J. Agric. Sci. Mansoura Univ., 34 (4): 3139 - 3151, 2009

COMPOSITIONAL AND FUNCTIOAL PROPERTIES OF BUTTERMILK PROTEIN PREPARATIONS

Ramadan, Fatma A. M.; I. H. I. Abd El-Ghany and M.A. Azzam
Dairy Science and Technology Department, Fac. of Agric.,Cairo Univ., Egypt

ABSTRACT

The compositional and functional properties (i.e., solubility ,water-holding and fat-absorption capacity, electroconductivity and emulsification capacity, and stability) as well as digestibility by trypsin of buttermilk solids (BMS) and buttermilk protein preparations (i.e., buttermilk whey protein concentrates, BMWPC ; buttermilk casein-co-precipitate, BMCCP ; total buttermilk protein, TBMP and buttermilk acid casein, BMAC) were studied. The effect of different pH levels on solubility, electroconductivity and emulsification capacity and stability was evaluated. All samples were similar in their chemical composition as they contained protein, lactose, fat and ash. BMS had higher fat, lactose and ash content and lower protein content compared with those of other dried buttermilk protein powders. Except electroconductivity and solubility, BMS showed limited functional properties in fat-absorption capacity (0.66 g fat/g protein) and emulsifying capacity (0.373-0.543, O.D) and stability (34-37 min.) at low pH (pH≤5) while , BMWPC had the highest fat-absorption capacity (2.5 g fat/g protein) and emulsifying capacity(0.400-0.800, O.D) and stability(38-44 min.) at the same pH , furthermore, its high digestibility by trypsin . This could be due to the heat treatment applied during preparation of BMWPC. So, BMWPC appears to be a promising and unique ingredient in the formulation of low pH foods.
Keywords: buttermilk, whey protein concentrates , functional properties

INTRODUCTION

In Egypt, large quantities of wastes derived from dairy industries are not used economically. One of these wastes is buttermilk. Buttermilk is an aqueous phase released during the manufacture of butter. It contains not only skim milk protein but milk fat , lactose , minerals and milk fat globule membrane(MFGM), mainly composed of proteins , phospholipids , sphingolipids and various glyco-proteins (Vyas *et al.,* 2002) . This characteristic composition makes buttermilk an interesting source of ingredients with unique functional properties among milk-derived products. The growing interest in the dairy industry for new products has led to an increase in the number of studies on buttermilk and its use in various dairy products (Raval and Mistry, 1999, O'Connel and Fox, 2000 ; Turcot *et al.,* 2001 ; Azzam, 2003 ; El-Sayed *et al.,* 2006 and Shazly *et al.,* 2008).

Because the commercial processes such as , ultra-filtration , reverse osmosis , ion exchange , gel filtration and spray draying , for recovering of these components from buttermilk are still expensive and not suitable for small dairy factories (Glover, 1985 and Morr, 1989) , this waste, up till now, still disposed directly into a sewage without utilization , then , causes many environmental problems .

The heat treatment for obtaining milk protein from this waste is partial solution for these problems, where still economical for small dairy producers

(Thompson and Reyes, 1980) . But, heating of milk has dramatic effects on the biochemical and physicochemical properties of milk proteins (Farrell, 1988). Heating induces denaturation, principally of the whey proteins and provides the energy to enable important chemical and physicochemical reactions in milk proteins (de Wit and Klareanbeek, 1984 and Parris *et al.,* 1991). Recently, a new technique for recovering the milk proteins is achieved. It is very simple, economic, without heat treatment, as it depends only on change of pH and gave total milk proteins which have much improved solubility and functional properties from over that of traditional co-precipitated protein (Metwally, 1997).

Despite the economic value of buttermilk solids, which is increasingly appreciated, with a potential worldwide production of 5.2×10^5 tonnes of solids per annum (IDF, 2002), no studies have focused on preparation of buttermilk proteins and the effect of preparation conditions on its functional properties. Therefore, the main objective of this work was to assess the compositional and functional properties of buttermilk proteins and to evaluate the effect of various preparation conditions on their functionality.

MATERIALS AND METHODS

Cultured buffalo's cream was churned to butter. Resultant buttermilk was divided into tow parts, the first was dried in oven at 40ºC for the preparation buttermilk solids (BMS), while the second was raw material to protein making. Sunflower oil was purchased from a local supermarket (Arma,Co.). All chemicals used were of reagent grade and were purchased from Sigma Aldrich (St. Louis, MO).

For the preparation of total buttermilk proteins (TBMP, casein and undenatured whey proteins) , buttermilk was adjusted to pH 10 by addition of 1N NaOH and heated to 50±1ºC to solubilize casein micelles . After that, pH was adjusted to 2.5 at 40ºC using 1N HCL to form a complex between the whey proteins and casein. The pH was readjusted to 4.6 by 1N NaOH to precipitate the complex proteins. The proteins were removed from the whey using cheese-cloth and then washed three times using warmed distilled water at pH 4.5. The buttermilk casein-whey protein co-precipitate(BMCCP, casein and denatured whey proteins) recovered from buttermilk by precipitation at pH 4.6 after heating at 90ºC for 15 min. at pH 7.5. Buttermilk acid casein (BMAC) was precipitated at pH 4.6 using 1N HCL, and the collected casein was washed three times using warmed distilled water at pH 4.5 . After collecting of casein , the resultant acid whey was heated at three pH levels 2.5 , 4.5 and 7.5 at 90ºC for 15 min., followed by cooling , isoelectric precipitation , and centrifugation to recover the aggregated protein . These products were designated as buttermilk whey protein concentrates (BMWPC) at three pH levels 2.5, 4.5 and 7.5 (Morr, 1985 and Metwally, 1997).

