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OXIDATIVE STABILITY OF PROCESSED CHEESE SPREADS AS AFFECTED BY ADDED WHEY PROTEINS/ CARRAGEENAN PARTICULATE

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ABSTRACT

The effect of added whey proteins/carrageenan particulate on the oxidative stability of processed cheese spreads was investigated. Processed cheese spreads (low fat, half fat and full fat) containing different levels of whey ptoteins/carrageenan particulate, (0, 2, 4 and 6%) were prepared. Spreads from different treatments were analysed for gross composition (TS, fat, TN and SN, lactose and acidity), SH groups, thiobarbeturic acid (TBA) values and conjugated dienes during a storage periods of 90 days in the refrigerator. Addition of particulates increased slightly the SH group contents of spreads with the increase in percentage of particulates added. However, the SH groups in spreads decreased with advanced storage. The TBA values of spreads increased slightly with advanced storage. However, the effect of added particulate on TBA values was negligible. On the other hand, the conjugated dienes contents increased with the increase in fat content in spreads and with advanced storage in all treatments. However, the increase in dienes in full fat spreads during storage was less pronounced in spreads containing high percentage of particulates. This suggests the whey protein particulate may have beneficial effect on the oxidative stability of full fat processed cheese spreads.

Keywords: Processed cheese spreads, whey proteins/carrageenan particulate, SH groups, conjugated dienes, TBA value.

INTRODUCTION

The manufacture of low fat processed cheeses and processed cheese spreads has several advantages to consumer health (Gliksman, 1995), and satisfy the increased demand for low fat cheeses (Montel, 2008) Several ingredients have been used to replace fat in low fat processed cheese including commercial fat replacers (Kebary *et al*, 1998 and Kebary *et al*, 2001), denatured whey protein and protein co-precipitate (Salem *et al.,* 1987), starches and modified starches (Brummel and Lee, 1990). Recently, Good quality low processed cheese spread was obtained from Ras cheese with rennet curd from skim milk with or without rice powder as a fat repalcers (El-Shibiny *et al.,* 2007).

Heat denaturation of whey proteins in the presence of carrageenans modifies the reaction and properties of the formed aggregates (Capron *et al,* 1999; Zhang & Foegeding, 2003). The whey proteins/carrageenan aggregates has been used successfully in the preparation of low fat yoghurt (Shenana *et al,* 2007). The heat denaturation of whey proteins increases their free SH groups which can act as antioxidants in the food systems. The oxidation of anhydrous milk fat was significantly limited by micro-

encapsulation in whey protein isolates (Moreau & Rosenberg, 1996). Also, whey protein coating reduced significantly thiobarbeturic acid-reactive substances and peroxide value formation in sausage during refrigerated storage (Shon & Chin, 2008).

In the present study, the addition different levels of whey protein/carrageenan aggregates has been evaluated in relation to oxidative stability of processed cheese spreads with different fat contents.

MATERIALS AND METHODS

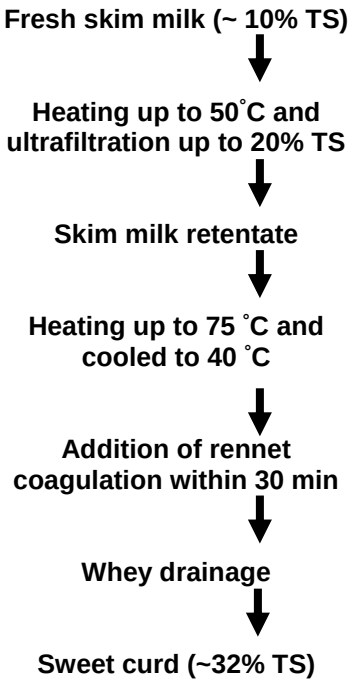
Materials

1- Ripened Ras cheese, obtained from Cairo market. The cheese was selected by the flavour characteristic of fully ripened Ras cheese. The cheese had 71.8% TS, 22.0% protein, 36.5% fat and 9.0% ash contents

2- Whey protein concentrate (WPC), was obtained from local market imported by El-Sayed Awad Al-Amreety Co. (Cairo) from VRS Foods Ltd. The product containg 37.5% protein, 1.5% fat and 7.1% ash contents

3- Joha S_9 emulsifying salts (BK Giulini Chemie GmbH, Landenburg, Germaby) was obtained from the local market.

