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EFFECT OF ADDING DENATURED WHEY PROTEINS TO CHEESE MILK OR CHEESE CURD ON SOME PROPERTIES OF RAS CHEESE

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ABSTRACT

The effect of adding denatured whey proteins (D.W.P.) in the manufacture of Ras cheese was investigated. Denatured whey proteins were prepared by heating whey (90°C/10 min), strained, and the precipitate was pressed. One and two percent of the DWP were added to the cheese milk or cheese curd and the manufacture of Ras cheese was then completed by the traditional method. The cheese was ripened for 120 days and cheese samples were taken and analysed at intervals for cheese yield, acidity, salt, fat and total nitrogen and non protein nitrogen, total volatile fatty acids, and free amino acids contents at the end of repining period. The results indicated that addition of D.W.P. had no effect on composition and improved slightly the sensory properties of Ras cheese.
Keywords: denatured whey protein- Ras cheese

INTRODUCTION

Whey is composed of an array of high and low molecular weight constituents including proteins, carbohydrates, minerals, enzymes, and vitamins. The main protein fractions of whey are betalactoglobulin, α-lactalbumin, serum albumin, lactoferrin, lactoperoxidase, and bioactive immunoglobulins. The amounts of these proteins in different whey products vary considerably according to the milk source, the treatment of received milk during processing, and how whey is processed. Differences are especially noted in the amount of immunoglobulins in whey products. This reveals differences in the degree of protein denaturation associated with varying processing techniques (Lucas 1999).

Whey proteins are highly functional and nutritious proteins used in a variety of products. Whey proteins can be used in sports and nutrition bars and beverages, infant formula, dairy foods, meat, and other foods. Concentrated whey, whey powder, lactose, lactalbumin, whey protein fractions, whey protein concentrates (W.P.C.), and whey protein isolates (W.P.I.) are few products that can be obtained from processing of liquid whey. The most commonly used forms of whey in the food industry are dried whey powder (about 11 to 13% protein), W.P.C. (34 to 89% protein), and W.P.I. (>90% protein). Whey proteins have many functional characteristics such as high solubility, dispersibility, water binding, foaming, whipping, emulsification, gelation, and buffering power and are used frequently in food applications (Evans *et al.,* 2009).

There are several dairy products that have been made with the inclusion of whey protein products. Isleten and Karagul-Yuceer (2006) manufactured nonfat yoghurt from reconstituted skim milk powder (S.M.P.)

fortified with whey protein isolate (W.P.I.) as yoghurt texture improver, whereas Pinto *et al.,* (2007) used whey protein concentrate in processed cheese spread manufacture. Also, El-Sheikh *et al.,* (2010) made Ricotta cheese from UF-whey protein concentrate (W.P.C.) fortified with 2, 4 and 6 % S.M.P., and acidified with 0.14 g/kg citric acid or 1.5 % Glucono-Delta-Lactone (G.D.L.). Henriqies *et al.,* (2010) investigated the production and incorporation of liquid W.P.C. in fresh cheese and set yoghurt, as well as their functional and sensorial properties. Recently, (Ismail *et al.,* 2011), used of D.W.P. to improve the properties of low fat Mozzarella cheese properties.

In Egypt, tons of whey are produced annually as by-product of cheese industry. In the past, whey was considered a waste product which dumps into sewage, a practice that creates environmental havoc. Recently, salted whey results from Ras cheese manufacture is used for production denatured whey protein in some small cheese factories, to be used as an ingredient in Mish cheese manufacture.

The aim of this research was to investigate the effect of blending different concentrations of denatured whey protein with cow's milk or cheese curd on some properties of Ras cheese.

