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SOME PROPERTIES OF CO-PRECIPITATES PREPARED FROM A MIX OF SKIM MILK AND UNSALTED OR SALTED RENNET WHEY

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Abstract: Protein co-precipitates were prepared by mixing skim milk with unsalted or salted whey in different ratios; 4:1, 3:1, 2:1, 1:1, and 1:2 (v/v). The pH of skim milk or the mixes with whey was adjusted to pH 7.5, then heated at 90ºC for 15 min. The protein was precipitated at pH 4.6. Salt (NaCl) was added at different levels (2, 4, 6, and 8%) to unsalted whey before mixing with skim milk. Samples of skim milk and whey were taken as control for comparison. The resultant co-precipitates were assessed for nitrogen distribution and functional properties. Results of N distribution revealed that, the amount of pH 4.6 precipitated N at pH 7.5 after heat decreased with decreasing the ratio of skim milk in the mix, while the amount of SN increased. Co-precipitate isolated from skim milk/unsalted whey mixes contained 10.78 to 27.08% whey protein corresponding to 15.28 to 25.31% in that isolated from skim milk/salted whey mixes depending on the ratio of skim milk in the mix. Functionality testing revealed that water solubility of co-precipitate decreased with decreasing the ratio of skim milk in the mix. The solubility of co-precipitates isolated from skim milk/unsalted whey mixes was relatively less than that of isolated from skim milk/salted whey mixes. Foaming capacity of co-precipitate decreased with the decreasing the skim milk ratio in the mix, particularly, in that prepared from skim milk/unsalted whey mixes. Addition of NaCl at different levels to unsalted whey before mixing with skim milk apparently improved the functionality of the co-precipitates, but the ratio of skim milk in the mix had the potential effect.

Key words: Protein co-precipitates; functional properties; heat treatment; protein recovery

Abbreviations: TN: total nitrogen; SN: soluble nitrogen; CN: casein nitrogen; NCN: non-casein nitrogen; NPN: non-protein nitrogen

Introduction

Whey contains more than half the solids present in the original whole milk including 20% of the proteins and most of lactose , minerals and water soluble vitamins. There has been recognition that the proteins and lactose in whey are valuable nutrients which should not be wasted (Schingothe, 1976; Mann, 1977; Gupta & Thapa, 1991; Boumba *et al,* 2001).

Further, the increase in the use of functional proteins in processed foods and development of new technologies i. e. ultrafiltration, and ion-exchange as an economic recovery of whey proteins are additional factors in encouraging the industrial use of whey (Marshall, 1982; Jelen, 1983; Maubois, 1983; Slack *et al.,* 1986; Philippopoulos & Papodokis, 2001).

The recognition of whey as a source of unique physiological and functional attributes has increased incorporation of whey and whey components into a variety of foods. Whey protein concentrate (WPC) and whey protein isolate (WPI) are high protein, low carbohydrates that are currently in demand due to increased awareness of nutrition and alternative methods for weight control. Dairy products, particularly whey proteins products, contain high concentrations of vitamins and minerals (Micintosh, *et al.,* 1998 and Russell, 2004)

Preliminary studies indicated poor functionality of whey protein powder prepared by acid-heat precipitation from unsalted or salted rennet whey. However, whey protein powder prepared from salted whey had relatively better function properties than that prepared from unsalted whey, but the values were less than those of other whey protein preparations i.e. whey protein concentrate and whey protein isolate (Ateteallah, 2007). However, the techniques used for the preparation of whey products i.e. ultrafiltration, ion-exchange and chemical complex formation are rather expensive. Another alternative for the recovery of whey protein is by co-precipitation with casein by heat treatment of skim milk (Grufferty & Mulvihill, 1987 and Murphy & Mulvihill, 1988). Studies indicated that co–precipitate with good functionality could be obtained by heat treatment of milk at 90ºC for 15 min at alkaline pH (pH 7.5).

The aim of the present study was to find out a simple and easy applicable method for the reuse of whey through preparation of co-precipitates from mixing skim milk with whey either unsalted or salted.

