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Recovery of milk fat globule membrane (MFGM) from buttermilk: effect of Ca-binding salts

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Research Article

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Abstract

In this Research Communication we present a study of the effect of Ca-binding salts on the recovery of milk fat globule membrane (MFGM) from buttermilk. Sodium phosphate buffer was used for the purpose of MFGM recovery from buttermilk for the first time and we showed that 0.1 M buffer at pH 7.2 was the most effective for the recovery of MFGM. The fact of high efficacy of sodium phosphate buffer in recovery of MFGM from buttermilk allowed us to suggest that MFGM in buttermilk is present in association with casein through Ca- bridges formed between phospholipids of MFGM and phosphate groups of casein, primarily with k-casein as the peripheral protein of casein micelles.

Buttermilk, which is a relatively inexpensive and widely available by-product of butter production, is a rich source of milk fat globule membrane (MFGM), a milk component which can be considered as a valuable nutraceutical (Spitsberg, 2005). In recent years new findings about health benefits of MFGM have been published (Snow *et al.*, 2010; Fuller *et al.*, 2013; Billeaud *et al.*, 2014; Hernell *et al.* 2016). Given a sustained interest in MFGM as a nutraceutical, the isolation of large amounts of this milk component is important. For obtaining viable amounts of MFGM from buttermilk Corredig *et al.*, 2003 proposed microfiltration of buttermilk in the presence of sodium citrate which causes dissociation of casein micelles and thereby provides better conditions for the isolation of MFGM fraction. Below we present the recovery of MFGM from buttermilk by using sodium phosphate buffer in comparison with sodium citrate and other Ca-binding compounds.

Materials and Methods

Fresh industrial buttermilk was provided by the butter producing facilities of Yosef Tal Factory (Afula, Israel). All chemicals were analytical grade, obtained from Aldridge-Sigma (Israel) and Merck (Germany).

SDS-PAGE

The 12% SDS-PAGE and Western blotting were done as described by Spitsberg *et al.* (1995). The quantification of protein was determined with Bradford Reagent (Bio Rad, USA) according to the protocol provided by the vendor.

Differential centrifugation

Differential centrifugation was performed using a Stratos (Germany) centrifuge at low temperature 2–4 °C. In the preliminary experiments we demonstrated that the MFGM from bovine milk was completely recovered in the sedimented pellet at 32 000 × ***g*** for 60 min using the angle rotor with 15 ml tubes.

Isolation of MFGM fraction from buttermilk

The pH of 0.5 l of buttermilk was adjusted to 4.6 with diluted HCl. The mixture was left for 30 min at room temperature and then was cooled to 2–4 °C and centrifuged at 10 000 × ***g*** at 2–4 °C for 15 min to collect the co-precipitated casein and MFGM. The 10 000 × ***g***-supernatant (first supernatant) was subjected to centrifugation at 32 000 × ***g*** for 1 h to collect MFGM not precipitated with casein and still present in the solution. The co-precipitated casein and MFGM was mixed with one of a number of different Ca-binding salts (listed in Table 1) in ratio 1 to 10 volume to volume. After that the mixture was centrifuged at 2000 × ***g*** at 2–4 °C for 15 min and the 2000 × ***g***-supernatant was centrifuged at 32 000 × ***g*** at 2–4 °C for 1 h to get the pellet of MFGM. The pellet of MFGM was collected and suspended in 5 ml of water and after that the protein concentration was measured with Bradford reagent

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Table 1. Effect of Ca-binding salts on recovery of MFGM from buttermilk

	Treatment	MFGM/100 ml BM (mg)
1	First supernatant	1.9
2	Na-citrate 0.1 M, pH 7.1	23.4
3	Na-oxalate 0.1 M, pH 7.1	10.67
4	Calgon (sodium hexametaphosphate) 0.1 M, pH 7.1 [b]	72.49
5	Na-phosphate 0.1 M, pH 7.2	78.9
6	Na-phosphate 0.1 M, pH 4.6	1.53
7	Na-phosphate 0.1 M, pH 5	1.64
8	Na-phosphate 0.1 M, pH 6	2.2
9	Na-phosphate 0.1 M, pH 7.4	32.44
10	Na-phosphate 0.1 M, pH 7.8	25.26
11	NaCl 0.1 M, pH 7.2	nd
12	Na_2SO_4 0.1 M, pH 7.2	nd

BM, buttermilk; nd, not detected.

and the final result was normalized to 100 ml of buttermilk. This suspension which, as we suggested, represents the recovered MFGM was analyzed by 12% SDS-PAGE.

