THE EFFECT OF ADDING ANHYDROUS ACETIC ACID TO PREPARE WHEY PROTEIN CONCENTRATES TO IMPROVE ITS FUNCTIONAL PROPERTIES.

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ABSTRACT

Two types of whey protein concentrates were prepared, One was from sour whey and the other from salted or unsalted sweet whey. They were acetylated by different concentrations and their effectiveness properties were studied. It was found that the sour whey(unsalted and acetylated) exceeded its counterpart of treatments in the percentage of moisture and ash. However, the salted and unsalted sweet whey(acetylated) had significant superiority over other treatments, in the following properties; total protein, foam size, gel formation, oil and water absorption, and the emulsification properties. Thus, the results showed that the 0.5٪ of acetylation for salted and unsalted sweet and sour whey proteins was the best in changing the functional properties of the acetylated whey protein concentrates compared to the acetylation percentage 0.3٪ and 0.9٪, respectively. In addition, the salt effect in most treatments has shown a significant effect. So, the above results showed that salted sour and sweet whey protein are the best in the process of acetylation because of its improvement of functional and chemical properties.

Key words: whey protein concentrates, acetylation, functional properties, Anhydrous Acetic Acid .

INTRODUCTION

Whey is a greenish yellow liquid resulting from the manufacturing of cheese or casein by adding rennet enzyme or one of organic acids such as lactic acid, citric acid, or acetic acid on by salting out. Whey contains high percentages of organic components such as whey proteins (alpha-lactalbumin and beta-lactoglobulin), albumin serum, protease peptone and immunoglobulin, it also contains lactose, soluble vitamins, salts, and organic ions. Whey is considered as one of cheese factories by-products, in which large quantities of whey were thrown away with sewerage water deranges which will deteriorate social health, contaminate the whole environment and cause harmful to the national economy. The nutritional and functional characteristics of whey proteins are related to the vital constructive function of these proteins(De Witt,1997). The functional properties of whey proteins are specified by previous treatments of whey such as denaturation of proteins and percentage of

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non-protein material. This leads to modulate whey proteins which causes changes in some of its properties and leads to an increase in its uses and opens a wide field for its nutritional application aspects. The basic aim of modulating proteins is to improving its nutritional value, and preventing the damaging reactions such as Millard reactions or the change of tissue formation case(Sun and Gunasekaran,2010). Johnson and Brekke (1983) mentioned that acetylating as the modulation or chemical transfer of amino groups in the lysine amino acid by the anhydrous acetic acid. This causes the exclusion of positive charges in the amino group in the sixth carbon atom in lysine which leads an increases in the negative charge of the whole protein. Thus, occurring of a covalent binding which neutralized the acetates with the amino groups of the protein that causes the lake of involution of protein molecule as results of reduction in the electrostatic overlaps between amino acids of different charges. Therefore, the protein solubility increases and the electrical equivalence point and gel power decreases by heating .When the solubility of protein improves, the water molecules penetration for the protein is facilitated. The characteristic of emulsifying and foam formation depend on the solubility of the protein. acetylation with anhydrous acetic acid for the whey protein concentrations increased the capacity of water absorption and improve thermal stability(Creamer,1994). Thus, this study aimed to conducting a chemical modulation of whey protein concentrates that was prepared by different methods and leads to changing of its capacity properties through acetylating and the studying of the effect of these changes on the active groups of amino acids for the whey proteins and their effectiveness on its capacity properties .

MATERIALS AND METHODS

1) **Whey samples** : Whey samples were obtained from cheese which was made from cow's milk in Tikrit University dairy plant. Salah Aldeen governorate, Republic of Iraq. The whey samples consist of :
   a) Whey samples resulting from fresh cheese made by rennet (Sweet whey protein concentrate) .
   b) Whey samples resulting from sour cheese made by acidified milk–Acid Whey Protein Concentrate .
2) **Whey Protein Concentrates**: Whey proteins and its concentrates were prepared according to Ali(2007) with slight modification. The pH of whey samples obtained from the first treatment(a)were adjusted to 4.6 using hydrochloric acid of 2 moles then heated to ambient temperature of 90C$^{\circ}$ for 20 minutes in water bath, the resulting precipitate was cooled to room temperature of 20C$^{\circ}$ then filtrated through butter-muslin cloth, and the residual was washed with warm distilled water for few minutes. The precipitates were placed in an oven at 40C$^{\circ}$temperature until drying, then grinded and placed in glass container.

The sour whey precipitates which were prepared as in (b) treatment was removed by centrifugal force of 11600g for 20 minutes. The precipitates were dried and kept in clean and sterilized glass container.

