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COMPOSITION AND MICROSTRUCTURE OF SOFT BRINE CHEESE MADE FROM INSTANT WHOLE MILK POWDER

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Abstract

Comparative studies were made on the composition, microstructure and organoleptic quality of soft brine cheese made from instant whole milk powder and from raw milk. The chemical analysis of young and ripened (one and two month) cheese revealed similarity except for a higher salt content in the cheese made from reconstituted milk at the end of ripening. Electron microscopic studies showed distinct differences in the structure of the protein matrices in the ripened cheese samples, i.e. a very homogeneous structure in the cheese made from raw milk compared with a slightly aggregated state of the protein in the cheese made from reconstituted milk. The organoleptic examinations resulted in an overall acceptable quality of the cheese made from reconstituted milk except for a higher saltiness.

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KEY WORDS: Soft brine cheese;Instant whole milk powder;Freeze-fracturing.

Introduction

The use of non-fat dried milk for cheese manufacture requires adequate reconstitution and recombination techniques, and is now common in a few developed countries.

In many developing countries, e.g. Egypt, the shortage in the milk supply requires increasing use of milk powder for dairy products. Because modern equipment and processing facilities for the reconstitution and recombination are often not available in private small dairy plants, the use of whole milk powders could be advantageous.

Reconstituted whole dried milk, however, tends to exhibit limited solubility which may result in fat destabilization and also sediment formation (14). In order to circumvent these problems, the use of instant whole milk powders could represent an alternative (22, 26).

The use of milk powder for the manufacture of Camembert cheese and the resulting changes in the microstructure during ripening have been described by Peters and Knoop (17, 18).

We now report results on the composition and structure of soft brine cheese made from instant whole milk powders.

Materials and Methods

Five imported instant whole milk powders of different origin were purchased from the local market in Egypt. The average composition of these powder samples was: 26-28 % butterfat, 25.5-26.5 % protein, 37-38 % lactose, 5.5-6.5 % ash and 2-3 % moisture. Each powder was reconstituted to 20 % total solids in water (30^{o}C) by only gentle mechanical stirring. For control, fresh cows' milk from the University of Zagazig Experimental Farm was used.

Batches of soft brine cheese were made from each of the reconstituted milks and from the fresh milk according to a method described earlier (10, 14): The milks were at first heated to 65^{o}C for 30 minutes, then cooled to 32^{o}C. Subsequently 2 % starter and 0.04 % calcium chloride were added. The curd was cut after 60 minutes and filled into wooden moulds, then pressed and brine salted (16 % salt, 24 hours). The young cheeses were packed in tins containing salted sweet whey (14 % salt) and stored for two months

at 12-14°C for ripening.

Fresh and reconstituted milks and their wheys were analysed for pH, °SH, fat and total nitrogen. The fat and protein retention was calculated. Young and ripened (1 and 2 months) cheese samples were examined for pH, buffer capacity, lactose, fat, moisture, calcium and phosphorus and salt (NaCl) (2, 5, 7). The protein breakdown of cheese was determined as soluble nitrogen (24), non-protein nitrogen (23), peptide nitrogen (6), and amino acid nitrogen (23). Cheese scoring was carried out after two months of ripening (3). Three replicate analyses of the samples by each the analytical techniques were carried out. Mean value $\bar{x}$ and standard deviation σ_x were calculated (25).

The young and ripened cheese samples were prepared for electron microscopy by application of the freeze-fracturing technique (4, 20). Small pieces (1-2 mm^3) of cheese were mounted on specimen holders, using glycerol as an intermediate layer for increasing mechanical stability. The specimens were quickly frozen by immersion into melting Freon 22 (- 160°C) and stored under liquid nitrogen. Freeze-fracturing was carried out in a BALZERS BA 360 M unit at an object temperature of - 120°C. For replication the freshly freeze-cleaved surface was immediately shadowed with 2 nm platinum/carbon under an angle of 45° and further stabilized by 20 nm of pure carbon. The replicas were floated onto distilled water and then transferred to 5 % sodium hypochlorite (undiluted bleach) for approximately 2 hours and passed again to distilled water. Fat was removed by a short treatment in pure acetone. Electron microscopy was carried out with a Siemens ELMISKOP I at 80 kV.

