

## **Evidence of association of milk fat globule membrane with protein matrix in dairy gels as revealed by confocal microscopy**

**Nathanon Trachoo**

### **Abstract**

Trachoo, N.

**Evidence of association of milk fat globule membrane with protein matrix in dairy gels as revealed by confocal microscopy**

Songklanakarin J. Sci. Technol., 2003, 25(6) : 791-797

Consumer needs for low caloric and reduced fat foods has increased in Thailand. Cholesterol in milk fat has been a drawback for milk products. Reduction of fat results in undesirable effects in some products. To reduce fat in yogurt, total solid is lowered to 9-10%. This adversely affects physical and sensory properties of yogurt. Syneresis, weak body, lack of flavor and poor texture and mouthfeel are common defects of nonfat and lowfat yogurt. Our previous study has shown that yogurt made from milk with high milk fat globule membrane (FGM) had less whey separation. The objective of this study was to investigate the association of FGM on protein matrix in dairy gels using confocal microscopy. Sweet buttermilk contains a large amount of FGM. A model system was used by addition of glucono- $\delta$ -lactone into yogurt mixes. When directly acidified, membrane-like materials were observed under the microscope. They functioned as bridges linking protein matrices together resulting in a more defined protein network. This suggested that FGM has a potential to improve textural properties of yogurt.

---

**Key words** : milk fat globule membrane, yogurt, confocal microscopy, protein matrix

---

Ph.D.(Food Science and Technology), Department of Food Technology and Nutrition, Faculty of Technology, Mahasarakham University, Mahasarakham 44000 Thailand

E-mail: nathanon.t@msu.ac.th

Received, 18 February 2003      Accepted, 27 August 2003

## บทคัดย่อ

ณัฐนนท์ トラชู

หลักฐานความสัมพันธ์ของเยื่อหุ้มเม็ดไขมันนมกับโครงร่างโปรตีนในเจลของนํ้านม:  
แสดงได้จากกล้องจุลทรรศน์แบบคอนโฟคอล

ว. สงขลานครินทร์ วทท. 2546 25(6) : 791-797

ความต้องการอาหารที่มีแคลอรีและไขมันต่ำของคนไทยกำลังมีเพิ่มมากขึ้น คอเลสเตอรอลในไขมันจากนํ้านมเป็นข้อเสียสำคัญของการบริโภคนํ้านมและผลิตภัณฑ์นม การลดไขมันในนํ้านมลงส่งผลเสียต่อการผลิตและคุณภาพของผลิตภัณฑ์บางชนิด โยเกิร์ตไขมันต่ำมักจะประกอบด้วยของแข็งทั้งหมดประมาณ 9-10% ซึ่งจะทำให้โยเกิร์ตที่ได้มีคุณภาพทางกายภาพและประสาทสัมผัสต่ำลง การแยกชั้นของเวย์ เนื้อสัมผัสที่นุ่มเกินไป และกลิ่นรสที่ด้อยลงเป็นข้อเสียที่มักพบบ่อยในโยเกิร์ตที่มีไขมันต่ำ จากการศึกษาที่ผ่านมาพบว่าโยเกิร์ตไขมันต่ำที่ผลิตด้วยนมที่มีเยื่อหุ้มเม็ดไขมันสูงจะเกิดการแยกชั้นของเวย์ลดลง วัตถุประสงค์ของงานวิจัยต้องการศึกษาความสัมพันธ์ของเยื่อหุ้มเม็ดไขมันในนมกับโครงข่ายโปรตีนในเจลของโยเกิร์ตเทียม โดยใช้กล้องจุลทรรศน์แบบคอนโฟคอล ทางเนยเหลวที่ผลิตจากการผลิตเนยเหลวมีเยื่อหุ้มเม็ดไขมันสูง จากผลการทดลองพบว่าเยื่อหุ้มเม็ดไขมันในเจลที่ผลิตจากทางเนยเหลวที่ผลิตจากการผลิตเนยเหลวทำหน้าที่เชื่อมต่อสายของโครงข่ายโปรตีน และอาจส่งผลให้เกิดลักษณะสัมผัสที่ดีขึ้นในโยเกิร์ต