MATERIALS AND METHODS

Materials:

Fresh cow's milk was obtained from El-Serw Animal Production Research Station, Animal Production Research Institute, Agriculture Research Center. Whey protein was prepared from Ras cheese whey. Yoghurt starter culture (Yo-Fex,YC-350,DVS) consists of *Streptococcus saliverus* ssp. *thermophilus* and *L. delbrueckii* ssp. *bulgaricus* were obtained from Chr. Hansen's Lab A/S Copenhagen, Denmark. As a coagulant, liquid calf rennet (107 IMCU/mL) was obtained from local market and was added to milk at a ratio of 30 mL 100 kg^{-1}milk. Dry coarse commercial food grade salt was obtained from El-Nasr Company of Alexandria. All chemicals were of laboratory grade.

Methods:

Ras cheese manufacture:

Preparation of denatured whey proteins:

Ras cheese whey (pH 5.97) was heated to 95°C for 10 min., cooled and the flocculated denatured whey proteins were recovered by straining through cloth bags for three hrs. The precipitate was transferred to wooden frames and pressed overnight.

Cheese manufacture:

Ras cheese was made from raw cow's milk as described by Hofi *et al.,* (1970). Five treatments of Ras cheese were prepared as follows:

Treatment A: Cheese made from fresh milk.

Treatment B: Cheese made from raw milk supplemented with 1% (w/w) denatured whey proteins preparation. The denatured whey proteins preparation was blended with milk using high speed blender at 2000 rpm for 5 min.

Treatment C: The same as treatment B except that 2% denatured whey proteins preparation was added to milk.

Treatment D: Denatured whey proteins preparation (1% w/w) was added to the obtained cheese curd, mixed thoroughly before hoping in the cheese frames.

Treatment E: The same as treatment D except that 2% denatured whey proteins preparation was added to cheese curd.

Half percent of yoghurt starter was added to five milk treatments. The resultant cheese were stored for ripening at 25°C and analyzed after 15, 30, 60, 90 and 120 days of storage period. All analyses were also performed in duplicate.

Methods of analysis:

Milk, denatured whey protein preparation (D.W.P.P.) and cheese samples were analyzed for titratable acidity (T.A.), total solids (T.S.), fat and pH values and salt contents according to the methods of Association of Official Analytical Chemists (AOAC, 2000). Total nitrogen (T.N.) and non-protein-nitrogen (N.P.N.) contents were determined by the Kjeldahl method according to Ling (1963). The cheese yield was determined by dividing the weight of cheese by the weight of milk used to make cheese, multiplied by 100. Total volatile fatty acids (T.V.F.A.) was determined as described by Kosikowski (1978), and expressed as mL of 0.1N NaOH, 100 g^{-1} cheese. Amino acids analysis was carried out according to Marino *et al.,* (2010). The cheese samples were scored for flavour (50 points), body and texture (35 points) and appearance and colour (15 points) by ten researchers in El-Serw Animal Production Research Station.

The obtained results were statistically analyzed using a software package (SAS, 1991) based on analysis of variance. When F-test was significant, least significant difference (LSD) was calculated according to Duncan (1955) for the comparison between means. The data presented, in the tables, are the mean (± standard deviation) of 3 experiments.

RESULTS AND DISCUSSION

Chemical composition of milk and denatured whey proteins preparation:

Data in table 1 show the Chemical composition of milk and DWPP used in Ras cheese manufacture. The addition of different levels of whey proteins (1 and 2%) to cow's milk slightly increased the milk acidity and T.S. contents and total protein percentages and decreased pH values whereas had no clear effect on fat content of milk.

Yield of Ras cheese:

The yield of fresh Ras cheese made from cow's milk and whey proteins are tabulated in table 2. Addition of D.W.P.P. to both milk and cheese curd significantly increased ($P<0.001$) the yield of Ras cheese. The increase in cheese yield was more pronounced with adding D.W.P.P. to cheese curd as compared to its addition to milk. The percentages of rising were 1.17, 3.07, 2.44 and 5.32% for treatments B, C, D and E respectively.

This may be due to resolubilization and loses of the added DWPP in whey. Also, the cheese yield increased with the increase of the added percentage of DWPP to either milk or cheese. Hinrichs (2001) stated that the yield of cheese increased by incorporating of whey proteins in cheese curd.