Results and Discussion

Milk reconstitution

When instant whole milk powder was added to the water and mildly agitated, it formed a homogeneous reconstituted milk, showing no clumps or butteroil lenses on the surface. Analyses of fresh milks and the corresponding wheys are shown in Table 1. The values for fresh milk may somewhat differ from milks produced in countries with a moderate climate but are not abnormal for conditions in Egypt. The higher content of fat and protein of reconstituted milks as compared with fresh milk resulted from using 20 % total solids which was necessary in order to obtain a satisfactory curd firmness. Fat and protein retention in cheese made from fresh milk was higher than in those made from reconstituted milks, although the latter had 20 % total solids. The average °SH value of the reconstituted milks is almost double that of fresh milk which has to be ascribed to the higher protein content. The comparably high σ_x for °SH in reconstituted milk results from differences between the five milk powders used.

A precipitate was observed in the whey of cheeses made from reconstituted milks. This could be due to the protein denaturation during the drying process, resulting in insoluble whey protein aggregates. The amount of insoluble whey is dependent upon the preheating conditions of the milk and milk concentrate (13). On the other hand, a slight increase in insoluble protein can occur as a result of the drying process in all milk powders (15).

The curds from the reconstituted milks were still softer than the curd from the fresh milk despite the 20 % total solids chosen. In this respect, instant dried milk and ordinary dried milk are similar in curd formation and insoluble whey contents. This phenomenon has been repeatedly described (12, 16).

Chemical composition of cheese

The average values of pH, buffer capacity, lactose, fat, protein, moisture, calcium, phosphorus and salt (NaCl) content of cheese made from fresh milk (variant I) and cheeses made from the five reconstituted milks (variant II) are shown in Table 2. Young cheese II has a buffer capacity and a lactose content higher than that of cheese I, due to the use of 20 % total solids. After one month of ripening lactose vanishes in both types of cheese and, at the same time, the buffering capacity of the cheeses and the pH values increase.

After two months, cheese I showed a higher pH value and a lower buffer capacity than cheese II. Changes in lactose, pH and buffer capacity are in accordance with those given by Czulak et al. (5). Fat, protein and moisture contents of cheese II were less than those of cheese I.

The average value of calcium and phosphorus of all cheeses was nearly the same and was

Table 1. Analysis of milks and wheys (Average of three replicates)

Samples	Index	Milk pH	Milk °SH	Milk Fat %	Milk Protein Nx6.38	Whey pH	Whey °SH	Whey Fat %	Whey Protein	Protein retention %	Fat retention %
Fresh milk	$\bar{x}$	6.43	7.80	1.10	3.38	5.80	5.30	0.30	0.42	87.10	90.01
	σ_x	0.09	0.40	0.41	0.32	0.13	0.30	0.04	0.06	0.88	0.50
Reconstituted milks (5 batches)	$\bar{x}$	6.38	14.85	5.31	5.74	5.52	4.81	0.69	1.53	81.61	87.02
	σ_x	0.11	2.61	0.65	0.11	0.42	0.20	0.10	0.03	2.11	3.04

Table 2. Chemical composition of cheese. (Average of three replicate analyses)

Age of cheese	Treatment	Index	pH	Buffer capacity in 0.5nNaOH	Lactose %	Fat in D.M. %	Protein (Nx6.38)	Moisture %	Calcium %	Phosphorus %	Salt (NaCl) %
Young	I	$\bar{x}$	5.17	2.71	0.82	45.79	21.82	58.20	0.96	0.68	2.93
		σ_x	0.11	0.84	0.04	0.24	0.16	1.22	0.11	0.06	0.20
	II	$\bar{x}$	5.11	3.19	1.36	41.32	20.79	53.19	0.89	0.69	3.86
		σ_x	0.14	0.92	0.03	0.65	0.13	2.26	0.13	0.05	0.67
One Month	I	$\bar{x}$	5.46	3.82	-	42.83	21.94	55.40	0.93	0.70	4.16
		σ_x	0.10	1.01	-	0.61	0.16	2.41	0.07	0.02	0.30
	II	$\bar{x}$	5.40	4.11	-	39.48	20.93	51.12	0.87	0.69	4.51
		σ_x	0.14	0.58	-	0.56	0.11	2.20	0.04	0.11	0.21
Two months	I	$\bar{x}$	5.61	2.99	-	40.06	21.76	52.30	0.98	0.71	4.96
		σ_x	0.07	1.07	-	0.35	0.12	1.58	0.09	0.03	0.16
	II	$\bar{x}$	5.50	3.78	-	38.58	20.86	50.16	0.96	0.69	6.01
		σ_x	0.09	0.93	-	0.57	0.14	1.25	0.08	0.12	0.22

