

Inclusion of whey proteins in rennet curd matrix.

Minia, J. Agric. Res. And Develop., 28 (2): 317-330.

By

S.I. Shalabi*; F.S. Ibrahim*; A.A. Abd El-Khair**; A.H. Ateteallah**

* *Dairy Sci. Dept., Faculty of Agric., Minia Univ., Egypt.*

** *Food & Dairy Sci. Dept., Faculty of Agric., Sohag Univ., Egypt.*

ABSTRACT

A study was conducted to assess rennet coagulation properties and nitrogen distribution as incorporating whey proteins into milk. Whey proteins were retained either by applying high heat treatment at 95°C for 5 min at pH 6.6 or by a direct addition of denaturated whey proteins. Curds were also made by inclusion of unsalted or salted whey in milk, either by mixing whey with skim milk or reconstitution of skim milk powder in whey. Results revealed that, heat treatment of milk increased RCT and decreased curd firmness, syneresis, and nitrogen loss in the whey. Rennet coagulability was improved by CaCl_2 addition. Direct addition of denaturated whey protein powder had no effect on RCT, but decreased the syneresis, and increased the nitrogen retained in the rennet curd. Whey proteins powder prepared from unsalted whey increased the firmness of the formed curd. All unheated mixes of skim milk with unsalted whey gave a good coagulability. Heat treatment of these mixes increased RCT and all mixes gave a soft curd and failed to form gel with 1:1 mixes. Addition of CaCl_2 slightly improved the rennet coagulability. The mixes of skim milk with salted whey exhibited a similar trend. Reconstitution of skim milk (12% w/v) in unsalted whey decreased clotting time than that in salted whey. However, a quite form gel was obtained in both cases, being a slightly firmer in that reconstituted in salted whey. Heat treatment of whey either unsalted or salted before reconstitution of skim milk decreased the syneresis and increased RCT, gel firmness and nitrogen retained in the gel. Increasing the levels of skim milk powder did not affect the rennet coagulability and using 30% level produced a cheese-like curd

Key words: Whey proteins; High heat treatment; Coagulation properties

Abbreviation key: RCT: Rennet clotting time; CF: Curd firmness, TN: Total nitrogen

INTRODUCTION

In traditional cheese-making casein forms the curd structure while whey proteins are lost in the whey. There are several technologies for re-integrating whey proteins into cheese during processing. The application and process adaptations depend mainly on the type of cheese and the desired texture. Whey proteins may be retained by applying high heat treatment in order to affix the whey proteins to caseins or by using membrane technology to reduce the aqueous phase. Alternatively, whey proteins may be removed from drained whey by ultrafiltration and then added to curd after special heat treatment or by recycling into cheese milk (Brown & Ernstorn, 1982; Lo and Bastian, 1998; Atra et al., 2005).

These methodologies can be a useful mean for recovering nutritional valuable whey proteins. Whey proteins are an excellent source of high sulfur containing amino acids and with casein which contains more tyrosine and less sulfur amino acids, the overall cheese value is enhanced (Wingerd, 1971 and Brown & Ernstorn, 1982) When whey proteins are integrated into fresh, soft, semi-hard and hard cheese, this not only improves the nutrient value and yield but also causes changes in functional properties

(Banks et al., 1994; de Wit, 1998; Lucey et al., 1999; Mead and Roupas, 2001). However, the characteristics of the produced cheeses using such techniques need to be assessed.

Several attempts were made for incorporation of whey proteins in different cheese varieties either in its native form or denaturated (**Lo and Bastian, 1998**). The influence of the addition of whey protein concentrates (WPC) on the yield and properties of some salt, semi-hard and hard cheese varieties were reviewed by **Hinrichs (2001)**.