Chemical composition of Ras cheese:

Results in table 2 illustrate the changes in titratable acidity (%) and pH values of Ras cheese during ripening. As ripening periods advanced, the acidity of cheese in all treatments increased gradually ($P<0.001$), while pH values significantly decreased ($P<0.001$).

Table (1): Chemical composition of milk and denatured whey proteins preparation used in Ras cheese manufacture.

Treatments	Acidity %	pH values	TS %	Fat %	Total Protein %	Salt %
Fresh cow's milk (A)	0.16	6.66	11.65	3.4	3.12	-
Fresh cow's milk+ 1% whey protein (B)	0.16	6.64	11.79	3.4	3.24	-
Fresh cow's milk+ 2% whey protein (C)	0.17	6.59	11.93	3.3	3.37	-
Whey Proteins	0.28	5.23	25.01	0.9	16.2	7.66

Table (2): Effect of adding denatured whey proteins to cow's milk on yield and some chemical properties of Ras cheese.

Treatments*	Storage periods (days)	Yield (%)	Acidity (%)	pH values	TS (%)	Fat (%)	Fat/DM (%)	Salt (%)
A	15	11.08	1.76	5.14	66.71	29.7	44.52	2.34
	30	-	1.93	5.01	69.08	30.5	44.15	3.11
	60	-	2.02	4.92	70.39	31.3	44.47	3.44
	90	-	2.15	4.84	72.46	32.1	44.30	3.78
	120	-	2.31	4.70	73.30	33.0	45.02	3.93
B	15	11.21	1.83	5.06	65.26	28.8	44.13	2.63
	30	-	2.08	4.93	67.72	29.9	44.15	3.39
	60	-	2.21	4.80	69.50	30.6	44.02	3.59
	90	-	2.34	4.70	70.89	31.4	44.29	3.91
	120	-	2.51	4.58	72.21	32.1	44.45	4.13
C	15	11.42	1.90	5.00	64.91	28.4	43.75	2.85
	30	-	2.19	4.85	67.09	29.5	43.97	3.61
	60	-	2.30	4.71	69.45	30.2	43.48	3.76
	90	-	2.45	4.55	70.67	31.2	44.15	4.05
	120	-	2.67	4.42	72.10	31.9	44.24	4.29
D	15	11.35	1.81	5.09	67.56	29.4	43.52	2.45
	30	-	2.02	4.97	70.41	30.0	42.61	3.16
	60	-	2.10	4.86	72.11	31.0	42.99	3.56
	90	-	2.22	4.77	73.29	32.1	43.80	3.88
	120	-	2.36	4.66	75.06	32.9	43.83	4.10
E	15	11.67	1.86	5.02	67.95	29.3	43.12	2.58
	30	-	2.10	4.89	70.83	30.3	42.78	3.23
	60	-	2.24	4.77	73.23	31.1	42.69	3.63
	90	-	2.38	4.65	74.41	31.9	42.87	3.97
	120	-	2.55	4.52	75.78	32.7	43.15	4.15

Also, the same table shows fast increase in acidity during the first month of ripening followed by slight and gradual increase in titratable acidity during the rest of ripening period. This coincides with the reported changes in cheese microflora being increased during the first month of ripening and then decreased. Similar results were found by El-Baz *et al.,* (2011). From the foregoing results, it is clear that the addition of D.W.P.P. to cow's milk and curd raised the acidity and reduced pH values of Ras cheese.

T.S., fat and salt contents of all samples significantly increased ($P<0.001$) as the ripening periods progressed (Table 2). The sharp increase in T.S. and fat contents could be partially attributed to the dry salting of cheese as well the relatively high temperature of the curing room (25°C). The obtained results are agreement with those reported by Mehanna *et al.,* (2009).

