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Use of Chitosan as an Edible Coating in RAS Cheese

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ABSTRACT

Ras cheese was coated by chitosan at different concentrations ranged from 0.5 to 2%.The moisture content of cheese significantly ($p< 0.05$) affected with chitosan treatment and progression of ripening. Fat and total nitrogen contents of experimental Ras cheeses were comparable with those of the controls and were not significantly different ($p>0.05$). The change in acidity and TVFA was significantly ($P < 0.05$) higher in chitosan-coated cheeses. Soluble nitrogen / total nitrogen (SN/TN) of Ras cheese increased significantly ($p<0.05$) with Chitosan treatment and progression of the ripening period. Viability of lactic acid bacteria was three folds higher in 2% chitosan- coated Ras cheese as compared to uncoated cheese (control). Proteolytic and lipolytic bacterial counts of chitosan coated Ras cheese and control are comparable. The initial mold and yeast counts were not detected in all experimental cheeses. At 120th day, fungal growth in 2% chitosan-treated Ras cheese was declined by 1.5 logarithmic orders of magnitude as compared with uncoated cheese (control). There were significant differences ($p>0.05$) in overall organoleptic quality as affected by chitosan coating and ripening period. 2% Chitosan coated cheese had recorded significantly ($P<0.05$) the highest rating for total organoleptic compared with control cheese.

Key words: chitosan coating, Ras cheese, cheese ripening

INTRODUCTION

In recent year, the food industry interest increased in, edible active bio-based films and coatings because of their potential non-toxic character (nontoxic as a pharmaceutical excipient Illum (1998) , sensorial attributes like color, transparency, roughness or stickiness edibility, antagonistic properties against pathogenic micro-organisms, non-polluting and low cost (Va´ sconez etal. 2009). For these reasons, they have attracted particular attention and have been considered in the food preservation because of their ability to be used as food coating materials for prolonging the shelf life of different food products (Aider, 2002).

Chitosan is a unique cationic polysaccharide in the nature. It is specificity bind to bacterial cell wall through electrostatic interaction and may exhibit efflux of amino acids and cations. Loss of these substrates depletes proton motive force, which ultimately interferes with cellular biosynthesis. These events result in the collapse of the membrane potential and ultimately cause cellular death (Liu et al., 2004).

Chitosan coatings were tested on different types of cheese aiming at prolonging their shelf life, such as Mozzarella (Altieri et al., 2005), Emmental (Coma et al., 2002), Regional Saloio (Cerqueira et al., 2009), Apulia spreadable cheese (Gammariello et al., 2008), Saloio cheese (Fajardo et al., 2010) and other dairy products (Coma, 2003).

Cheeses such as Ras cheese (Egyptian hard cheese) are susceptible to mold growth when kept under refrigeration which represents a potentially quality problem during ripening and refrigeration

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storage (Pitt, 1993 and Saleh, 2003). Cheeses contamination with mold develops undesirable organoleptic attributes, appearance and off flavor. Also, it may create public health hazards based on its potential production of mycotoxins especially aflatoxins group (Marquadt&Frolich, 1992 and Thomas, 1994) due to the highly diffusivity into a cheese matrix (Shih &Marth, 1972; Blanco et al 1988, and El-Deep et al 1992). Thus, this work was carried out to investigate the possibility of using Chitosan as a coating in Ras cheese during ripening and its effect on the characteristics of cheese quality.

MATERIALS AND METHODS

Reagents:

Fresh Cow's milk was obtained from the herd of Tokh Tanbisha farm, Minufiya University, Shibin El-Kom, Egypt. *Lactococcus lactis* subsp. *Lactis* CH1 was 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. Calf rennet powder (Ha- La) produced by CHR – Hansen's Lab., Denmark, and fine table salt were obtained from local market. Chitosan powder from Crab Shells, is a linear copolymer composed of β (1 (4) -linked 2-acetamido-2-deoxy-β-d-glucopyranose and 2-amino-2-deoxy-β-dglucopyranose units provided by ROTH Bestellen Sie Zum Nulltarif, Germany.