In the present study, whey proteins were incorporated into cheese matrix either in denaturated form (by heat induced complexes between casein and whey proteins or by direct addition of denaturated whey protein particles) or in the native form by direct inclusion of whey in cheese milk (by mixing the whey with skim milk or skim milk powder). As the essential step in the manufacture of most cheese varieties is the milk coagulation, mainly by rennet. The effects of these treatments on milk rennet coagulation properties and nitrogen loss in the whey were investigated

MATERIALS AND METHODS

Materials:

Cheese milk:

Whole cow's milk was obtained from the herd of Faculty of Agric., Minia University. The milk was separated, skim milk was used as cheese milk to eliminate the fat interference.

Skim milk powder:

Low- heat skim milk powder imported from USA was used

Unsalted whey:

It was obtained after Ras cheese manufacture at the Dairy Laboratory, Faculty of Agric., Minia university

Salted whey:

It was obtained after Domiati cheese manufacture at the Dairy Laboratory, Faculty of Agric., Minia university

Rennet:

A commercial standard calf liquid rennet obtained from the local market was used.

Preparation of whey proteins:

The acidification and heating method described by **Modler and Emmaons (1976)** was followed for the preparation of whey proteins from unsalted and salted whey.

Experimental design:

Five experiments were carried out as follows:

Experiment 1:

Whey proteins were retained by applying high heat treatment in order to affix the whey proteins to caseins. In this experiment, skim milk was heat treated at 90°C for 5 min at its natural pH (6.6)

Experiment 2:

Denaturated whey protein particles (powder) prepared from unsalted or salted whey were added to skim milk in four levels i.e. 0.5, 1.0, 1.5, and 2.0% (w/v).

Experiment 3:

Whey either unsalted or salted was mixed with skim milk in the ratios of 1:1, 1:2, 1:3, and 1:4 (v/v). The mixes were heat treated at 90°C for 5 min, and then CaCl₂ (0.02%.) was added.

Experiment 4:

Skim milk powder was reconstituted in heat treated (90°C for 5 min) unsalted or salted whey in a level of 12% (w/v). The pH was adjusted to 6.6, and then CaCl₂ (0.02%.) was added.

Experiment 5:

Skim milk powder was reconstituted in heat treated (90°C for 5 min) unsalted or salted whey in ascending levels; 20, 25, and 30% (w/v). The pH was adjusted to 6.6.

For the first four experiments the rennet was added at the rate of 1 ml/ liter. While, for the fifth experiment rennet was added in proportional to protein content.

Analytical Methods:

Chemical analysis:

Total nitrogen (TN) was determined by the semi-micro kjeldahl method and pH of samples was measured using Orion pH-meter

Coagulation time determination:

Rennet coagulation time (RCT) was taken as the time for the first sign of clotting to appear after the addition of 0.2 ml of rennet solution to 15 ml of milk sample at 37°C.

Curd firmness determination:

The penetration method described by **Shalabi (1987)** was used for determination of curd firmness

Syneresis determination:

The volume of whey expelled within 60 min for rennet curd cut after 2.5 times the rennet coagulation time, was measured as described by **Marshall (1982)**.

RESULTS AND DISCUSSION

Results in Table 1 show a decrease in the amount of N lost in the whey and decreased even more by CaCl₂ addition. This illustrated that about 25% of whey N was retained in the rennet gel which was increased to 35% by CaCl₂ addition. Data show also that heating of milk adversely affected the rennet coagulation properties. The RCT increased from 10 min in the unheated sample to 32 min after heating. Addition of 0.02 CaCl₂ offset the adverse effect of heating and caused a decrease in RCT to 15 min. Although, changes in salt equilibrium are involved in the ill effect of heat treatment on rennet coagulability. It was reported that the principal factor is the intermolecular disulphide bond formation between k-casein and whey proteins (**Sawyer, 1969; Shalabi & Wheelock, 1976, 1977; Singh et al., 1988 and Corredig & Dalgleish, 1999**).

