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Effect of Flour on the Microstructure of Goat Milk Yoghurt

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Abstract: The objective of this study was to determine the effect of six types of flours, namely corn flour, rice flour, glutinous rice flour, soy flour, cassava flour and oat flour. These flours were used to replace Whole Milk Powder (WMP) at 20, 15, 15, 15, 10 and 5%, respectively. Goat Milk Yoghurt (GMY, 18% total solid) fortified with flour was compared to control group (non fortified flour) in microstructure. Microstructure was determined by Transmission Electron Microscopy (TEM). The results showed that all flours can prevent whey separation and improve texture of GMY as compared to control group. TEM micrographs demonstrated that the microstructure of GMY gel with all the flours were more firm than the control group and showed the interaction of flour with casein micelles and their participation in the formation of the gel structures. It exhibited protein network (casein micelle chain), fat globules and bacteria in all samples and showed that flour types have an influence upon the association of casein micelles and thus affect microstructure.

Key words: Goat milk yoghurt, flour, transmission electron microscope, corn flour, rice flour

INTRODUCTION

Yoghurt is a dairy product of high nutritional value and is hence beneficial to human health. Barnett and Foster (1993) reported that the last 20 years have seen yogurt become one of the healthiest most natural foods available. Now, new styles and formulations changes have been introduced to enhance the product's healthy image, e.g., lower fat and calorie levels, vitamins, increased fiber, etc. Production of yoghurt from Goats' Milk (GM) has provided an alternative for the enhancement of the consumer's health and as a consequence it contributes significantly to human nutrition because of its higher digestibility (small fat globules) and lower potential for allergic reactions compared to Cow Milk (CM) (Uysal-Pala *et al*., 2006). However, GM has low levels of α-s_1-casein might be produced curds of weaker and less firm qualities than CM. GMY differs from CM yoghurt in some important properties such as the firmness of the coagulum which tends to be soft and less viscous (Vargas *et al*., 2008).

Non-dairy ingredients, especially polysaccharides such as locust bean gum, xanthan gum, guar gum, pectin and starches can also be used in yoghurt in conjunction with dairy ingredients or on their own to modify the rheological properties (Oh *et al*., 2007). Thus, one way to improve the texture of GMY, aside from the addition of WMP is the use of flour and stabilizers which increase firmness and prevent syneresis. Flour is a food carbohydrate which provides soluble macromolecules exhibiting properties typical of such materials; high viscosity, adhesion and surface coating. It contains not only polysaccharides (cellulose, hemicelluloses and pectins) and proteins but also dietary fiber useful for the human digestive system. Moreover, it is natural stabilizer which enhances and maintains the desirable characteristics in yoghurt for example, body and texture, viscosity/consistency, appearance and mouthfeel (Tamime and Robinson, 2007).

Microscopy at a higher resolution helps to understand what happens to the original biological materials in milk when they are processed into yoghurt (Kalab, 1979). For instance, Kalab *et al*. (1975) studied the effects of three different thickening agents (carrageenan, waxy maize starch and gelatin) on the microstructure of yoghurt which were examined by scanning through electron microscopy and TEM. They reported that starch and carrageenan contributed for a considerable change to the microstructure. GMY is very interesting from a structural viewpoint because of milk

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compositions. Electron microscopy reveals some peculiarities in the development of GMY structure. TEM is a very useful technique in other food science microscopy, particularly with viscous foods such as stirred yogurt. Consequently, it has become a suitable tool for observing GMY microstructures because of its high resolution and its ability to characterize internal structures of the protein chains and clusters (Barrantes *et al*., 1996). This study aimed to evaluate the effect of six types of flours on the microstructure of GMY fortified with flour and compared to GMY without flour supplementation.

MATERIALS AND METHODS

Yoghurt preparation: Seven different formulations were prepared by mixing all ingredients (goat milk, WMP, modified starch, pectin 020 (Burapacheep, Thailand), flour) in different proportions. Fresh raw goat milk was preheated separately to 50-60°C before the ingredient were added then the GMY mixes were heated to 80°C for 5 min (Tamime and Robinson, 2007) and cooled to 42°C in an ice-water bath. The mixtures of GMY were gently stirred and augmented with a starter cultures (YC-380, Christian Hansen's Laboratory, Inc (Milwaukee, WI)). Afterwards, the mixtures were transferred to sterilized containers which were kept at 42°C in an incubator until a pH value of approximately 4.6 was reached. After fermentation, the yoghurts were stored at 4°C.

