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Review

Processing of high-protein yoghurt – A review

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A B S T R A C T

High-protein yoghurt has gained increased consumer interest over the recent years, partly driven by improvements in taste and texture; there is also greater scientific evidence on dairy protein health benefits. The protein content of yoghurt can be increased prior to fermentation by addition of dairy powder, evaporation or membrane filtration, or after fermentation by straining, mechanical separation, or membrane filtration. Concentration of yoghurt after fermentation produces large volumes of acid whey, a major concern for the dairy industry; by concentrating prior to fermentation, production of acid whey is avoided. Different processing techniques influence yoghurt composition, structure, rheology, and sensory properties. This review discusses the challenges, opportunities, the influence of macro components in milk and different processing techniques on composition, structure, rheology, and sensory properties of high-protein yoghurt, along with their benefits and drawbacks for the dairy producer.
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Contents

1. Introduction

High-protein yoghurts and fermented milks with a variety of names, have existed for a long time in many countries. Labneh (Eastern Mediterranean), Torba (Turkey), Stragisto (Greece), Chakka

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(India), and Ymer (Denmark) are all examples of concentrated or strained fermented milks with different geographical origins (Tamime, Hickey, & Muir, 2014). In the USA, consumers were accustomed to thinner, more liquid yoghurt before the introduction of high-protein yoghurt, marketed as "Greek yoghurt" or "Greek-style yoghurt". Primarily, increased protein content contributed to texture benefits like thicker and creamier products, and the increased amount of scientific documentation claiming health benefits of dairy proteins, drove the "Greek yoghurt" market in the USA (Fekete, Givens, & Lovegrove, 2013; Pasiakos, 2015; Phillips, Tang, & Moore, 2009). In Europe, the growth of high-protein yoghurt has been slower than in the US market; however, there has been an increased intake of high-protein yoghurt in Northern Europe (Scandinavia, the Netherlands, Belgium, UK, and Germany) in recent years (Mellentin, 2013, 2014).

The rise in consumer demand for high-protein yoghurt is expected to continue (Mellentin, 2016), and the protein trend is also connected to the weight wellness trend and consumer interest in products with limited additives ("clean label") (Mellentin, 2013). High-protein yoghurts with a high content of whey proteins could be beneficial in infant, elderly, or sports nutrition due to the ability of whey proteins to increase plasma amino acids (Boirie et al., 1997; Hall, Millward, Long, & Morgan, 2003), and trigger muscle protein synthesis (Garlick, 2005; Tipton et al., 2007). Furthermore, high-protein yoghurts could be beneficial in calorie-restricted diets, because the energy intake from protein seems to have a greater effect on satiety than intake of fat or carbohydrate (Benelam, 2009). High-protein yoghurts with different whey protein to casein ratios can be produced.

Tamime et al. (2014) surveyed the information contained on labels of 109 commercial concentrated fermented milks with different geographical origins. The protein content varied from approximately 8%–4.5%. Some samples had even lower protein content than 4.5%. The authors proposed that yoghurts labelled "Greek" or "Greek-style" yoghurt with a protein content of <5% were misnamed. It is worth discussing whether including protein content in legislative provisions could clarify the distinction between varieties of high protein yoghurts to guide both manufacturers and consumers.

According to the Codex standard for fermented milk (Codex standard 243–2003), yoghurt contains a minimum of 2.7% milk protein and less than 15% fat. Concentrated fermented milk is a fermented milk where the protein has been increased prior to or after fermentation to a minimum of 5.6% (Codex Alimentarius, 2011). There is no legal standard to define "high-protein yoghurt". However, the "concentrated fermented milk" term may comprise "high-protein yoghurt". Based on the Codex standard definition of "concentrated fermented milk", it is hereby proposed that "high-protein yoghurt" is a yoghurt containing a minimum of 5.6% protein, and less than 15% fat. The protein content can be obtained prior to fermentation by fortification with milk powder, evaporation, or membrane filtration, or after fermentation by straining (draining), mechanical separation, or membrane filtration. In the following, the term "high-protein yoghurt" includes yoghurt processed by increasing the protein content either before or after fermentation.