I - Fresh milk cheese II - Reconstituted milk cheeses (5 batches)

Table 3. Protein degradation of cheese (Average of three replicate analyses)

Age of cheese	Treatment	Index	Total nitrogen (T·N) %	Soluble nitrogen (S·N) % of T·N	Non-protein N % of T·N	Non-protein N % of S·N	Peptide N % of T·N	Peptide N % of S·N	Amino acid N % of T·N	Amino acid N % of S·N
Young	I	$\bar{x}$	3.42	10.15	6.02	44.16	0.95	8.12	0.88	8.51
		σ_x	0.16	1.80	0.91	5.66	0.24	2.25	0.14	0.76
	II	$\bar{x}$	3.26	12.89	5.95	47.07	0.61	4.40	0.45	3.47
		σ_x	0.13	1.02	0.38	4.39	0.14	1.00	0.11	0.81
One month	I	$\bar{x}$	3.44	18.96	10.12	51.89	1.56	9.11	2.98	15.80
		σ_x	0.16	2.45	1.07	4.47	0.91	0.03	0.58	2.16
	II	$\bar{x}$	3.28	16.43	8.67	53.24	1.22	7.01	2.75	15.33
		σ_x	0.11	1.66	1.21	6.32	0.36	2.26	0.27	1.26
Two months	I	$\bar{x}$	3.41	24.69	14.01	57.25	3.11	12.43	4.01	16.04
		σ_x	0.12	1.89	1.09	4.24	0.48	3.72	1.45	3.53
	II	$\bar{x}$	3.27	19.48	12.46	63.90	2.50	11.28	3.73	19.52
		σ_x	0.14	1.12	1.27	5.60	0.49	2.65	0.75	4.24

I - Fresh milk cheese II - Reconstituted milk cheeses (5 batches)

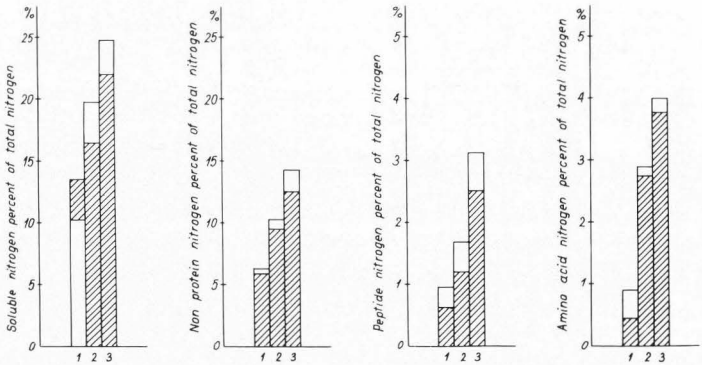

Fig. 1. Ripening indices of cheese made from fresh (□) and from reconstituted (▨) milk. 1: young cheese; 2: one month matured cheese; 3: two months matured cheese

Table 4. Scoring of cheese made from fresh milk (I) and reconstituted milks (II) after two months of ripening