All protein preparations (TBMP, BMCCP, BMAC and $BMWPC_{2.5\ ,4.5,7.5}$) were dried in oven at 40ºC . Because all powders vary in their levels of protein, analysis of samples was conducted based on equivalent protein basis.

All samples were analyzed in triplicates.

Dry matter (DM) was determined by draying each sample for 5 h in a vacuum oven at 100ºC (American Dairy Products Institute, 1990). Fat content was determined using the Mojonnier Ether extraction method as described by Marshall (1992). Ash content was determined by ignition for 16 h at 550ºC in an electric muffle furnace (AOAC, 1995). Total protein content was measured by determining total nitrogen content using the Kjeldahl method according to AOAC (1995). Conversion factor used was 6.38. The protein profile was established by polyacrylamide gel electrophoresis according to Lee *et al.,*(1975) using Tris-buffer gradient gels. Proteolysis of protein powders by trypsin was carried out according to Datta Roy (1981). Content of lactose was calculated by difference [DM – (TP + fat + ash)] as proposed by Guzman-Gonzalez *et al.,* (1999) . The pH of 1% protein (wt/wt) reconstituted powder were determined using a pH meter (3305-JENWAY.UK).

For Examining the functional properties, an equivalent weight of 1 g protein from any powder was dissolved either in 100 ml deionized water for determine the water holding capacity (WHC) by centrifugation method according to AACC (1981) or in deionized water or phosphate buffer for determine the solubility at initial normal pH, pH 3 , 5 and 8 according to Haque and Mozaffer (1992) , electroconductivity using conductometer (3112-JENWAY.UK) and emulsifying capacity(EC) and stability(ES)at pH 3 , 5 and 8 according to Pearce and Kinsella ,(1978) . The same previous weight was thoroughly vortexed 10 ml sunflower oil for determine the fat absorption capacity (FAC) according to Ahmedna *et al.,* (1999).

Statistical analysis for the obtained data was carried out using 2 × 3 factorial design. Duncan's test was used to make the multiple comparisons (Steel and Torri , 1980).Significant differences were determined at $P < 0.05$.

RESULTS AND DISCUSSION

The major composition of buttermilk solids (BMS) and the buttermilk protein preparation powders is presented in Table (1) . The protein content was lower in BMS (31.17%), than in protein powders obtained from the same buttermilk. This was due to increment of the other components in buttermilk as much as its protein content. On the other hand, it was observed difference among the buttermilk protein powders in their protein content , since , buttermilk casein-whey protein co- precipitate(BMCCP) was the highest (82.5%) while, buttermilk whey protein concentrate ($BMWPC_{4.5}$) was the lowest (69.54%) . The high protein content of BMCCP is due to the used heat treatment during its preparation, which causes association of all whey proteins with milk fat globule membrane (MFGM) and casein micelle (Dalgleish and Banks, 1991) , whereas by using the high temperature with pH 4.5 for precipitation of buttermilk whey proteins, caused loss part of the covered proteins (Abd El-Salam *et al.,* 1975 and Modler and Harwalkar , 1981) .

Differences were observed for the fat content among samples. Buttermilk protein powders contained 2.4 to 7.11% fat, whereas, BMS had

higher fat content (18.5%). This was due to the lack of fat removal process during the manufacture of buttermilk on site , which did not include a centrifugation step to remove the excess fat.

Ash content was between 2.5 and 5% , and lactose between 12 and 45% .It could be observed differences in lactose content, which were strongly related to the differences in protein content.

An initial pH of the BMS solution (1% protein) was 5.31, whereas the pH of the buttermilk protein powders was lower than 5 because of the acidification occurring during their preparation (Sodini *et al.,* 2006).

Table (1): Gross composition (%)on a DM basis, and initial pH of the samples

Samples*	Total protein	Fat	Ash	Lactose	pH**
BMS	31.17^{D}	18.50^{A}	5.11^{A}	45.22^{A}	5.31
BMCCP	82.50^{A}	2.55^{C}	2.80^{C}	12.15^{C}	4.39
TBMP	79.88^{AB}	2.41^{C}	2.45^{C}	15.26^{BC}	4.56
BMAC	80.04^{AB}	2.72^{C}	2.75^{C}	14.49^{BC}	4.51
$BMWPC_{7.5}$	73.17^{BC}	7.11^{B}	4.18^{AB}	15.54^{BC}	4.60
$BMWPC_{4.5}$	69.54^{C}	6.39^{B}	3.97^{B}	20.10^{B}	4.55
$BMWPC_{2.5}$	74.50^{ABC}	6.81^{B}	4.23^{AB}	14.46^{BC}	4.53
LSD at 0.05%	9.12	1.17	1.11	6.28	-

***Samples : BMS = Buttermilk solids , BMCCP = buttermilk casein-whey protein co-precipitated ,**

TBMP = total buttermilk protein ,BMAC = buttermilk acid casein , BMWPC = buttermilk whey protein concentrate at pH 7.5 , 4.5 and 2.5. **pH was determined in a 1% protein solution .

The solubility is an important functionality for protein preparations, which governs many other functional properties (Kinsella, 1976). The solubility of the different powdered samples at various pH levels is indicated in Table (2).

