

## Rheological Properties and Microstructure of Buffalo Yoghurt

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### ABSTRACT

The rheological properties and microstructure of buffalo yoghurt were investigated and compared to bovine yoghurt. Buffalo yoghurt exhibited a greater degree of thixotropy and a higher level of syneresis. These differences can be linked to the underlying microstructure of the yoghurt, which is more porous and contains bigger fat globules compared to bovine yoghurt. This study shows how milk properties other than the total solids content influence yoghurt quality.

### INTRODUCTION

The rheological properties and structural arrangement of food components are critical parameters that determine the quality of semi-solid foods, such as yoghurt. The properties of the raw components also have a large effect on the product. Several milk types that are currently used to produce yoghurt differ in their composition and properties. These include bovine milk and small volumes from buffalo or other species<sup>1</sup>.

Buffalo milk is considered more nutritious than bovine milk due to its higher concentration of fat, protein, total solids and minerals i.e. calcium and phosphorous<sup>2,3</sup>, it

is consequently processed differently prior to yoghurt making with only pasteurisation and no homogenisation or fortification, due to the high solids content<sup>4</sup>. In contrast, bovine milk is typically fortified and homogenised to produce a homogenous product with a desirable firmness and texture<sup>1,5</sup>.

To date few studies have examined the rheological properties of buffalo yoghurt, with no study of the microstructure of buffalo yoghurt. Comparisons to the well-studied bovine system are also limited. Therefore, this study aimed to characterize the physiochemical and microstructural properties of buffalo yoghurt and to compare these properties with bovine yoghurt produced following standard industrial procedures.

### MATERIALS AND METHODS

#### Production of yoghurt

Yoghurt was produced from either raw unhomogenised buffalo milk (Shaw River, Victoria, Australia) or pasteurised homogenised bovine milk fortified with skim milk and whole milk powder (Pura Brand, Victoria, Australia). The chemical composition of the bovine milk after

fortification was similar to commercial bovine yoghurt; with a similar level of protein and total solids to buffalo milk (Fig. 1). Buffalo and fortified bovine milk were processed in the same way, as shown in Fig. 2, using the commercial starter culture ABT-5 (Bayswater, Victoria, Australia).

Chemical analysis

The total solids content was analysed following the AOAC method<sup>6</sup>, while the concentration of protein, fat and lactose were determined following the method as described by Pesce and Strande<sup>7</sup>, Atwood and Hartmann<sup>8</sup> and Gosling and co-authors<sup>9</sup> respectively.

Size distribution of milk fat globules

The size distribution of milk fat globules was assessed using a Mastersizer 2000 (Malvern Instruments, Malvern, UK) as described previously<sup>11</sup>.

Rheological properties

Rheological properties of yoghurt were determined following the method described by Purwandari and Vasiljevic<sup>10</sup> using a controlled stress rheometer (AR-G2, TA instruments Ltd., New Castle, U.S.A.) fitted with a cone plate (40 mm diameter / 4° angle).

Microstructural analysis

The microstructure of milk samples was analysed using an inverted confocal scanning laser microscope (CLSM) (Leica TCS SP2; Leica Microsystems, Heidelberg, Baden-Wurtemberg, Germany), while microstructure of yoghurt samples was assessed using cryo scanning electron microscopy (cryo-SEM) (Quanta, FEI Company, Hillsboro, Oregon, U.S.A.) equipped with a solid state backscattered electron detector (SSD)<sup>12</sup>.

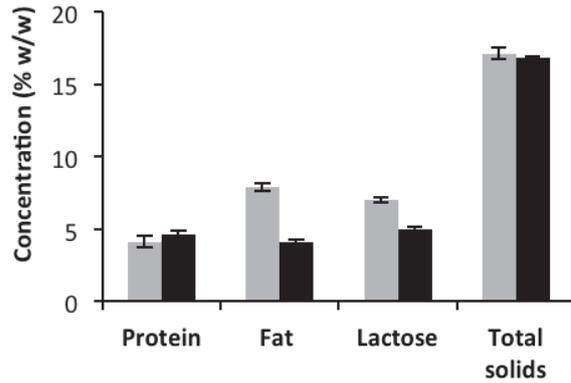


Figure 1. Chemical composition of buffalo milk (■) and fortified bovine milk (■) (n=6). Error bars show the standard deviations of the mean (n=6).

