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Microstructure and physicochemical properties reveal differences between high moisture buffalo and bovine Mozzarella cheeses

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ABSTRACT

Mozzarella cheese is a classical dairy product but most research to date has focused on low moisture products. In this study, the microstructure and physicochemical properties of both laboratory and commercially produced high moisture buffalo Mozzarella cheeses were investigated and compared to high moisture bovine products. Buffalo and bovine Mozzarella cheeses were found to significantly differ in their microstructure, chemical composition, organic acid and proteolytic profiles but had similar hardness and meltability. The buffalo cheeses exhibited a significantly higher ratio of fat to protein and a microstructure containing larger fat patches and a less dense protein network. Liquid chromatography mass spectrometry detected the presence of only β-casein variant A2 and a single β-lactoglobulin variant in buffalo products compared to the presence of both β-casein variants A1 and A2 and β-lactoglobulin variants A and B in bovine cheese. These differences arise from the different milk composition and processing conditions. The differences in microstructure and physicochemical properties observed here offer a new approach to identify the sources of milk used in commercial cheese products.

1. Introduction

Mozzarella belongs to the *pasta-filata* family, where the cheese is stretched or plasticised in hot water (Jana & Mandal, 2011; Kindstedt, 1993). Traditionally produced in Italy from the milk of water buffalo, this cheese is now manufactured worldwide and can be produced from several sources of milk including bovine, goat or sheep (Kindstedt, Caric, & Milanovic, 2004). The cheese can be classified into two types based on moisture content. These are low moisture Mozzarella cheese (LMMC) with a moisture content between 45 and 52% w/w and high moisture Mozzarella cheese (HMMC) with a moisture level > 52% w/w (Jana & Mandal, 2011; Kindstedt et al., 2004).

The moisture content is a major determinant of the quality and functional properties of Mozzarella cheese (Kindstedt, 1993; McMahon & Oberg, 1998; Rowney, Roupas, Hickey, & Everett, 1999). A high moisture Mozzarella cheese has a soft texture and milky flavour but a poor shreddability. Consequently this cheese is mostly used as a table cheese that is consumed within a few days of production. Low moisture Mozzarella cheese has a firmer body, better shreddability, a longer shelf-life and is normally used as an ingredient for pizza toppings (Kindstedt, 2012). Despite the importance of moisture, most studies have focused on low moisture products made from bovine and buffalo milk (Guinee, Feeney, Auty, & Fox, 2002; Jana & Upadhyay, 1997; Ma, James, Zhang, & Emanuelsson-Patterson, 2013; Rowney et al., 1999; Yazici & Akbulut, 2007) due to the more widespread use of this cheese. A greater understanding of the physicochemical properties of the high moisture Mozzarella cheeses, particularly the differences in these properties arising from different milk sources, is important as this knowledge can be used to assist with quality control and new product development.

The microstructure of a cheese is a key factor in determining the resulting functional properties (Everett & Auty, 2008; Ong, Dagastine, Kentish, & Gras, 2013; Rowney et al., 1999). This structure is known to be affected by processing conditions (Ma, James, Zhang, & Emanuelsson-Patterson, 2011; Ribero, Rubiolo, & Zorrilla, 2009), such as the mechanical and thermal treatments that occur during Mozzarella production that alter the arrangement of fat and protein. Different microscopic techniques have been used to characterise the microstructure of low moisture Mozzarella cheese, including confocal laser scanning microscopy (CLSM), transmission

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electron microscopy (TEM), scanning electron microscopy (SEM) and cryo-SEM (Ma et al., 2013; Reid & Yan, 2004; Ribero et al., 2009; Tunick, Van Hekken, Cooke, & Malin, 2002). The microstructure of a high moisture Mozzarella cheese, however, has not been investigated. Furthermore, each of the above techniques has its own advantages and limitations (Ong, Dagastine, Kentish, & Gras, 2011). A combination of multiple microscopy techniques therefore allows a greater understanding of cheese structure.

Studies comparing high moisture buffalo and bovine cheeses are limited. Pagliarini, Monteleone, and Wakeling (1997) have observed that the high moisture Mozzarella cheese made from full fat buffalo milk has significantly different sensorial properties to the equivalent bovine cheese. The buffalo product was identified by its cohesiveness, acid and salty flavour and yoghurt odour, while the bovine cheese was identified by its sweetness together with a milky and creamy flavour and fibrous and elastic texture. Physically, the curds from the two milk types have been found to differ; the curd from buffalo milk exhibited a greater firmness (indicated by a higher storage modulus G′) with a higher calcium concentration and a higher yield than the curd produced from bovine milk (Hussain, Bell, & Grandison, 2011; Hussain, Yan, Grandison, & Bell, 2012), while the porosity remained similar (Hussain, Grandison, & Bell, 2012). Interestingly, if curd was prepared from ultrafiltered bovine milk that had been standardised to a similar fat and protein concentration as buffalo milk, this product was still less firm than the buffalo equivalent and significantly more porous (Hussain, Bell, & Grandison, 2013a, 2013b), indicating that gross composition is insufficient to explain the differences between these products and highlighting the need to consider differences in individual fat and protein components. The microstructure, texture and other physicochemical properties of the final Mozzarella cheeses made from these two milk types, however, were not further investigated in these studies.