The solubility varied significantly according to the type of powder and the pH of the solution. The solubility range of the powdered samples was in the order: BMAC < BMCCP < $BMWPC_{4.5}$ < $BMWPC_{2.5}$< $BMWPC_{7.5}$ < TBMP < BMS .The solubility of the BMS powder was the highest among the other samples at initial pH (5.31). Whereas, an initial pH for BMS solution was above the isoelectric point (pI), thus a protein had a net negative charge, more water interacts with the protein charges (Mann and Malik, 1996; Darewiez, 2001 and Fachin and Viotto, 2005). Also, the solubility of BMWPC at its initial pH was superior from that of BMCCP. This might be due to devoid of casein as insoluble protein fraction at low pH (Chobert *et al.,* 1988; Chansiri *et al.,* 1999 and Sodini *et al.,* 2006) .The solubility of all powdered samples increased by increasing the pH up to 8. For instance, the solubility increased from 6.06 at pH 5 to 19.86 % at pH 8 for BMAC. Whereas , the proteins are more soluble in low(acids) or high (alkaline) pH values because of the excess of charges of the same sign, producing repulse among the molecules and, consequently, contributing to its largest solubility (Wong *et al.,* 1996) .

On the other hand, the solubility varied significantly between the undenatured and denatured buttermilk protein. For instance, the solubility at

pH 8 for TBMP was 59.87 %, whereas it was 23.42 % for BMCCP at the same pH. During preparation of BMCCP by heating, the protein is denatured. Denaturation decreases protein solubility, compared to native protein, and leads to aggregation and difficulty of reversal upon cooling (Mine, 1995 and Kim, 1998) .

Table (2): Solubilityof buttermilk solids and buttermilk protein powders at different pH-values.

Samples	pH-values				Mean
	Initial pH	3	5	8	
		Solubility (%)			
BMS	24.59^{H}	35.94^{E}	21.18^{J}	67.44^{A}	37.29
BMCCP	7.37^{S}	11.83^{Q}	9.67^{R}	23.42^{I}	13.07
TBMP	18.69^{M}	29.08^{F}	20.21^{JK}	59.87^{B}	31.96
BMAC	5.22^{T}	7.58^{S}	6.06^{T}	19.86^{KL}	9.68
$BMWPC_{7.5}$	17.46^{N}	25.95^{G}	18.73^{M}	43.99^{C}	26.53
$BMWPC_{4.5}$	9.83^{R}	12.69^{PQ}	11.79^{Q}	29.02^{F}	15.83
$BMWPC_{2.5}$	13.61^{OP}	19.03^{LM}	14.77^{O}	37.11^{D}	21.08
Mean	13.82	20.30	14.60	40.10	

BMS = Buttermilk solids , BMCCP = buttermilk casein-whey protein co-precipitated , TBMP = total buttermilk protein , BMAC = buttermilk acid casein , BMWPC = butter milk whey protein concentrate at pH 7.5 , 4.5 and 2.5. LSD at 0.05% of pH = 0.41 ; samples = 0.54 ; pH × samples = 1.08 .

The water holding capacity (WHC) of proteins has an important role in the physical (e.g., elasticity, swelling), chemical (e.g., emulsification) and sensory (e.g., juiciness) attributes of foods .BMAC had the greatest WHC, followed by BMCCP, $BMWPC_{4.5}$, ($BMWPC_{2.5}$ = $BMWPC_{7.5}$ =TBMP) and BMS (Table 3) . A similar finding has been reported by Kamal (1998), who suggested that the pasteurized or sterilized-buffalo milk casein had the lowest WHC, as compared with that of the raw buffalo milk casein .This is due to the heat treatment applied during preparation of casein, induced alteration in the surface characteristics of casein micelles and association of denatured whey protein (β-lactoglobulin) with casein micelles thus, increases in WHC and decrease in solubility (Kinsella and fox, 1987). Hence, there are inverse relationship between solubility and WHC(Modler and Harealkar, 1981).For instance, the solubilites of BMAC, BMCCP and TBMP were 5.22 , 7.37 and 18.69 %, respectively in distilled water, while their WHCs were 221 , 193 and 84 %, respectively . This relationship was confirmed for several protein preparations such as wheat germ protein (Vani and zayas, 1995); municipal bean protein (El-Adawy, 2000) and soy protein isolate (Wong and Kitts, 2003).

Fat absorption capacity (FAC) or the ability of proteins to bind fat by nonpolar amino acids present in the side chains of protein (Susheelamma and Rao, 1974) is an important functional property in fabricated foods, where it improves the texture and mouth feel (Kinsella, 1976). FAC of the different powders is showed in Table (3).

FAC varied from 66 to 276 % according to the type of the powder. $BMWPC_{4.5}$ had the greatest FAC, followed by $BMWPC_{7.5}$, $BMWPC_{2.5}$ TBMP,BMCCP and (BMAC = BMS). The superior FAC of BMWPC can be

explained by sulfhydryl groups, which indicate a denatured and unfolded protein molecule with hydrophobic regions for interaction with fat (Wong and Kitts, 2003) . Also, Voutsinas and Nakai, (1983) found that high solubility negatively affected the FAC of proteins. One possible reason for the adverse effect of high solubility on the FAC of proteins is the conformation of the soluble proteins which does not permit their binding sites (hydrophobic side chains) to be sterically available for interaction with oil hydrocarbon chains. The ability of BMS to bind fat was the weakest among all samples. Ahmedna *et al*.(1999) and Wong and Kitts (2003) also found BMS to have a lower FAC than nonfat dried milk and soy protein isolate . One explanation for the difference in FAC between samples is that BMS had a higher fat content among all powdered samples, which therefore would result in a decrease potential to bind more fat (Lin and Zayas, 1987). The presence of milk fat globule membrane in BMS did not enhance its FAC over BMAC, which has no milk fat globule membrane content present.