3) **Changing the activity of whey protein concentrates properties**: The following chemical methods were used to modulating whey proteins to form the derivatives for the secondary groups through changing hydrogen bonds or the hydrophobic forces. This depends on the properties of the chemical compound that was used and the reaction conditions. In general, the modification that was used in this study was depended on the formation of subside amino group derivatives products through using the acetylation process. In this procedure(Kebary et al.1993) the suspension of sour and sweet whey protein concentrates were prepared with 2.5%(w/v)in distilled water at room temperature of about 25C°. The hydrogen exponent(pH) was adjusted to 7.5 using sodium hydroxide 2N. Anhydrous acetic acid were added to the suspension at concentrations of (0.3 , 0.5 and 0.9)gm of acetic acid/gm of protein. The suspension was kept for one hour, and the final pH was adjusted to 7.5. A dialysis of the samples with distilled water was conducted at room temperature over 24 hours period of time using a dialysis bag type(*Medical Dialysis Bag, Hi Media, INDIA*) with change of water each 6 hours . The Samples were dried in the oven at ambient temperature of 40C° and kept until usage .
4) **Chemical Analyses**: The moisture, ash and total protein (6.38 x Total N.) of whey protein concentrates were estimated according to AOAC(2003).
5) **Functional properties of whey protein concentrates** :
    - **a- Gelatination**: The method clarified by Ju and Kilara(1998) which was done by taking 3.2 gm, of acetylated whey protein concentrates and adding to it (26.6) ml of distilled water and 3.3 ml of calcium chloride (1 mol). The sample was left for 15 minutes and heated at 80C° for 30 minutes in water bath. Samples were then cooled in ice flack for 15 minutes and kept at 4C° for 24 hours . The gel force was measured by a penetrometer of the type Humboldt MFG-250 of American origin. The penetrometer is a digitally device which explains the material solidness, and it is an indirect measurements the penetration depth (mm) in the sample with a duration of 5 seconds according to the method mentioned in AOAC(1990) .
    - **b- Foams**: The method of Phillips et al. (1990) was used to estimate the of foaming size and its stability of whey proteins , 2.3 gm of whey proteins was added to 35 ml of distilled water and heated to 60C° for a period of 15 minutes. The suspension is then stirred for a period of 15 minutes using an electrical laboratory stirrer. The mixture was then transferred to graded cylinder of 100 ml capacity and the size of the foaming was rerecorded. As for the foaming stabilize, the size of

foaming was measured after 10, 20 and 30 minutes. The foaming size and stability was estimated at pH of 5.0, 7.0, and 8.0 .

c- **Emulsifying capacity**: The emulsifying capacity was estimated according to the method clarified by Dipak and Kumar(1986) by taking 1 ml of whey protein concentrates resolved in 100 ml of distilled water and the pH was adjusted to 5.0, 7.0 and 8.0 using 0.1N hydrochloric acid and sodium hydroxide for each sample . Then, the sunflower oil was added at a steady state with stirring manually until the viscosity is increased. This is calculated on the basis of emulsifying oil milliliters with(100) ml of protein solution. In order to calculate the emulsifying stability the emulsion is left for a period of 1, 24, 42 and 72 hours according to the emulsion stability on the basis of remaining emulsion oil quantity(ml/gm protein).

6) **Water/Oil Absorption Capacity** : The method described by Lin and Humbert (1974) was followed by taking 10 ml of each water and oil and adding 1 gm of acetylated whey protein concentrates. This was continuously stirred for a period of 1 minutes with the adjusting the pH to 5 by using by hydrochloric acid 1N. The solution was placed in the tube of the centrifuge devise and left for (30) minutes at room temperature and the centrifuge was conducted at 3500 g for a period of (30) minutes. The separated liquid size is recorded using a graded cylinder of 10 ml capacity . The free liquid size and residual size in each of the water and oil that were absorbed by the sample were measured.

7) **Statistical Analysis**: The data were analyzed according to the practical experiments system using ANOVA as mentioned by Alrawi and Khalaf Allah(1980. The means were tested according to the Duncan's multiple range test under the level ($P \leq 0.05$). The program SAS(2002) was used to conduct the statistical analysis of the data .

RESULTS AND DISCUSSION

Table(1) revealed that the moisture percentage was significantly high in the unsalted sour acetylated whey protein concentrates with 7.04٪. This percentage differed slightly from salted sour acetylated whey protein concentrates, whereas its percentage decreased in the two types of sweet whey. It was noticed that using the whey protein acetylated led to an increase in the water absorption property as a results of high dissociation in the protein molecule and subsequently permits the water molecules passing to it. In addition to that, the water absorption capacity and the protein solubility increased by a sour hydrogen exponent and this results were agreed with what was mentioned by Jandal et al.(2012), Liu and Hung (1998) and Kebary et al.(1993). However, the effect of protein and salt interaction, showed that there is a big effect on moisture content because of the high ability of the two types of acetylated whey protein to retain water .