The objective of this study was to characterise the microstructure of high moisture buffalo Mozzarella using both CLSM and cryo-SEM techniques and the physicochemical properties of cheese produced both commercially and within a controlled laboratory environment. The study aimed to obtain a better understanding of this cheese and to compare the properties of the high moisture buffalo Mozzarella cheese with bovine Mozzarella cheese to allow a greater insight into the effect of milk type on the quality and functional properties of the cheese. Herein, the term Mozzarella cheese is used to indicate the high moisture variant and wherever different, the details of the cheese are clearly stated.

2. Materials and methods

2.1. Production of buffalo Mozzarella cheese in the laboratory

Buffalo Mozzarella cheese was produced following a previously described method (Fainberg, 2012; Yazici & Akbulut, 2007) with some parameters optimized as a result of laboratory screening experiments. Pasteurised buffalo milk was obtained from a local farm (Shaw River, Yambuk, Australia). The milk was used for cheese making within one day of receipt. Four litres of buffalo milk was warmed to 37 °C before the addition of starter culture TCC-20 (0.072 $g{\cdot}L^{-1}$–0.4 $U{\cdot}L^{-1}$, CHR-Hansen, Bayswater, Australia) containing a mixture of *Streptococcus thermophilus* and *Lactobacillus helveticus*. When the milk pH dropped to 6.5, 0.2 mL per L of Chymosin (40 $IMCU{\cdot}L^{-1}$, Chymax-plus, CHR-Hansen, Bayswater, Australia) was added and the milk allowed to set for approximately 30 min until an appropriate curd firmness, assessed by a knife test, was obtained. The curd was then cut into small cubes, approximately 2 cm in size and left to heal for 10 min. The curd was gently stirred (~30 s) followed by cooking at 42 °C. During cooking, the curd was stirred for 10 min followed by resting for 10 min. This stirring step was repeated until the curd pH reached 5.2, which normally took around 1.5–2 h. The whey was drained and the curd milled and dry-salted with 2% w/w salt. The curd was then submerged in hot tap water 1:1.5 w:w at 85–90 °C and incubated for 3 min to allow the heat to penetrate into the curd. Half of this water was then decanted and fresh hot water poured onto the curd and left to incubate for another 3 min before stretching. A wooden paddle was used to assist the stretching step in hot water. The cheese curd was moulded into small balls, approximately 80–100 g in size. The cheese balls were finally stored in chilled water in a cold room at 4 °C until further analysis. The cheese production was repeated in three trials on different days and at least two cheese samples were analysed in each trial for each analysis. The shelf life of high moisture traditional buffalo Mozzarella cheese is approximately five to sevendays after production (Altieri, Scrocco, Sinigaglia, & Del Nobile, 2005), therefore the laboratory buffalo Mozzarella cheese was characterised on day 1 (BM Lab-D1) and day 7 (BM Lab-D7) of storage.

2.2. Commercial buffalo and bovine Mozzarella cheese collection

The commercial cheeses analysed included two buffalo (BM) and two bovine (CM) cheese products manufactured in Australia, coded as BM-cheese A, B and CM-cheese A and B and one buffalo Protected Designation of Origin cheese produced in Italy (Zanetti Mozzarella di Bufala Campana, purchased in Cora supermarket, Pacé, France). The products manufactured in Australia were used for the characterization of microstructural and physicochemical properties, while the buffalo cheese purchased in France was only used for the purpose of microstructural comparison. Six cheese samples were analysed for each commercial Mozzarella cheese, except for the moisture and microstructural investigations where three and four samples were used, respectively.

2.3. Chemical compositional analysis

The protein, fat and moisture content of the milk and cheese was determined using the Kjeldahl method (IDF, 2008), the gravimetric method (IDF, 2004a) and the oven drying method (IDF, 2004b) respectively. The minerals, calcium, phosphorous, sodium and ash contents were determined using inductively coupled plasma optical emission spectrometry (Varian ICP - OES 720, Varian Inc., Palo Alto, CA, USA) following an established method (Rice, 2008). The concentration of sugars (lactose, glucose and galactose) was determined by a high performance liquid chromatography (HPLC, Shimadzu Prominence system, Rydalmere, Australia) using a refractive index detector and a 300 × 7.8 mm Rezex RCM-Monosaccharide Ca^{2+} column (Phenomenex, Lane Cove, Australia), as described previously (Gosling et al., 2009). The organic acid profile was determined using an HPLC system equipped with a photo diode array ultra violet detector and a Bio-Rad Aminex HPX 87H cation exchange column connected to a cation H^{+} guard column (Bio Rad Laboratories Pty Ltd., Hercules, CA, USA), as previously described (Nguyen, Ong, Lefevre, Kentish, & Gras, 2014). The cheese pH was measured using an electrode pH meter (Orion 720A, Wallsend, Australia).

2.4. Texture analysis

The texture of the Mozzarella cheese was determined following the method described by Zisu and Shah (2005) with some modifications. The measurement was performed using a TA.XT-2 texture analyser (Stable Microsystems, Godalming, England) equipped with a 20 N load cell and a 25.4 mm diameter cylindrical probe. A cylindrical portion was excised from the central part of the cheese ball using a cork borer 20 mm in diameter. A sample 20 mm in height was then obtained in the middle part of the cylindrical portion. The cheese sample was kept in an enclosed container to prevent dehydration and held at 20 °C for at least 2 h prior to measurement, in order to allow equilibration to a temperature similar to that of consumption. The contact area and the contact force were set at 1 mm^2 and 5 g, respectively. The instrument