Experimental procedures:

Ras cheese was made from heat treated milk (72°C / 15 Sec) and cooled immediately to 35°C, as described by Hofi et at (1970). Starter culture was used at the level of 1% for the ripening of cheese milk, after 30 min., Pre-dissolved rennet powder was added at the level of 3.0g/100kg milk. After the dry salting step that continued 3 days. Salted cheese blocks were cleaned and coated by chitosan at different concentrations ranged from 0.5 to 2%. All cheese treatments were stored at 12-14 °C and relative humidity 80% and sampled when fresh (5 days) and at monthly intervals up to four months. The whole experiment was duplicated.

Chemical analysis:

Moisture, fat, total and soluble nitrogen and titratable acidity were determined according to A.O.A.C (2000), while total volatile fatty acids were determined by the steam distillation method described by Kosikoweski (1966).

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.

Microbiological analysis:

Lactic acid bacteria were determined on MRS plates according to the pour plate method, with overlay, after incubation at 30 °C for 2-3 day as described by De Man et al. (1960). Proteolytic bacterial count was examined using skim milk agar as described by Frank et al. (1993). Lipolytic bacterial count was determined according to Harrigan (1998) using Victoria Blue Butter Fat Agars. Yeast and mold were enumerated using potato dextrose e agar as suggested by Harrigan and McCance (1990).

Sensory evaluation:

The organoleptic properties of resultant Ras cheese were assessed by 20 panelists from the staff members of Dairy Tech. Dept., Food Tech. Research Institute, Agric. Res. Center, Giza, Egypt, and staff members at Department of Dairy Science and Technology, Faculty of Agriculture, Minufyia University according to score sheet described by Abdou et al. (1977).

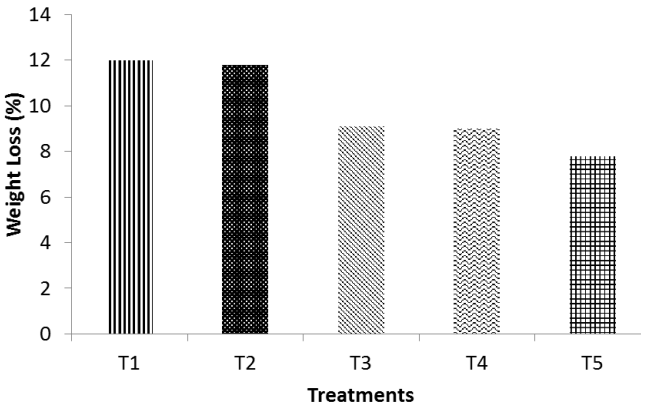
Statistical analysis:

Factorial experiment was used to analyze all the data, 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 $p \leq 0.05$.

RESULTS AND DISCUSSION

Change in cheese weight loss was expressed as a percentage of the initial weight of the cheese samples and presented in Figure-1.

Figure-1. Cheese loss (%) of chitosan coated Ras cheese during storage



The overall weight loss was significantly ($p<0.05$) Table 5 greater in control (T1) and T2 samples compared to, T3, T4, and T5 treatments, showing that all three treatments are of benefit in controlling weight loss during storage. The T5 treatment had the best effect (7.8%). Similar findings were reported the

Table-1 Effect of treatment of Ras cheese with chitosan and ripening period on moisture, fat and total nitrogen content

treatments	Moisture (%)					Fat / DM (%)					Total nitrogen/DM (%)				
	Storage period (days)														
	0	30	60	90	120	0	30	60	90	120	0	30	60	90	120
T_1*	41.50	40.20	39.30	38.35	38.02	49.10	49.16	49.20	49.18	49.12	5.70	5.72	5.73	5.75	5.76
T_2	41.60	40.40	39.35	38.50	38.20	49.10	49.18	49.22	49.20	49.20	5.72	5.73	5.76	5.78	5.79
T_3	41.56	40.60	39.50	38.70	38.30	49.15	49.18	49.21	49.23	49.25	5.70	5.71	5.72	5.74	5.76
T_4	41061	40.80	39.68	38.88	38.59	49.15	49.20	49.26	49.28	49.30	5.69	5.71	5.72	5.74	5.75
T_5	41.62	41.40	41.02	39.80	39.55	49.18	49.19	49.21	49.25	49.28	5.70	5.72	5.72	5.73	5.75

*T1: Ras cheese not coated (Control); T2: Ras cheese coated by 0.5 % chitosan; T3: Ras cheese coated by 1.0 % chitosan; T4: Ras cheese coated by1.5 %chitosan; T5: Ras cheese coated by 2.0 % chitosan

Table-2. Ripening indices of Rras cheese made with chitosan during ripening period.