4- Sweet curd, prepared by rennet coagulation of ultrafiltered skimmed milk retentate as shown in the following flow chart:



Methods

A split plot design (4x3) was followed to study the effect of fat content and percentage of whey protein particulate on the composition, SH groups and oxidative stability of processed cheese spreads.

1.1. Preparation of whey protein/carrageenan co-particulate

Whey from Emmental-type cheese (pH 6.2-6.3) was obtained from Arab Dairy Co. (Kaha, Kalubiea, Egypt). Whey was strained through cheese cloth to remove cheese fines and other suspended materials, and the fat was separated using ALfA Laval cream separator. The defatted whey was then ultrafiltered using carbosep laboratory unit (SFEC, France) equipped with 0.7 m^2 inorganic zirconium oxide membrane (molecular cut off 50,000). Ultrafiltration was carried out in a batch mode at 40°C and 0.5 and 0.3 MPa inlet and outlet pressure, respectively. Ultrafiltration was continued to concentration factor x 8. The obtained retentate was used in the preparation of whey protein/carrageenan co-particulate.

The method of El-Sheikh *et al,* (2001) for the preparation of whey protein particulate was adopted with some modifications as follows: (a) sodium carrageenan was added to the whey retentate at the rate of 0.1% (w/w). The pH was adjusted to pH 5 using 6N HCl. (b) the whey retentate containing the carrageenan was heated at 85°C for 30 min, and then homogenized at 60°C using 2 stage laboratory homogenizer (Rannie, Copenhagen) at 20 and 5 MPa for the 1st and 2nd stage, respectively, and (c) the homogenized retentate/carrageenan mixture was then centrifuged at *5000* G for 10 min. The precipitate was considered as particulated whey proteins concentrate (PWPC). The preparation had 12.1% TS , 6.72% protein and 0.79% ash contents.

1.2. Manufacture of processed cheese spreads

The amounts of ingredients (Table 1) for the manufacture of processed cheese spreads were calculated in order to give three levels of fat in the final product ***i.e*** full-fat (~20%), half-fat (~10%) and low-fat (~7%). The particulated whey protein was prepared and added to each category at 4 levels namely; 0, 2, 4 and 6%, respectively. The ingredients were mixed, placed in the processing kettle (Stephans Universal machine, Switzerland) of 2.5 kg capacity and then heated by direct steam up to 90°C with continuous mixing at 1400 rpm for 5 min. Heating was discontinued, the hot cheese melt was packaged manually in wide-open screw capped glass bottles (100 ml capacity), and stored at 5°C until analysed.

Determination of gross composition

Samples were analysed in triplicates for total solids by the direct oven method and fat content by Gerber method as described by Ardo and Polychroniadou (1999). Titratable acidity, and ash and lactose contents were determined according to AOAC (1990). The total nitrogen (TN) was determined according International Dairy Federation (IDF, 1991), and soluble nitrogen (SN) as described by Ling(1963).

Determination of total and free sulfhydryl groups

The method of Ellman (1959) was followed for the determination of total and free sulfhydryl groups.

Determination of conjugated dienes.

The conjugated dienes were determined of fat extracted from cheese spreads using the official method for spectrophotometric determination of dienes (AOAC, 1990).

Determination of thiobarbeturic acid value

The official method (AOAC, 1990) for the determination of the thiobarbeturic acid value was followed.

Table 1: Amounts of ingredients (g) used in the preparation of processed cheese spreads with different fat contents.