Table (3): Water and fat binding capacity of buttermilk solids and buttermilk protein powders .

Samples	WHC g H_2O/g protein	FAC g fat/g protein
BMS	0.81^{D}	0.66^{F}
BMCCP	1.93^{B}	0.95^{E}
TBMP	0.84^{CD}	1.14^{D}
BMAC	2.21^{A}	0.73^{F}
$BMWPC_{7.5}$	0.86^{CD}	2.43^{B}
$BMWPC_{4.5}$	1.06^{C}	2.76^{A}
$BMWPC_{2.5}$	0.94^{CD}	2.11^{C}
LSD at 0.05%	0.23	0.19

WHC = water holding capacity , FAC = fat-absorption capacity ,BMS = Buttermilk solids , BMCCP = buttermilk casein-whey protein co- precipitated , TBMP = total buttermilk protein , BMAC = buttermilk acid casein , BMWPC = buttermilk whey protein concentrate at pH 7.5 , 4.5 and 2.5.

The results of electroconductivity determination (Table 4) have shown that BMS solutions had the highest electroconductivity, followed by BMCCP, $BMWPC_{7.5}$, $BMWPC_{2.5}$, $BMWPC_{4.5}$, TBMP and BMAC, depending on pH. It is known that minerals of protein are more soluble at low pH (St-Gelais *et al.,* 1995). Therefore, when dissolving of BMS or any protein powdered in acidic buffer solution (pH ≤ 5), the amounts of Ca and P liberated from the protein will be considerable and causes an increase of electroconductivity (Zhuang *et al.,* 1997 and Therdhai and Zhou, 2001). High milk fat globule membrane (MFGM) content in BMS solution provide electroconductivity, whereas, MFGM is source of phosphorus which are completely soluble at pH ≤ 5 (Mabrook and Pettry, 2003) .

An o/w emulsion is a suspension of fat droplets in water that is stabilized by a surface-active agent or emulsifier at the o/w interface. Therefore, the ability of a protein to act as an emulsifier will depend primarily on its solubility, lipid-to-protein ratio and degree of surface denaturation. The emulsifying capacity(EC) in 30% oil in water emulsion has been determined with 1% protein solution at various pH levels for the 7 powdered samples

(Table 5) . The EC range of the powdered samples was in the order : BMS > $BMWPC_{7.5}$ > $BMWPC_{2.5}$ > $BMWPC_{4.5}$ > BMCCP > TBMP > BMAC .These differences are due to the proportion of milk fat globule membrane (MFGM) to protein content, which is higher in BMS than in the other protein preparations, as reported by Kanno, 1989 and Sodini *et al.,* 2006) .In general, the absorption of casein , whey and MFGM protein at the interface of an o/w emulsion has been shown to be dependent on the ratio of the three proteins present in the protein powders (Corredig and Dalgleish, 1998).On the other hand, the poor EC of BMAC can be attributed to a high casein concentration where, the effective number of protein aggregates is low (Roesch *et al.,* 2004) .

Table (4): Conductivity at different pH-values, of buttermilk solids and buttermilk protein solutions.

Samples	pH-values 3	5	8	Mean
	Conductivity(mS/cm)			
BMS	3.59^{B}	4.00^{A}	2.18^{F}	3.26
BMCCP	3.07^{C}	3.51^{B}	2.10^{F}	2.89
TBMP	1.69^{HI}	1.95^{G}	1.12^{J}	1.59
BMAC	1.22^{J}	1.58^{I}	0.96^{K}	1.25
$ABMCWPC_{7.5}$	2.46^{E}	2.88^{D}	1.73^{H}	2.36
$ABMCWPC_{4.5}$	2.33^{E}	2.79^{D}	1.64^{HI}	2.25
$ABMCWPC_{2.5}$	2.39^{E}	2.81^{D}	1.77^{H}	2.32
Mean	2.39	2.79	1.64	

BMS = Buttermilk solids , BMCCP = buttermilk casein-whey protein co-precipitated, TBMP = total buttermilk protein , BMAC= buttermilk acid casein , BMWPC = buttermilk whey protein concentrate at pH 7.5, 4.5 and 2.5. LSD at 0.05% of pH = 0.06 ; samples = 0.09 ; pH × samples = 0.15 .

At pH ≤ 5 $BMWPC_{7.5,\ 2.5\ and 4.5}$ had the greatest EC, followed by BMS , BMCCP , TBMP and BMAC (Table 5) .This means that there was no effect of low pH on the EC of the $BMWPC_{7.5,\ 2.5\ and 4.5}$. Chobert *et al.* (1988) were attributed that to the low level of insoluble protein at pH 4.6 in whey protein powder. In addition sulfhydryl groups in whey proteins that increase surface hydrophobicity, thus, the EC (Wong and Kitts, 2003).

Regarding emulsifying properties of buttermilk casein powders, the results showed that BMCCP had the highest EC, compared with TBMP and ABMC at the pH range studied. This result was attributed to the heat treatment applied during preparation of BMCCP, which induced whey protein complexation with casein and MFGM protein, improves the EC of the resulting isolated protein (Reimerdes and Lorenzen, 1983 and Grufferty and Mulvihill, 1991 and Kamal, 1998).

The emulsifying stability (ES) of all powdered samples was measured in a 30% o/w emulsion by monitoring changes in turbidity over time. $BMWPC_{7.5,\ 2.5\ and 4.5}$ was found to be the most effective at stabilizing the emulsion over a 60 min period, followed by BMCCP, TBMP, BMS, and BMAC (Table 5).Whey proteins form more stable emulsions than caseins (Phillips, 1981) presumably because surface films of whey protein are more viscous than those of caseins (Castle *et al.,* 1987) . The complexed whey protein in

BMCCP may be responsible for the greater stability of emulsion prepared from this protein compared to emulsion prepared from BMAC (Grufferty and Mulvihill, 1991) .All samples had higher ES at pH 8 than those at pH 3 and 5.

