control cheese (uncoated)
C2 : cheese coated with 2.0 % chitosan solution
T1 ، T2 ، T3 and T4 : cheese treatments coated by replacing 0.25 % 50 % ، 75 % and 100 % of chitosan solution with jojoba oil respectively.

Table (4): Changes in titratable acidity and ripening indices with Chitosan and /or Jojoba oil of Ras cheese coated

Cheese Treatment	Ripening Period (month)								
	SN (%)			TVFA (ml 0.1 NaOH/100g)			Titratable Acidity (%)		
	0	3	6	0	3	6	0	3	6
C1	0.338	0.435	0.718	26.4	56.5	92.1	0.41	1.67	1.69
C2	0.318	0.418	0.561	26.7	63	94.3	0.42	1.52	1.71
T1	0.387	0.425	0.568	26.7	65	97.3	0.42	1.63	1.75
T2	0.389	0.443	0.615	27.4	86.4	101	0.43	1.71	1.78
T3	0.390	0.445	0.719	27.6	88.3	115.8	0.44	1.73	1.80
T4	0.398	0.453	0.769	28.5	90.20	118.3	0.44	1.75	1.82

* See table (1)

Table (6): Effect of jojoba oil and chitosan on organoleptic properties of Ras cheese

Cheese Treatments	Ripening Period Months																											
	Flavor (out of 50)							Body and Texture (out of 40)							Appearance (out of 10)							Total Score (100)						
	0	**1**	**2**	**3**	**4**	**5**	**6**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**0**	**1**	**2**	**3**	**4**	**5**	**6**
C1	3 5	40	41	43	43	44	45	32		34	3 4	35	31	3 3	6	7	6	6	7	7	8	73	7 9	81	84	8 5	8 2	86
C2	3 6	3 7	3 8	38	40	44	44	3 2	33	34	34	35	35	36	7	6	6	8	7	7	7	7 5	7 6	78	80	8 2	86	87
T1	34	42	43	43	4 3	45	45	32	33	33	35	36	37	3 6	7	6	6	5	5	7	7	7 3	81	82	83	85	8 8	89
T2	35	43	43	45	45	47	4 6	33	33	35	3 6	36	37	37	7	6	8	8	7	8	8	7 5	82	86	89	8 8	92	91
T3	35	43	43	45	4 5	47	4 7	32	33		38	38	37	3 6	7	7	9	8	9	9	8	74	8 3	8 8	89	9 2	93	9 3
T4	34	4 2	45	45	4 7	48	4 7	33	35	36	37	37	39	3 8	7	7	8	8	9	8	8	7 4	8 4	8 9	90	93	9 5	9 4

* See table (1)

Table (7): Statistical analysis of properties of Ras cheese as affected by Chitosan and Jojoba Oil

Cheese Properties	Effect of treatment							Effect of ripening per. Month							
	Mean Squares	Multiple Comparisons						Mean Squares	Multiple Comparisons						
		C1	C2	T1	T2	T3	T41		0	1	2	3	4	5	6
Chemical properties:-															
Moisture (%)	34.498*	C	B	B	B	AB	A	45.481*	A			B			c
Acidity	0.015*	C	AC	B	AB	A	A	2.681*	C			B			A
Fat (%)	24.381*	A	A	AB	AB	BC	c	58.381*	C			B			A
Salt (%)	0.348*	A	A	A	AB	AB	B	1.894	C			B			AB
Ash (%)	1.995*	A	A	A	A	AB	B	0.332*	C			B			AB
TN (%)	44.248*	A	A	A	AB	BC	C	138.604*	C			B			A
SN (%)	0.024*	E	D	C	B	A	A	0.887*	C			B			A
TVFA (ml 0.1N NaOH/100gm)	0.967*	B	B	B	B	A	A	19.561*	C			B			A
Weight Loss	0.269*	A	B	BC	CD	D	E	0.100*							
Organoleptic properties:-															
Flavor (50)	136.900*	D	C	BC	B	A	A	68.421*	F	E	D	C	B	A	A
Body and texture (40)	16.933*	B	B	A	A	A	A	23.747*	B	AB	AB	A	A	A	A
Color and appearance (10)	10.017*	C	B	AB	A	A	A	1.857	A	A	A	A	A	A	A
Total Score (100)	249.833*	E	D	C	B	A	A	167.040*	D	D	C	B	B	A	A