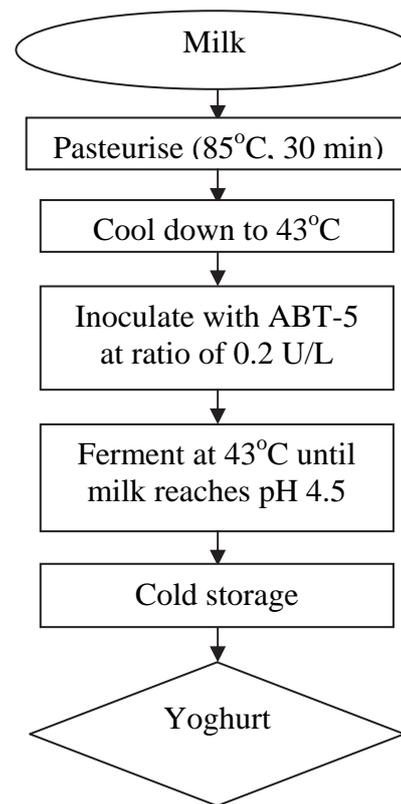


Figure 2. Process flow chart for yoghurt production.

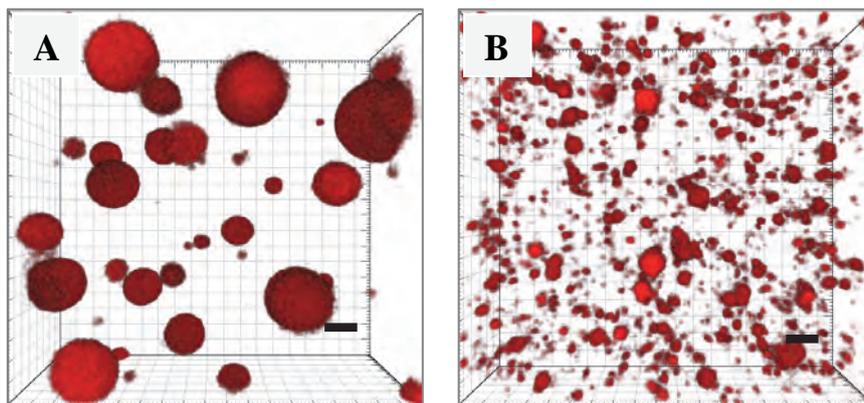


Figure 3. Microstructure of milk showing fat globules within buffalo milk (A) or fortified bovine milk (B) samples observed by CLSM. The samples were stained with Nile Red, diluted 5 times with agarose gel and observed using a  $\times 100$  objective. The scale bars are 4  $\mu\text{m}$  in length.

#### Determination of syneresis

Yoghurt syneresis was determined using a previously described method<sup>10</sup> and expressed as a weight percentage of the whey separated from the gel over the initial weight of the yoghurt.

### RESULTS AND DISCUSSION

#### Microstructure and size distribution of milk fat globules

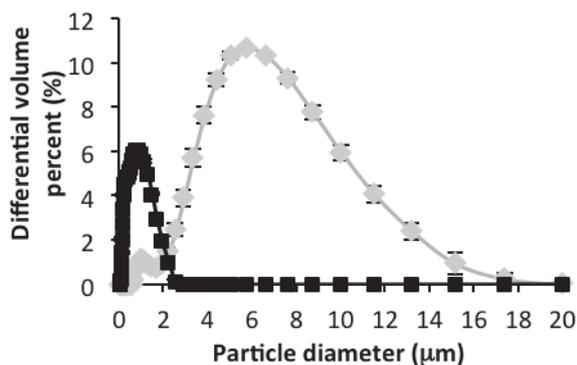


Figure 4. Size distribution of milk fat globules in buffalo ( $\blacklozenge$ ) or fortified bovine milk samples ( $\blacksquare$ ). Error bars show the standard deviation ( $n=6$ ).

The microstructure of the fat within buffalo or fortified bovine milk observed by CLSM is quite different, as shown in Fig. 3, where the buffalo fat globules are clearly

much larger than the more numerous small fat droplets in fortified bovine milk. These differences are also apparent in the size distribution data obtained by light scattering in Fig. 4, where buffalo fat globules are typically 5.5  $\mu\text{m}$  in diameter and bovine fat globules 0.6  $\mu\text{m}$  in diameter.