Materials and Methods

Materials:

Milk:

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

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

Experimental design:

In set of experiments skim milk was mixed with unsalted and salted whey in different ratios for preparation of co-precipitate. To study the effect of salt level, NaCl was added at different levels (2, 4, 6, and 8%) to unsalted whey before mixing with skim milk

Preparation of co-precipitate:

The method described by Murphy and Mulvihill (1988) was followed. Rennet whey (salted or unsalted) and skim milk were mixed in different ratios 4:1, 3:1, 2:1, 1:1, and 1:2 (v/v) and the pH was adjusted to 7.5 using 2N NaOH and the mixtures were heated at 90ºC for 15 min in water bath, then cooled to 25ºC. Co-precipitate was obtained by acidification to pH 4.6, filtered through cheese cloth, then air dried for 2-3 days at room temperature.

Determination of solubility:

Solubility of co-precipitate was determined by the nitrogen solubility index procedure following the method of Paulsen *et al.* (1960), modified by Modler and Emnons (1976). Protein Dispersions (1% w/v) were assessed at pH values ranging from 2 to 7 using 0.1 N. HCl or 0.1 N. NaOH, the samples were maintained at 4ºC for 24 h, after which the pH was readjusted. The samples were centrifuged at 3000g for 20 min at room temperature, then filtered through filter paper Whatman No.1 and the protein content of the filtrates was determined by the semi micro-Kjeldahl method as described in the AOAC (1975).

Determination of foaming properties:

Foaming properties were determined using the method described by Mohanty et al. (1988) with some modifications. Sample (2 g) was dispersed in distilled water and pH was adjusted to a pH ranged from 2 to 7 using 2N HCl or NaOH. The final volume of each individual sample was made to 200-ml with distilled water and solutions were equilibrated at room temperature for 5 min. The foaming properties were assessed by whipping the sample in Domestic Food Blender for 5 min at the high speed. The foam was transferred to 500 ml graduated cylinder and the initial volumes of foam and liquid were noted. Foam properties were assessed as: volume expansion (overrun) and foaming stability as the time required for the foam to collapse.

Results and Discussion

Nitrogen recovery from skim milk/ whey mixes heated at alkaline pH:

Nitrogen distribution of skim milk, unsalted, Salted rennet whey and the mix of each whey with skim milk are shown in Tables 1 and 2. The content of casein N decreased with decreasing ratio of skim milk in the mix with unsalted or salted whey. The values as a percentages of total nitrogen in the original solutions decreased from 75.85% and 77.33% without adding whey (skim milk samples) to 52.85% and 55.19% when skim milk ratio decreased to 1 and whey ratio increased to 2 i.e. 1:2 skim milk/unsalted or salted rennet whey mix, respectively. In contrast to casein N content, the percentages of non casein nitrogen (NCN) increased with decreasing the skim milk ratio in the mix with unsalted or salted rennet whey. However, less variations in the NPN content of both mixes were observed. The NCN values increased from 24.15% and 22.67% in skim milk samples to 47.14% and 44.80% in 1:2 mixes of skim milk with unsalted or salted rennet whey, respectively. Murphy and Mulvihill, (1988) reported no changes in NCN with the variation in skim milk ratio with acid whey.

Nitrogen distribution in the heated solutions are also shown in Tables 1 and 2 . The precipitated N (precipitated protein) values at pH 4.6 showed a gradual decrease with decreasing the ratio of skim milk in the heated mixes with either unsalted or salted whey as compared with skim milk samples. However, when these values were calculated as a percentage of total N of the original samples expressed as N recovery (protein recovery), no practical changes were apparent with the decrease in the ratio of skim milk in the mixes with whey either unsalted or salted at 2:1 ratio, indicating good protein recovery with the dilution of skim milk with whey. In skim milk samples, the N recovery (protein recovered) was 85.01% (Table 1) and 91.24 (Table 2). These values being 84.41% (Table 1) and 84.20% (Table 2) with 2:1 ratio of skim milk/unsalted or salted whey, respectively. In 1:1 and 1:2 mixes an apparent decrease in the N recovered was observed. The values were 78.60% and 73.38% in 1:1 and 1:2 mixes of skim milk/unsalted whey (Table 1). The corresponding values with salted whey mixes were 77.28% and 73.90%, respectively, which could be also considered as a reasonable recovery.

