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UTILIZATION OF WHEY PROTEIN IN YOGURT

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ABSTRACT

The aim of this work was to study the possibility of modifying three types of whey protein concentrations (WPC) chemically to improve their functional properties and use them to make fortified yogurt. Salted and acid whey protein concentrations were obtained by acid/heat or ultra filtration treatments. It was modified by acetic acid anhydride at the rate of 0.3, 0.5 and 0.7g/g protein. Acetylation of salted whey protein concentrate (SWPC), acid whey protein concentrate (AWPC) and filtrated whey protein concentrate (FWPC) improved its functional properties such as water absorption, emulsification capacity and gelation. The best treatment was acetylated WPC at 0.5g acetic acid anhydride/ g protein. Concerning the type of whey protein concentration FWPC exhibited the best functional properties followed by SWPC and then AWPC. Fortification of cows milk with modified WPC to make yogurt caused a pronounced increase in total solids, ash, pH and curd tension, while decreased syneresis. Also, addition of acetylated FWPC and SWPC up to 2% increased the acceptability of fortified yogurt.

INTRODUCTION

Manufacture of cheese results in production of up to 4 - 9 kg of liquid whey for every kilogram of the final product. One hundred kilograms of liquid whey, containing approximately 3.5 kg of biological oxygen demand (BOD) and 6.8 kg of chemical oxygen demand (COD), has the polluting strength equivalent to sewage produced by 45 people (Henning, 1998). Yet, this so called waste contains many valuable constituents. These include especially proteins that possess important nutritional and biological properties – particularly with regard to promotion of health, as well as prevention of diseases and health conditions. Antimicrobial and antiviral actions, immune system stimulation, anticarcinogenic activity and other metabolic features have indeed been associated with such whey proteins, as a-lactalbumin, b-lactoglobulin, lactoferrin, lactoperoxidase, and bovine serum albumin. (Madureira *et al.*, 2007). Thus, whey protein concentrates are common emulsifying, gelling and bulking ingredients in numerous foods as meat, bakery, and dairy products to increase firmness or cause gel formation (Meydani & Ha, 2000). However, current commercial utilization of WPC is still limited partially because of the great variability in its functional properties due to differences in whey source, composition and processing parameters (Kinsella & Whitehead, 1989). Previous studies have reported that various functional properties of WPC such as solubility, heat gelation, foaming and emulsion capacity can be influenced by the whey processing conditions (Mangino *et al.*, 1987; Morr & Foegeding, 1990). Among the different process parameters, heating plays a determinant role in whey protein functionality and pH effect (Kessler & Beyer, 1991 and Singh & Creamer, 1991). The source of whey has been shown also to affect the properties of the WPC (De la Fuente *et al.*, 2002 and Ji & Haque, 2003). Heat stability of whey proteins decreases with increasing protein concentration (Modler *et al.*, 1987). Yogurt products have achieved considerable economic importance worldwide owing to their high nutritional image. This positive image could be expanded further by adding nutraceutical (nutritional and pharmaceutical) ingredients such as whey proteins to the yoghurt (Guggisberg *et al.* 2007). Also, whey protein concentrate (WPC) has been added to milk for yogurt manufacture to reduce whey separation and increase the firmness and viscosity of yogurt (Robinson & Tamime, 1986). Traditionally, skim milk powder is used for milk fortification. However, availability and the low cost of WPC make them attractive and they are now commonly used to replace skim milk powder in yoghurt formulation. Significant variation in the functionality of WPC in yoghurt has been reported. Yoghurt fortified with various samples of commercial WPC had water holding capacity ranging from 45% to 63% Brookfield viscosity from 10 to 30Pa s, apparent viscosity from 0.16 to 1.1 Pa s (Guinee *et al.*, 1995), complex viscosity from 13 to 24 Pa s (Sodini *et al.*, 2005), and firmness from 50 to 79 g (Modler *et al.*, 1983). Syneresis is considered a primary defect and a limiting factor in consumers' acceptability of the Yogurt (Kailasapathy, 2006). Another aspect that can compromise acceptability is acidity, which can also affect the viability of probiotic bacteria in lactic beverages (Thamer & Penna, 2006).

The aim of this work was to prepare whey protein concentrates from salted and acid whey by heat/acid precipitation, filtration and evaluate their chemical composition. Then, investigate the possibility of improving the WPC by acetylation and evaluate its chemical and physicochemical properties, study the effects of adding the modified WPC on yogurt quality and monitor changes during 12 days of refrigerated storage.

MATERIAL AND METHODS

Salted whey of Domiati cheese, acid whey resulted from Ras cheese and raw cow's milk were obtained from Misr Milk and Food Co.