Table (6) illustrates the digestive action of trypsin on buttermilk solids and buttermilk protein preparations during 60 min. It is clear that the tryptic digestion of all powdered samples occurred, but of buttermilk whey protein concentrate powder ($BMWPC_{2.5}$) had the highest digestibility followed by $BMWPC_{7.5,\ 4.5}$, BMCCP, TBMP, BMS and BMAC. This is due to the used heat treatment during its preparation, which caused disassociation of proteins then increase of digestion rate (Hassan *et al*., 2002).

Table (5) : Emulsifying capacity (EC)* and stability (ES) of buttermilk solids and buttermilk protein powders .

pH Values	EC at time interval 0	10	20	30	40	50	60(min)	ES (min)
Buttermilk solids (BMS)								
3	0.543^{I}	0.526	0.453	0.332	0.206	0.132	0.105^{FGH}	37.19^{M}
5	0.373^{LM}	0.349	0.331	0.299	0.217	0.121	0.047^{GH}	34.33^{P}
8	1.803^{A}	1.711	1.653	0.936	0.888	0.780	0.728^{A}	50.32^{F}
Mean	0.906						0.293	40.61
Buttermilk casein-whey protein co-precipitated (BMCCP)								
3	0.457^{J}	0.453	0.447	0.401	0.365	0.285	0.113^{FGH}	39.85^{K}
5	0.361^{MNO}	0.344	0.329	0.284	0.154	0.111	0.053^{GH}	35.16^{N}
8	0.826^{D}	0.793	0.723	0.686	0.592	0.413	0.346^{CDE}	51.64^{D}
Mean	0.547						0.171	42.22
Total buttermilk protein (TBMP)								
3	0.366^{MN}	0.316	0.294	0.274	0.153	0.104	0.081^{FGH}	38.53^{L}
5	0.352^{NO}	0.344	0.331	0.267	0.158	0.101	0.050^{GH}	34.97^{NO}
8	0.806^{E}	0.717	0.674	0.601	0.543	0.457	0.332^{CDE}	51.01^{E}
Mean	0.508						0.154	41.50
Buttermilk acid casein (BMAC)								
3	0.349^{O}	0.318	0.295	0.187	0.134	0.099	0.047^{GH}	34.67^{OP}
5	0.272^{P}	0.255	0.205	0.151	0.126	0.072	0.025^{H}	33.04^{Q}
8	0.676^{G}	0.643	0.613	0.536	0.420	0.395	0.254^{DEFG}	48.06^{G}
Mean	0.432						0.109	38.59
buttermilk whey protein concentrate ($BMWPC_{7.5}$)								
3	0.808^{E}	0.664	0.623	0.558	0.429	0.315	0.266^{DEF}	44.72^{H}
5	0.423^{K}	0.381	0.373	0.351	0.266	0.198	0.120^{FGH}	41.88^{I}
8	1.202^{B}	1.103	1.043	0.864	0.772	0.704	0.678^{AB}	68.95^{A}
Mean	0.811						0.355	51.85
buttermilk whey protein concentrate ($BMWPC_{4.5}$)								
3	0.648^{H}	0.600	0.593	0.456	0.333	0.258	0.172^{EFGH}	40.84^{J}
5	0.389^{L}	0.359	0.334	0.214	0.196	0.110	0.085^{FGH}	38.39^{L}
8	0.918^{C}	0.869	0.777	0.686	0.553	0.479	0.453^{CD}	59.23^{C}
Mean	0.652						0.237	46.15
buttermilk whey protein concentrate ($BMWPC_{2.5}$)								
3	0.701^{F}	0.648	0.535	0.478	0.384	0.290	0.199^{EFGH}	41.89^{I}
5	0.410^{K}	0.399	0.376	0.298	0.225	0.181	0.107^{FGH}	40.59^{J}
8	0.923^{C}	0.887	0.849	0.716	0.657	0.572	0.494^{BC}	64.55^{B}
Mean	0.678						0.267	49.01

***EC : Expressed as optical density at 500 nm. LSD at 0.05% for EC of samples at 0 min = 0.01 and at 60 min = 0.12 ; LSD at 0.05% for EC of samples × pH at 0 min = 0.016 and at 60 min = 0.21 ; LSD at 0.05% for ES of samples = 0.24 ; samples × pH = 0.42 .**

Table (6) : Tryptic digestion* of buttermilk solids and buttermilk protein powders

Samples	Time of digestion (min) Zero	60	Hydrolysis* rate(%)
BMS	0.041	0.232	465.85^{E}
BMCCP	0.060	0.392	553.33^{C}
TBMP	0.043	0.265	516.28^{D}
BMAC	0.044	0.126	268.18^{F}
$BMWPC_{7.5}$	0.064	0.551	760.94^{B}
$BMWPC_{4.5}$	0.066	0.568	760.60^{B}
$BMWPC_{2.5}$	0.054	0.468	766.66^{A}
LSD at 0.05%		1.19	

***Expressed as optical density at 280 nm.**
****Rat of hydrolysis = O.D at zero min – O.D at 60 min / O.D at zero min × 100**

Patterns of electrophoresis were performed to buttermilk solids (BMS) and buttermilk protein preparations (Fig 1) .