The values of protein recovery from skim milk are in agreement with those reported by Grufferty and Mulvihill (1987) for heat treated skim milk at 90ºC for 15 min and at pH 7.5. However, the values of protein (N recovery) from the heated mixes of skim milk and unsalted or salted whey are in agreement with the values reported by Murphy and Mulvihill (1988) for skim milk/ acid whey mixes. In the whey samples the protein recovery by precipitation at pH 4.6 after the heating was 42.85% from unsalted whey (Table 1) and 47.79% from salted whey (Table 2). In both types of heated mixes; skim milk/ unsalted whey and skim milk/ salted whey; there were some of the nitrogen fraction remained soluble (un-recovered) in the pH 4.6 filtrate (pH 4.6 soluble N). These are perhaps un-complexes whey proteins, proteose peptone components and non protein nitrogen. Muller et al., (1967) reported that also some of the caseins remain soluble in the pH 4.6 supernatants. Table 1, shows no substantial changes in the values of pH 4.6 soluble N of heated skim milk mixes with unsalted whey. While for the mixes with salted whey there was a gradual increase in pH 4.6 soluble N with decreasing the ratio of skim milk (Table 2). Meanwhile, in both mixes when the pH 4.6 soluble N values were calculated as a percentage of total N in the original solutions, there was a remarkable increase in these percentages with decreasing the ratio of skim milk in the mix (Tables 1 and 2). Generally, whey samples either salted or unsalted contained a higher percentages of un-precipitated protein compared with skim milk or skim milk / rennet whey mixes. This suggests that casein had a destabilizing influence on whey proteins (Kenkare et al., 1965; Morr & Josephson, 1968 and Murphy & Mulvihill, 1988).

Table (1): Nitrogen distribution* (mg/100 ml) in skim milk, unsalted whey, mixes of both and nitrogen content after heating at 90 ºC for 15 min., at pH 7.5

Ratio of skim milk/ unsalted whey	Original mix				After heating			
	TN	NCN	CN	NPN	SN pH 4.6	NPN	Precipitated N	N recovery %
Skim milk	574.0	138.6 (24.15)	435.4 (75.85)	42.23 (7.34)	75 (13.06)	45.75	488	85.01
4:1	518.0	129.5 (25.00)	388.5 (75.00)	42.17 (8.14)	72 (13.90)	43.25	446	86.10
3:1	490.0	127.4 (26.00)	368.6 (74.00)	48.61 (9.92)	68 (13.88)	43.25	422	86.12
2:1	462.0	127.6 (27.49)	334.4 (72.38)	47.22 (10.22)	72 (15.59)	43.50	390	84.41
1:1	327.0	122.5 (37.46)	204.5 (62.53)	40.22 (12.30)	70 (21.4)	43.00	257	78.60
1:2	263.0	124.0 (47.14)	139.0 (52.85)	42.97 (16.34)	70 (26.62)	42.00	193	73.38
Whey	122.5	122.0	0	37.62 (30.71)	70 (57.14)	40.00	52.5	42.85

*average of three replicates

- Data in parentheses as a percentage of TN.

Table (2): Nitrogen distribution* (mg/100 ml) in skim milk, salted whey, mixes of both and nitrogen content after heating at 90 ºC for 15 min., at pH 7.5

Ratio of skim milk/ salted whey	Original mix				After heating			
	TN	NCN	CN	NPN	SN pH 4.6	NPN	Precipitated N	N recovery %
Skim milk	525.00	119.00 (22.67)	406.00 (77.33)	42.00 (8.00)	46.0 (8.76)	33.75	479.00	91.24
4:1	442.75	122.50 (27.67)	320.25 (72.33)	36.75 (8.30)	57.2 (12.91)	39.00	385.55	87.09
3:1	420.00	115.50 (27.50)	403.50 (72.50)	36.75 (8.75)	53.0 (12.62)	39.00	367.00	87.38
2:1	379.75	115.00 (30.28)	264.75 (69.72)	36.72 (9.67)	60.0 (15.80)	38.00	319.75	84.20
1:1	301.00	112.00 (37.21)	189.00 (62.72)	32.00 (10.63)	68.4 (22.72)	42.25	232.60	77.28
1:2	256.67	115.00 (44.80)	141.67 (55.19)	33.25 (19.95)	67.0 (26.10)	42.25	189.67	73.90
Whey	117.60	117.60	0	36.14 (30.71)	61.4 (52.21)	35.50	56.20	47.79

*average of three replicates

- Data in parentheses as a percentage of TN.