Starter culture

The starter culture for yogurt production consists of *Streptococcus salivarius subsp. Thermophilus* (ST) and *Lactobacillus delbrueckii subsp. Bulgaricus* (LB) strains were secured from C.H.P.Hansen`s Laboratorium A/S 1250 Copenhagen, Denmark.

Preparation of whey protein concentration.

Both, acid and salted whey were filtered through double layered cheese cloth. The pH of whey was adjusted at 4.6 and heated at 90 °C for 15 min. in a water bath, then cooled to room temperature. Then, the precipitate salted whey was washed several times with distilled water to remove the salt and this concentrated was designated as salted whey protein concentrated (SWPC). While, the precipitate of acid whey was obtained by centrifugation and this concentrate was designated as acid whey protein concentrate (AWPC).

Acid whey protein was concentrated four folob using plates-module with Of- membrane of Firma Kalle Wesbaden, type E10, F.R. Germany. This concentrate was designated as filtrated whey protein concentrate (FWPC).

Dialysis membrane-Medical International LTD,239 Liverpool ROAO, London NI ILX. (Isabelle *et al.*,2006)

Chemical modification (acetylation) :

A 25% (w/v) suspension of whey protein concentrate was prepared in distilled water at room temperature, and the pH adjusted to 7.5 by the addition of 2N NaOH. Acetic anhydride was added to the suspension over 30 - 90 min. at the levels of 0.3, 0.5 and 0.7 g acetic acid/ g protein, the weight of acetic anhydride was determined by its specific gravity. The pH was maintained in the range of 7.5 to 8.5 at room temperature by addition of 2N NaOH. The final pH was adjusted to 7.5 and slurry was left for 2h at room temperature then dialyzed against distilled water for 24h with changing the distilled water 4 times. The slurry was frozen overnight and freeze dried at – 60 °C (Lab Conco Freeze Dry 64312 Kansas, Missouri) then ground to pass through a 0.25 mm size sieve (Rahma & Narasinga Rao, 1983).

Manufacture of yogurt :

Fresh cow milk (3% fat) was divided into 7 equal portions. One portion was used as a control . The other six portion were fortified with SWPC or FWPC, which added at the ratios of 1%, 2% and 3% respectively. All batches were heated to 85 °C for 20 min. then cooled to 40 °C. Yogurt batches were inoculated with 1% *Streptococcus salivarius subsp. Thermophilus* (ST) + 1% *Lactobacillus delbrueckii subsp. Bulgaricus* (LB) . The inoculated batches were packed in plastic cups (100g) and incubated at 42 °C until complete setting (3-3.5 h). Resultant yogurt was stored for 12 day at 6 °C (Gauche *et al.*, 2009).

Chemical analysis:

moisture, ash, crude protein and fat were determined according to the methods of the A.O.A.C. (2005).

Functional properties methods:

Water absorption measured according to the procedure of Sosulski (1962).

Emulsification capacity was determined according to the method as described by Beuchat *et al.* (1975).

Gelation:

The ability of modified whey protein samples to form gel was measured according to the method described by Shigeru & Kinsella (1985).

Syneresis:

Syneresis was determined according to the method of Dannenberg & Kessler (1988).

Sensory evaluation:

Sensory evaluation was conducted by 12 consumer panelists according to the method mentioned by Nelson & Trout (1980).

RESULTS AND DISCUSSION

Acetylation has been used to expand the range of functional properties of plant proteins. Therefore, it has been concerned in this work to investigate the effect of acetylation on whey protein functionality. Whey proteins were precipitated by acid/heat treatment of salted and acid whey making salted and acid whey protein concentrates (SWPC and AWPC respectively), while, the third type of whey protein was obtained by ultra filtration of acid whey (FWPC). These three types of WPC were acetylated by using different levels pf acid anhydrides 0.3, 0.5 and 0.7g/g protein.

The extent of acetylation was determined by measuring the amount of free lysine available to react with 2,4,6, trinitrobenzen-sulfonic acid in untreated and acetylated whey protein concentrates and the results are illustrated in Fig (1).