Treatments	Acidity (%)					SN / TN (%)					TVFA (ml. 0.1 NAOH/ 100g)				
	Storage period (days)														
	0	30	60	90	120	0	30	60	90	120	0	30	60	90	120
T1*	0.43	1.41	1.62	1.69	1.72	5.30	15.80	18.30	25.90	30.50	27.60	45.80	68.10	86.80	98.20
T2	0.44	1.48	1.65	1.70	1.76	5.31	16.40	18.90	26.95	32.50	27.70	46.70	68.90	87.50	99.30
T3	0.44	1.49	1.69	1.75	1.81	5.31	17.90	20.11	28.30	36.40	27.80	48.20	69.95	89.40	101.40
T4	0.43	1.52	1.71	1.79	1.84	5.33	18.50	22.13	29.60	38.30	28.00	50.10	70.90	92.30	103.80
T5	0.45	1.56	1.75	1.86	1.95	5.32	20.90	24.80	32.80	42.80	28.10	53.20	72.90	95.40	105.90

*See foot note table (1)

lower weight loss of semi-hard cheeses coated with chitosan-based edible film (Fajardo et al., 2010). Also, different materials (e.g. alginate, gellan, k-carrageenan, galactomannan) comparing with uncoated cheeses has been reported in the literature (Cerqueira et al., 2010; Kampf and Nussinovitch, 2000).

Table 1 presents the moisture content of Ras cheese samples. The moisture content of cheese significantly ($p<0.05$) affected with chitosan treatment and progression of ripening. The decrease in moisture was more pronounced in the uncoated sample (control) compared to Chitosan coated cheese. At 120 days of ripening, Ras cheese coated by 2% Chitosan (T5) had the greatest moisture content 39.55%, while uncoated cheese (T1) showed the lowest 38,02%. The enhanced moisture retention in Chitosan-treated cheese during proceeding maturation suggests that Chitosan could serve as a barrier and resistance against water vapor permeability (Papaioannou et al., 2007 and Cerqueira at all. 2009). Despite the fact that chitosan has a limited moisture barrier properties (Bordenave et al., 2007). It is possible that hydrophilic Chitosan may interact with hydrophobic cheese matrix substrates at the surface, thereby enhances the moisture barrier capacity (Wong et al. 1992).

Table-1& 5 showed fat and total nitrogen contents of experimental Ras cheeses were comparable with those of the controls and were not significantly different ($p >0.05$).

Acid content is one of the most important factors in determining cheese flavor. As it is evident from Table-2, changes in titratable acidity and total volatile fatty acids increased significantly ($P < 0.05$) with the extension of the ripening period (Table 2 and Table 5). The change in acidity and TVFA was significantly ($P < 0.05$) higher in chitosan-coated cheeses and the highest inT5 coated with (2 % chitosan). After 120th days of storage, the acidity and TVFA of treatment T5 was the highest (1.95 and 105.90), and lowest with uncoated cheese (1.72 and 98.20) which shows that chitosan coating of cheese could effectively contribute to the flavor retention. The generations of volatile free fatty acids of the chain length C2-C8 were reported to contribute to the cheese flavor (Singh et al. 2003)

Table-2 shows changes in soluble nitrogen / total nitrogen (SN/TN) (as an index of cheese ripening). (SN/TN) increased significantly ($p < 0.05$) with Chitosan treatment and advanced with the ripening period) (Table 5). The increase in chitosan coated cheese was more compared to uncoated cheese (control). The increase in SN/TN could be attributed to the higher acidity of coated cheese, which provides more convenience condition to perform certain cellular activities, i.e., proteolysis (Fox, 1969).