Results

SDS-PAGE and immuno-blotting of the isolated MFGM

Judgment on the presence of MFGM in a sample of buttermilk was based on the specific position of certain proteins in gel after running 12% SDS-PAGE (Fig. 1). These proteins are xanthine oxidase (XO), glycoprotein CD36, butyrophilin (BTN), and fatty-acid-binding protein (FABP) (Spitsberg *et al.*, 1995; Mather, 2000; Spitsberg, 2005). Analysis of the MFGM fraction clearly showed the presence of XO, CD36, BTN, and FABP (Fig. 1, right-hand side). In addition, the immune-blot (Fig. 1, left-hand side) revealed the presence of FABP in the MFGM isolated from buttermilk. There was no essential difference in protein composition between MFGM obtained with the different Ca-binding salts which we used.

Recovery of MFGM

The pellet of MFGM from the first supernatant contained only 1.9 mg of protein per 100 ml of buttermilk, suggesting that most MFGM was co-precipitated with casein. Treatment of the precipitate of casein-MFGM with the different Ca-binding salts (Table 1) showed that sodium phosphate buffer 0.1 M at pH 7.2 was the most effective in the release of MFGM from the precipitate providing 78.9 mg MFGM/100 ml of buttermilk. Polyphosphate Calgon 0.1 M produced 72 mg MFGM/100 ml of buttermilk at pH 7.1. Treatment of the precipitate with sodium citrate 0.1 M at pH 7.1 produced only 24.6 mg MFGM/100 ml and with sodium oxalate 0.1 M at pH 7.1 even less, 10.56 mg/100 ml of buttermilk. Sodium phosphate 0.1 M at pH 4.6 yielded 1.53 mg MFGM/100 ml of buttermilk. Sodium chloride and sodium sulfate were not effective in the recovery of MFGM from the precipitate.

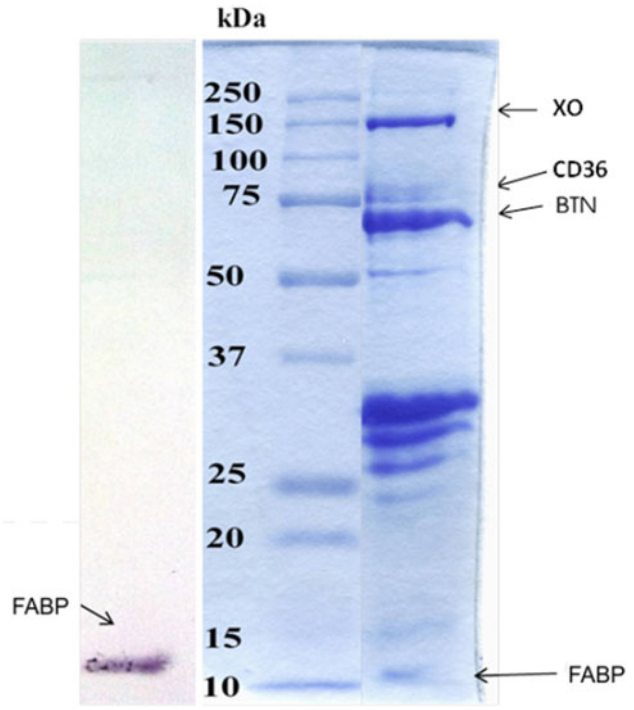

Fig. 1. 12% SDS – PAGE of MFGM recovered from buttermilk. On the right: gel electrophoresis, left lane is protein standard, right lane is MFGM isolated from buttermilk with sodium phosphate buffer at pH 7.2. On the left: immuno-blot of the part of the gel presented on the right side; rabbit polyclonal antibody to FABP was used as described by Spitsberg *et al.* (1995). XO, xanthine oxidase; CD36, glycoprotein CD36; BT, butyrophilin; FABP, fatty-acid-binding protein.