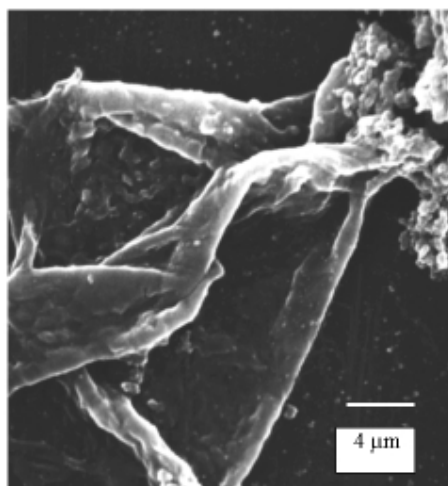
ภาควิชาเทคโนโลยีการอาหารและโภชนาศาสตร์ คณะเทคโนโลยี มหาวิทยาลัยมหาสารคาม อำเภอเมือง จังหวัดมหาสารคาม 44000

The previous studies (Kalab, 1980; Tamime *et al.*, 1989; Tamime *et al.*, 1991) demonstrated the presence of milk fat globule membrane (FGM) in protein matrix of yogurt. Part of FGM is protein and some other trace materials are phospholipids. These proteins may be incorporated in protein matrix of yogurt (Aguilera and Kinsella, 1991, Corredig and Dalgleish, 1996) and may help improve texture (Trachoo and Mistry, 1998). Phospholipids such as sphingomyelin were believed to be tumor suppressor fats (Parodi, 1996). Normally sphingomyelin and other phospholipids account for 0.2-1.0% of the total lipids in milk, where they are associated with FGM. During milk processing, especially homogenization, this membrane is disrupted and the phospholipids relocate into aqueous phase. Sweet buttermilk is a rich source of FGM (Christie *et al.*, 1987), therefore it can be a potential raw material for yogurt manufacturing.

According to our previous study which the microstructure of yogurt made from skim milk fortified with sweet buttermilk powder and ultra-

filtered sweet buttermilk was investigated, it was found that there were some membrane-like materials associated with the yogurt protein matrices (Trachoo and Mistry, 1998). By observing under a scanning electron microscope (SEM), the size of the membrane-like materials was much bigger than casein micelles (Figure 1). Unfortunately, SEM did not allow us to observe the membranous materials underneath the surface of the specimen because SEM lacks such ability. Confocal laser scanning microscope (CLSM) was used in the studies of yogurt microstructure by Hassan and Frank (Hassan and Frank, 1997, Hassan *et al.*, 1995). With CLSM, non-invasive serial optical sectioning of intact or even living specimens can be obtained without out-of-focus blur (Sheppard and Shotton, 1997).

The objective of this research was to investigate the association of FGM with protein matrix of dairy gels made from different dairy ingredients (sweet buttermilk, sweet buttermilk powder, nonfat dry milk, whole milk and skim milk) using CLSM.



**Figure 1. Photomicrograph of membranous material in yogurt at 5,000 magnification**

#### Materials and Methods

Sweet buttermilk powder was obtained from Wisconsin Whey International Inc., Madison, WI. Cream was obtained from raw milk by centrifugation. The cream was then churned to separate out sweet buttermilk, which was used for yogurt production. Low heat nonfat dry milk (NDM), sweet buttermilk powder, whole milk and skim milk were commercially obtained.

#### Yogurt sample preparation

The yogurt mixes made with sweet buttermilk, sweet buttermilk powder, skim milk, whole or nonfat dry milk contained 10-11% total solids.

Table 1 shows the composition of raw materials. The mixes were added with Nile red (10 g/L) (Sigma Chemical Co., St. Louis, MO) solution (0.7 ml/L milk) and 2% (wt/vol) glucono- $\delta$ -lactone (Sigma Chemical Co., St. Louis, MO) and incubated in a glass chamber at 40°C for 4.5 hr, then cooled to room temperature.

#### Confocal scanning microscopy

The gel samples were observed using an MTC-600 confocal scanning laser microscope (Bio-Rad Inc., Hertfordshire, England) with 60 times oil immersion objective (1.4 numerical aperture) and an Ar/Kr laser (BioRad Inc.) using two different modes of operation. Stained fat

**Table 1. Composition<sup>1</sup> of raw materials used for gel preparation**

Raw material	Total Solid (%)	Total Proteins (%)	Fat (%)	Ash (%)	Carbohydrates <sup>2</sup> (%)
Nonfat dry milk	96.83	36.22	1.03	7.91	51.67
Whole milk	12.34	3.32	3.65	0.64	4.73
Skim milk	9.81	3.27	0.11	0.81	5.62
Sweet butter milk	9.86	3.51	0.21	0.91	5.23
Sweet butter milk powder	95.83	31.45	7.43	6.93	50.02

<sup>1</sup>Mean values of sample duplicates.