Whey protein content:

The whey protein content of the pH 4.6 precipitated complex was calculated based on the initial whey protein content in the original solutions (NCN-NPN) and the whey protein content in the supernatant after heat treatment (pH 4.6 SN-NPN), the calculated values are presented in Table 3. The data show that about 64.54% to 69.65% of the whey proteins were precipitated with casein (complex with casein) in the case of the mixes of skim milk/unsalted whey, depending on the ratio of skim milk in the mix. In the mixes of skim milk/ salted whey, the precipitated whey proteins ranged from 67.31 to 83.44% of the original whey proteins, depending on the ratio of skim milk in the mix. Assuming that all of casein was precipitated by acidification to pH 4.6 before and after heating, so the differences theoretically represents whey protein in the co-precipitate. Therefore, co-precipitate isolated from skim milk/unsalted whey mixes contained 10.78% to 27.98% whey protein, and that isolated from skim milk/salted whey mixes contained 15.28% to 25.31% whey proteins, depending in the ratio of skim milk in the mix. Murphy and Mulvihll (1988) showed some differences between experimentally determined whey protein content in the co-precipitate and the theoretically calculated.

Functional properties of co-precipitates:

Solubility:

Water solubility index at pH 7.0 of co-precipitates prepared from heat treated (90ºC for 15 min, at pH 7.5) skim milk and skim milk/rennet whey (unsalted or salted) mixes are shown in Table 4. Whey samples, unsalted or salted, were also heated (90ºC for 15 min) at alkaline pH (7.5) and the whey proteins were precipitated at pH 4.6, for comparison. Generally, there was a decrease in the solubility of the co-precipitates with decreasing the skim milk ratio in the mix. Co-precipitate solubility dropped from 67.17% in that prepared from heated skim milk to 51.33% in that prepared from heated 4:1 skim milk/unsalted whey mix, and from 70.23% to 55.56% in the corresponding salted whey mix. A further drop in the solubility of the co-precipitate, but to a lesser extent was observed with 3:1, 2:1, 1:1 and 1:2 skim milk/ whey mixes either unsalted or salted. The solubility of the protein isolated from heat treated whey (unsalted or salted) was very low (13.89% and 17.81%, respectively). This suggests that increasing whey proteins content in the co-precipitate decreased the solubility (Fig.1). Murphy and Mulvihill (1988) observed similar trend with skim milk/ acid whey mixes. However, the obtained solubility values were lower than those reported by the mentioned authors, perhaps due to differences in the method used for the measurement of solubility.

Foaming properties:

Foaming capacity and foaming stability of the obtained co-precipitates are also shown in Table 4. The foaming capacity of co-precipitates decreased from 160% in that obtained from skim milk to 138% in co-precipitate of 4:1 mix with unsalted whey. A similar decrease was recorded thereafter in co-precipitates obtained by 3:1 (134%) and 2:1 (124%) mixes. It decreased further in co-precipitates of 1:1 (119%) and 2:1 (116%) mixes; these values were more or less close to protein isolated from heated unsalted whey (115%). However, the foaming capacity of co-precipitates prepared from skim milk/salted whey mixes was remained unchanged up to 2:1 dilution with salted whey (168%-162%). A slight decrease was observed with co-precipitates from 1:1 (158%) and 1:2 (142%) skim milk/ salted whey mixes. It appears, therefore, that the presence of salt in the whey counteracted the weak effect of complexed whey proteins. The formed foam was stable up to 60 min (time for the foam to collapse) with co-precipitates prepared from heated skim milk, 4:1, 3:1, and 2:1 skim milk/ unsalted whey mixes. A drop to 24 and 22 min was observed with co-precipitates from

1:1 and 1:2 skim milk/ unsalted heat treated mixes, respectively. On the other hand, the foaming stability of co-precipitates obtained from heated skim milk/salted whey mixes were decreased as the ratio of skim milk decrease in the mix. The decrease was not great up to 2:1 mix, then followed by a sharp drop thereafter. The foam collapsed after 10 min with 1:1 and 1:2 mixes, which is more or less correspond to the values obtained with protein isolated from heated whey either unsalted or salted (Table 4).

Table (4): Solubility and foaming properties* at 20ºC, and pH 7.0 of protein isolated from skim milk, whey, and mixes of both after heating at 90ºC, at pH 7.5

Ratio of skim milk/ whey	Unsalted whey			Salted whey		
	Solubility (%)	Foaming capacity (%)	Foaming stability (min)	Solubility (%)	Foaming capacity (%)	Foaming stability (min)
Skim milk	67.17	160	60	70.23	168	60
4:1	51.33	138	60	55.56	166	60
3:1	50.98	134	60	54.72	164	40
2:1	50.18	124	60	53.23	162	50
1:1	49.70	119	24	50.17	158	10
1:2	45.72	116	22	47.41	142	10
Whey	13.89	115	10	17.81	118	10

*average of three replicates.