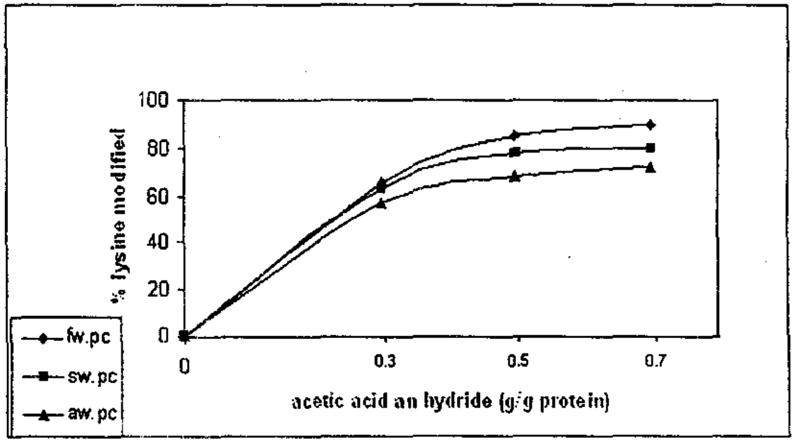

Fig. (1): The extent of acetylation was determined by measuring the amount of free lysine available

The modification rates of whey protein concentrates (SWPC, AWPC and FWPC) increased by increasing the levels of acetic acid anhydride. These results are in agreement with those reported by Dua & Mahajan, (1996). Moisture, lactose, total protein, fat, and total ash contents of acetylated salted, acid and filtered whey protein concentrates (ASWPC, AAWPC and AFWPC, respectively) are shown in Table (1). Acetylation caused an increase in moisture and total ash. Moisture contents of acylated SWPC were slightly higher than those of corresponding treatments of acylated FWPC and AWPC. The increase in ash content of acetylated WPC may be due to adjusting the pH during modification process with enhance the salt formation (sodium acetate). These results are in agreement with those reported by El-Naggar, (1997). On the other hand, acetylation of all whey protein concentrates (SWPC, AWPC and FWPC) caused a slight decrease in total protein, lactose and fat contents. This reduction of protein, lactose and fat contents of the modified whey protein concentrates increased with increasing the acetic acid anhydrides percentage.

Table (1): Moisture, lactose, total protein, fat, and total ash contents of modified whey protein concentrates (SWPC), (AWPC) and (AFWPC).

Properties %	SWPC				AWPC				FWPC			
	Acetic anhydride g/g protein											
	Control	0.3	0.5	0.7	Control	0.3	0.5	0.7	Control	0.3	0.5	0.7
Moisture	6.15	6.44	7.00	7.07	5.15	5.24	5.37	5.58	5.15	5.18	5.37	5.73
lactose	14.92	12.78	10.65	9.47	12.98	12.25	11.61	11.08	17.98	16.34	15.70	15.04
protein	68.19	67.81	67.12	66.64	72.34	71.68	69.35	68.40	65.82	64.89	63.59	62.46
Fat	2.20	2.10	1.90	1.81	1.98	1.49	1.20	1.15	1.30	1.25	1.20	1.15
Total ash	8.43	10.78	13.21	14.90	7.44	9.22	12.36	13.67	9.64	12.22	14.03	15.50

Functional properties:

The water absorption capacities, emulsification capacity and gelation of acetylated whey protein concentrates (ASWPC, AAWPC and AFWPC) are shown in Figs. (2, 3 and 4). The water absorption capacities of whey protein concentrates increased as the rats of modification increased, while the emulsification capacity increased and gelation improved with the levels of acetic acid anhydrides up to 0.5g/g protein. Also, AFWPC exhibited the best functional properties followed by ASWPC and then AAWPC.

Increase the water absorption capacity of modified whey protein concentrates may be attributed to acetylation decreases the number of positive charge on protein molecules.(Dua & Mahajan, 1996) and (El-Adawy, 2000). Also, acetylation tends to cause unfolding of protein chain, thereby exposing hydrophilic sections of peptides, which improves the emulsification properties of the protein (Feeny *et al.*, 1982).

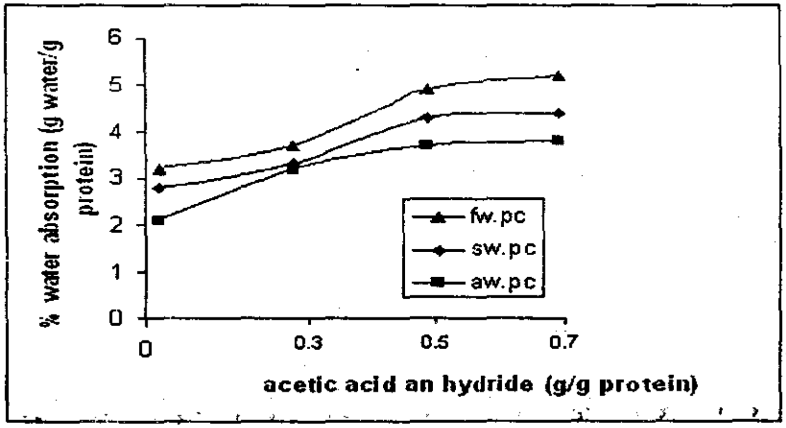

Fig. (2): The water absorption capacities of acetylated whey protein concentrates (ASWPC, AAWPC and AFWPC)