Table 1: Effect of heat treatment of skim milk at 90°C for 5 min on coagulation properties and nitrogen loss in the whey

Treatments	Coagulation properties			TN in whey (mg/100ml)
	RCT (min)	CF (gm)	Syneresis (ml/500ml)	
Skim milk, (unheated control)	146	10	22.46	328
Skim milk, heated	123 (25%)	32	14.26	215
Skim milk, heated then 0.02% CaCl ₂ added	106 (35%)	15	17.02	286

TN: 546 mg/100 ml of milk

A decrease in the firmness of formed rennet gel was apparent as a result of heat treatment. This was improved by CaCl_2 addition. It has been also reported that rennet gel strength was decreased by heat treatment as a result of incorporation of whey proteins into the gel matrix (**Ashworth & Nebe, 1970 and Singh et al., 1988**). Also, the amount of whey expelled from the rennet curd after 60 min from cutting (**Marshall, 1982**) was decreased after heat treatment, but was improved by CaCl_2 addition. **Schmidt and Morris (1984)** reported that complexes of casein and heat denaturated whey proteins appear to possess increased the water holding capacity. Table 1 also shows a decrease in the amount of N lost in the whey and decreased even more by CaCl_2 addition. This illustrates that about 25% of whey N was retained in the rennet gel which was increased to 35% by CaCl_2 addition.

Results in Table 2 show that these additions had no effect on RCT. Surprisingly; whey protein particles from unsalted whey caused a noticeable increase in the firmness of the formed curd with the increase in the amount of added whey proteins, while the effect with that from salted whey was very slight. **Steff et al., (1999 a & b)** reported that particulated whey proteins produced from whey and added to cheese milk changed the structure of the resulting rennet-induced gel and, therefore, the renneting properties. Whey proteins were entrapped into the pores of casein network and were not actively involved in strand formation. The strength of the resulting gel is crucially influenced by the size of the particles. If they are bigger than the holes in the casein network, then the particles disrupt the regular structure of the network which leads to a weaker gel. Therefore, the observed effect on the rennet gel firmness could possibly due to that the size of the whey protein particles entrapped into the rennet gel were within the hole size of casein network. Table 2 also shows a noticeable decrease in syneresis especially with 2% added whey powder. This was expected as the presence of whey proteins in rennet gel matrix increases its water binding capacity (**Schmidt & Morris, 1984 and Mead & Roupas, 2001**). With both type of whey protein powder, increasing the level of added whey protein was accompanied by a concomitant increase in the amount of N retained in the curd, reaching 24.28% and 30.57% with 2% unsalted and salted whey protein particles, respectively. The large particles seem to be lost in the whey, hence, they were included in the whey analysis (Table 2). The ability to incorporate whey N into rennet curd while retaining good rennet coagulability has obvious possibilities for increasing cheese yield.

Table 2: Effect of direct addition of denaturated whey protein particles on rennet coagulation properties of milk and nitrogen retained in the curd

Treatments	Added whey proteins %	Coagulation properties			Nitrogen distribution		
		RCT (min)	CF (gm)	Syneresis (ml/500ml)	TN	N loss in whey	Retained N in curd
Skim milk	0	10	22.46	328	514	164	350
Skim milk + whey proteins from unsalted whey	0.5	8	22.29	287	567	188	388 (10.85)
	1.0	8	28.00	291	588	191	396 (13.14)
	1.5	8	28.47	293	609	201	407 (16.28)
	2.0	8	30.02	224	630	195	435 (24.28)
Skim milk +	0.5	9	23.13	300	573	184	389 (11.14)

whey proteins from salted whey	1.0	9	24.00	295	601	198	403 (15.14)
	1.5	9	24.01	293	628	198	430 (22.85)
	2.0	9	24.82	268	655	198	457 (30.75)

Data in parenthesis represent percentage of increase in retained N

Marshall (1986) reported an increased yield of 4.5% for Cheshire cheese made from milk heated at 97°C for 15 sec, enriched with Ca and adjusted to pH 6.4. Also, Med and Roupas (2001). Reported an increase in yield of Pizza cheese with addition of whey protein particles to cheese milk up to 0.4% (w/v), but cheese had poor functionality especially with high level of added whey protein particles (0.4%).