Addition of whey proteins to cow's milk slightly decreased T.S. and fat contents of Ras cheese. This may due to the high water holding capacity of DWPP which may increase the moisture holding in cheese curd. On the other hand, mixing of whey proteins with cheese curd slightly increased T.S. and fat contents of cheese. This can be explained on the basis that the mechanical treatment of the curd during mixing with D.W.P.P. might increase the exudation of the aqueous phase during the cheese processing. Adding whey proteins to both milk and curd increased salt content of cheese due to the high salt content of D.W.P.P. (Table 1). Ismail *et al.,* (2011) found that the addition of D.W.P.P. to buffaloe's milk increased the T.S. values of Mozzarella cheese.

Results of total nitrogen (T.N.), TN/DM, non-protein-nitrogen (N.P.N.), N.P.N//T.N. and T.V.F.A. values of Ras cheese are shown in table (3). Addition of D.W.P.P. to cow's milk and cheese curd significantly increased ($P<0.001$) T.N., T.N./D.M., N.P.N., N.P.N./T.N. and T.V.F.A. values of cheese. The increasing trend was higher with adding whey proteins to curd than that of adding to milk. Because of high protein content of whey proteins (16.2%), it is expected to raise the TN and NPN values of cheese. As a general, during ripening stage T.N., T.N./D.M., N.P.N., N.P.N./T.N. and T.V.F.A. values of cheese gradually increased.

Free Amino Acids (FAA):

Free amino acids contents of 120 days ripened Ras cheese from different samples are cleared in table (4). In general, concentration of proline acid was the predominant one of free amino acids contents in all cheese samples. Addition of denatured whey proteins to cow's milk or cheese curd increased concentrations of various amino acids of cheese.

Table (3): Effect of adding D.W.P.P. to cow's milk on T.N. and some ripening indices of Ras cheese.

Treatments	Storage periods(days)	T.N. (%)	T.N./D.M. (%)	N.P.N. (%)	N.P.N./T.N. (%)	T.V.F.A.*
A	15	3.39	5.08	0.12	3.57	11.4
	30	3.81	5.51	0.15	4.04	15.6
	60	3.87	5.50	0.18	4.57	20.0
	90	3.94	5.44	0.19	4.94	23.8
	120	4.02	5.48	0.21	5.32	26.4
B	15	3.45	5.29	0.13	3.68	11.6
	30	3.87	5.71	0.16	4.21	15.6
	60	3.92	5.64	0.18	4.72	20.2
	90	3.98	5.61	0.21	5.22	24.0
	120	4.07	5.64	0.23	5.58	26.7
C	15	3.50	5.39	0.13	3.83	11.6
	30	3.91	5.83	0.17	4.32	15.9
	60	3.95	5.69	0.19	4.78	20.3
	90	4.03	5.70	0.21	5.31	24.2
	120	4.12	5.71	0.24	5.73	27.0
D	15	3.61	5.34	0.14	3.93	11.8
	30	4.13	5.86	0.17	4.24	15.8
	60	4.19	5.81	0.20	4.72	20.3
	90	4.25	5.80	0.22	5.25	24.1
	120	4.29	5.17	0.24	5.71	27.1
E	15	3.69	5.43	0.15	4.06	12.2
	30	4.22	5.96	0.18	4.34	16.2
	60	4.27	5.83	0.21	4.85	20.5
	90	4.32	5.80	0.23	5.37	24.4
	120	4.38	5.78	0.25	5.80	27.4

*** expressed as mL 0.1 NaOH 100 g $^{-1}$ cheese**

Table (4): Amino acid concentrations (ug/ml) of Ras cheese at the end of storage periods

Amino acids	Treatments				
	A	B	C	D	E
Aspartic	4.36	18.65	17.13	9.61	10.27
Threonine	11.91	112.76	41.40	68.51	4.98
Serine	12.79	15.12	47.37	38.98	8.79
Glutamic acid	27.06	140.22	75.14	77.08	37.40
Glycine	8.24	13.51	9.07	9.67	9.17
Alanine	12.08	30.35	13.67	20.66	19.36
Valine	16.53	83.48	70.83	40.12	42.98
Methionine	75.56	605.67	220.93	259.56	246.57
Isoleucine	23.57	86.68	159.06	38.76	30.45
Leucine	60.90	175.66	125.62	95.41	78.95
Tyrosine	134.00	376.50	169.41	136.67	195.38
Phenylalanine	94.19	206.78	156.5	99.69	121.19
Histidine	36.55	147.04	98.00	85.00	83.20
Lysine	122.64	354.74	196.02	240.64	230.69
NH4+	85.37	207.03	134.39	187.10	119.00
Arginine	35.38	123.72	36.6	42.83	65.62
Proline	956.96	2107.96	997.41	1220.04	1091.19