A plain yoghurt with a high consumer acceptance should in general have a smooth, uniform and spoonable texture, and be free from lumps, graininess, and visual whey separation (Lucey & Singh, 1997; Lucey, 2004); and should have a clean and typical yoghurt flavour. Acetaldehyde, diacetyl, and lactic acid are considered as the major aroma components of yoghurt, but also other components, like acetone, acetoin, and acetic, formic, butanoic, and propanoic acids, have been listed as contributors to yoghurt flavour (Routray & Mishra, 2011). In a sensory evaluation of a wide range of commercially available plain yoghurts, strained "Greek-style yoghurts" with different fat levels were distinguished from the other yoghurt samples (stirred or set-type) by having a thicker and firmer consistency (Brown & Chambers, 2015). Full-fat (8.8 or 20%) "Greek-style yoghurts" differed from the low-fat (2%) and non-fat (<0.05%) "Greek-style yoghurts" by having a less chalky mouthfeel like dry, powdery sensation in the mouth. All "Greek-style yoghurts" had a relatively high degree of smoothness irrespective of fat content. Desai, Shepard, and Drake (2013) reported that a full-fat strained yoghurt received a higher overall impression score than low-fat and non-fat "Greek yoghurts" in a consumer acceptance test. The full-fat yoghurt was characterised by the descriptive panel as having high sensory intensities of milk fat flavour, viscosity, firmness, and denseness, and moderate amounts of sweet and sour taste. Although full-fat high-protein yoghurts have preferable sensory properties, the largest dairy companies offer a wide range of non-fat and low-fat high-protein yoghurts to meet consumer demands.

Sensory and texture attributes such as creaminess, viscosity, and smoothness are important drivers of liking of high-protein yoghurts (Desai et al., 2013) and low-fat yoghurts (Frøst & Janhøj, 2007). Sensory and physical properties of a high-protein yoghurt are influenced and controlled by the composition of the yoghurt milk base and by the processing parameters and conditions. During the last decade, the amount of research focusing on high-protein acid milk gels and yoghurts has increased.

Different challenges and opportunities related to product and process can occur in the production of high-protein yoghurts: Production of acid whey from concentrating fermented yoghurt; Sensory defects such as graininess, bitterness, too acidic flavour, and whey separation; Technological challenges related to concentrating proteins prior to fermentation with the use of membrane filtration. This review aims to overview the influence of the macro components in the yoghurt milk base on structure, rheology, and sensory properties of high-protein yoghurt. Different processing techniques for production of high-protein yoghurts and their impacts on yoghurt composition, structure, rheology and sensory properties are discussed, along with their benefits and disadvantages for the dairy producer.

2. Influence of milk macro components on high-protein yoghurt

2.1. Protein

Protein is the crucial milk macro-component in the formation of an acid milk gel such as yoghurt. Several authors have reviewed the formation of acid milk gels in general (Dalgleish & Corredig, 2012; Livney, Corredig, & Dalgleish, 2003; Lucey & Singh, 1997; Lucey, 2002; van Vliet, Lakemond, & Visschers, 2004) and of yoghurts in particular (Heertje, Visser, & Smits, 1985; Lee & Lucey, 2010; Lucey, 2004; Sodini, Remeuf, Haddad, & Corrieu, 2004).

The variation in protein content and protein composition among commercial yoghurts and concentrated fermented milks (Tamime et al., 2014) leads to a great variation in physical and sensory properties among yoghurts on the market. In general, an increase in the protein content of a yoghurt milk base yields a yoghurt with increased firmness, viscosity and G', mainly due to the increased amount of protein participating in the gel network (Abrahamsen & Holmen, 1980; Biliaderis, Khan, & Blank, 1992; Mistry & Hassan, 1992; Schkoda, Hechler, & Hinrichs, 2001a). However, protein and total solids content are often dependent variables in experiments; thus, the effect of the protein content is often confounded with the total solids content (Sodini et al., 2004).