Treatment	No.	Finish and appearance (20)	Body and texture (30)			Flavour (50)		Comments	Total (max. 100)
			Holes (5)	Colour (10)	Consistency (15)	Aroma (25)	Taste (25)		
I	1	20	5	10	14	20	20	White, soft, pure, slight salty.	89
	2	20	5	9	13	18	19	Creamy, slight soft, acid, salty.	84
	3	20	5	10	14	19	20	White, soft, pure, salty.	88
	$\bar{x}$	20	5	9.7	13.7	19	19.7		87
II	1	20	5	9	12	18	20	Creamy, smooth, slight salty.	84
	2	20	5	9	12	16	17	Creamy, porous, brittle, distinct salty	79
	3	20	5	8	11	17	18	Slight creamy, tough, distinct salty.	79
	$\bar{x}$	20	5	8.7	11.7	17	18.3		80.7

constant throughout ripening. This result is in agreement with those of Poznanski and Rymaszenski (19) and Raadsveld and Klomp (21).

Cheese II contained more salt than cheese I, due to the quicker penetration of the brine into cheese made from dried milks (17, 18).

Protein degradation of cheese

The breakdown of protein during cheese ripening was measured by the ripening indices as illustrated in Table 3 and Fig. 1.

In young cheese, a slight increase of soluble nitrogen was found in cheese II compared with cheese I. After one month and more pronounced at the end of two months cheese made from fresh milk had more soluble nitrogen than that made from reconstituted milk, i.e. 24.69 % v. 19.48 %.

The non-protein nitrogen values were the same in both cheeses at the beginning of ripening and increased during the ripening to be higher in cheese I than in cheese II, i.e. 14.01 % and 12.46 %, respectively.

Peptide nitrogen and amino acid nitrogen values were higher in cheese I than cheese II throughout the ripening period and affected the cheese flavour. The relative content of peptide nitrogen related to total nitrogen was 3.11 % in cheese I and 2.50 % in cheese II, while amino acid nitrogen relative to total nitrogen was

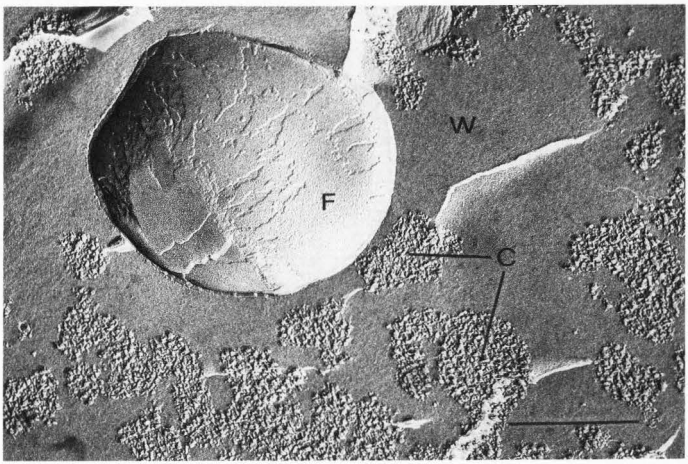

Fig. 2. Young cheese made from fresh milk. The casein micelles (C) are coagulated and form an open network. In the whey phase (W) only rarely can single protein particles be seen. Generally, no adsorption of casein at the surface of fat globules (F) occurs. Bar, 0.5µm.

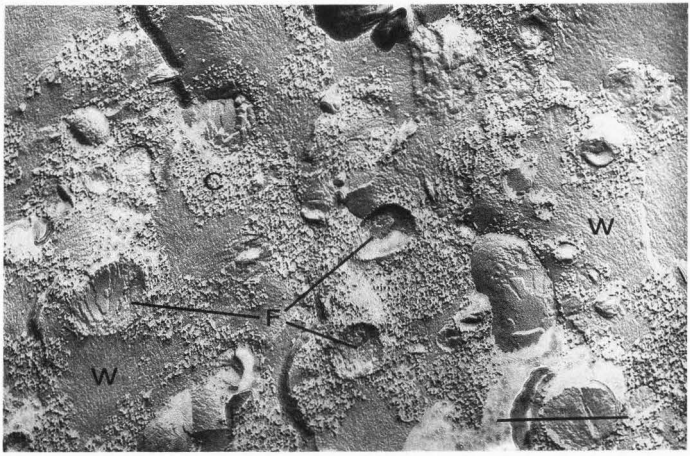

Fig. 3. Young cheese made from reconstituted milk. In contrast to young cheese made from fresh milk (Fig. 2), the fat globules (F) are distinctly smaller and show a strong association with the casein (C). W: whey phase. Bar, 0.5µm.