% particulate	Ras cheese	Retentate (sweet curd)	Emulsifier salt	Particulate	Added water (ml)	Condensed water (ml)
Low fat spreads						
0.0	579.3	413.6	43.75	0.0	375.0	100.0
2	579.3	413.6	43.73	21.0	375.0	100.0
4	579.3	413.6	43.73	42.0	375.0	100.0
6	579.3	413.6	43.73	63.0	375.0	100.0
Half fat spreads						
0.0	358.6	761.4	51.0	0..0	400.0	100.0
2	358.6	761.4	51.0	21.0	400.0	100.0
4	358.6	761.4	51.0	42.0	400.0	100.0
6	358.6	761.4	51.0	63.0	400.0	100.0
Full fat spreads						
0.0	251.0	869.0	51.0	0.0	350.0	100.0
2	251.0	869.0	51.0	21.0	350.0	100.0
4	251.0	869.0	51.0	42.0	350.0	100.0
6	251.0	869.0	51.0	63.0	350.0	100.0

RESULTS AND DISCUSSION

Gross composition

Table 2, shows that the TS content of processed cheese spreads from different treatments increased with advanced storage. This can be attributed to slight water evaporation and dryness of the spreads during cold storage. The used packages were not sealed which might allowed for water evaporation from the packaged product. Similar results were reported (El-Neshawy *et al,* 1987; Hamed *et al,* 1997). The fat content of spreads from different treatments decreased slightly during storage in accordance with results from previous studies (El-Neshawy *et al,* 1987; Hamed *et al,* 1997) while Shehata *et al,* (1982) found no significant changes in the fat content of processed cheese during storage. The decrease in the fat content may be attributed to formation of free oil and its subsequent adherence to the package. Addition of particulate had no probable effect on changes of the TS and fat contents of spreads of different fat contents during storage.

Table 2: Changes in the total solids and fat/dry matter contents (%) of processed cheese spreads during storage as affected by the addition of whey protein/carrageenan aggregates.

% particulate	Fresh		15 days		30 days		60 days		90 days	
	TS	Fat/DM	TS	Fat/DM	TS	Fat/DM	TS	Fat/DM	TS	Fat/DM
Low fat spreads										
0.0	26.37	18.96	26.52	18.85	27.78	18.0	29.25	17.09	29.82	16.77
2.0	26.49	18.88	26.85	18.62	27.83	17.97	29.17	17.14	29.78	16.79
4.0	25.87	19.33	26.12	19.14	27.63	18.1	29.09	17.19	29.81	16.77
6.0	26.51	18.86	26.35	18.98	27.96	17.88	29.27	17.08	30.23	16.54
Half fat spreads										
0.0	28.12	24.89	28.3	24.73	29.64	23.62	31.18	22.45	32.52	21.59
2.0	28.35	24.69	28.38	24.67	29.68	23.58	30.71	22.79	32.1	21.81
4.0	28.4	24.65	28.53	24.45	30.21	23.17	31.23	22.41	32.7	21.41
6.0	28.06	24.95	28.26	24.77	29.42	23.79	30.92	22.64	32.71	21.4
Full fat spreads										
0.0	31.32	38.31	31.55	38.03	32.45	36.98	33.89	35.41	34.91	34.37
2.0	31.55	38.03	31.85	37.68	32.64	36.76	34.06	35.23	35.0	34.28
4.0	31.8	37.74	32.08	37.41	33.00	36.36	34.1	35.19	35.15	34.13
6.0	31.99	37.51	32.15	37.25	33.05	36.31	34.26	35.03	35.26	34.03

Processed cheese spreads with high fat content had less lactose content than the low fat spreads (Table 3). During storage, the lactose content of spreads from different treatments decreased slightly which had been attributed to the limited growth and activity of the spread microflora (Al-Khamy *et al,* 1997). The changes in acidity during storage (Table 3) support this explanation, as it increased with advanced storage. However, the increase in acidity can be attributed to the increased solids and protein content of spreads during storage in addition to the possible hydrolysis of the emulsifying salts and the limited growth and activity of the spreads microflora. However, the fat content and the added whey protein particulate had no probable effect of the changes in acidity of spreads during storage. The ash content of processed cheese spreads from different treatments was not affected by the added particulate or fat content (Table 6).. However, it increased slightly during storage probably due to the decrease in the moisture content.

The TN and SN of processed cheese spreads from different treatments (Table 4) were almost unaffected by the added whey protein particulate due to the small amounts added from this ingredient in the formulation. During storage, the TN and SN increased slightly which may be attributed to moisture losses and increased TS. However, the SN/TN increased slightly during storage suggesting increased peptization of cheese proteins during storage.