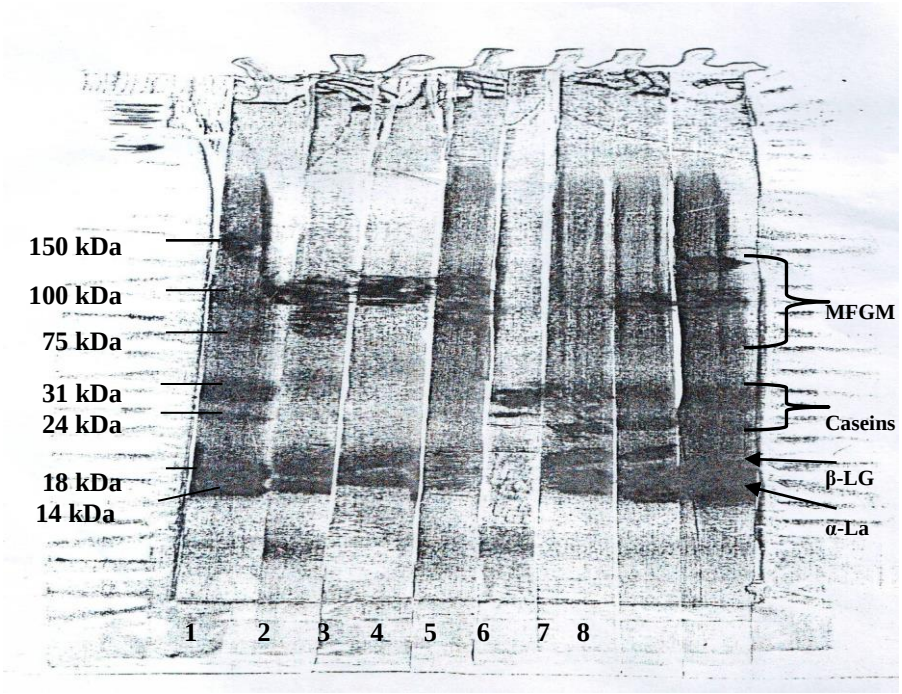

Fig: (1) SDS-PAGE of the BMS and BM protein powders. Lanes: 1= molecular weight standard ; 2 = $BMWPC_{2.5}$; 3 = $BMWPC_{7.5}$; 4 = $BMWPC_{4.5}$; 5 = BMAC ; 6 = TBMP ; 7 = BMCCP ; 8 = BMS

CONCLUSION

This study showed that buttermilk could be successfully used as a source of proteins, and can be used as functional ingredients in formulated food products. BMS was demonstrated to possess limited functional

properties beyond electroconductivity and solubility compared with BMWPC and BMCCP. The type, surface charges and hydrophobicity of protein present were closely related to the functional properties examined in this study. For instance, hydrophobicity of BMWPC enhanced FAC and emulsifying stability while, BMAC was less effective at forming and stabilizing o/w emulsion because of a decreased tendency to be absorbed onto the o/w interface. Inverse relationship between WHC and solubility was established in this study. BMAC had the greatest WHC and lowest solubility. Also, the presence of MFGM in BMS did not enhance the functional properties of BMS, except electroconductivity, over that observed for BMWPC, which it is expected that, has lower MFGM content. The ratio between the casein, whey protein and MFGM content in BMS likely determines its functional properties. In general, the functional properties of buttermilk proteins in food matrices are still worth further study.

REFERENCES

AACC. (1981). Method for water hydration capacity of plant protein material. 26: 291-293.

Abd El-Salam, M.H. and El-Shibiny, S.(1975). Preparation and properties of β-casein from buffaloe's milk. J. Dairy Res., 42: 163.

Ahmedna, M.; Prinyawiwatkul, W. and Rao, R. (1999). Solubilized weat protein isolate: Functional properties and potential food applications. J. Agric. Food Chem., 47: 1340.

American Dairy Products Institute. (1990). Standards for grades of dry milks,bulletin 916. AD PI, Chicago, IL.

Association of official Analytical Chemists. (1995). Official Methods of Analysis. 16_{th} ed. AOAC.Gaithersburg, MD.

Azzam, M.A.A. (2003). Production of low-calorie ice milk. J. Agric. Sci. Mansoura Univ., 28(12) : 8139.

Castle, J.; Dickinson, E.; Murray, B.S. and Stainsby, G. (1987). Mixed-protein films adsorbed at the oil-water interface. In: proteins at Interfaces: Physicochemical and Biochemical Studies, pp. 118-134, Brash, J.L., Horbett, T.A., eds. (ACS Symposium, Series No. 343) Washington DC: American Chemical Society.

Chansiri, G.; Lyons, R.T. ; Patel, M.V. and Hern, S.L. (1999). Effect of surface charge on the stability of oil/water emulsions during steam sterilization. J. Pharmaceutical Sciences, 88: 454.

Chobert, J.M.; Bertrand-Harb, C. and Nicolas, M.G. (1988). Solubility and emulsifying properties of caseins and whey proteins modified enzymatically by trypsin. J. Agric. Food Chem., 36: 883.

Corredig, M. and Dalgleish, D.G. (1998). Buttermilk properties in emulsions with soybean oil as affected by fat globule membrane-derived proteins. J. Food Sci., 63: 476.

Dalgleish, D.G. and Banks, J.M. (1991). The formation of complexes between serum proteins and fat globules during heating of whole milk. Milchwissenschaft, 46: 75.

Darewiez, M. (2001). Effect of enzymatic modification of beta-casein on its structure and some functional properties. Rozprawy-I- monographic-dissertations and monographs. No. 5-88.