Results in Table 3 show that all unheated mixes of skim milk with unsalted whey gave good rennet coagulation properties. The RCT ranged from 10 min in pure skim milk to 15 min in 1:1 mix. The rennet gel formed was quite firm. The curd firmness was reverse proportional with the ratio of whey inclusion in cheese milk. The higher the whey ratio in the mix the lower was the firmness. The rate of whey separation from the rennet curd showed a slight decrease with the increase of the ratio of whey in the mix. The higher the whey ratio in the mix the lower the syneresis was found. RCT for any particular mix increased after heat treatment at 90°C for 5 min. All heat treated mixes gave soft gels and failed to form an accepted gel with 1:1 mix. In case of heat treated mixes with 0.02% CaCl₂, the RCT values were still more than those of the unheated mixes and the formed gel were still far less firmness. Also, more water was retained in the formed gel as a result of the heat treatment. This was expected and may be due to whey protein increases the water holding capacity (**Schmidt and Morris, 1984**).

Table 3: Rennet coagulation properties and nitrogen distribution of skim milk/ unsalted whey

Treatments	Mix ratio	Coagulation properties			Nitrogen distribution		
		RCT (min)	CF (gm)	Syneresis (ml/500ml)	TN	N loss in whey	Retained N in curd
Skim milk	-	10	24.00	338	576	174	402
Skim milk/unsalted whey	4:1	10	20.00	354	553	188	365
	3:1	12	20.00	255	532	184	348
	2:1	12	16.00	358	491	188	303
	1:1	15	11.40	388	427	188	239
Skim milk/unsalted whey after heat treatment at 90°C/ 5 min	4:1	19	8.56	152	553	137	416
	3:1	20	8.04	173	532	147	385
	2:1	22	7.00	Weak gel	491	143	348
	1:1			Very week gel	427		
Skim milk/unsalted whey after heat	4:1	13	10.67	178	553	130	423
	3:1	16	10.03	183	532	137	395

treatment at 90°C / 5 min and 0.02% CaCl ₂ added	2:1	16	8.30	174	491	137	354
	1:1	19	7.00	Weak gel	427	123	304

Similar trend was observed for the mixes of skim milk with salted whey (Table 4). The changes in RCT were from 8 min in skim milk to 19 min in 1:1 mix. The curd firmness decreased from 21 g in skim milk to only 11.5 g in 1:1 mix and syneresis increased from 308 ml to 320 ml. Also, heat treatment caused a remarkable increase in RCT with a marked reduction in both of gel firmness and syneresis. A weak gel was formed in heated mix (1:1) with a marked decrease in syneresis. Addition of 0.02% CaCl₂ to heated mixes caused an apparent enhancement in the rennet coagulability, but the improvement was less than that of unheated mixes.

Table 4: Rennet coagulation properties and nitrogen distribution of skim milk/ salted whey

Treatments	Mix ratio	Coagulation properties			Nitrogen distribution		
		RCT (min)	CF (gm)	Syneresis (ml/500ml)	TN	N loss in whey	Retained N in curd
Skim milk	-	8	21.00	308	532	170	362
Skim milk/ unsalted whey	4:1	9	17.08	315	505	184	321
	3:1	10	14.40	316	481	198	283
	2:1	12	14.00	318	474	205	269
	1:1	19	11.50	320	345	239	106
Skim milk/ unsalted whey heat treated at (90°C for 5 min)	4:1	10	10.38	190	505	126	379
	3:1	15	9.55	195	481	131	350
	2:1	20	9.55	275	474	133	341
	1:1	32	7.50	Weak gel	345	130	215
Skim milk/ unsalted whey heat treated at (90°C for 5 min) and 0.02% CaCl ₂ added	4:1	10	14.53	190	505	126	379
	3:1	15	13.14	235	481	126	355
	2:1	24	10.67	290	474	129	345
	1:1	30	9.58	345	345	130	215

Obviously, mixing of skim milk with whey would result in a decrease in the TN content of the mixes (Tables 2 & 3). However, the changes in the amount of N lost in the resultant whey were less remarkable, particularly with mixing with unsalted whey. This decreased the amount of N retained in the curd. Heat treatment reduced N losses in the whey and increased the amount of N retained in the curd.