Effect of salt level:

Nitrogen recovery:

Results in Table 5 show that the variation in the levels of NaCl in the whey before mixing with skim milk had no apparent effect on the N content of the mixes either before or after the heating. There was a constant level of NCN and NPN in each of the mixes made. These mixes were adjusted to pH 7.5, heated at 90ºC for 15 min and precipitated by acidification to pH 4.6 . There was also a constant concentration of uncomplexed proteins remaining in the pH 4.6 filtrate (pH 4.6 soluble N). The values of pH 4.6 precipitated N as a percentages of TN remained unchanged for each mix with the different levels of salt. However, as it was expected, the protein recovery increased with increasing the ratio of skim milk in the mix regardless the salt level.

Table (5): Nitrogen content* (mg/100 ml) of skim milk, salted rennet whey, mixes of both and pH 4.6 filtrate and precipitate after heating at 90ºC for 15 min, at pH 7.5

Ratio of rennet whey/ skim milk	Salt level in added whey	TN	NCN	CN	NPN	After heating	
						pH 4.6 soluble N of %TN	N recovery%
1:1	0	274	126	148	49	21.40	78.60
	2	274	126	148	49	21.94	78.06
	4	274	126	148	49	20.41	79.59
	6	274	126	148	49	22.96	77.04
	8	274	126	148	49	23.47	76.53
1:3	0	581	143	438	42	13.88	86.12
	2	581	143	438	42	13.25	86.75
	4	581	143	438	42	14.00	86.00
	6	581	143	438	42	13.25	86.75
	8	581	143	438	42	13.25	86.75
1:4	0	591	157	434	37	13.90	86.10
	2	591	157	434	37	10.66	89.34
	4	591	157	434	37	10.00	90.00
	6	591	157	434	37	10.66	89.34
	8	591	157	434	37	10.66	89.34
Skim milk	-	703	143	660	37	9.96	90.04
Whey	-	176	176	0	43	33.82	68.18

*average of three replicates

- N recovery % = TN – pH 4.6 SN/ TN

Functional Properties:

Water solubility index and foaming properties of protein isolates (co-precipitates) at pH 7.0, and 20ºC are illustrated in Figs. 1 and 2. The results clearly demonstrated that the presence of salt (NaCl) generally improved both the solubility and foaming properties of the prepared co-precipitates. The improvement effect varied with the increase in the salt level in the whey used for preparation of co-precipitates, and was quite noticeable with whey containing 8% salt. The observed variations between co-precipitates prepared from different mixes of whey/skim milk regardless the salt level, were expected as the properties of co-precipitates depend to a great extent on the ratio of skim milk in the mix.

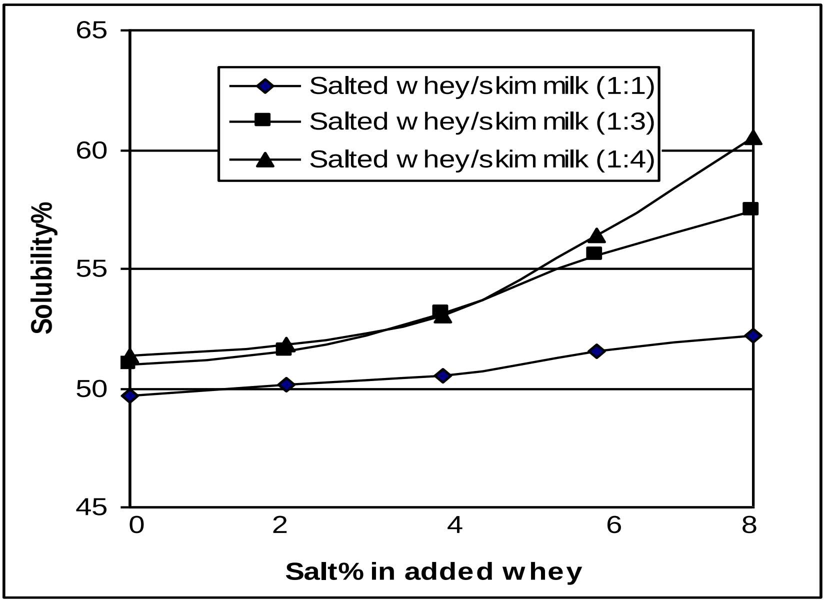

Fig. (1): Effect of salt level in added whey to skim milk on solubility of co-precipitates