T3-4

SH groups Contents

Analysis of free and total SH groups in the prepared spreads gave almost the same values (Table 5). This indicated that in freshly prepared spreads the SS groups were almost completely reduced to free SH groups due to the heat treatment received during processing. Addition of increasing quantities of whey protein particulate in the spreads formulations increased slightly the SH content of spreads (Table 5). This can be attributed to the small quantities whey proteins from the particulate in cheese formulations compared to proteins from the other ingredients (cheese and retentate). Also, the SH groups increased with the increase in the TS of cheese. The SH group contents of processed cheese spreads from different treatments decreased during storage being more pronounced during the 2nd month of storage. This decrease may be attributed to the oxidation of the SH groups during storage being active as antioxidant in the system. The percentage losses of SH was nearly the same in the spreads from the different treatments.

Table 5: Changes in total and free SH groups (µmole/g) of processed cheese spreads containing different levels of whey protein/carrageenan aggregates during storage

% particulate	Fresh		30 days		60 days		90 days	
	Total	Free	Total	Free	Total	Free	Total	Free
Low fat spreads								
0.0	34.86	34.17	34.63	33.93	28.74	28.69	28.12	28.07
2.0	35.84	35.15	35.25	34.88	29.37	29.32	28.12	28.07
4.0	36.18	35.49	35.95	35.27	29.22	29.17	29.11	29.06
6.0	36.28	35.59	36.14	35.47	29.37	29.32	28.93	28.98
Half aft spreads								
0.0	36.67	35.98	36.42	35.68	30.21	30.16	29.48	29.43
2.0	37.20	36.51	36.92	36.33	29.66	29.61	29.55	29.50
4.0	37.38	36.69	37.07	36.52	30.69	30.64	30.29	30.24
6.0	37.59	36.90	37.40	36.74	30.69	30.64	30.25	30.20
Full fat spreads								
0.0	38.54	37.86	38.13	37.43	32.05	32.00	31.24	31.19
2.0	38.68	37.99	38.33	37.84	32.71	32.66	31.83	31.78
4.0	38.90	38.21	38.70	38.02	33.49	33.44	32.71	32.66
6.0	39.25	38.56	39.25	38.65	33.89	33.84	33.23	33.18

Conjugated dienes contents

The first step in fat oxidation is the formation of conjugated dienes. Therefore, changes in conjugated dienes in fat rich products is considered as an index of fat oxidation. Table 6, shows, the conjugated dienes of freshly prepared spreads increased with the increase of the fat content. This can be explained on the basis that milk fat contains natural dienes (Gunstone, 2004). Therefore, the increase in the milk fat content increased the dienes content of freshly prepared spreads. However, during storage the increase in dienes indicated fat oxidation. The effect of added whey protein particulate on the formation of dienes was only apparent in full fat spreads while it had no probable effect in the low and half fat spreads. In full fat spreads, the formation of dienes decreased with the increase in the whey protein

particulate. This suggests the beneficial effect of the added particulate in reducing fat oxidation in full fat spreads.

Table 6: Changes in ash (%) and diene (%) contents of processed cheese spreads during storage as affected by the addition of whey protein/carrageenan aggregates.

%	Fresh		15 days		30 days		60 days		90 days	
particulate	Ash	Diene	Ash	Diene	Ash	Diene	Ash	Diene	Ash	Diene
Low fat spreads										
0.0	2.69	0.073	2.69	0.058	2.72	0.073	2.75	0.083	2.81	0.094
2.0	2.67	0.057	2.67	0.069	2.68	0.079	2.72	0.087	2.78	0.099
4.0	2.69	0.069	2.69	0.075	2.71	0.084	2.73	0.091	2.79	0.099
6.0	2.69	0.071	2.69	0.077	2.71	0.094	2.74	0.099	2.80	0.115
Half fat spreads										
0.0	2.71	0.082	2.71	0.092	2.73	0.109	2.76	0.114	2.81	0.127
2.0	2.71	0.086	2.71	0.096	2.73	0.105	2.75	0.109	2.81	0.120
4.0	2.70	0.088	2.70	0.102	2.73	0.119	2.76	0.128	2.81	0.138
6.0	2.71	0.089	2.71	0.103	2.73	0.116	2.76	0.124	2.81	0.130
Full fat spreads										
0.0	2.72	0.109	2.72	0.145	2.74	0.168	2.77	0.198	2.83	0.205
2.0	2.71	0.11	2.72	0.138	2.74	0.165	2.78	0.191	2.82	0.205
4.0	2.72	0.108	2.72	0.142	2.74	0.153	2.78	0.168	2.82	0.183
6.0	2.72	0.104	2.72	0.134	2.74	0.140	2.77	0.154	2.82	0.178