Results in Table 5 show that reconstituted SMP in unsalted whey clotted in 10 min, while that reconstituted in salted whey coagulated in 30 min, perhaps due to the presence of salt which delayed coagulation time (Fox et al., 2000). In both cases a

quite firm gel was formed being slightly firmer than that reconstituted in salted one. It has been reported that the addition of NaCl or CaCl₂ increases the gel firmness up to 100 mM but markedly decrease it at higher concentration, possibly via displacement of micellar Ca (Fox et al., 2000).

Table 5: Rennet coagulation properties and nitrogen distribution of skim milk powder reconstituted in unsalted or salted whey

Treatments	Coagulation properties			TN	TN in whey	Retained N in the curd
	RCT (min)	CF (gm)	Syneresis (ml/500ml)			
SMP (12%) in unsalted whey	10	21.09	208	802	375	427
SMP (12%) in heated unsalted whey	24	20.04	195	802	286	516
SMP (12%) in salted whey	30	22.55	195	829	349	480
SMP (12%) in heated salted whey	42	17.10	141	829	403	525

Results in Table 6 show that all SMP levels gave good rennet coagulability. The RCT ranged from 4 to 7 min, and curd firmness from 32.5 to 34.87 g in SMP reconstituted in unsalted whey. The corresponding values for that reconstituted in salted whey were 6-9 min and 27.37 to 39.23 g in order. In both cases a remarkable decrease was observed in syneresis with the increase of SMP level. In fact with the level of 30 % SMP the curd was more or less cheese like.

The results clearly demonstrated that 4:1 or 3:1 mix of skim milk with unsalted or salted whey could be possibly used for cheese manufacture. Also, reconstitution of SMP could be another useful mean for manufacture of cheese.

Table 6: Rennet coagulation properties and nitrogen distribution of skim milk powder reconstituted in unsalted or salted whey in different levels

Treatments	SMP Level %	Coagulation properties			TN	TN in whey	Retained N in curd
		RCT (min)	CF (gm)	Syneresis (ml/500ml)			
SMP/ unsalted whey	20	4	32.50	150	1180	470	710
	25	5	33.60	110	1411	592	819
	30	7	34.87	72	1567	592	975
SMP /salted whey	20	6	27.37	168	1061	481	580
	25	7	32.62	112	1266	591	675
	30	9	39.32	92	1411	591	820

- Rennet was added in proportional to the protein content

CONCLUSION

A new combination of whey and milk for cheese production procedure was proposed, which makes possible a significant increase in the cheese yield by incorporating the whey proteins.. Mixing of skim milk with unsalted or salted whey in ratios of 4:1 or 3:1 could be possible used for manufacture of cheese. Reconstitution of skim milk powder in whey could be another useful mean for cheese manufacture.

The study offers a simple and early applicable way for recovering nutritional valuable whey proteins and to overcome some of environmental problems caused by discharge the whey into draining system.