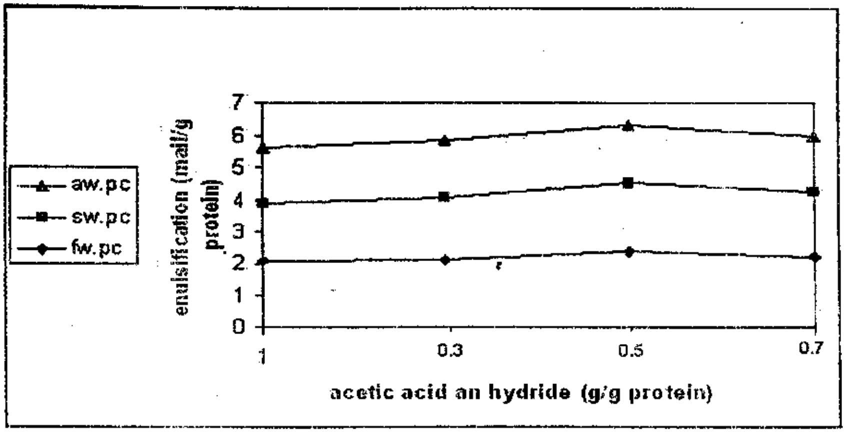

Fig. (3): The emulsification capacity of acetylated whey protein concentrates (ASWPC, AAWPC and AFWPC)

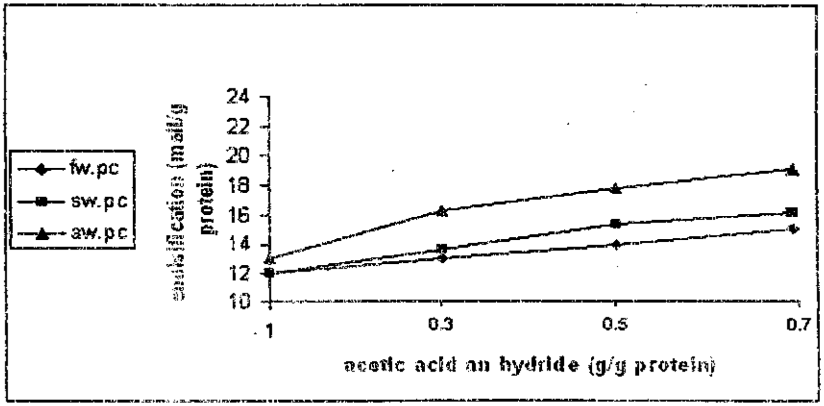

Fig. (4): The water absorption capacities, emulsification capacity and gelation of acetylated whey protein concentrates

As shown in Table (2), the values of pH, Syneresis, and curd tension of yogurt were inversely proportional to the amount of ASWPC and AFWPC (1%, 2% and 3%) added. The pH values were directly related to the percentage of ASPWC and AFWPC addition. Due to the buffer properties of whey protein, the pH values increased with rising levels of whey protein (Guggisberg et al., 2007). The pH values and curd tension increased with rising levels of ASWPC and AFWPC, while, syneresis decreased gradually as the amount from AFWPC and ASWPC were increased and as storage period progressed up to 12 days at 6 °C. Gruenér & Ismónd, (1977) reported that the obvious increase in emulsification capacity of acetylated proteins may be due to the increase of protein solubility and molecular flexibility which facilitates rearrangement of the protein at the interface.

Table (2): Total solids, protein, ash, pH, curd tension and syneresis of all yogurt batches made from fortified cow's milk with acetylated either filtrated whey protein concentrate (AFWPC) or salted whey protein concentrate (ASWPC

treatment		Total solids	Total protein	Ash	PH	Curd tension	Syneresis
control	fresh	11.81	3.46	0.618	4.08	10.4	43.5
	12 day	11.89	3.56	0.620	4.01	١ز١٢	35.8
As1	fresh	13.08	4.17	0.751	4.16	17.2	39.1
	12 day	13.16	4.28	0.754	4.00	20.0	32.0
As2	fresh	13.93	4.81	0.883	4.25	20.1	35.0
	12 day	13.99	4.94	0.886	4.11	23-9	27.5
As3	fresh	14.88	5.49	1.018	4.31	22.5	31.7
	12 day	14.95	5.60	1.021	4.47	23.4	27.0
Af1	fresh	13.29	4.12	0.762	4.15	17.4	38.2
	12 day	13.42	4.20	0.764	4.09	20.3	33.8
Af2	fresh	14.08	4.76	0.909	4.28	22.2	33.6
	12 day	14.19	4.86	0.907	4.14	25.6	27.2
Af3	fresh	15.10	5.38	1.051	4.35	25.1	31.3
	12 day	15.23	5.52	1.055	4.28	28.6	26.2

ASWPC was added to yogurt at the rate of 1%, 2% and 3% as As1, As2 and As3 respectively.