Sensory properties of Ras cheese:

Scores for the different organoleptic attributes of Ras cheese from different treatments are showed in table 5. As ripening period advanced, scores for colour & appearance, body & texture and flavour of cheese of treatments A, B and C were significantly increased ($P<0.001$). For samples D and E improvement trend stopped after 90 days of ripening period. Addition of whey proteins to milk and curd slightly raised scores of organoleptic properties of cheese. However, at the end of ripening periods (120 days) samples D and E which made by mixing curd with 1 and 2% whey proteins gained the lowest scores.

Table (5): Effect of mixing various concentrations of denatured whey proteins to cow's milk or curd on organoleptic properties of Ras cheese

Treatments*	Storage periods (days)	Colour & Appearance (15)	Body& Texture (35)	Flavour (50)	Total (100)
A	15	11	26	38	75
	30	13	30	40	83
	60	13	32	43	88
	90	13	32	45	90
	120	13	32	46	91
B	15	11	27	38	76
	30	13	30	41	84
	60	13	33	44	90
	90	13	33	46	92
	120	13	33	47	93
C	15	11	27	38	76
	30	13	31	43	87
	60	13	33	45	91
	90	13	33	47	93
	120	13	33	47	93
D	15	11	27	40	78
	30	13	31	44	88
	60	13	33	46	92
	90	13	33	47	93
	120	13	33	45	91
E	15	11	27	39	77
	30	12	31	44	87
	60	13	32	47	92
	90	13	33	47	93
	120	13	32	44	89

Finally, it is concluded from this study that Ras cheese with acceptable properties to 90 days of ripening periods could be made from cow's milk or its curd mixed with 1 and 2% denatured whey proteins.