<sup>2</sup>Calculated by difference.

globules and phospholipids were observed under fluorescence mode with 568 nm excitation wavelength and the normal microstructure of yogurt protein matrices were studied using reflectance mode (Hassan and Frank, 1997). Pictures generated with the two different modes were captured and saved onto a Zip<sup>®</sup> disk for further processing. Pictures of stained fat materials and yogurt protein matrix were combined using computer software, Confocal Assistant version 3.10.

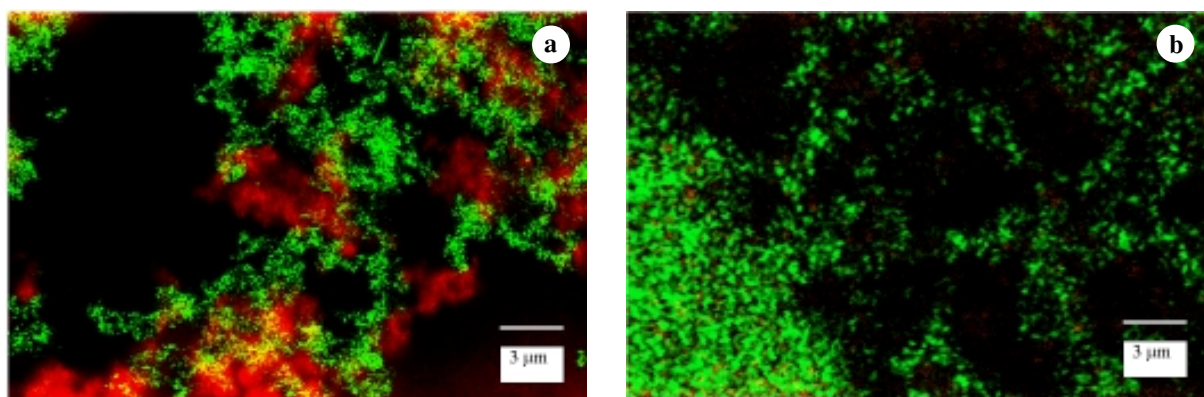
### Results and Discussions

Gel formation was achieved by addition of glucono- $\delta$ -lactone to yogurt mixes and incubation at 40°C for 4.5 h allowing glucono- $\delta$ -lactone to be hydrolyzed into solution to yield gluconic acid (Hashizume and Sato, 1988). This simulates the gel formation in yogurt (Hassan *et al.*, 1995) without using starter culture, thus reducing the problem of starter culture contamination. Nile red was previously used to stain fat globule in milk (Hassan and Frank, 1997). In this study, fading of Nile Red stain was a major problem during the confocal laser scanning operation for all samples especially in lower fat content yogurts. Capturing a picture in fluorescence mode first and then in reflectance mode reduced the problem. In some cases where the samples were low in fat content such as those made from skim milk, NDM or sweet buttermilk powder, time required to work with these samples before capturing had to be minimum. This suggests that anti-fading agent may be needed for long exposure of the laser beam. Also, it is possible to use some other dyes for staining FGM.

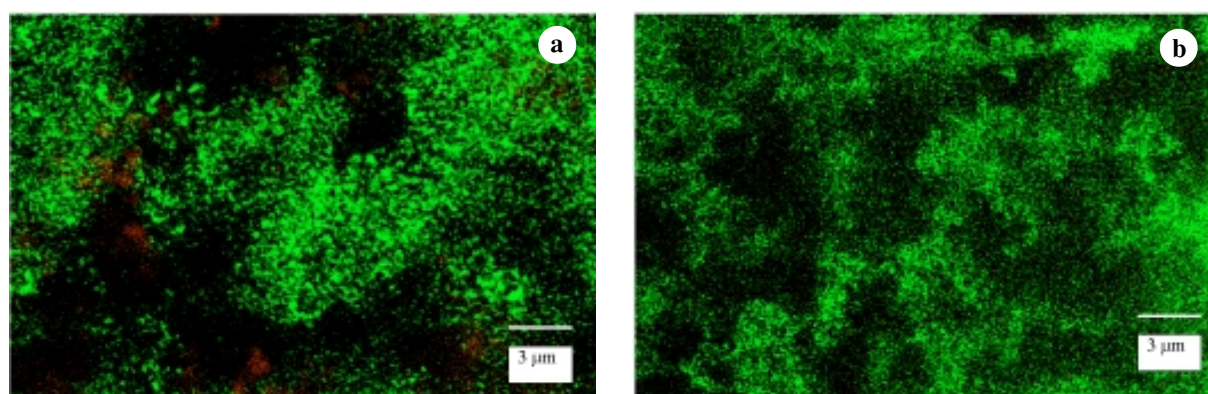
To differentiate protein matrix from the fat materials in the gels, artificial colors, green and red were assigned to protein matrix and fat materials, respectively. Pictures were taken at magnification of 3,000 with the help of digital zoom. The stained fat materials in gel samples made from raw materials from which most fat was removed, such as nonfat dry milk, sweet buttermilk and sweet buttermilk powder, are most likely to be FGM or part of FGM (phospholipids), thus these images