Datta Roy, D.C. (1981). Proteolysis of buffaloe's milk by trypsin in vitro. Indian J. Dairy Sci., 34: 233.

de Wit, J.N. and Klarenbeek, G.K. (1984). Effects of various heat treatments on structure and solubility of whey proteins. J. Dairy Sci., 67: 2701.

El-Adawy, T.A. (2000). Functional properties and nutritional quality of acetylated and succinylated mung bean protein isolate. Food Chemistry, 70:83.

El-Sayed, S.M.; Hamed, I.M.; Asker, A.A. and Hamzawi, L.F. (2006). Plasma lipid profile of rats fed on processed chesse spread enriched with buttermilk concentrate. Egyptian J. Dairy Sci., 34: 169.

Fachin, L. and Viotto, W.H. (2005). Effect of pH and heat treatment of cheese whey on solubility and emulsifying properties of whey protein concentrate produced by ultrafiltration. International Dairy Journal, 15: 325.

Farrell, H.M., Jr. (1988). Physical equilibrium: Proteins. Page 461 in Fundamentals of Dairy Chemistry. N.P. Wong, R. Jenness, M. Keeny, and E.H. Marth, ed. Van Nostrand Reinhold Co., New York, NY.

Glover, F.A. (1985). Ultrafiltration and reverse osmosis for the dairy industry. Technical bulletin 5, The National Institute for Research in Dairying Reading, England.

Grufferty, M.B. and Mulvihill, D.M. (1991). Emulsifying and foaming properties of protein isolates prepared from heated milks. Journal of the Society of Dairy Technology. 44(1): 13.

Guzman-Gonzalez, M.; Morais, F. ; Ramos, M. and Amigo, L. (1999). Influence of skimmed milk concentrate replacement by dry dairy products in a low fat set-type yoghurt model system.I: Use of whey protein concentrates, milk protein concentrates and skimmed milk powde. J.Sci. Food Agric., 79: 1117.

Haque, Z.U. and Mozaffar, Z. (1992). Casein hydrolysate: 2. Functional properties of peptides.Food Hydrocolloids, 5: 559.

Hassan, Z.M.R.; Metwally, A.I. and Awad, R.A. (2002). Functional properties of enzymatically modified buffalo milk protein products. Annals Agric. Sci., 47(2): 551.

International Dairy Federation. (2002). World Dairy Situation. FIL-IDF, Brussels, Belgium.

Kamal, N.M. (1998). Comparative studies on buffalo, cow and camel casein1. Effect of heat treatments on some functional properties of casein. J. Agric. Sci. Mansoura Unvi., 23(1): 339.

Kanno, C. (1989). Emulsifying properties of bovine milk fat globule membrane in milk fat emulsion: Condition for the reconstitution of fat globules. J. Food Sci., 54: 1534.

Keenan, T.W. and Dilewski, D.P.(1995). Intracellular origin of milk lipid globules and the nature and structure of the milk lipid globule membrane. Advanced Dairy Chemistry – 2. lipids, P.F. Fox, (Ed), p. 89-130. Chapman & Hall, London.

Kim, J.C. (1998). Milk protein stainless steel interaction relevant to the initial stage of fouling in thermal processing. J. Food Process Engineering, 21(5): 369.

Kinsella, J.E. (1976). Functional properties of proteins in foods: A survey. CRC Crit. Rev. Food Sci. Nutr., 7: 219.

Kinsella, J.E. and Fox, P.F. (1987). Water sorption by milk proteins. Bulletin of the InternationalDairy Federation, 209: 12.

Lee, D.N. ; Moor, E.E. and Merson, R.L. (1975). Electrophoresis of cottage cheese whey proteins and their polymers . J. Dairy Sci. 58:658.

Lin, C.S. and Zayas, J.F. (1987). Functionality of defatted corn germ proteins in a model system:fat binding capacity and water retention. J.Food Sci., 52: 1308.