Discussion

Recovery of MFGM from the mixture of precipitated casein and precipitated MFGM using Ca-binding salts demonstrated that the most effective in recovery was sodium phosphate buffer at pH 7.2 with the yield of 78.9 mg MFGM per 100 ml of buttermilk, followed by polyphosphate, Calgon, with the yield at pH 7.1 of 72.49 mg MFGM per 100 ml of buttermilk. The pH 7.1–7.2 was optimal for the recovery of MFGM, and its shift to alkaline or acidic side led to reduction of the recovery of MFGM. Here it is worthwhile to mention that Holzmuller and Kulozik (2016) using a new analytical method, SDS-PAGE non-stain method, found that buttermilk contains, by protein, 90 mg MFGM per 100 ml of buttermilk, and our best results are quite close to this value.

Our findings may suggest that in buttermilk casein and MFGM form a complex *via* Ca – bridges between phosphorylated casein and phosphate of phospholipids of MFGM. Casein is present in milk and buttermilk as micelles (Dalgleish, 1998). These micelles are colloidal particles formed by casein aggregates wrapped up in soluble κ-casein molecules. Calcium, naturally present in casein micelle (Holt *et al.*, 1986), can form a complex between MFGM and casein micelle through its binding to phospho-casein and phospholipids of MFGM. In the presence of sodium phosphate buffer the assembly of MFGM – casein micelle can dissociate into its components, especially at pH 7.2–7.4. At this pH the ion $(HPO_4)^{2-}$ is predominant in the solution and it can play a main competitive role in withdrawing Ca from the assembly of MFGM and Ca-casein: interaction of $(HPO_4)^{2-}$ with Ca leads to the formation of a hardly soluble compound

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$CaHPO_4$. Reduction of recovery of MFGM at pH 8 can probably be explained by non-favorable interaction of $(HPO_4)^{2-}$ with the highly negatively charged complex casein – MFGM. At pH values below 7 the major phosphate ion in phosphate buffer is $(H_2PO_4)^{-}$. This ion is not a strongly competitive binder of Ca because Ca $(H_2PO_4)^2$ is a compound that is quite soluble in water.

We think that Ca-bridges may play a significant role in the interaction between casein and milk fat globules (MFG) in the process of natural lactation that favors the exocytosis of milk fat globule.

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References

Billeaud C, Puccio G, Salida E, Guillois B, Vaysse C, Pecquet S and Steenhout PH (2014) Safety and tolerance evaluation of milk fat globule membrane-enriched infant formulas: a randomized controlled multicenter non-inferiority trial in healthy term infants. *Clinical Medicine Insights: Pediatrics* **8**, 51–60.

Corredig M, Roesch RR and Dalgleish DG (2003) Production of a novel ingredient from buttermilk. *Journal of Dairy Science* **86**, 2744–2750.

Dalgleish DG (1998) Casein micelles as colloids: surface structures and stabilities. *Journal of Dairy Science* **81**, 3013–3018.

Fuller KL, Kuhlenschmidt TB, Kuhlenschmidt MS, Jimenez-Flores R and Donavan R (2013) Milk fat globule membrane isolated from buttermilk or whey cream and their lipid components inhibit infectivity of rotavirus in vitro. *Journal of Dairy Science* **86**, 2725–2743.

Hernell O, Timby N, Domellöf M and Lönnerdal B (2016) Clinical benefits of milk fat globule membranes for infants and children. *Journal of Pediatrics* **173**, 60–65.

Holt C, Davis DT and Law AJR (1986) Effects of colloidal calcium phosphate content and free calcium concentration in the milk serum on the dissociation of bovine casein micelles. *Journal of Dairy Research* **53**, 557–572.

Holzmuller W and Kulozik U (2016) Quantification of MFGM proteins in buttermilk and butter serum by means a stain free SDS-PAGE method. *Journal of Food Composition and Analysis* **69**, 102–109.

Mather IM (2000) A review and proposed nomenclature for major proteins of the milk-fat globule membrane. *Journal of Dairy Science* **83**, 203–247.

Snow DR, Jimenez-Flores R, Ward RE, Cambell J, Young MJ, Nemere I and Hintze KJ (2010) Dietary milk fat globule membrane reduces the incidence of aberrant crypt foci in Fischer-344 rats. *Journal of Agricultural and Food Chemistry* **58**, 2157–2163.

Spitsberg VL (2005) *Invited review*: Bovine milk fat globule membrane as a potential nutraceutical. *Journal of Dairy Science* **88**, 2289–2294.

Spitsberg VL, Matitashvili E and Gorewit RC (1995) Association of fatty acid binding protein and glycoprotein CD36 in the bovine mammary gland. *Journal of Biochemistry* **230**, 872–878.

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