REFERENCES

- Ashworth, U.S. and Nebe, J. (1970).** Comparison of rennet curd tension with undenatured whey proteins as a measure of heat treatment. *J. Dairy Sci.* 53, 415-419.
- Atra, R.; Vatai, G.; Bekassy-Molnar, E. and Balint, A. (2005).** Investigation of ultra- and nanofiltration for utilization of whey protein and lactose. *J. Food Engineering*, 67 (3): 325-332.
- Banks, J. M., A.J.R. Law, J. Leaver, and D. S. Horne (1994).** Sensory and functional properties of cheese: incorporation of whey proteins by pH manipulation and heat treatment. *J. Soc. Dairy Technol.* 47:124–131.
- Brown, R.J. and Ernstrom, A. (1982).** Incorporation of ultrafiltration concentrated whey solids into Cheddar cheese for increased yield. *J. Dairy Sci.* 65: No. (12)
- Corredig, M. and Dalgleish, D. G. (1999).** The mechanisms of the heat induced interaction of whey proteins with casein micelles in milk. *Int. Dairy J.* 9: 233-236.
- de Wit, J.N (1998).** Nutritional and functional characteristics of whey proteins in food products. *J. Dairy Sci.*, 81:597.
- Fox, P.F.; Guinee, T.P.; Cogan, T.M. and McSweeney, P.H. (2000).** Fundamentals of Cheese Science. A Wolters Kluwer Company.
- Hinrichs, J. (2001).** Incorporation of whey proteins in cheese. *Int. Dairy J.* 11: 495-503.
- Lo, C. G., and Bastian E. D. (1998).** Incorporation of native and denatured whey proteins into cheese curd for manufacture of reduced fat, Havarti-type cheese. *J Dairy Sci* 81:16–24.
- Lucey et al. (1999).** Effect of heat-whey protein addition on the rheological properties and structure of acid skim milk gels. *Int. Dairy J.* 9:275.
- Marshall, K.R. (1982).** In: " Developments in Dairy Chemistry" P. F. fox, ed p. 339. Applied Science Publishers Ltd., London.
- Marshall, R.J. (1986).** Increasing cheese yields by high heat treatment of milk. *J. Dairy Res.* 53: 313-322.
- Mead, D. and Roupas, P. (2001).** Effect of incorporation of denatured whey proteins on chemical composition and functionality of Pizza Cheese. *Aust. J. Dairy Technol.* 65: 19-23.
- Modler, H.W. and Emmaons, D.B. (1976).** Properties of whey protein concentrate prepared by heating under acidic conditions. *J. Dairy Sci.* 60: 177-184.
- Sawyer, W.H. (1969).** Complex between β -lactoglobulin and k-casein. A review *J. Dairy Sci.* 52:1347-1355.
- Schmidt, R.H. and Morris, H.A. (1984).** Gelation properties of milk proteins. Soy proteins and blended protein system. *Food Tech.* 38: 85-96.

- Shalabi, S. I. (1987).** Milk clotting activity of commercial rennet substitute. A comparative study. Minia J. Agric. Res. & Dev. 9: 441-460.
- Shalabi, S. I. and Wheelock, J.V. (1976).** The role of α -lactalbumin in the primary phase of chymosin action on heated casein micelles. J. Dairy Res. 44: 351-355.
- Shalabi, S. I. and Wheelock, J.V. (1977).** Effect of sulphydryl-bloking agents on primary phase of chymosin action on heated casein micelles. J. Dairy Res. 43: 331-335.
- Singh, H.; Shalabi, S. I.; Fox, P.F.; Flynn, A. and Borry, A. (1988).** Rennet coagulation of heated milk: influence of pH adjustment before or after heating. J. Dairy Res. 55: 205-215.
- Steff, A.; Hafenmair, M.; Hechler, A. and Hinrichs, J. (1999a).** Influence of whey protein particles on the renneting properties of milk. Milchwissenschaft 54: 510-513.
- Steff, A.; Schreiber, R.; Hafenmair, M. and Kessler, H.G. (1999b).** Effect of denatured whey proteins on the rennet induced aggregation of casein micelles. Int. Dairy J. 9: 401-402.
- Wingerd, W.H. (1971).** Lactalbumin as food ingredient. J. Dairy Sci. 54: 1234.

