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# INCLUSION OF WHEY PROTEINS IN RENNET CURD MATRIX

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## ABSTRACT

**Whey proteins were retained in rennet curd 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 denatured 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.**

## INTRODUCTION

In traditional cheese-making casein forms the curd structure while whey proteins are almost 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 the functional properties (Banks et al., 1994; de Wit, 1998; Lucey et al., 1999; Mead and Roupas, 2001).

Several attempts were made for incorporation of whey proteins in different cheese varieties in its either 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 soft, semi-hard and hard cheese varieties were reported by Hinrichs (2001).

In the present study, whey proteins were incorporated into rennet curd 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). The effects of these treatments on milk rennet coagulation properties and nitrogen loss in the whey were investigated

## MATERIALS AND METHODS

### Materials:

**Milk samples:**

Whole cow's milk was obtained from the herd of Faculty of Agric., Minia University. The milk was; skimed to eliminate fat interference.

**Skim milk powder:** Low- heat skim milk powder imported from USA was used

**Unsalted whey:** was obtained after Ras cheese manufacture at the Dairy Laboratory, Faculty of Agric., Minia University

**Salted whey:** was obtained after Domiati cheese manufacture at the Dairy Laboratory, Faculty of Agric., Minia University

**Rennet:** 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.

### Analytical Methods:

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

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 was determined by:** the penetration method described by Shalabi (1987)

**Syneresis was determined as:** the volume of whey expelled within 60 min from rennet curd, cut after 2.5 times the rennet coagulation time, as described by Marshall (1982).

## RESULTS AND DISCUSSION

### Heating of Milk:

Results in Table 1 show 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 $CaCl_2$ 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) | 10 | 22.46 | 328 | 164 |
| Skim milk, heated | 32 | 14.26 | 215 | 123 |
| Skim milk, heated then 0.02% $CaCl_2$ added | 15 | 17.02 | 286 | 106 |

- Average of three replicates
- TN: 546 mg/100 ml of milk

A decrease in the firmness of formed rennet gel was apparent because of heat treatment. This was improved by $CaCl_2$ addition. It has been also reported that rennet gel strength was decreased by heat treatment because 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 an increase in water holding capacity. Table 1 also shows a decrease in 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.

**Addition of whey protein particles:**

Results in Table 2 show that direct additions of whey protein particles 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

**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 protein particles % | 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 protein particles 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 + whey proteins particles from salted whey | 0.5 | 9 | 23.13 | 300 | 573 | 184 | 389 (11.14) |
| | 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) |

- Average of three replicates
- Data in parenthesis represent percentage of increase in retained N

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 whole 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 (Schmidit & 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.

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%).

**Recycling of whey:**

**a- Mixing whey with Skim milk:**

Whey either unsalted or salted was mixed with skim milk in the ratios of 1:1 , 1:2 , 1: 3 and 1: 4 ((u/v). The mixes were heated at 90°C for 5 min and then $CaCl_2$ (0.02%) was added.

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. 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% $CaCl_2$, the RCT values were still more than those of the unheated mixes and the formed gel were still far less firm. In addition, more water was retained in the formed gel because of the heat treatment. This was expected and may be due to whey protein increased the water holding capacity (Schmidt and Morris, 1984).

**Table 3: Rennet coagulation properties and nitrogen distribution of skim milk/ unsalted whey mixtures before and after heating**

| 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 treatment at 90°C / 5 min and 0.02% $CaCl_2$ added | 4:1 | 13 | 10.67 | 178 | 553 | 130 | 423 |
| | 3:1 | 16 | 10.03 | 183 | 532 | 137 | 395 |
| | 2:1 | 16 | 8.30 | 174 | 491 | 137 | 354 |
| | 1:1 | 19 | 7.00 | Weak gel | 427 | 123 | 304 |

- Average of three replicates

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. In addition, 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% $CaCl_2$ 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 mixtures before and after heating.**

| 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 |

- Average of three replicates

Obviously, mixing of skim milk with whey would result in a decrease in the TN content of the mixes (Tables 3 & 4). However, the changes for 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.

**b- Reconstitution of skims milk powder in whey**

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 $CaCl_2$ 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 |

average of three replicates

Increasing the level of skim powder up to 30%, 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. In addition, 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 |

- Average of three replicates
- 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.

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# Inclusion of whey proteins in rennet curd

# تضمين بروتينات الشرش فى الخثرة الأنفحية

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**قسم الصناعات الغذائية والألبان - كلية الزراعة ـ 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 الطبيعى (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 الإضافة المباشرة لبروتينات الشرس المدنترة بنسب تراوحت بين ٠,٠٥ ــ 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 سواءا المحضرة من شرش غير مملح أو مملح. وقد كانت هناك زيادة ملحوظة في درجة صلابة الخثرة الناتجة من إضافة بروتينات الشرس المحضرة من الشرس غير المملح. هذا وقد اسفرت نتائج إدماج الشرش فى اللبن الفرز إلى أن جميع مخاليط اللبن الفرز الغير معاملة حراريا سواءا مع الشرش غير المملح أو الشرش المملح أعطت خثرة جيدة بينما أدت المعاملة الحرارية لهذه المخاليط إلى زيادة زمن التجبن وأعطت خثرة ضعيفة وفى 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 حراريا. استغرق زمن التجبن نحو

١٠ق عند استرجاع اللبن الفرز المجفف (١٢% ـ و/ح) في الشر ش غيــر المملــح مقابل ٣٠ ق عند الاسترجاع في الشر ش المملح وفى كلتا الحالتين كانت الخثرة الناتجة ذات خواص جيدة خاصة عن الاسترجاع في الــشر ش غيــر المملــح. أدت المعاملــة الحرارية للبن الفرز المسترجع سواء في الشرش غير المملح أو المملح إلى عدم تكون خثرة ، أما المعاملة الحرارية للشر ش غير المملح أو المملح قبل الاســترجاع أعطــى خثرة متماسكة وكانت كمية النيتروجين المحتجزة داخل الخثرة كبيــرة. هــذا وقــد أدت زيادة نسب اللبن الفرز المجفف إلى ٢٠ ،٢٥ ،٣٠ % عند الاسترجاع إلــى انخفــاض زمن التجبن وزيادة كمية النيتروجين المحتجزة داخل الخثرة وكانت الخثــرة متماســكة وعند زيادة النسبة إلى ٣٠% كانت الخثرة الناتجة شبيهة بالجبن . وخلصت الدراســة إلى أن استرجاع اللبن الفرز المجفف في الشر ش يمكن اســتخدامه كوســيلة بــسيطة وسهلة التطبيق لإعادة استخدام الشر ش في صناعة الجبن .