Table 1. *Moisture percentage of two types of Acetylated Whey protein Concentrates**

Types of Protein concentrates	Salted		Unsalted		Protein effect	
	Range	Average± S.E.	Range	Average± S.E.	Range	Average± S.E.
Sweet Whey	6.20-5.65	5.86 ± 0.21 c	5.57-5.30	5.44 ± 0.13 d	6.20-5.30	4.65 ± 0.13 b
Sour Whey	6.70-6.02	6.34 ± 0.35 b	7.32-6.78	7.04 ± 0.27 a	7.32-6.02	6.69 ± 0.19 a
Saltiness Effect	6.70-5.65	6.10 ± 0.15 a	7.32-5.30	6.24 ± 0.37 a		

Numbers having similar letter within the same column are not significantly different at P ≤ 0.05. Average are three replicates.

Table(2) showed that the whey protein acetylation has led to an increase in the percentage of ash. The ash percentage in the unsalted sour acetylated whey protein concentrates was increased significantly by 6.84٪ whereas this percentage in the unsalted sweet acetylated whey protein concentrates was the least percentage 3.74٪. Our results were agreed with the results found by Jandal et al.(2012),Yusuf *et al.*(1997) and Araji (2007). The increase of ash percentage in unsalted sour whey in this transferring is due to part of the glutinous calcium salts into the ionic form which increases the ash percentage and this is in agreement with Fox (2001). Also the effect of salt and protein was overlapped It is noticed that it have a big effect on ash as a results of transference to the ionic form by the whey proteins and especially the salted sour ones.

Table 2. *Ash percentage of two types of Acetylated Whey protein Concentrates.*

Types of Protein concentrates	Salted		Unsalted		Protein effect	
	Range	Average± S.E.	Range	Average± S.E.	Range	Average± S.E.
Sweet Whey	4.90-4.35	4.56 ± 0.29 c	3.87-3.60	3.74 ± 0.13 d	4.90-3.60	4.15 ± 0.20 b
Sour Whey	6.40-5.72	6.04 ± 0.35 b	7.12-6.58	6.84 ± 0.27 a	7.12-5.72	6.44 ± 0.21 a
Saltiness Effect	6.40-4.35	5.29 ± 0.35 a	7.12-3.60	5.29 ± 0.69 a		

*Numbers having similar letter within the same column are not significantly different at P ≤ 0.05. * Average are three replicates.

Table(3) illustrates the total protein percentage of acetylated whey protein concentrates. It is noticed that the addition of 0.5 percentage gave the highest average for salted and unsalted sweet whey (٪ 14.19 and ٪ 13.93) in accordance with its counterparts of treatments. The acetylation process increases the protein content in the whey protein concentrates (Onwulata and Huth, 2008). Concerning of the unsalted sour whey, the counterpart treatment has given the least average of 12.13 percentage among all treatments of this property . It has been noticed that the overlap of the salting process effect has exceeded the un salting in the acetylated whey proteins and the precipitation of protein by the salting gratificant ion process and this causes dehydration. Our results were in agreement with Jandal *et al.*(2012), Khader *et al.*(2001) and

Zedan *et al.*(2001) in which the total protein percentage increased after acetylation and disagree with Ali (2007).

Table 3. *Effect of Acetylation Treatments on Total Protein for WPC .*

Type of protein concentrate	Added percentage of Anhydrous Acetic Acid ٪	Salted		Un salted		Effect of overlap between protein and added percentage	
		Range	Average ± S. E.	Range	Average ± S. E.	Range	Average ± S. E.
Sweet Whey	Control	13.40-12.90	13.13± 0.15b	13.20-12.90	13.03± 0.09bc	13.40-12.90	13.08± 0.08b
	0.3	14.36-13.78	14.01± 0.18a	14.50-13.44	13.88± 0.32a	14.95-13.44	13.95± 0.17a
	0.5	14.43-13.88	14.19± 0.16a	14.55-13.47	13.93± 0.32a	14.55-13.47	14.06± 0.17a
	0.9	14.31-13.81	14.08± 0.15a	14.50-13.42	13.83± 0.34a	14.50-13.42	13.95± 0.17a
Sour Whey	Control	12.37-12.15	12.25± 0.07d	12.30-12.00	12.13± 0.09d	12.37-12.00	12.19± 0.06d
	0.3	12.87-12.30	12.58± 0.17bd	12.77-12.23	12.49± 0.16cd	12.87-12.23	12.53± 0.10cd
	0.5	12.94-12.33	12.63± 0.18bd	12.85-12.24	12.53± 0.18a	12.94-12.84	12.58± 0.11c
	0.9	12.57-12.11	12.37± 0.14d	12.59-12.21	12.41± 0.11d	12.59-12.11	12.39± 0.08cd
Effect of overlap between added percentage and Saltiness	Control	12.40-12.15	12.69± 0.21a	13.20-12.00	12.58± 0.21a		
	0.3	14.36-12.30	13.30± 0.34a	14.50-12.23	13.18± 0.35a		
	0.5	14.43-12.33	13.41± 0.37a	14.55-14.24	13.23± 0.35a		
	0.9	14.31-12.11	13.22± 0.39a	14.50-14.21	13.12± 0.36a		
Effect of overlap between Protein and Saltiness	Type of protein concentrate	Sweet Whey		Sour Whey			
		Range	Average ± S. E.	Range	Average ± S. E.		
	Salted	14.43-12.90	13.85± 0.14a	14.55-12.90	13.67± 0.17a		
	Unsalted	12.94-12.11	12.46± 0.08b	12.85-12.00	12.39± 0.08b		