Fortification of the yoghurt milk base with milk powders prior to fermentation is a processing option in the manufacturing of high-protein yoghurt. Available milk powders vary widely in their composition. Milk protein concentrate (MPC) is used in the commercial production of high-protein yoghurts. Fortification of the yoghurt milk base with higher-protein MPCs provides protein enhancement without adding significant amount of lactose (Agarwal, Beausire, Patel, & Patel, 2015). Mistry and Hassan (1992) reported that non-fat set yoghurts fortified with MPC with acceptable sensory properties had protein contents less than 8% and lactose contents of at least 5%. Yoghurts with a protein content above 8% gave a grainy texture. The desired level of protein could also be reached by fortifying the yoghurt milk base with micellar casein concentrate (MCC).

Bong and Moraru (2014) produced high-protein (~9.5% true protein), non-fat stirred yoghurt by fortification of skim milk with MCC-88 (88% total protein in dry matter) or MCC-58 (58% total protein in dry matter). Yoghurts produced by MCC fortification were compared with a lab-produced strained yoghurt (cheesecloth), and a commercial “Greek-style yoghurt”, both containing the same amounts of protein. As expected the amount of lactic acid (g 100 g^{-1}) at the final pH (~4.3) was significantly higher in the MCC-fortified yoghurts than in the strained yoghurt due to higher buffer capacity in the MCC-fortified yoghurt. Yoghurt fortified with MCC-58 and the commercial yoghurt had similar G' and flow behaviour, suggesting similar textural properties. The strained yoghurt had the highest serum-holding capacity, followed by the commercial “Greek-style yoghurt” and the MCC-58 yoghurt. The better serum-holding capacity of the strained yoghurt was linked to the lower casein to serum protein ratio of this yoghurt, and thus the increased cross-linking of the gel network and the high water-binding capacity of denatured whey proteins. The MCC-58 yoghurt had better serum-holding capacity than the MCC-88 yoghurt, which was explained by the higher total solids content of the MCC-58 yoghurt (~19% versus 15% total solids). Based on these findings, the authors proposed that MCC-58 could be a suitable protein source in the production of high-protein yoghurt. However, the authors did not study the sensory properties of the yoghurts. Jørgensen et al. (2015) observed that a high-protein (~8%), low-fat stirred yoghurt produced from casein concentrate from microfiltration (MF) of skim milk had a coarse and granular appearance and a mealy consistency. This yoghurt had a undenatured (native) whey protein to casein ratio (10:90) similar to that of the MCC-58 yoghurt produced by Bong and Moraru (2014), indicating that the MCC-58 yoghurt probably had less acceptable consistency.

Several authors have investigated the effect of whey protein addition on the rheological properties of acid gels and yoghurts. In general, increased amount of denatured whey proteins has been reported to increase the final G', maximum compression force, obtained by penetration test, and/or viscosity of acid milk gels (Chever, Guyomarc'h, Beaucher, & Famelart, 2014; Guyomarc'h, Queguiner, Law, Horne, & Dalgleish, 2003b; Lucey, Munro, & Singh, 1999), yoghurts with protein content <5.6% (Krzeminski, Großhable, & Hinrichs, 2011; Kücükcetin, 2008b; Laiho, Williams, Poelman, Appelqvist, & Logan, 2017; Puvanenthiran, Williams, & Augustin, 2002; Remeuf, Mohammed, Sodini, & Tissier, 2003; Zhao, Wang, Tian, & Mao, 2016), and high-protein yoghurts (>5.6% protein) (Jørgensen et al., 2015). On the contrary, Guzmán-González, Morais, Ramos, and Amigo (1999) and Modler and Kalab (1983) reported that adding whey protein concentrate (WPC) decreased yoghurt viscosity and firmness. In most of these studies, except for those by Guyomarc'h et al. (2003b) and Jørgensen et al. (2015), the whey protein source was a WPC or a whey protein isolate, usually obtained from cheese whey. WPCs vary in their compositions as for instance degree of β-lactoglobulin (β-LG) lactosylation and mineral content (Holt et al., 1999a,b), and this could be one reason for the conflicting results of Guzmán-González et al. (1999), and Modler and Kalab (1983). This was also underpinned by the results of Modler and Kalab (1983), as yoghurt prepared from fresh skim milk fortified with ultrafiltered WPC was firmer than yoghurt prepared from milk fortified with WPC desalinated with electrodialysis or ion exchange.