TBA values

The TBA is another index for fat oxidation. Table 7, shows the changes in TBA values of spreads from different treatment. The results clearly indicate that the TBA values increased slightly with advanced storage. However, changes in the fat content or added percentage of whey protein particulate had no probable effect on the changes of TBA values of spreads.

Table 7: Changes in the thiobarbturic acid (optical density) of processed cheese spreads containing different levels of whey protein/carrageenan aggregates during storage

% particulate	Fresh	30 days	60 days	90 days
Low fat spreads				
0.0	0.026	0.033	0.037	0.038
2	0.022	0.027	0.031	0.033
4	0.025	0.030	0.033	0.034
6	0.034	0.036	0.041	0.41
Half fat spreads				
0.0	0.31	0.036	0.039	0.041
2	0.31	0.035	0.035	0.040
4	0.20	0.030	0.034	0.036
6	0.021	0.031	0.035	0.037
Full fat spreads				
0.0	0.025	0.034	0.038	0.037
2	0.023	0.034	0.038	0.039
4	0.022	0.038	0.042	0.043
6	0.025	0.029	0.035	0.036

Conclusions

Addition of whey protein/carrageenan particulate increased slightly the SH groups of processed cheese spreads, and decreased the formation of dienes in full fat spreads. This suggests the whey protein particulate may have beneficial effect on the oxidative stability of full fat processed cheese spreads.

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ثبات مفرودات الجبن المطبوخ ضد التأكسد وتأثره باضافة محببات بروتينات الشرش/الكاراجينان

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إستهدف البحث دراسة أثر اضافة محببات بروتينات الشرش/الكاراجينان علي ثبات مفرودات الجبن المطبوخ ضد التأكسد٠ صنعت مفرودات الجبن المطبوخ بنسب دهن مختلفة (منخفض الدسم، نصف دسم ، كامل الدسم) وأضيف اليها نسب مختلفة (صفر، ٢%، ٤%، ٦%) من محببات بروتينات الشرش/الكاراجينان٠ خزنت المفرودات المصنعة علي درجة حرارة الثلاجة وتم تحليلها علي فترات لمدة ٩٠ يوما٠ تم تحليل المفرودات لتركيبها العام (نسبة المادة الصلبة، الدهن، النيتروجين الكلي والذائب، واللاكتوز، الحموضة) كما تم تقدير كل من مجموعات السلفهيدريل الكلية، ثنائية الروابط المزدوجة المرتبطة Dienes وقيم حمض الثيوباربتيوريك٠ وقد أشارت نتاج الدراسة الي أن مجموعات السلفهيدريل تزيد قليلا باضافة محببات بروتينات الشرش/الكاراجينان وبزيادة نسبة الماء الصلبة في المفرودات٠ وقد تناقصت محتوي مجموعات السلفهيدريل أثناء التخزين وخاصة بعد الشهر الثاني من التخزين ولم تكن هناك فروق تذكر في معدل تناقص هذه المجموعات في المفرودات من مختلف المعاملات٠ كذلك وجد أن محتوي المكونات ثنائية الروابط المزدوجة المرتبطة يزيد مع زيادة مدة التخزين وكان أثر اضافة محببات بروتينات الشرش علي تطور هذه المكونات واضحا فقط في المفرودات المحتوية علي نسبة دهن عالية حيث تناقص معدل زيادها مع زيادة الكمية المضافة من المحببات٠ كذلك زادت قيم حمض الثيوباربتيورك قليلا أثناء التخزين ولم تكن هناك فروق واضحة بين تلك القيم في المفرودات من المعاملات المختلفة٠ وقد يكون لاضافة المحببات أثر مفيد في زيادة الثبات ضد التأكسد في المفرودات المحتوية علي نسبة دهن عالية٠

Table 3: Changes in lactose content and acidity(%) of processed cheese spreads during storage as affected by the addition of whey protein/carrageenan aggregates.