Mabrook, M.F. and Pettry, M.C. (2003). Effect of composition on the electrical conductance of milk. J. of Food Engineering 60: 321.
Mann, B. and Malik, R. (1996). Studies on some functional characteristics of whey protein-poly saccharide complex. J. Food Sci. and Technology, 33(3): 202.
Marshall, R.T. (1992). Standards methods for the examination of dairy products, 16th Ed. The American Public Health Association Inc., Washington, DC.
Metwally, A.I. (1997). Comparative study between functional properties of spray dried buffalo's ulterafiltrated skim milk retentate and respective total milk proteinate . Annals Agric. Sci. Ain Shams Univ., Cairo, Egupt. 42(1): 313.
Mine, Y. (1995). Recent advances in understanding off egg white protein functionally. Trends in Food Sci. and Technology, 6(7): 225.
Modler, H.W. and Harwalkar, V.R. (1981). Whey protein concentrate prepared by heating under acidic conditions. 1. Recovery by ulterafiltration and functional properties. Milchwissen- schaft, 36: 537.
Morr, C.V. (1985). Functionality of heated milk proteins dairy and related food. J. Dairy Sci., 68: 2773.
Morr, C.V. (1989). Whey proteins: Manufacture. Developments in Dairy Chemistry-4, Functional Milk Proteins. Elsevier Applied Sci., London, England.
O'Connell, J.E. and Fox, P.F. (2000). Heat stability of buttermilk. J. Dairy Sci., 83: 1728.
Parris, N.; Purcell, J.M. and Patshkin, S.M. (1991). Thermal denaturation of whey proteins in skim milk. J. Agric. Food Chem. 39: 2167.
Pearce, K.N. and Kinsella, J.E. (1978). Emulsifying properties of proteins evaluation of turbiditimetric technique. J. Agric. Food Chem.,26: 716.
Philips, M.C. (1981). Protein conformation at lipid interfaces and its role in stabilizing emulsionsand foams. Food Technology, 35: 50.
Raval, D.M. and Mistry, V.V. (1999). Application of ultrafiltered sweet buttermilk in the manufacture of reduced fat process cheese. J. Dairy Sci., 82: 2334.
Reimerdes, E.M. and Lorenzen, P.C. (1983). New results about milk protein emulsifiers. In: Physicochemical Aspects of Dehydrated protein-rich Milk products, pp 70-93. proceedings of the IDF Symposium, Helsingor, Denmark.
Roesch, R.R.; Rincon, A. and Corredig, M. (2004). Emulsifying properties of fractions prepared from commercial buttermilk by microfiltration. J. Dairy Sci., 87: 4080.
Scott, L.L.; Duncan, S.E.; Sumner, S.S.; Waterman, K.M. and Kaylegian, K.E. (2003). Influence of emulsifying component composition on creams formulated with fractionated milk fat. J.Agric. Food Chem. 51: 5933.
Shazly, A.B.; Mahran, G.A.; El-Senaity, M.H., Abd El-Aziz, M. and Fatouh, A.E. (2008). Improving low-fat processed chesse spread using whey protein concentrate or buttermilk curd. Egyptian J. Dairy Sci., 36: 83 .
Sodini, I.; Morin, P.; Olabi, A. and Jimenez-Flores, R. (2006).Compositional and functional properties of buttermilk: A Comparison between sweet sour and whey buttermilk. J. Dairy , Sci., 89: 525.
Steel, R.G.D. and Torrie, J.H. (1980). Principle and procedures of statistics. A-biometrical approach.2nd,ED Mc Graw-Hill Book Co.,New York.
St-Gelais, D.; Champagne, C.; Erepmoc, F. and Audet, P. (1995). The use of electricalconductivity to follow acidification of dairy blends. International Dairy J. 5: 427.

Susheelamma, N.S. and Rao, M.V.L. (1974). Surface-active principle in black gram (*Phaseolasmungo*) and its role in the texture of leavened foods containing legume. J. Sci. Food Agric.,25: 665.
Therdhai, N. and Zhou, W. (2001). Artificial neural network modeling of the electrical conductivity properties of recombined milk. J. Food Engineering 50: 107.
Thompson, L.U. and Reyes, E.S. (1980). Modification of heat coagulated whey protein concentrates by succinylation. J. Dairy Sci., 63: 715.
Turcot, S.; Turgeon, S.L. and St-Gelais, D. (2001). Effect of buttermilk phospholipids concentrations in cheese milk on production and composition of low fat Cheddar cheese. Lait. 81:429.
Vani, B. and Zayas, J.F. (1995). Weat germ protein flour solubility and water retention. J. Food Sci., 60: 845.
Voutsinas, L.P. and Nakai, S. (1983). A simple turbidimetric method for determining the fatbinding capacity of proteins. J. Agric. Food Chem. 31: 58.
Vyas, H.K.; Astaire, J.C. and Jimenez-Flores, R. (2002). Buttermilk fractionation by microfiltration. J. Dairy Sci., 85 : 1639.
Walstra, P.; Geurts, T.J.; Noomen, A.; Jellema, A. and van Boekel, M.A.J.S. (1999).DairyTechnology: Principles of milk properties and processes. Marcel Dekker, New York, NY.
Wong, P.Y.Y. and Kitts, D.D. (2003). A comparison of the buttermilk solids functional properties to nonfat dried milk, soy protein isolate, dried egg white, and egg yolk powders. J. Dairy Sci., 86: 746.
Wong, W.S.; Camirond, W.M. and Pavlath, A.E. (1996). Structures and functionality of milk proteins. Critical Reviews in Food Sci. and Nutrition, 36(8): 807.
Zhuang, Y.; Zhou, W. ; Ngayen, M.H. and Hourigan, J.A. (1997). Determination of prolein content of whey powder using electrical conductivity measurement. Indian Dairy J. 7: 647.

الخواص التركيبية والوظيفية لبروتينات لبن الخض

فاطمه على متولى رمضان ، إسماعيل حسين إسماعيل عبد الغنى و محمد أحمد عزام
قسم علوم الألبان – كلية الزراعة – جامعة القاهرة

يهدف هذا البحث الى دراسة التركيب الكيماوى و الخواص الوظيفية لجوامد و بروتينات لبن الخض.

وقد دلت النتائج على ما يلى :

- تميزت عينات جوامد لبن الخض عن بروتيناتة المحضرة منه بارتفاع نسبة كل من الدهن و الرماد و اللاكتوز و انخفاض محتواها من البروتين.
- زيادة درجة ذوبان جوامد لبن الخض و درجة التوصيل الكهربى لمحاليلها عن محاليل البروتينات الاخرى.
- تميزت بروتينات شرش لبن الخض بارتفاع قدرتها على الأرتباط بالدهن (٢,٥ جرام دهن / جرام بروتين) و مقدرتها الإستحلابيه العالية (OD ٠,٤٠٠ – ٠,٨٠٠) وارتفاع ثبات المستحلب (min ٣٨ ـ ٤٤) عند pH ≥ ٥ بالإضافة إلى أرتفاع معدل هضمها بأنزيم التربسين عن جوامد لبن الخض و المستحضرات البروتينية الاخرى .

و من هذة الدراسة يمكن استخدام بروتينات لبن الخض فى توليفات غذائية مختلفة.