AFWPC was added to yogurt at the rate of 1%, 2% and 3% as Af1, Af2 and Af3 respectively.

Sensory evaluation are summarized in Table (3). The results table indicated that, treatments AF1, AF2 and AS2 were rated to have optimum scores of flavor, body & texture, appearance and acidity. However, only treatments AS3 and AF3 were less flavor and acidity than treatment AF2 through AS1. However, after 12 days of refrigerated storage, flavor, body & texture, appearance and acidity were declined in all treatments. Overall, panelist found treatments AF2 and AS2 to be the most desirable. Therefore, it could fortified cows milk with AFWPC and ASWPC up to 2% without detrimental effects on yogurt quality. These results are in agreement with those reported by Guggisberg, et al., (2004).

Table (3): Sensory evaluation syneresis of all yogurt batches made from fortified cow's milk with acetylated either filtrated whey protein concentrate (AFWPC) or salted whey protein concentrate (ASWPC).

Treatments	Flavor (45)		Body&Texture (30)		Apperance (15)		Acidity (10)		Total score (100)	
	Fresh	Fresh 12day	Fresh	Fresh 12day	Fresh	Fresh 12day	Fresh	Fresh 12day	Fresh	Fresh 12day
control	41.2	34.1	24.5	21.3	11.1	8.2	8.5	5.9	85.3	68.5
As1	41.1	33.2	24.2	21.1	11.2	8.1	9.0	6.8	85.5	69.2
As2	42.1	32.2	26.1	24.2	12.8	10.8	9.3	7.9	90.3	74.9
As3	39.1	30.1	24.1	19.3	10.3	8.1	8.2	5.8	82.7	64.3
Af1	42.3	35.2	26.2	24.2	12.4	9.6	9.1	7.3	90.0	76.3
Af2	42.9	34.1	27.1	25.2	13.5	11.5	9.3	8.1	92.6	78.2
Af3	39.3	33.3	24.1	20.1	10.9	7.9	8.3	5.6	83.6	67.9

ASWPC was added to yogurt at the rate of 1%, 2% and 3% as As1, As2 and As3 respectively.

AFWPC was added to yogurt at the rate of 1%, 2% and 3% as Af1, Af2 and Af3 respectively.

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أستخدام بروتينات الشرش في تدعيم الزبادى

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تهدف هذه الدراسة الى امكانية تحسين الخواص الوظيفية لبروتينات الشرش المختلفة عن طريق التعديل الكيماوى ، و أستخدامها فى صناعة الزبادى. و لقد تم تحسين بروتينات الشرش المفصوله باستخدام المعا ملة الحرارية/الحامضية أو المفصوله بواسطة تقنية الترشيح الفائق عن طريق معاملتها بحمض الخليك اللامائى بتركيزات ۰,۳ و۰,۵ و۰,۷ جم حمض/جم بروتين.

و نتيجة لعملية الأستلة لكل من مركز بروتينات الشرش المملح و مركز بروتينات الشرش الحامضى و مركز بروتينات الشرش المرشحة، تحسنت خواصها الوظيفية و منها القدرة على أمتصاص الماء و السعة الأستحلابية و تكوين الجيل. و كانت أحسن نتائج الأستلة لبروتينات الشرش عند تركيز ۰,۵ جم حمض الخليك اللامائى /جم بروتين. أما فيما يخص نوع مركز بروتينات الشرش فقد أظهرت النتائج أن مركز بروتينات الشرش المرشحة هى أحسن المركزات المختبرة من حيث الخواص الوظيفية يليها مركز بروتينات الشرش المملح ثم مركز بروتينات الشرش الحامضى. و عند تدعيم اللبن البقرى بمركز بروتينات الشرش المحسنة لصناعة الزبادى ، حدثت زيادة محسوسة فى المواد الصلبة الكلية و الرماد و رقم الأس الأيدروجينى و صلابة الخثرة ، بينما نقصت كمية الشرش المفصولة من الزبادى . ووصلت نسبة التدعيم بمركز بروتينات الشرش المحسن سواء المرشح أو المملح فى الزبادى الى ۲% ، بدون التأثير على خواصه الحسية بالسلب.