*Numbers having similar letter within the same column are not significantly different at $P \leq 0.05$. * Average are three replicates.

Table(4) shows the effect of acetylation treatments on the size of foaming in the whey protein concentrates. It has been noticed that the adding percentages of 0.3٪and 0.5٪ for salted and unsalted sweet whey have been exceeded the other treatments of this property, and the highest average was reached 20.02 for the percentage (0.5٪) for salted whey protein, and followed by the treatment of the same percentage for unsalted protein .

Also, for the treatment of unsalted sour whey proteins to the corresponding sample, it had given the least average among its treatment counterparts in which it reached 8.97. These results were in agreement with results of Jandal *et al.*(2012), Ali(1997), Araji (2007) and Kebary *et al.*(2001).

As for the overlap between protein effect and salt effect, the treatment of 0.5٪ has exceeded in all overlaps, and the salt effect also gave significant differences compared with unsalty.

Table 4. *Effect of Acetylation Treatments on Foaming size property(ml/gm protein) for WPC.*

Type of protein concentrate	Added percentage of Anhydrous Acetic Acid ٪	Salted		Un salted		Effect of overlap between protein and Addition percentage	
		Range	Average ± S. E.	Range	Average ± S. E.	Range	Average ± S. E.
Sweet Whey	Control	12.30-11.80	12.03± 0.15f	11.20-11.00	11.10± 0.06g	12.30-11.00	11.57± 0.22f
	0.3	21.80-19.00	20.81± 0.91a	20.75-19.70	20.25± 0.30a	21.80-19.00	20.53± 0.45a
	0.5	22.00-20.50	21.02± 0.49a	21.05-20.90	21.00± 0.50a	22.00-20.50	21.01± 0.22a
	0.9	19.20-18.70	18.93± 0.15b	18.30-17.70	18.03± 0.18b	19.20-17.70	18.48± 0.23b
Sour Whey	Control	9.44-9.18	9.31± 0.08h	9.01-8.90	8.97± 0.04h	9.44-8.90	9.14± 0.08g
	0.3	16.66-16.00	16.30± 0.17c	16.20-15.70	16.00± 0.15d	16.60-15.70	16.15± 0.12d
	0.5	18.30-17.65	18.00± 0.19b	17.60-16.25	17.00± 0.31c	18.30-16.25	17.50± 0.21c
	0.9	15.90-15.10	15.50± 0.23d	14.18-13.75	14.00± 0.13e	15.90-13.75	14.75± 0.36e
Effect of overlap between added percentage and Saltiness	Control	12.30-9.18	10.67± 0.61c	11.20-8.90	10.04± 0.48c		
	0.3	21.80-16.00	18.50± 1.09ab	20.75-15.70	18.13± 0.96ab		
	0.5	22.00-17.65	19.51± 0.72a	21.05-16.25	19.00± 0.91a		
	0.9	19.20-15.10	17.22± 0.78ab	18.30-13.75	16.02± 0.91b		
Effect of overlap between Protein and Saltiness	Type of protein concentrate	Sweet Whey		Sour Whey			
		Range	Average ± S. E.	Range	Average ± S. E.		
	Salted	22.00-11.80	18.20± 1.12a	11.00-21.05	17.60± 1.18ab		
	Unsalted	18.30-9.18	14.78± 0.99bc	17.60-8.90	13.99± 0.94c		

*Numbers having similar letter within the same column are not significantly different at $P \leq 0.05$. * Average are three replicates.