#### pH of buffalo or fortified bovine yoghurt during fermentation

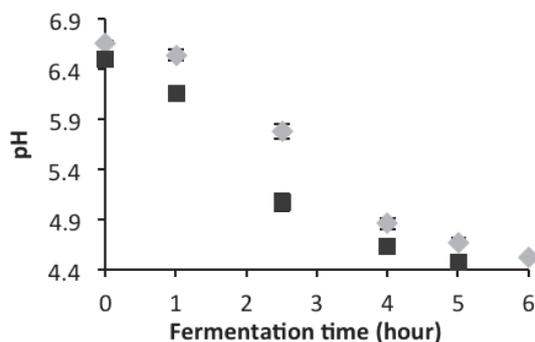


Figure 5. Changes in pH measured during the fermentation of buffalo milk ( $\blacklozenge$ ) or fortified bovine yoghurt ( $\blacksquare$ ). Error bars show the standard deviation of the mean ( $n=6$ ).

During fermentation there was a decrease in pH measured within buffalo and fortified bovine milk samples (Fig. 5). The

time required for milk to reduce to pH 4.5 was one hour longer for buffalo milk compared to fortified bovine milk. This longer fermentation time was due to the initial pH of buffalo milk, which was higher than bovine milk.

Rheological properties of buffalo or fortified bovine yoghurt

Buffalo yoghurt exhibited shear-thinning and thixotropic properties, similar to fortified bovine yoghurt, as shown in Fig. 6 where the degree of thixotropy is indicated by the area between the upper and lower curves.

Buffalo yoghurt was significantly more thixotropic than fortified bovine yoghurt ( $P < 0.05$ ). The thixotropy also increased significantly ( $P < 0.05$ ) after an extended cold storage of 28 days. The greater thixotropy

of buffalo yoghurt indicates a poorer structure that is less able to recover from deformation, especially after storage.

Microstructure of buffalo or fortified bovine yoghurt

The cryo-SEM images of the microstructure of buffalo yoghurt (Fig. 7) show the presence of large fat globules located within the protein network, which also contains a large number of serum pores. Most of the large fat globules act as inert filler but some disrupt the protein network. The homogenised fat globules in bovine yoghurt are significantly smaller than in buffalo yoghurt, consistent with observations made by CLSM and dynamic light scattering in Fig. 3 and Fig. 4. All of these smaller bovine fat globules appeared embedded within the protein network.

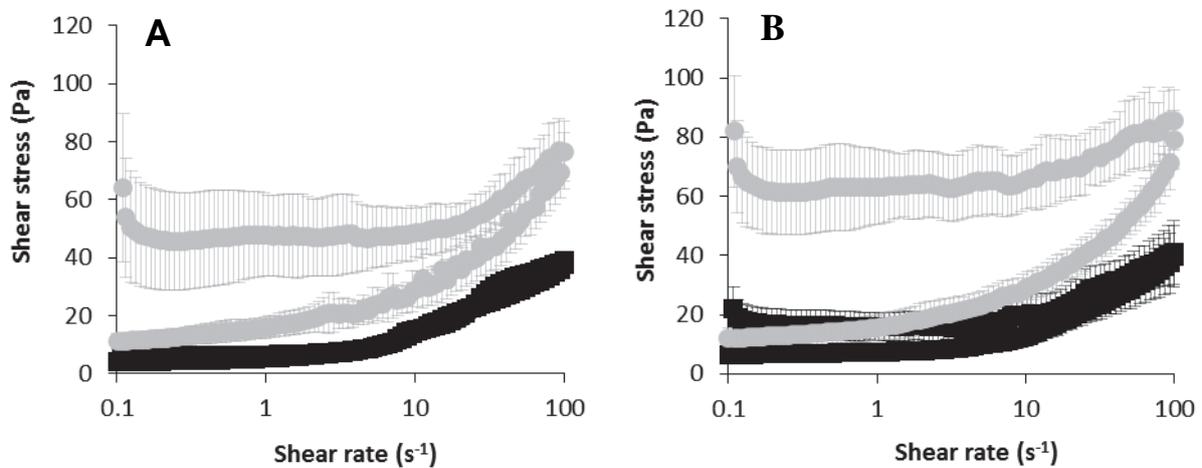


Figure 6. Changes in the thixotropy of buffalo (◆) or fortified bovine yoghurt (■) as a function of shear rate measured at day 1 (A) or day 28 (B). Error bars show the standard deviation of the mean (n=6).