Table-3. Microbiological counts in Ras cheese coated with chitosan during ripening

Treatments	Lactic acid bacterial count (CFU × 10^5 /g)				
	Storage period (days)				
	0	30	60	90	120
T1	2.9	4.9	7.3	5.7	3.2
T2	3.0	5.3	7.4	5.7	3.4
T3	3.1	5.5	7.7	6.0	3.7
T4	3.0	5.8	8.2	6.7	4.1
T5	3.1	6.7	8.9	7.4	6.6
	Proteolytic bacterial count (CFU × 10^4 /g)				
T1	1.5	2.6	3.8	4.5	2.2
T2	1.5	2.8	3.9	4.7	2.2
T3	1.61	3.1	4.3	4.8	2.4
T4	1.5	3.5	4.9	5.6	2.7
T5	1.7	3.8	5.6	6.4	3.1
	Lipolytic bacterial count (CFU × 10^4 /g)				
T1	0.6	1.5	3.2	2.1	1.1
T2	0.6	1.7	3.2	2.3	1.2
T3	0.5	1.8	3.3	2.6	1.4
T4	0.6	2.3	3.7	2.8	1.5
T5	0.5	2.6	4.3	3.0	1.7
	Mold and yeast count (CFU × 10^3 /g)				
T1	-*	2.40	5.60	5.30	11.40
T2	-	1.90	3.30	6.20	7.50
T3	-	1.20	2.20	4.90	3.30
T4	-	-	1.2	1.50	1.80
T5	-	-	-	0.40	0.45

* : Not detected

Table-3 shows the results of lactic acid bacteria viability during storage. The populations of lactic acid bacteria showed a gradual increase after storage times of 30, 60, and 90 days, with counting values (CFU x 10^5/g) at 90th days, varying from 5.7 in the control (T1) to 7.4 at 2% Chitosan-coated cheese (T5). After 120th days, the counts were reduced to 3.2 x 10^5 and 6.6 x 10^5 CFU/g for control (T1) and (T5), respectively. This result indicates that the viability was 3 folds higher in the Chitosan- coated Ras cheese as compared to uncoated cheese (control). The survivability of microaerophilic lactic acid bacteria encountered in chitosan-treated cheese may be attributed to limited aeration within the cheese ecosystem. Also, Chitosan coating causes reduction of oxygen partial pressure (pO_2) of the cheese (Cerqueira et al. (2010). These results are in accordance with those reported by Altieri et al. (2005), Del Nobile et al. (2009) and Di Pierro et al. (2011).

Proteolytic and lipolytic bacterial counts of chitosan coated Ras cheese and control are shown in Table 3. The proteolytic activity of chitosan coated Ras cheese and control increased till 90th days of storage, with an overall increase of 6 folds in (T5) and 4 folds in (T1) compared to their initial counts, respectively. At 120th days of storage, Proterolytic bacterial count of all experimental cheeses declined and they were comparable for T1,T2.T3 and T4, whereas T5 reduced to 3.1 x104 CFU/ g. Lipolytic bacterial counts followed the same trend as proteolytic activity except their peaks were counted at 60th day of storage and ranged from 3.2 x104 to 4.3104 CFU/ g. Lactic acid bacteria yielded significant proteolytic and lipolytic activities make an important contribution to the overall flavor development in cheese during ripening (Savijoki et al. 2006).

Table-4. Changes in total organoleptic quality score of Ras cheese coated by chitosan during ripening.

Treatments	Organoleptic scores (out of 100)				
	Storage period (days)				
	0	30	60	90	120
T1	55.20	62.50	70.80	75.60	80.90
T2	55.80	63.90	72.80	76.90	82.70
T3	56.10	65.80	75.90	79.70	86.80
T4	55.90	68.90	79.60	83.60	88.90
T5	56.80	73.10	84.70	88.20	92.50

*See footnote table (1).

The initial mold and yeast counts were not detected in all experimental cheeses Table 3. Visible mold and yeast growth was observed in control and beginning from 30th day of ripening. The mould and yeast count of control sample increased drastically from 2.40x10^3 to 11.40x10^3 cfu g-1. At 90th day of ripening, Chitosan- coated Ras cheese (T5) exhibited fungal count 0.40 x 10^3, whereas, At 120th day, the chitosan was able to retard fungal growth by reduction approximately 1.5 logarithmic units compared with that of control. This condition could be attributed to low O_2 concentration within Chitosan-coated Ras cheese samples as compared with control samples. Antimicrobial activity of chitosan against microorganisms was in the following order: Yeasts and moulds>Gram-positive and Gram-negative bacteria (Aider, 2010, Sagoo et al. (2002 Furthermore, Fajardo et al. (2010) demonstrated that the combination of chitosan and natamycin exert an inhibitory effect on moulds/yeasts in Saloio cheese. El-Diasty et al., (2012) indicated that treatment of Kareish cheese with the addition of chitosan suppressed the mould and yeast growth and prolonged the shelf-life.