The gel force is considered one of the important properties used in many nutritional products. The results showed that the fact of the acetylation of whey protein concentrates has led to a slight significant increase Table(5). Thus, the comparison sample in unsalted sour whey proteins gave the highest average which (30.22 mm), therefore, exceeded all other treatments, and was reduced in the addition percentage 0.5٪ for salted sweet whey with an average 9.63mm. The comparison sample also exceeded by giving in the highest average of 27.09mm because the best gel was given by the protein was at the sour

conditions after protein precipitation. It had been noticed that adding calcium chloride to whey protein concentrates increased gel force (Schmidt *et al.*, 1984), whereas it caused reduction in gel force and that was noticed in sweet whey proteins gained from cheese by rennet (Brandenberg *et al*., 1993).

Table 5. *Effect of acetylation treatments on gelatination property (mm) for WPC.*

Type of protein concentrate	Added percentage of Anhydrous Acetic Acid ٪	Salted		Un salted		Effect of overlap between protein and Addition percentage	
		Range	Average ± S. E.	Range	Average ± S. E.	Range	Average ± S. E.
Sweet Whey	Control	17.10-16.80	16.97± 0.09e	20.10-20.00	20.03± 0.03 d	20.10-16.80	18.50± 0.69 c
	0.3	12.00-8.00	10.00± 1.16 i	13.00-10.00	11.33± 0.88 h	13.00-8.00	10.67± 0.72 f
	0.5	10.30-9.00	9.63± 0.38 i	12.13-11.20	11.68± 0.27 h	12.13-9.00	10.66± 0.50 f
	0.9	12.20-11.40	11.77± 0.23 h	14.70-12.60	13.53± 0.62 g	14.70-11.40	12.65± 0.49 e
Sour Whey	Control	27.19-26.97	27.09± 0.06 b	30.35-30.00	30.22± 0.11 a	30.35-26.97	28.65± 0.70 a
	0.3	16.10-15.30	15.72± 0.23 e	19.20-18.30	18.76± 0.26 d	19.20-15.30	17.24± 0.61cd
	0.5	15.30-14.50	14.97± 0.24 f	17.20-16.40	16.80± 0.23 e	17.20-14.50	±0.44 18.88d
	0.9	20.30-19.70	20.03± 0.18 d	21.10-22.30	21.80± 0.36 c	22.30-19.70	20.92± 0.43 b
Effect of overlap between added percentage and Saltiness	Control	27.19-16.80	22.03± 2.26 ab	30.35-20.00	25.13± 2.28 a		
	0.3	16.10-8.00	12.86± 1.38 c	19.20-10.00	15.05± 1.71 c		
	0.5	15.30-9.00	12.30± 1.21 c	17.20-11.20	14.24± 1.16 c		
	0.9	20.30-11.40	15.90± 1.85 c	22.30-12.60	17.67± 1.88 bc		
Effect of overlap between Protein and Saltiness	Type of protein concentrate	Sweet Whey		Sour Whey			
		Range	Average ± S. E.	Range	Average ± S. E.		
	Salted	17.10-8.00	12.09± 0.92 b	20.10-10.00	14.14± 1.08 b		
	Unsalted	27.19-14.50	19.45± 1.45 a	30.35-16.40	21.89 ± 1.55 a		

*Numbers having similar letter within the same column are not significantly different at $P \leq 0.05$. * Average are three replicates.

Table(6) clarifies that acetylation treatments on the whey protein concentrates, have the ability to absorbed oil. It has been revealed that acetylation treatment for salted whey protein concentrates led to significant increase in the ability to absorbed oil in to the concentrates. These values of absorbed oil of un-acetylated samples were 2.20, 2.14, 1.45 and 1.38 ml./gm protein, respectively. These values became higher after acetylation of salted sweet whey protein concentrates as 2.84, 3.24 and 2.63 ml./gm protein,

respectively. The acetylation effect according to the same percentages of unsalted sweet whey protein concentrates in the property of oil absorption led to significant increase as compared to the untreated sample but more significant than salted sweet whey proteins. The effect of acetylation on oil absorption of salted and unsalted sour whey protein concentrates, was an increase in oil absorption property of concentrates compared to the untreated sample with less percentage compared to salted and unsalted sweet whey protein concentrates. From these rustles it has been noticed that acetylation of whey protein concentrates with a percentage of 0.5٪ has significantly exceeded all treatments in the oil absorption property .The oil absorption property for unsalted sweet whey protein concentrates exceeded all treatments .