By MF, milk can be fractionated into a casein-rich retentate and a permeate with native undenatured whey proteins, commonly referred to as native whey, ideal whey, virgin whey, or serum proteins. Native whey is, opposed to cheese whey, free from somatic cells, lactic acid bacteria, bacteriophages, remnants of rennet, cheese fines, and the glycomacropeptide from κ-casein (κ-CN), and has a neutral pH and taste (Maubois, 2002). Dispersions of native whey protein powders made from MF of milk have been reported to give a significantly higher gel strength than dispersions of whey protein powders from cheese whey (Heino, Uusi-Rauva, Rantamäki, & Tossavainen, 2007). Heino et al. (2007) attributed the excellent gelling properties of native whey protein powders to the lack of glycomacropeptide and the high amount of native whey proteins. Recently, Jørgensen et al. (2015) investigated the effect of adding native whey protein concentrate (NWPC) to casein concentrate from MF of skim milk on the rheological, structural, and sensory properties of stirred yoghurt. They observed that reducing heat treatment from 95 °C for 5 min to 75 °C for 5 min of yoghurt milk bases with high whey protein to casein ratios (25:75–35:65) gave viscous, stirred high-protein yoghurts (~8%) with rather small coagulum particle size, relatively smooth sensory consistency and shiny appearance. Thus, keeping considerable amounts of the whey proteins in their undenatured form (~40–50%) improved the sensory properties of these high-protein yoghurts. The G' and the firmness of the yoghurts were reduced compared with those of the yoghurts from milk bases where almost all the whey proteins were denatured by heat treatment at 95 °C for 5 min. Such reduction in firmness and thickness measured with a texture analyser is shown in Fig. 1. Chever et al. (2014) also observed reduced viscosity, firmness, and coagulum particle size of stirred, high-protein acid gels (9.2% protein) when an increasing amount of whey protein was kept in its undenatured form. Schmidt, Sistrunk, Richter, and Cornell (1980) reported that heat treatment of a yoghurt milk base (6.4% protein) at 90 °C for 30 min resulted in a grainy body of set yoghurt, while a reduction in the heat treatment temperature to 80 or 85 °C for 30 min yielded a smooth and firm-bodied yoghurt. However, the improved sensory properties could likely not be explained by the presence of undenatured whey proteins, because heat treatment at 80 °C for 30 min is expected to completely denature ß-LG (Dannenberg & Kessler, 1987).

From a nutritional perspective, it could be interesting to produce high-protein yoghurts with a considerable amount of undenatured whey proteins from native whey (Gryson et al., 2014; Hamarsland et al., 2017; Sousa et al., 2012; Walrand et al., 2016). Guggisberg, Eberhard, and Albrecht (2007) and Patocka, Cervenkova, Narine, and Jelen (2006) observed a reduction in G' of yoghurts when whey proteins were added to yoghurt milk after heat treatment to retain the whey proteins in their undenatured state. Addition of whey proteins to the yoghurt after fermentation resulted in two separate phases comprising fluid whey and a coagulated protein mass (Patocka et al., 2006). The yoghurts produced by these authors were not sensory evaluated. Jørgensen et al. (2014), however, investigated the effect of adding NWPC to a casein-concentrated (made from MF of skim milk) yoghurt milk base (~8% true protein and whey protein to casein ratio 30:70) before heat treatment, after heat treatment, or to the fermented yoghurt before cooling, on the sensory properties of yoghurt. They observed that adding NWPC to the yoghurt milk base after heat treatment or to the yoghurt after fermentation yielded yoghurts with unacceptable sensory properties