indicate the location of FGM and the association of FGM with protein matrix.

Gel samples made with sweet buttermilk apparently had a large amount of fat materials (Figure 2a) although most fat was previously removed. These fat-containing materials seemed to connect protein chains together providing an improved protein network. This may be due to the protein in FGM on milk fat globules being capable of binding to casein micelles during acid gel formation (Aguilera and Kinsella, 1991) resulting in a stronger gel. A research result showed that fat globules coated with protein participate in milk gel formation (Aguilera and Kessler, 1988, Aguilera and Kinsella, 1991). Ultrafiltered sweet buttermilk, which also contains large amount of free FGM, was used to make yogurt with low syneresis and firm texture (Trachoo and Mistry, 1998). Gel sample made with sweet buttermilk powder had lower fat materials compared to acidified sweet buttermilk gel (Figure 2b). This result corresponds with the work of Christie and co-workers (Christie *et al.*, 1987) who reported that they did not detect phospholipids in sweet buttermilk powder although they found are increased level of phospholipids in sweet buttermilk. Most fat materials in samples made from sweet buttermilk powder did not link protein network together (Figure 2b). Casein micelles in this sample were closely surrounded by fat materials. Heat treatment during the process of sweet buttermilk powder manufacture caused whey protein incorporation into FGM (Lee and Sherbon, 2002) and denaturation (Kinsella and Whitehead, 1989). When gel formation occurred after the addition of glucono- $\delta$ -lactone, FGM (if it exists) and casein are thus likely to co-precipitate to form the gel matrix. In other words, FGM in sweet buttermilk powder were not available to freely bind to casein micelles in the matrix, unlike those found in the sample made with liquid sweet buttermilk. The amount of fat containing materials in this acidified gel from sweet buttermilk powder was far less than in that made from sweet buttermilk. This may be due to the drying process during the manufacture of the sweet buttermilk powder. Atomization and heat treatment may break down



**Figure 2.** Optical thin sections of acidified gels made from sweet buttermilk (a) and sweet buttermilk powder at 3,000 magnification (b)



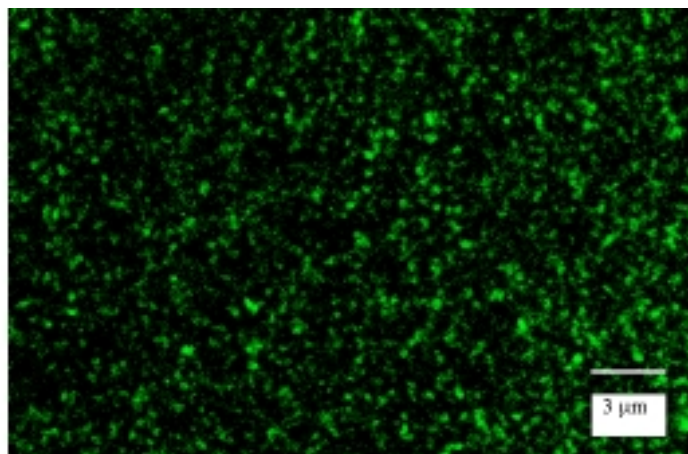
**Figure 3.** Optical thin sections of acidified gels made from whole milk (a) and skim milk (b) at 3,000 magnification

FGM into small pieces. Samples prepared from whole milk contained fairly small fat materials which looked like dots in the picture (Figure 3a). This is due to homogenization which breaks down the fat globules into smaller sizes. After homogenization, casein protein in milk absorbed onto fat globules and may act as a binder between these small fat globules (Darling and Butcher, 1978). Therefore, the small fat globules with casein on their surface acting as casein micelles or sub-micelles may aggregate together during casein precipitation. Also, whey protein was believed to absorb to the fat serum interface during homogenization (Lee and Sherbon, 2002). This may be the reason why yogurt made from whole milk had

a better mouthfeel and less whey separation. Fat materials were hardly found in samples prepared with NDM or skim milk (Figures 3b and 4). This was because of the low fat content in the skim milk and NDM. Although sweet buttermilk and sweet buttermilk powder contains as little fat as skim milk and NDM, they contain large amounts of FGM which, as shown in this experiment, binds or links to protein matrix in acidified gels, and potentially in yogurt as well.