These results were in agreement with Jandal et al. (2012), Kebary *et al*. (1993), Liu and Hung (1998) and Lupano *et al*. (1996). Theses authors have been proved that the cause of increasing of oil absorption value that using acetylated whey proteins was the reason behind bigger dissociation in the protein molecule and consequently to an increase to water or oil molecule passage to the inside of molecule which led to increases its ability to absorb water or oil. The results showed the overlapping between protein and saltiness have revealed a significant superiority of salted sweet whey proteins which differed from the rest of treatments except salted sour whey proteins. The percentage of salted sweet whey proteins was higher in value and reached 2.73٪ . However, the least value was for unsalted sour whey protein(2.02٪) which did not differ from the sweet whey proteins treatment. The salted sweet whey proteins significantly exceeded the unsalted whey proteins in the overlap of saltiness effect by2.41٪ and 2.33٪, respectively. The unsalted sweet whey proteins were significantly exceeded by 0.5 percentage, hence, it reached 3.34٪ over other whey proteins in the tribal –overlapping which did not differ from the salted sweet whey proteins .

Table 6. *Effect of acetylation treatments on the ability to Absorbed oil property (ml/gm protein) for WPC .*

Type of protein concentrate	Added percentage of Anhydrous Acetic Acid ٪	Salted		Un salted		Effect of overlap between protein and Addition percentage	
		Range	Average ± S. E.	Range	Average ± S. E.	Range	Average ± S. E.
Sweet Whey	Control	2.35-2.05	2.20±0.09 e	2.16-2.10	2.14± 0.02 e	2.35-2.05	2.17± 0.04 E
	0.3	2.91-2.76	2.84± 0.04 b	2.76-2.62	2.67± 0.04 bc	2.91-2.62	2.76± 0.05 b
	0.5	3.27-3.22	3.24 ± 0.02 a	3.70-3.04	3.34± 0.19 a	3.70-2.04	3.29± 0.09 A
	0.9	2.76-2.55	2.63± 0.06 c	2.53-2.36	2.44± 0.05 d	2.76-2.36	2.54± 0.06 C
Sour Whey	Control	1.50-1.35	1.45± 0.05 f	1.40-1.35	1.38± 0.02 f	1.50-1.35	1.42± 0.03 F
	0.3	2.37-2.24	2.32± 0.04 de	2.30-2.19	2.25± 0.03 de	2.37-2.19	2.29± 0.03 de
	0.5	2.54-2.30	2.42± 0.07 d	2.40-2.22	2.30± 0.05 de	2.54-2.23	2.36± 0.05 d
	0.9	2.24-2.15	2.18± 0.03 e	2.19-2.11	2.14± 0.03 e	2.24-2.11	٢.١٦ ٠.٠٢± E
Effect of overlap between added percentage and Saltiness	Control	2.35-1.35	1.83± 0.17 c	2.16-1.35	1.76± 0.17 c		
	0.3	2.91-2.24	2.58± 0.12 ab	2.76-2.19	2.46 ± 0.01 ab		
	0.5	3.27-2.30	2.83± 0.19 a	3.70-2.23	2.82± 0.25 a		
	0.9	2.76-2.15	2.41± 0.11 ab	2.53-2.11	2.29± 0.07 b		
Effect of overlap between Protein and Saltiness	Type of protein concentrate	Sweet Whey		Sour Whey			
		Range	Average ± S. E.	Range	Average ± S. E.		
	Salted	3.27-2.05	2.73± 0.12 a	3.70-2.10	2.65± 0.14 a		
	Unsalted	2.54-1.35	2.09± 0.12 b	2.40-1.35	2.02± 0.11 b		

*Numbers having similar letter within the same column are not significantly different at $P \leq 0.05$. * Average are three replicates.

Table(7) refers to the fact that salted sweet whey exceeded significantly with an addition of 0.5٪ with the highest average of (4.21) ml/gm protein .However, the control experiment decreased by 3.10٪, and the salted sour whey exceeded by addition of 0.5٪ with the highest average of 3.60. However, the comparison of additional percentage decreased with an average of 2.30. Concerning the unsalted sweet whey, the additional percentage exceeded 0.5٪ by the highest average of 4.00, whereas it decreased for the comparison by 2.78. Also, the unsalted sour whey, the addition percentage exceeded significantly by an average of 3.30, however, it decreased for the comparison

by an average of 2.24. It is noticed that, these rustles of acetylation of whey protein concentrates by a percentage of 0.5٪ significantly exceeded all other treatments for water absorption and the salted sweet whey protein concentrates value was superior in all treatments. These results were agree with the findings of Jandal *et al.*(2012) and Kebary *et al.* (1993) who proved that the increase of water absorption values using acetylated whey proteins is the cause behind the dissociation in protein molecule and consequently the increase of oil or water passage inside the molecule which increases the ability of water or oil absorption. From the same Table it is shown that there was a significant differences for the overlap of protein effects. Therefore, the two types of sweet proteins exceeded the types of sour whey, and the salted sweet whey proteins exceeded other whey proteins by a percentage of 0.5٪ and reached 4.11٪ .