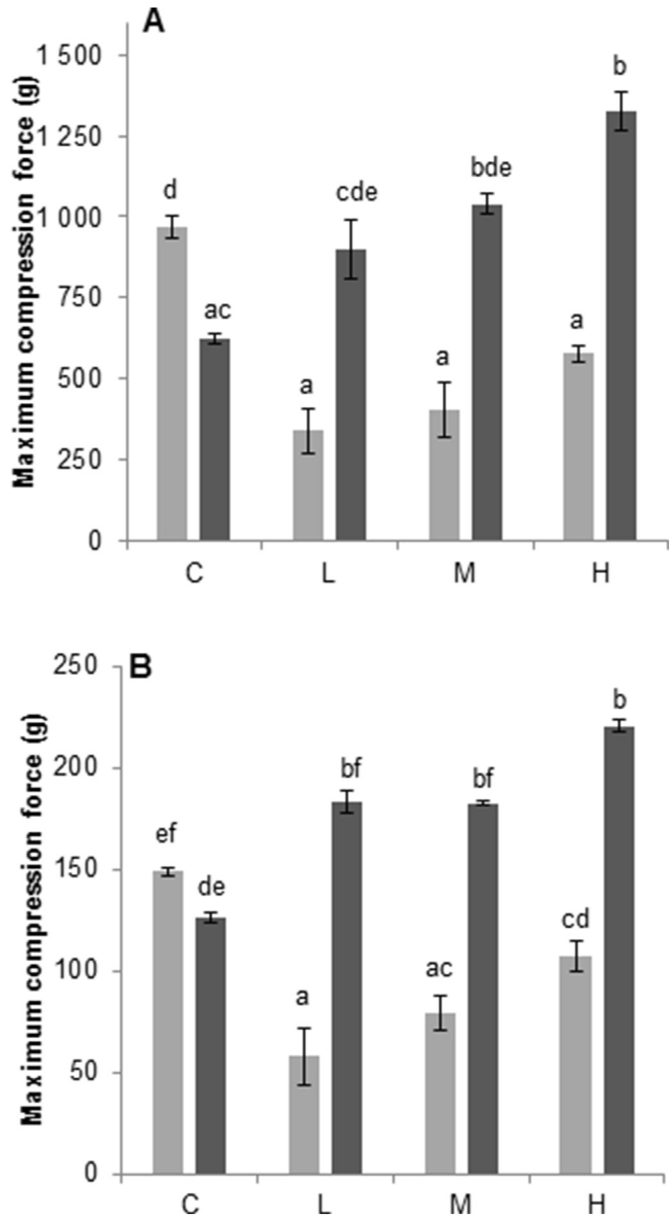

Fig. 1. Firmness (mean ± SD from the mean, n = 2) of set yoghurts (A) and thickness of stirred yoghurts (B) measured with texture analyser. Yoghurt milk bases were heat treated at (■) 75 °C for 5 min or (■) 95 °C for 5 min. Notations C, L, M and H refer to the added level of native whey protein concentrate in terms of whey protein:casein-ratios in the yoghurt milk bases; control (no addition, 10:90); low (25:75); medium (35:65); and high (45:55), respectively. Yoghurts with no common letters differ according to Tukey's pairwise comparison ($P < 0.05$). Note the different scale values of the y-axes (Jørgensen et al., 2015).

(mealy and granular). However, the addition of NWPC to the yoghurt milk base followed by a reduced heat treatment (75 °C/5 min) compared with the conventional heat treatment (95 °C/5 min) gave a smooth and shiny yoghurt.