### Conclusions

The associations of fat materials with protein matrices in acidified gels prepared with sweet



**Figure 4. Optical thin sections of acidified gel made from nonfat dry milk at 3,000 magnification**

buttermilk, sweet buttermilk powder, whole milk, skim milk or NDM were studied using confocal laser scanning microscopy. It was found that gel prepared with sweet buttermilk made from un-homogenized cream contained a large amount of membranous material. This membrane-like material may function as bridges linking protein matrix together. In whole milk, homogenization may contribute to the reformation of fat globules; therefore the fat materials may associate with protein matrix in different ways from that found in gel prepared with sweet buttermilk. Samples made with skim milk and NDM contained less fat material, therefore the association of fat materials with protein matrices was not significant.

#### References

- Aguilera, J.M., and Kessler, H.G. 1988. Physicochemical and rheological properties of milk fat globules with modified membranes. *Milchwissenschaft*, 43: 410.
- Aguilera, J.M., and Kinsella, J.E. 1991. Compression strength of dairy gels and microstructural interpretation. *J. Food Sci.*, 56: 1224-1228.
- Christie, W.W., Noble, R.C., and Davies, G. 1987. Phospholipids in milk and dairy products. *J. Soc. Dairy Technol.*, 40: 10-12.
- Corredig, M., and Dalgleish, D.G. 1996. Effect of different heat treatments on the strong binding interactions between whey proteins and milk fat globules in whole milk. *J. Dairy Res.*, 63: 441-449.
- Darling, D.F., and Butcher, D.W. 1978. Milk-fat globule membrane in homogenized cream. *J. Dairy Res.*, 45: 197-208.
- Hashizume, K., and Sato, T. 1988. Gel forming characteristics of milk proteins 1. Effect of heat treatment. *J. Dairy Sci.*, 71: 1439.
- Hassan, A.N., and Frank, J.F. 1997. Modification of microstructure and texture of rennet curd by using a capsule-forming non-ropy lactic culture. *J. Dairy Res.*, 64: 115-121.
- Hassan, A.N., Frank, J.F., Farmer, M.A., Schmidt, K. A., and Shalabi, S.I. 1995. Formation of yogurt microstructure and three-dimensional visualization as determined by confocal scanning laser microscopy. *J. Dairy Sci.*, 78: 2629-2636.
- Kalab, M. 1980. Possibilities of an electron-microscopic detection of buttermilk made from sweet cream in adulterated skim milk. *Scanning Electron Microscopy*, 3: 645-651.
- Kinsella, J.E., and Whitehead, D.M. 1989. Proteins in whey: chemical, physical, and functional properties. *Adv Food Nutr Res*, 33: 343-438.

- Lee, S.J., and Sherbon, J.W. 2002. Chemical changes in bovine milk fat globule membrane caused by heat treatment and homogenization of whole milk. *J Dairy Res*, 69: 555-567.
- Parodi, P.W. 1996. Milk fat components: possible chemopreventive agents for cancer and other diseases. *Aust. J. Dairy Technol.*, 51: 24-32.
- Sheppard, C.J.R., and Shotton, D.M. 1997. *Confocal Laser Scanning Microscopy*. BIOS Scientific Publishers, New York.
- Tamime, A.Y., Kalab, M., and Davies, G. 1989. Rheology and microstructure of strained yoghurt (Labneh) made from cow's milk by three different methods. *Food Microstruct.*, 8: 125-135.
- Tamime, A.Y., Kalab, M., Davies, G., and Mahdi, H.A. 1991. Microstructure and firmness of Labhen (high solids yoghurt) made from cow's, goat's and sheep's milk by a traditional method or by ultrafiltration. *Food Struct.*, 10: 37-44.
- Trachoo, N., and Mistry, V.V. 1998. Application of ultrafiltered sweet buttermilk and sweet buttermilk powder in the manufacture of nonfat and low fat yogurt. *J. Dairy. Sci.*, 81: 3163-3171.