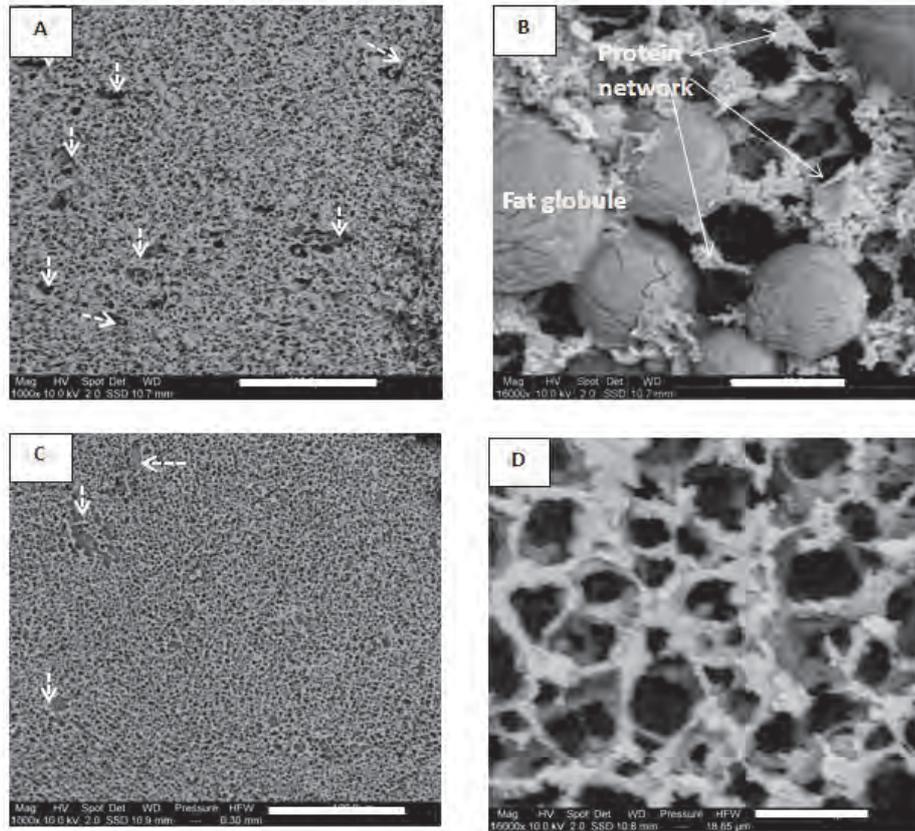


Figure 7. Microstructure of buffalo (A, B) or fortified bovine yoghurt (C, D) after 1 day of storage as observed by cryo-SEM. Images were captured using a solid state detector at 1,000x magnification (left), and 16,000x magnification (right). The scale bars are 100  $\mu$ m in length (left) or 5  $\mu$ m in length (right). Arrows indicate the presence of serum pores within the network.

### Syneresis of buffalo or fortified bovine yoghurt

Buffalo yoghurt exhibited a significantly higher level of syneresis than bovine yoghurt ( $P < 0.05$ ) (Fig. 8). This finding is significant, as syneresis is perceived as a major physical defect of yoghurt.

The greater syneresis observed in buffalo yoghurt is likely due to the lower surface area of the fat globules in buffalo milk compared to the equivalent volume of fat in fortified bovine milk (Fig. 3-4, 7). The reduced surface area of buffalo fat globules may limit the interaction between protein and fat within the network, leading to a decreased water holding capacity. The disruption of the protein network by large

fat globules and more porous microstructure may also contribute to syneresis.

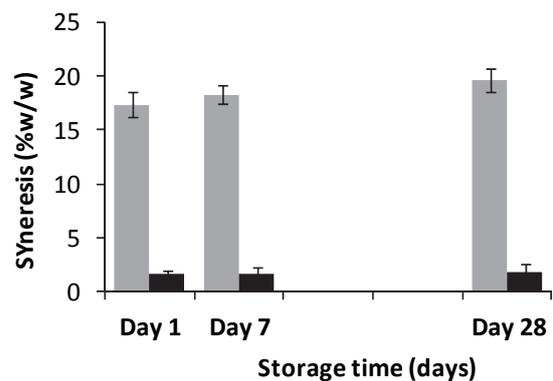


Figure 8. Changes in the syneresis of buffalo yoghurt (■) or fortified bovine yoghurt (■) during storage. Error bars show the standard deviation of the mean ( $n=6$ ).

## CONCLUSION

Buffalo yoghurt has rheological properties and a microstructure that differ to bovine yoghurt. It exhibits a higher degree of syneresis and a greater degree of thixotropy and these defects correlate with a more porous microstructure that is disrupted by larger fat globules than in bovine yoghurt. Our results show that the protein network structure, the fat content and properties including the fat globule size and surface area may contribute significantly to the structure and properties of yoghurt.

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