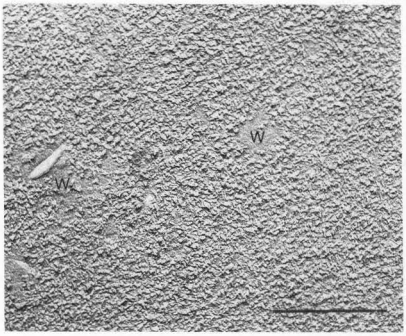

Fig. 4. Two months matured cheese made from fresh milk. The casein forms a nearly continuous mass of finely dispersed single protein particles. Occasionally small, particle-free areas of whey (W) occur. Bar, 0.5 µm.

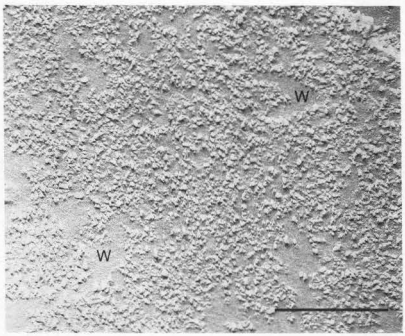

Fig. 5. Two months matured cheese made from reconstituted milk. The casein matrix is distinctly less homogeneous than in the cheese made from fresh milk (see Fig. 4) and shows a fine network-like structure with the whey phase (W) still clearly detectable. Bar, 0.5 µm.

4.01 % in cheese I and 3.73 % in cheese II after two months of ripening.

These analytical results show that the changes of protein degradation were more pronounced in cheese made from fresh milk.

Cheese scoring

Two months matured cheeses were scored for organoleptic properties. 20 points were given for finish and appearance, 30 points for body and texture (including holes, colour and consistency) and 50 points for flavour (aroma and taste) as shown in Table 4. (The point numbers are the maximum scores for the best cheese). The external finish and appearance were similar in both treatments and no holes were observed. Colour of cheese I was white while that of cheese II was light creamy to creamy. The consistency of cheese I was soft and typical, while cheese II showed a tendency to be tough and brittle and sometimes had a porous texture. Control cheese I had a better flavour than cheese II.

The organoleptic properties of all cheeses were similar except for the porous structure and slightly higher saltiness of cheese II compared with cheese I.

Electron microscopy

Representative micrographs of the young cheese samples are shown in Figs. 2 and 3. The cheese made from raw milk (Fig. 2) is characterized by a loose network of partly coalesced casein micelles with whey forming a continuous phase. Because the raw milk was not homogenized and therefore the fat globules largely retained the original fat globule membrane casein is normally not associated with fat globules. The young cheese made from reconstituted milk (Fig. 3) shows a casein distribution which is roughly similar to that of young cheese made from raw milk. The main structural difference is the strong interaction between casein and fat globules which is a result of the homogenization applied during processing of the milk powders. As a result the small fat globules are largely embedded in the casein aggregates.

It is known that the development of the microstructure of various types of cheese during ripening is characterized by a varying degree of disintegration of casein micelles resulting in a penetration of whey into the disintegrated casein mass (11).

The microstructure of the cheeses after two months of ripening is shown in Fig. 4 (cheese made from raw milk) and Fig. 5 (cheese made from reconstituted milk). The main characteristic for both cheeses is the degradation of the casein into a more or less uniform matrix which is typical also for other cheese varieties (1, 8-10, 20). The protein matrix of the cheese made from raw milk (Fig. 4) is characterized by a very uniform distribution of small protein particles and by a rare occurrence of small areas (0.1-0.2 µm in diameter) of whey where protein particles are not detectable.

In contrast to this type of protein matrix structure, the cheese made from reconstituted milk (Fig. 5) shows a markedly less homogeneous distribution of protein particles. They appear to be still slightly aggregated thus making the whey phase clearly visible.