Table 7. *Effect of acetylation treatments on Water Absorption property (ml/gm protein) for WPC* .

Type of protein concentrate	Added percentage of Anhydrous Acetic Acid ٪	Salted		Un salted		Effect of overlap between protein and Addition percentage	
		Range	Average ± S. E.	Range	Average ± S. E.	Range	Average ± S. E.
Sweet Whey	Control	3.20-2.99	3.10± 0.06 fg	2.85-2.70	2.78± 0.04 h	2.70-3.20	2.94±0.08 fe
	0.3	3.88-3.76	3.83± 0.04 c	3.71-3.62	3.67± 0.03 cd	3.62-3.88	3.75± 0.04 b
	0.5	4.21-4.00	4.21± 0.12 a	4.12-3.93	4.00± 0.06 b	3.93-4.43	4.11± 0.08 A
	0.9	3.45-3.25	3.34± 0.06 e	3.27-3.23	3.25± 0.01 fe	3.23-3.45	3.29± 0.03 cd
Sour Whey	Control	2.33-2.28	2.30± 0.02 i	2.25-2.24	2.24± 0.03 i	2.24-2.33	2.27± 0.01 G
	0.3	3.40-3.22	3.30± 0.05 e	2.99-2.91	2.95± 0.02 g	2.91-3.40	3.13± 0.08 de
	0.5	3.70-3.51	3.60± 0.06 d	3.38-3.20	3.30± 0.05 e	3.20-3.70	3.45± 0.08 C
	0.9	3.12-2.86	3.03± 0.08 g	2.77-2.50	2.65± 0.08 h	2.50-3.12	2.84± 0.01 F
Effect of overlap between added percentage and Saltiness	Control	3.20-2.28	2.70± 0.18 ef	2.85-2.24	2.51± 0.12 f		
	0.3	3.88-3.22	3.56± 0.12 ac	3.71-2.91	3.31± 0.16 bd		
	0.5	4.43-3.51	3.91± 0.13 a	4.12-3.20	3.65± 0.16 ab		
	0.9	3.45-2.86	3.18± 0.08 cd	3.27-2.50	2.95± 0.14 de		
Effect of overlap between Protein and Saltiness	Type of protein concentrate	Sweet Whey		Sour Whey			
		Range	Average ± S. E.	Range	Average ± S. E.		
	Salted	4.43-2.99	3.62± 0.13 a	4.12-2.70	3.42± 0.14ab		
	Unsalted	3.70-2.28	3.06± 0.15bc	3.38-2.24	2.79± 0.12 c		

*Numbers having similar letter within the same column are not significantly different at P ≤ 0.05. * Average are three replicates.

Table(8) showed that the effects of acetylation on emulsification property. It was noticed that, the salted sweet whey exceed significantly was obtained of 0.5٪ of anhydrous acetic acid and the highest average of emulsification of 52.67 (ml/gm), whereas the additional percentage for the control treatment was 36.03. Also, for the salted sour whey, the same percentage exceeded with the highest average of 36.00 (ml/gm) but reduced in the control sample. Concerning the unsalted sweet whey, the addition of 0.5٪ exceeded with the highest average of 43.33 (ml/gm), but was reduced in the control sample. The addition of 0.5٪ from unsalted sour whey, showed significant increase by an average of 30.39 (ml/gm) and reduced for the control trial. These results were agreed with *Mc*Clement (1999) and Damodaran (1997). The studies of these authors have clarified that, the cause of emulsification increase that using acetylated whey proteins causes bigger dissociation in the protein molecule and consequently an increase of protein solubility which increases the flexibility of molecules movement on the surfaces. And that the acetylation process increases the emulsification capacity property. Onwulata and Huth(2008) mentioned that emulsification increases with the increase in hydrogen exponent which affects the protein indissolubility ability, since the hydrogen exponent plays an important role in the increase and decrease of the emulsification capacity. Thus, emulsification decreased in the acidic medium and increased in the basic medium, this is due to the balance of water or oil desiring groups. This is a results of forming charged layers surrounding the oil pellets which causes dissonance or formation of watery layer around the surface that separates between the two layers, which in turn reduces energy and obstructs the coherence of oil pellets. The surface activity of proteins is due to its behavior of hydrophilic and hypophilic which assist of oil-water absorbing during emulsification. It has been found that proteins help to disrupt water drops during emulsification by reducing the surface tension between oil and water. Whey proteins contribute in the stability of oil droplets against amalgamation and proteins absorption results a sticky layer which prevents amalgamation during emulsification. The results of the analysis had shown that there is an overlap between protein and saltiness. The salted sweet whey proteins were significantly increased and differed from other treatments. The percentage of salted sweet whey proteins were the highest values (44.83٪) . The salted sweet whey proteins of 0.5٪ were significantly higher (48٪) over other whey proteins in the triple-overlap .