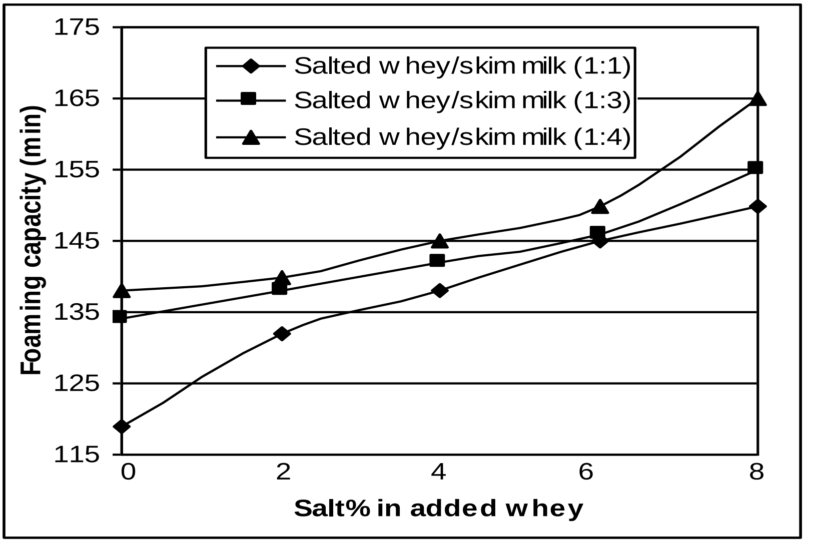

Fig. (2): Effect of salt level in added whey to skim milk on foaming capacity of co-precipitates

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خواص المترسبات المترافقة المحضرة من مخلوط اللبن الفرز مع الشرش غير المملح أو الشرش المملح

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تم تحضير المترسبات المترافقة بخلط اللبن الفرز مع الشرش غير المملح أو المملح بنسب 4: 1 ، 3: 1 ، 2: 1، 1: 1 ، 1: 2 (ح/ح) بالإضافة إلى عينة لبن فرز وعينة شرش ثم ضبط رقم الـ pH إلى 7,5 والتسخين على درجة حرارة 90ºم لمدة 15 ق ثم التبريد وضبط الـ pH إلى 4,6 . حيث أشارت نتائج توزيع النيتروجين إلى أن كمية النيتروجين الراسب عند pH 4,6 بعد التسخين على درجة حرارة 90ºم لمدة 15 ق على pH 7,5 قد انخفضت بانخفاض نسبة اللبن الفرز في المخلوط بينما ازدادت كمية النيتروجين الذائب . وقد سجل اللبن الفرز أعلى قيم للنيتروجين الراسب بينما أعطى الشر ش سوءا غير المملح أو المملح أدنى قيم. وقد احتوى المترسب المترافق المحضر من مخلوط اللبن الفرز مع الشر ش غير المملح على 10,78 - 27,08 % بروتينات شرش مقابل 15,28 - 25,31 % في نظيره المحضر من مخلوط اللبن الفرز مع الشرش المملح. هذا وقد أظهرت نتائج اختبارات الخواص الوظيفية للمترسبات المترافقة إلى انخفاض كل من القابلية للذوبان في الماء والقدرة على تكوين الرغوة وذلك بانخفاض نسبة اللبن الفرز في المخلوط وكانت القابلية للذوبان والقدرة على تكوين الرغوة في المترسب المترافق المحضر من مخلوط اللبن الفرز مع الشرش غير المملح اقل نسبيا من نظيرها في المترسب المترافق المحضر من مخلوط اللبن الفرز مع الشرش المملح . كما أظهرت النتائج أن إضافة كلوريد الصوديوم بمستويات 2، 4، 6، 8 % إلى الشرش غير المملح قبل الخلط مع اللبن الفرز قد أدى إلى تحسين الخواص الوظيفية للمترسب المترافق الناتج إلا أن نسبة اللبن الفرز في المخلوط كانت هي الأكثر تأثيرا .