The relatively loose and porous structure of the protein matrix of the cheese made from reconstituted milk must be ascribed to structural changes of the protein during the drying process of the milk (17, 18).

As a consequence of this loose structure the penetration of salt into the cheese made from reconstituted milk is more intensive than into the control cheese (see Tables 2 and 4). This has already been described for Camembert cheese (17, 18).

The structural differences of the protein matrices in ripened cheeses also may be related to the different consistencies (Table 4), especially toughness and brittleness and also the porous texture of the cheese made from reconstituted milk.

Conclusion

These studies have demonstrated that it is possible to produce a soft brine cheese of acceptable quality by use of instant whole milk powder. An increased saltiness may occur in comparison with cheese made from fresh milk due to a looser structure of the protein matrix.

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Discussion with Reviewers

D.N. Holcomb: The ^{O}SH of the reconstituted milk is almost double that of fresh milk. Is this difference solely due to the higher protein content of the reconstituted milk? Also, why is α_x so high for ^{O}SH in reconstituted milk? Is there any relationship between cheese properties and ^{O}SH?
Authors: The difference between ^{O}SH of fresh milk and mean ^{O}SH of the five batches of reconstituted milk must be primarily due to the different protein contents. The high α_x for ^{O}SH in reconstituted milk reflects differences between the five different milk powders used. Pronounced relationships between cheese properties and ^{O}SH were not obvious.

M. Kalab: Is it safe to assume that a close association between fat globules and casein in a soft brine cheese indicates that reconstituted nonfat dry milk had been used to make such a cheese? When you mention "representative" micrographs, do you mean that casein was attached to all fat globules, to most fat globules, or to some fat globules?
Authors: In the present study we used instant whole milk powder in which the fat phase had been homogenized. Since casein becomes closely associated with fat globules during homogenization of milk, such fat globules also subsequently become an integral part of the casein network during clotting processes. Original, unhomogenized fat globules do not show such an association with the casein network because the native fat globule membrane is a phospholipid - containing biomembrane - like interfacial layer with quite different properties. Of course, this original milk fat globule membrane may be partly destroyed during mechanical and thermal processing of the raw milk or during the cheese manufacture and will then allow casein to become adsorbed to the fat phase to a very limited extent. This explains why in the cheese made from reconstituted milk nearly all fat globules were closely associated with the casein whereas in the cheese made from fresh milk most fat globules were not.

M. Kalab: Have you found that freeze-fracturing is more reliable than embedding in a resin, sectioning, and staining to explain the microstructure of cheese?
Authors: According to our experience freeze - fracturing is a very reliable technique for studying the microstructure of cheese, either in a young or a ripened state. In the plane of fracturing which is preserved by the platinum/carbon-replication not only size, shape and substructure of the casein aggregates, but also free protein particles of molecular dimensions and also the fat phase can be inspected in detail. Thin-sectioning techniques appear especially suitable e.g. for three dimensional structure analysis (stereo micrographs, serial sections), for additional light microscopy (phase contrast) or for localizing certain constituents by specific staining or labelling techniques.

M. Kalab: Replicas initially floating on water are known to sink into acetone, warp, and roll. Which technique do you use and would you recommend to retrieve the cleaned replicas?
Authors: We normally transfer replicas from pure acetone to a 1:1 mixture of acetone and water and then to pure water. Often a direct transfer from acetone to water is successful but the replica may disintegrate into smaller pieces. If replicas do not completely unfurl on the water surface we put them back into acetone and repeat this procedure.

N.F. Olson: Other researchers (Green et al., J. Dairy Res. 48, 343) have observed increased coarseness of curd as the concentration of cheese milk was increased. Could the high concentration of dry milk used in treatment II have caused some or all of the coarse structure in this cheese?
Authors: It cannot be excluded that the higher concentration of dry milk used in these studies has partly contributed to the coarser structure. But according to other studies (ref. 17 and 18) this coarseness has to be mainly ascribed to effects resulting from the heating conditions during drying of milk.

Additional Reference

27. Green ML, Turvey A, Hobbs DG. (1981). Development of Structure and Texture in Cheddar Cheese. J. Dairy Research 48. 343-355.