Transmission Electron Microscope (TEM): Plain GMY fortified by flour was prepared for electron microscopy observations which were performed by TEM utilizing JOEL microscopes JEM-1230 (Jeol Ltd. Tokyo, Japan). The samples of formula were prepared by modification of the procedures of Kalab and Larocque (1996) and Harte *et al*. (2002). GMY samples were mixed with liquid 3% (w/v) agar. After solidification, the samples were cut slices of approximately 1×2×1 mm^3, prefixed with 5% Glutaraldehyde (GA) in Phosphate Buffer (PB) (pH 7) for 2-3 h and kept in a refrigerator for 2-3 h. After 3 cleansing (10 min each) in 0.1 M PB, the samples were post fixed in 1% osmium tetraoxide, kept in ice for cooling for 3 h and washed in distilled water for 10 min (2 times). The specimens were dehydrated by serial 10 min and washed in 30, 50, 70, 90 and 95% (1 time) and 100% (2 times) ethanol. The samples were embedded in Spurr's resins with 100% ethanol + QY-1 (Glycidye Butyl Ether) (1:1) for 15-30 min, with QY-1 for 15-30 min and with QY-1 +Spurr's resin (1:1) for 1 h. The specimens were polymerized in Spurr's resin 100% for 1 h twice then incubated at 60°C overnight. An ultra-microtome (Ultracut, Leica and model UCT 51) was used to cut the specimens into 50-70 nm from which were adhered to a copper grid. The samples were then double stained with uranyl acetate (5%) for 30 min then washed in distilled water and lead citrate (2.5%) for 10 min and rinsed with water. The ultra-thin sections were observed with TEM (JEOL JEM-1230) at 80 kV.

RESULTS

The results showed that all flours can prevent whey separation and improve texture of GMY as compared to control group. TEM micrographs demonstrated that the microstructure of GMY gel with all the flours were more firm than the control group and showed the interaction of flour with casein micelles and their participation in the formation of the gel structures. It exhibited protein network (casein micelle chain), fat globules and bacteria in all samples and showed that flour types have an influence upon the association of casein micelles and thus affect microstructure.

DISCUSSION

TEM revealed interesting features in the microstructure of GMY (Fig. 1) and its micrographs exhibited the protein network (casein micelle), fat globules in control. Unfortified flour GMY consisted of casein

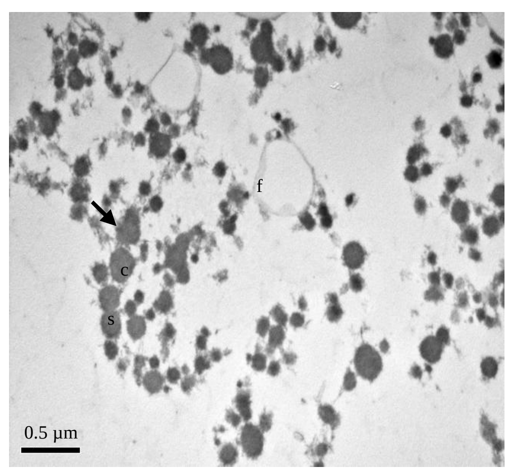

Fig. 1: Transmission electron micrograph of control GMY exhibits the microstructure of casein micelle chains and clusters. Arrows point to a spikes on the casein micelle surface. f: fat globule; c: casein micelle; s: simple casein micelle. These illustrations show the less aggregate forms of casein micelle chain and arranged in longitudinal polymers. Scale bar = 0.5 µm

micelles fused together and linked in long and loose chains which were finely clustered. Thus, GM has low levels of α-s_1-casein which has an effect on the GMY structure such as the firmness of the coagulum which tends to be soft and less viscous. The results of the current study was similar with that of Haque and Aryana (2002) who reported that in absence of any sweetener, the yogurt matrix consisted predominantly of casein micelles arranged in longitudinal polymers.

In Fig. 1 arrows point to a spike or hair line on the simple casein micelle surface, this structure develops in milk which has been heated to a minimum temperature of 80°C. At this condition β-lactoglobulin forms a complex with κ-casein at the casein micelle surface. Therefore, this complex gives the casein micelles a hairy or spiky appearance in thin sections. Other researchers have reported similar observations. Aguilera and Stanley (1999) and Tamime and Robinson (2007) for example reported that in heated milk, the yogurt gel is formed as casein micelles gradually increase in size and form a chain matrix. An even distribution of the proteins is achieved and the aqueous phase is immobilized within the network. When the pH drops below the isoelectric point of casein, the β-lactoglobulin/κ-casein complex promotes the formation of smaller diameter micelle aggregates and a gel matrix with increased water holding capacity.

GMY made with corn flour and glutinous rice flour (Fig. 2a and b) caused casein micelles to arrange themselves in double longitudinal polymers indicating an increased tendency to polymerize some micelles that are tightly fused together. Therefore, it had more interconnected clusters of densely aggregated proteins. Figure 2a shows the microparticulated protein (p) particle of corn flour and glutinous rice flour which can produce the gel responsible for firmness of GMY. In this image, GMY extended with corn flour shows the formation of a fibrillar microstructure which connected large clusters of casein micelle chains, similar to the result obtained by Williams *et al.* (2003) who reported that the viscosity of stirred yoghurt increased when 1% (w/w) modified waxy corn starch was added to yoghurt milk but the yoghurt developed a grainy texture with the an increased density of the protein network along with an increase in the starch level.