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Table 8. *Effect of acetylation treatments on emulsification property(ml/gm protein) for WPC.*

Type of protein concentrate	Added percentage of Anhydrous Acetic Acid ٪	Salted		Un salted		Effect of overlap between protein and Addition percentage	
		Range	Average ± S. E.	Range	Average ± S. E.	Range	Average ± S. E.
Sweet Whey	Control	36.10-35.99	36.03± 0.04 f	30.10-30.00	30.03± 0.03 g	36.10-30.00	33.03± 1.34 c
	0.3	45.40-44.30	44.97± 0.34 bc	41.00-38.45	40.12± 0.83 e	45.40-38.45	42.54± 2.83 b
	0.5	53.00-52.20	52.67± 0.24 a	45.00-42.00	43.33± 0.88 cd	53.00-42.00	48.00± 2.13 a
	0.9	47.00-44.50	45.67± 0.73 b	43.00-40.80	41.60± 0.70 de	47.00-40.80	43.63± 1.02 b
Sour Whey	Control	25.10-25.00	25.05± 0.03 h	21.00-12.02	21.01± 0.07 i	25.10-21.00	23.03± 0.90 e
	0.3	30.00-27.80	29.00± 0.64 g	25.74-24.50	25.18± 0.36 h	30.00-24.50	27.09± 0.92 d
	0.5	38.00-34.00	36.00± 1.16 f	28.50-23.00	30.39± 1.02 g	38.00-28.50	33.20± 1.43 c
	0.9	30.60-29.40	30.12± 0.37 g	28.00-25.60	26.68± 0.70 h	30.60-25.60	28.40± 0.85 d
Effect of overlap between added percentage and Saltiness	Control	36.10-25.00	30.54± 2.46 bc	30.10-21.00	25.52± 2.02 c		
	0.3	45.40-27.80	36.98± 3.59 ab	41.00-24.50	32.65± 3.37 bc		
	0.5	53.00-34.00	44.33± 3.76 a	45.00-28.50	36.86± 2.96 ab		
	0.9	47.00-29.40	37.89± 3.41 ab	43.00-25.60	34.14± 3.37 bc		
Effect of overlap between Protein and Saltiness	Type of protein concentrate	Sweet Whey		Sour Whey			
		Range	Average ± S. E.	Range	Average ± S. E.		
	Salted	53.00-35.99	44.83±1.79 a	45.00-30.00	38.77±1.59 B		
	Unsalted	38.00-25.00	30.40±1.22 c	32.00-21.00	25.81±1.05 D		

*Numbers having similar letter within the same column are not significantly different at $P \leq 0.05$. * Average are three replicates.

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تأثير إضافة حامض ألخليك اللامائي إلى مركزات بروتينات الشرش المحضرة لتحسين خواصها الوظيفية .

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المستخلص

حضر نوعان من مركزات بروتينات الشرش احدهما الشرش ألحامضي والأخر الشرش الحلو المملح وغير المملح ، حيث تمت استلتهما بتركيزات مختلفة ودرست خواص الفعالية لهما . وجد إن الشرش ألحامضي غير المملح المؤستل قد تفوق على إقرانه من المعاملات في نسبة الرطوبة والرماد ، بينما الشرش الحلو المملح وغير المملح المؤستل كان له النصيب الأكبر في التفوق المعنوي($P\leq 0.05$) لباقي المعاملات وفي صفات البروتين الكلي وصفة حجم الرغوة وصفة تكوين الهلام وصفات القدرة على امتصاص الزيت والقدرة على امتصاص الماء وكذلك صفة الاستحلاب. ومن هنا نجد بان نسبة الاستلة ٠.٥٪ لبروتينات الشرش الحلو والحامض المملح وغير المملح هي الأفضل في تغيير الخواص الوظيفية لمركزات بروتينات الشرش المؤستلة وذلك لتفوقها معنوياً ($P\leq 0.05$)على نسبة الاستلة 0.3 و٠.٩٪ وان تأثير الملح قد أعطى في اغلب المعاملات تأثيرا معنوياً لذلك نجد من خلال النتائج أعلاه بان بروتينات الشرش الحلو والحامض المملح هي الأفضل في عملية الاستلة لتغيير الخواص الوظيفية والكيماوية له .

الكلمات المفتاحية: مركزات بروتينات الشرش ، الاستلة ، الخواص الوظيفية ، حامض ألخليك اللامائي .