Casein micelles are polydisperse and vary in diameter from 50 to 500 nm as measured with electron microscopy (Fox & Kelly, 2004). Skim milk with an average micelle diameter ranging from 149 to 222 nm (Devold, Brovold, Langsrud, & Vegarud, 2000), can be fractionated into a retentate containing “large” casein micelles (~186 nm) and a permeate containing “small” casein micelles (~130 nm) with the use of MF (Jørgensen et al., 2016). Jørgensen, Abrahamsen, Rukke, Johansen, and Skeie (2017) reported that a yoghurt milk base with small casein micelles (~129 nm) gave high-protein set yoghurts (5.6% crude protein) with higher G' and higher firmness than a yoghurt milk base with large casein micelles (~183 nm). It was proposed that this increased gelation capacity could be attributed to an increased amount of κ-CN in small casein micelles. Donato, Guyomarc'h, Amiot, and Dalgleish (2007) observed a higher content of soluble complexes of whey proteins and κ-CN in heated milk with a naturally high content of κ-CN. Jørgensen et al. (2017) did not measure the distribution of bound and soluble complexes of whey proteins and κ-CN. However, a higher content of soluble complexes in the yoghurt milk base with small casein micelles could possibly provide more points of attachment during acidification, as previously reported by Anema, Lee, Lowe, and Klostermeyer (2004) and Guyomarc'h et al. (2003b). On the other hand, Horne (2003) observed no effect of casein micelle size on stiffness of gels made with glucono-δ-lactone (GDL). These gels were, however, made of non-heat-treated milk. Smaller casein micelles have previously been reported to produce firmer rennet gels (Gustavsson et al., 2014; Logan et al., 2015; Walsh et al., 1998).

The referred works support the hypothesis that firmness and G' of acid gels and yoghurts increase with increasing protein content and increasing amount of denatured whey proteins. In addition, research suggests that smaller casein micelles and a shift from bound towards soluble aggregates of whey proteins and κ-CN in the heat-treated milk could enhance the protein network of acid milk gels and yoghurts. Yoghurt milk bases with increased ratios of denatured whey protein to casein at constant total protein contents seems to yield firmer yoghurts with stronger protein networks (Jørgensen et al., 2015; Krzeminski et al., 2011; Kücükcetin, 2008b; Laiho et al., 2017). These studies covered ratios of denatured whey protein to casein from 10:90–60:40. Lucey et al. (1999) suggested that undenatured whey proteins act as inert fillers in the gel matrix, while denatured whey proteins associated with the casein micelles act as a bridging material by interacting with other denatured whey proteins. Increased amounts of protein particles participating in the gel network lead to increased branching, and consequently gels with higher G' (Lucey, Teo, Munro, & Singh, 1997). Guyomarc'h, Law, and Dalgleish (2003a) observed that an increase in whey protein:casein ratio of a heat-treated (95 °C, 10 min) milk-based mixture (4.7% total protein) increased the amount and average size of soluble aggregates of denatured whey proteins and κ-CN. An increase in the whey protein:casein ratio from ~15:85 to ~33:67 increased the average size of soluble aggregate from 3.5×10^6 to 5×10^6 Da. The ratio of whey proteins to κ-CN in the aggregates increased with increasing amount of whey protein in the mixture, while the proportion of κ-CN involved was rather consistent. The authors estimated that the soluble aggregates could be globular particles of more than 10 nm in diameter or hundreds of nanometers long linear particles. Increased amount and size of soluble aggregates could explain the observed higher G' values of acid gels of milk-based dairy systems with increased whey protein:casein ratios (Guyomarc'h et al., 2003b). Furthermore, a firmer yoghurt gel yields increased coagulum particle size of the stirred yoghurt with increased sensory roughness, coarseness, lumpiness, and graininess (Jørgensen et al., 2015; Krzeminski et al., 2013, 2011; Kücükcetin, 2008b; Laiho et al., 2017; Tomaschunas, Hinrichs, Köhn, & Busch-Stockfisch, 2012).

2.2. Fat content of high-protein yoghurt

The fat content of yoghurt varies from 0 to 10%, but is usually between 0.5 and 3.5% fat (Lucey & Singh, 1997). Traditional concentrated (strained) yoghurts, such as Labneh, typically have a fat content of 6–11% (Nsabimana, Jiang, & Kossah, 2005; Tamime & Robinson, 2007). Today, the largest dairy companies offer a wide range of concentrated yoghurts in the US and European markets, typically containing 0, 2, or 4% fat. According to the US Food and Drug Administration (USDA, 2016), these yoghurts are designated as non-fat (<0.5% fat), low-fat (0.5–2.0%), and regular (≥3.25%).