As shown in Fig. 2b casein aggregates of nonspecific shape and dimensions, fat the encapsulation of fat particles in aggregated protein particles. TEM images show that the gelatinized flour trapped in the polymer matrix represents a highly dense GMY as polysaccharides in this flour may interact with other components of the casein micelles during processing. These interactions may lead to changes in bioavailability of nutrients, texture or flavor of the product.

The microstructure of oat GMY (Fig. 2c) was composed of many interconnected chains of irregularly shaped casein micelle structures. The micrographs revealed a denser casein micelle structure with trapped fat globules and an aggregation of fine proteins or polysaccharide matrix. Oat flour is the sort of β-glucan which are cell wall polysaccharides of endosperm and aleurone cells of most commercial cereals. Khurana and Kanawjia (2007) reported β-glucan was used to prepare low fat yoghurt and as the amount of β-glucan increased

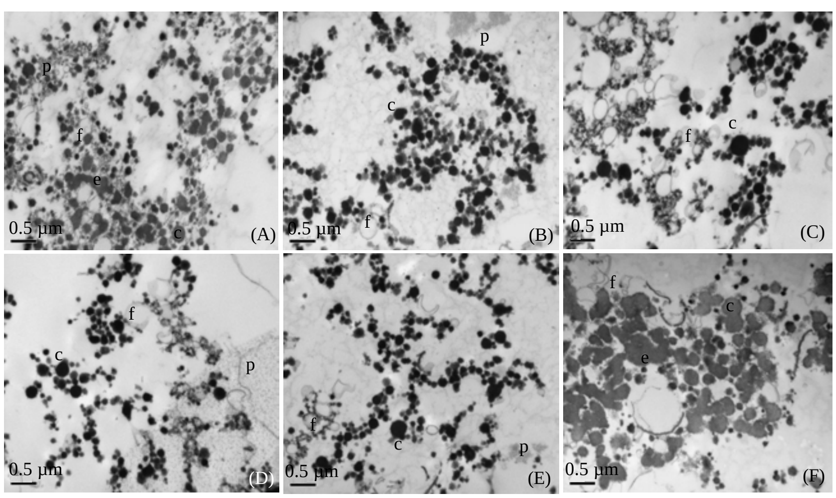

Fig. 2: Illustrates the microstructure of casein micelle chains and clusters in GMY fortified with A) corn flour; B) Glutinous rice flour; C) Oat flour; D) Soy flour; E) Rice flour and F) tapioca flour; f: fat globule; c: casein micelle; e: complex casein micelles seen in double longitudinal polymers; p: protein. This picture shows protein matrix (p) and minute fat globules (f) in association with fat globules with/within casein particle chains. Scale bar = 0.5 µm

a correspondent rise in yogurt consistency and firmness as well as a decrease in syneresis. It appears that the molecular features of oat flour added to GM have a large impact on body and texture of GMY.

Figure 2d shows the interaction of the casein micelles complex with soy proteins and fat globules. Fernandez-Garcia *et al.* (1998) reported that soy fibers caused a significant decrease in viscosity due to partial syneresis and led to lower overall flavor and texture scores as a grainy flavor and a gritty texture were intense in all fiber-fortified yogurts.

Figure 2e shows GMY made with rice flour exhibited the most relaxed and loose structures of casein micelles and some of the fine proteins and fat globules integrated onto the chains of casein micelles. These characteristics had an influence on body and texture of yoghurt, loose structures resulted in low viscosity and gel strength.

GMY fortified with tapioca flour (Fig. 2f) exhibited a more continuous and better defined network, showed large casein aggregates and fat globules encapsulated in casein micelles chains. Thus, from this appearance tapioca GMY attained high score in viscosity, body and texture from sensory evaluation. This is similar to the result reported by Sandoval-Castilla *et al.* (2004) who examined the use of tapioca starch as a fat replacer along with other whey-protein-based fat replacers. They reported that yoghurt with tapioca starch showed higher firmness than full-fat yoghurt. The microstructure of the yoghurt with tapioca starch showed some solubilized starch molecules integrated into the casein micelle network as well as starch gel fragments forming independent structures.

CONCLUSION

The results of this study showed that the use of flour to replace WMP affected the microstructures of GMY. The difference between yogurts made from WMP and supplements of six flours was in the microstructure of the GMY fortified with flour which was denser, firmer and more viscous and had a lower tendency toward whey separation than the control group.

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