Changes in total organoleptic quality of chitosan coated cheeses and control during ripening are presented in Table 4 and assessed statistically in Table 5. The results showed that there were

Table-5 Statistical analysis of Ras cheese properties.

Cheese properties	Effect of cheese treatments						Effect of ripening period (days)					
	Mean squares	Multiple comparison					Mean squares	Multiple comparison$^{\Delta}$				
		T1$^{\Delta}$	T2	T3	T4	T5		0	30	60	90	120
(%)Moisture	2.817 *	C**	C	BC	B	A	23.741*	A	B	C	D	E
(%) Fat / DM	0.339	A	A	A	A	A	0.083	A	A	A	A	A
TN/ DM (%)	1.618	A	A	A	A	A	4.321	A	A	A	A	A
Titratableacidity (%)	0.243 *	C	C	B	B	A	2.327 *	E	D	C	B	A
SN/TN (%)	0.112 *	E	D	C	B	A	1.058 *	E	D	C	B	A
TVFA (ml. 0.1 NAOH/ 100g)	463.076 *	E	D	C	B	A	9235.995 *	E	D	C	B	A
Organoleptic scores (out of 100)	113.251 *	E	D	C	B	A	1855.867 *	E	D	C	B	A
Weight loss (%) after 120 days	2.012 *	A	B	C	D	E	_____	-	-	-	-	-

Δ: See foot note table (1).

* : Significant at 0.05 levels.

** : For each effect the different letters in the means of the multiple comparisons are different from each. Letter A is the highest mean followed by B, C…..etc.

significant differences (p>0.05) in overall organoleptic quality as affected by chitosan coating and.ripening period. Initially, organoleptic scores were between 55 and 56.80 and increased as the ripening period progressed and reached between 80.90 and 92.50 at120th days of storage. Chitosan coated cheese (T5) had signed significantly (P<0.05) the highest rating for total organoleptic compared with control cheese. Chitosan coatings have been demonstrated to be effective in enhancing the oragnoleptic properties of cheese (Coma et al. 2003 and Gammriello et al. 2010).

These results demonstrate that the possible use of chitosan as a coating in Ras cheese. Chitosan at 2% concentration may be useful to produce high quality Ras cheese without detrimental effect on lactic acid bacteria.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Arabic summary

استخدام الشيتوزان فى تغليف الجبن الراس

تم تغليف الجبن الراس بتركيزات مختلفة من الشيتوزان تراوحت من صفرإلى 2,5٪. و أظهرت النتائج أن نسبة الرطوبة بالجبن تتأثر معنويا (P <0.05) بالمعامله بالشيتوزان و أثناء التسويه. وأن محتويات النيتروجين الكلى و الدهون بالجبن الراس المعامله لا تختلف كثيرا (P> 0.05) عن الجبن الراس الغير معامل (كنترول). كان التغيير في حموضة و الأحماض الدهنيه الطياره TVFA معنويا (P <0.05) أعلى في الجبن المغلفة بالشيتوزان. النيتروجين القابل للذوبان / النيتروجين الكلى (SN / TN) من الجبن الراس زاد معنويا (P <0.05) مع المعامله بالشيتوزان وأثناء التسويه . أعداد البكتيريا حمض اللاكتيك أعلى ثلاثة أضعاف في الجبن الراس المغلفة بالشيتوزان (2٪) بالمقارنة مع الجبن غير معامله (كنترول). أعداد البكتريا المحللة للبروتين و المحلله للدهن بالجبن الراس المعامله بالشيتوزان كانت متقاربه. لم يتم الكشف عن الفطر والخميرة فى الأيلم الأولى من التسويه في كل الجبن. في اليوم ال120من التسويه ، أنخفضت أعداد الفطريات و الخمائر فى الجبن الراس المعامله بالشيتوزان(2٪) بنسبة 1,5 دوره لوغاريتميه بالمقارنة مع الجبن (كنترول). كانت هناك فروق معنويه (P> 0.05) في الصفات الحسية التي تأثرت بتركيز الشيتوزان وفترة التسويه. و حصلت الجبن المغلفة بالشيتوزان (2٪) معنويا (P <0.05) على أعلى تقدير لمجموع الصفات الحسية مقارنة مع الجبن الغير معامله.