Table (6): Statistical analysis of Ras cheese treatments

Analysis	Effect of cheese treatments					
	A	**B**	**C**	**D**	**E**	**LSD**
Yield %	11.08^{e}	11.21^{d}	11.42^{b}	11.35^{c}	11.67^{a}	0.054***
Acidity%	1.83^{b}	2.19^{a}	2.28^{a}	2.10^{a}	2.22^{a}	0.260**
pH	4.92^{a}	4.81^{c}	4.70^{e}	4.87^{b}	4.77^{d}	0.029***
T.S.%	70.39^{bc}	69.12^{cd}	68.84^{d}	71.69^{ab}	72.44^{a}	1.302**
Fat%	31.32^{a}	30.54^{c}	30.25^{d}	31.08^{ab}	31.06^{b}	0.258**
Fat/D.M.	44.49^{a}	44.21^{ab}	43.92^{ab}	43.35^{ab}	42.92^{b}	1.303**
Salt%	3.32^{b}	3.43^{b}	3.71^{a}	3.43^{ab}	3.51^{ab}	0.387**
T.N.%	3.81^{e}	3.86^{d}	3.90^{c}	4.09^{b}	4.17^{a}	0.024**
T.N./D.M.	5.40^{d}	5.58^{c}	5.60^{b}	5.66^{c}	5.76^{a}	0.024**
N.P.N.%	0.0170^{c}	0.182^{c}	0.188^{b}	0.194^{b}	0.203^{a}	0.013***
N.P.N./T.N.%	4.48^{d}	4.68^{c}	4.79^{b}	4.77^{b}	4.88^{a}	0.026***
T.V.F.A.	19.44^{c}	19.62^{c}	19.80^{b}	19.82^{b}	20.14^{a}	0.256***
Colour & Appearance	12.60^{a}	12.60^{a}	12.60^{a}	12.60^{a}	12.40^{a}	1.303*
Body & Texture	30.40^{a}	31.20^{a}	31.40^{a}	31.40^{a}	31.00^{a}	2.605*
Flavour	42.40^{a}	43.20^{a}	44.00^{a}	44.40^{a}	44.20^{a}	2.605*
	Effect of storage time (days)					
	15	**30**	**60**	**90**	**120**	**LSD**
Acidity%	1.83^{c}	2.04^{bc}	2.17^{b}	2.31^{a}	2.28^{ab}	0.261**
pH	5.06^{a}	4.93^{b}	4.81^{c}	4.70^{d}	4.57^{e}	0.029**
T.S.%	66.48^{e}	69.03^{d}	70.94^{c}	72.34^{b}	73.69^{a}	1.303***
Fat%	29.12^{e}	30.05^{d}	30.84^{c}	31.72^{b}	32.50^{a}	0.258***
Fat/D.M. %	43.53^{a}	43.53^{a}	43.81^{a}	43.88^{a}	44.14^{a}	1.303*
Salt%	2.77^{c}	3.30^{b}	3.30^{b}	3.92^{a}	4.12^{a}	0.387**
T.N.%	3.53^{e}	3.99^{d}	4.04^{c}	4.10^{b}	4.18^{a}	0.024***
T.N./D.M.%	5.31^{d}	5.77^{a}	5.69^{b}	5.67^{b}	5.56^{c}	0.024**
N.P.N.%	0.133^{e}	0.166^{d}	0.192^{c}	0.212^{b}	0.234^{a}	0.013***
N.P.N./T.N.%	3.81^{e}	4.23^{d}	4.73^{c}	5.22^{b}	5.63^{a}	0.026***
T.V.F.A.	11.72^{e}	15.82^{d}	20.26^{c}	24.10^{b}	26.92^{a}	0.257***
Colour & Appearance	11.00^{b}	12.80^{a}	13.00^{a}	13.00^{a}	13.00^{a}	1.303*
Body & Texture	26.80^{b}	30.60^{a}	32.60^{a}	32.80^{a}	32.60^{a}	2.605*
Flavour	38.60^{c}	42.40^{b}	45.00^{b}	46.40^{a}	45.80^{a}	2.605**

Significant different at p < (*0.05, **0.01, ***0.001). For each effect the different letters in the means the multiple comparison are different from each. Letters a is the highest means followed by b, c …..etc.

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تأثير إضافة بروتينات الشرش المدنترة للبن البقري أو الخثرة على بعض خواص الجبن الراس

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أجري هذا البحث بهدف محاولة استخدام بروتينات الشرش المدنترة و التي تعد منتج ثانوي بمصانع الجبن في صناعة الجبن الراس. حيث أضيفت هذه البروتينات إلي اللبن البقري و أيضاً إلي خثرة الجبن بنسب ١ و ٢%. و تشير النتائج إلي أن إضافة بروتينات الشرش المدنترة إلي اللبن البقري أو الخثرة أدت إلي زيادة قيم التصافي، الحموضة، الملح، النيتروجين الكلى، النيتروجين الكلى/ المادة الصلبة، النيتروجين غير البروتينى ، النيتروجين غير البروتينى / النتروجين الكلي، الأحماض الدهنيه الكلية الطيارة و الأحماض الأمينية الحرة للجبن الراس الناتج. و قد لوحظ أن إضافة بروتينات الشرش للبن البقري خفضت قليلاً من نسب المواد الصلبة و الدهن بالجبن في حين أن إضافة هذه البروتينات لخثرة الجبن أدت إلي زيادة طفيفة في محتوى الجبن من المواد الصلبة و الدهن. كما أدت إضافة بروتينات الشرش للبن البقري أو الخثرة إلي تحسن في الخواص الحسية للجبن الراس.

قام بتحكيم البحث

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