تأثير إدماج بروتينات الشرش في اللبن على خواصه التجبنية

صموئيل اسكندر شلبي* - فوزي سيد إبراهيم* - عبد لعال عابدين على عبد الخير** - عطيت الله حسن عطيت الله**

*قسم علوم الألبان - كلية الزراعة - جامعة المنيا

**قسم الصناعات الغذائية والألبان - كلية الزراعة - جامعة سوهاج

تم إدماج بروتينات الشرش في اللبن الفرز إما في صورة بروتينات مدنترة (معقدات بالحرارة مع الكازين) أو بالإضافة المباشرة لبروتينات الشرش المدنترة أو على حالتها الطبيعية باسترجاع اللبن الفرز المجفف في الشرش أو بخلط الشرش مع اللبن الفرز المجفف بنسب مختلفة. وقد أشارت النتائج إلى أن المعاملة الحرارية للبن عند رقم الـ pH الطبيعي (٦,٦) على درجة حرارة ٩٠°م لمدة ٥ ق أدى إلى زيادة زمن التجبن مع ضعف قوة الخثرة الناتجة وانخفاض النيتروجين الفاقد في الشرش ، وقد أدت إضافة كلوريد الكالسيوم بنسبة ٠,٠٢% إلى حدوث تحسن واضح في خواص التجبن. أما بالإضافة المباشرة لبروتينات الشرش المدنترة بنسب تراوحت بين ٠,٠٥ - ٢% فإنها لم تؤثر على زمن التجبن ولكنها أدت إلى انخفاض معدل انفصال الشرش وزيادة النيتروجين المحتجز داخل الخثرة وتناسب ذلك مع زيادة نسبة بروتينات الشرش المضافة سواء المحضرة من شرش غير مملح أو مملح. وقد كانت هناك زيادة ملحوظة في درجة صلابة الخثرة الناتجة من إضافة بروتينات الشرش المحضرة من الشرش غير المملح. هذا وقد أسفرت نتائج إدماج الشرش في اللبن الفرز إلى أن جميع مخاليط اللبن الفرز الغير معاملة حرارياً سواء مع الشرش غير المملح أو الشرش المملح أعطت خثرة جيدة بينما أدت المعاملة الحرارية لهذه المخاليط إلى زيادة زمن التجبن وأعطت خثرة ضعيفة وفي حالة المخلوط (١:١) لم تتكون خثرة ، وبالرغم من حدوث بعض التحسن في خواص الخثرة الناتجة مع إضافة كلوريد الكالسيوم إلا أن مستوى جودة الخثرة ظل أقل من نظيره في المخاليط غير المعاملة حرارياً. استغرق زمن التجبن نحو ١٠ ق عند استرجاع اللبن الفرز المجفف (١٢% - و/ح) في الشرش غير المملح مقابل ٣٠ ق عند الاسترجاع في الشرش المملح وفي كلتا الحالتين كانت الخثرة الناتجة ذات خواص جيدة خاصة عن الاسترجاع في الشرش غير المملح. أدت المعاملة الحرارية للبن الفرز المسترجع سواء في الشرش غير

المملح أو المملح إلى عدم تكون خثرة ، أما المعاملة الحرارية للشرش غير المملح أو المملح قبل الاسترجاع أعطى خثرة متماسكة وكانت كمية النيتروجين المحتجزة داخل الخثرة كبيرة. هذا وقد أدت زيادة نسب اللبن الفرز المجفف إلى ٢٠، ٢٥، ٣٠ % عند الاسترجاع إلى انخفاض زمن التجبن وزيادة كمية النيتروجين المحتجزة داخل الخثرة وكانت الخثرة متماسكة وعند زيادة النسبة إلى ٣٠% كانت الخثرة الناتجة شبيهة بالجبن . وخلصت الدراسة إلى أن استرجاع اللبن الفرز المجفف في الشرش يمكن استخدامه كوسيلة بسيطة وسهلة التطبيق لإعادة استخدام الشرش في صناعة الجبن .