% particulate	Fresh		15 days		30 days		60 days		90 days	
	Lactose/ DM	Acidity	Lactose/ DM	Acidity	Lactose /DM	Acidity	Lactose DM	Acidity	Lactose/ DM	Acidity
Low fat spreads										
0.0	12.09	1.32	12.03	1.32	11.38	1.48	10.56	1.62	9.76	1.66
2.0	12.04	1.32	11.88	1.32	11.32	1.48	10.42	1.62	9.67	1.66
4.0	12.37	1.30	12.25	1.30	11.44	1.41	10.59	1.60	9.76	1.65
6.0	12.07	1.32	12.11	1.32	11.30	1.48	10.56	1.62	9.66	1.66
Half fat spreads										
0.0	11.38	1.32	11.31	1.32	10.69	1.48	9.91	1.62	9.04	1.66
2.0	11.29	1.30	11.28	1.31	10.65	1.41	10.09	1.61	9.10	1.64
4.0	11.27	1.30	11.22	1.31	10.43	1.41	9.86	1.61	8.93	1.64
6.0	11.40	1.30	11.32	1.30	10.71	1.41	9.93	1.61	8.90	1.64
Full fat spreads										
0.0	10.25	1.32	10.17	1.31	9.73	1.48	9.06	1.62	8.39	1.66
2.0	10.17	1.32	10.08	1.32	9.68	1.48	8.98	1.62	8.34	1.66
4.0	10.09	1.32	10.01	1.32	9.55	1.48	8.89	1.62	8.22	1.66
6.0	10.07	1.30	10.02	1.30	9.56	1.41	8.90	1.61	8.28	1.65

Table 4: Changes in TN, SN and SN/TN ratio (%) of processed cheese spreads containing different levels of whey protein particulates(average of three replicates)

% particulate	Fresh			30 days			60 days			90 days		
	T N	SN	SN/TN	T N	SN	SN/TN	T N	SN	SN/TN	T N	SN	SN/TN
Low fat spreads												
0,0	1.67	0.155	9.27	1.71	0.162	9.47	1.73	0.177	10.15	1.77	0.193	10.86
2	1.56	0.157	10.07	1.61	0.162	10.02	1.64	0.180	10.90	1.68	0.205	12.12
4	1.61	0.158	9.86	1.64	0.165	10.06	1.66	0.188	11.24	1.71	0.218	12.66
6	1.81	0.167	9.24	1.86	0.172	9.26	1.91	0.443	9.96	1.94	0.220	11.52
Half fat spreads												
0.0	1.99	0.17	8.54	2.02	0.175	8.66	2.04	0.192	9.41	2.09	0.215	10.32
2	1.45	0.142	9.77	1.58	0.152	9.58	1.64	0.172	10.69	1.70	0.200	12.07
4	1.75	0.155	8.88	1.83	0.17	9.31	1.86	0.190	10.22	1.92	0.218	11.40
6	1.9	0.162	8.51	1.97	0.177	8.97	2.01	0.192	9.58	2.04	0.217	10.65
Full fat spreads												
0.0	2	0.208	10.45	2.03	0.205	10.12	2.06	0.227	11.26	2.12	0.253	12.18
2	1.78	0.193	10.87	1.86	0.207	11.11	1.88	0.230	12.40	1.93	0.265	13.94

4	1.78	0.205	11.55	1.83	0.212	11.58	1.87	0.238	12.86	1.95	0.280	14.48
6	1.87	0.2	10.71	1.92	0.21	10.94	1.99	0.237	12.13	2.06	0.265	13.11

5164 5165 5166 5167 5168 5169 5170 5171 5172