The fat content of concentrated yoghurts contributes to the sensory profile and to the textural and rheological properties of the product. In a consumer survey with female consumers (n = 520, ≥18 y) who had consumed "Greek yoghurt" at least once within the last three months, 54% of the respondents consumed low-fat yoghurts, 26% consumed non-fat yoghurts, and 20% consumed full-fat yoghurts (Desai et al., 2013). Preference mapping using descriptive panel results and consumer acceptance testing (n = 155) of ten "Greek yoghurts" (from non-fat to full-fat with protein content from 5.8 to 10.6%) revealed that milk fat flavour was an important driver of liking. The yoghurt with the highest fat content had the highest sensory intensity of milk fat flavour, viscosity, and cohesiveness according to the descriptive panel results, and also received the highest overall impression score in consumer acceptance testing (Desai et al., 2013). For yoghurts with a protein content similar to that of raw milk, fat content is positively associated with sensory properties like creamy flavour, visual gel firmness, and mouthfeel (Folkenberg & Martens, 2003a). In a blind tasting (n = 69) of the same yoghurts, the participants preferred the high-fat yoghurts to the low-fat yoghurts (Folkenberg & Martens, 2003b).

Homogenisation of a yoghurt milk base increases the total surface area of the fat globules. The new surface layer of the fat globules is made up of casein micelles and fragments of casein micelles, whey proteins, and milk fat globule membrane material (Sharma, Singh, & Taylor, 1996). The new membrane material allows the fat globules to interact as pseudocasein particles in the protein gel network (Fox, Guinee, Cogan, & McSweeney, 2000), increasing the number of interacting particles of the yoghurt gel. Thus, increasing the fat content of homogenised yoghurt milk bases increases the G' of the yoghurt (Lucey, Munro, & Singh, 1998). If the milk is not homogenised or the homogenised fat is added after fermentation, the fat globules act as structure breakers and reduce the viscosity of the yoghurt (Schkoda, Hechler, & Hinrichs, 2001b; van Vliet & Dentener-Kikkert, 1982).

An increased fat content reduces the coagulum particle size of the yoghurt and increases the viscosity and G' (Brauss, Linforth, Cayeux, Harvey, & Taylor, 1999; Krzeminski et al., 2011). A small coagulum particle size is correlated with perceived increased smoothness and reduced graininess (Cayot, Schenker, Houzé, Sulmont-Rossé, & Colas, 2008; Jørgensen et al., 2015; Krzeminski et al., 2013). Sensory properties like smoothness, sufficiently high viscosity, fatty afterward mouthfeel, fat-related flavours, and also sweetness, are related to perceived creaminess of yoghurt (Frøst & Janhøj, 2007). The positive effect of fat on sensory and physical properties of yoghurts can, to a certain extent, be compensated for by an increased protein content in reduced-fat yoghurts (Tomaschunas et al., 2012). However, although increased protein content increases the fat-related attributes like creamy taste and texture and a fatty mouthfeel, very high intensities of these attributes can only be achieved with a high fat content (Tomaschunas et al., 2012).

2.3. Lactose

There seems to be limited research on the direct influence of lactose on the rheological and structural properties of yoghurt. However, lactose has been shown to influence the degree of heat denaturation of whey proteins (Anema, 2000; Anema, Lee, & Klostermeyer, 2006), which in turn influences the rheology and structure of yoghurt (Anema et al., 2004; Dannenberg & Kessler, 1987; Jørgensen et al., 2015; McKenna & Anema, 1993). Anema et al. (2006) studied the effect of lactose on heat denaturation of β-LG and α-lactalbumin (α-LA) by recombining low-heat skim milk powder (SMP) in lactose solutions of 5, 10, and 15% to a protein content equal to that of a 9.6% total solids skim milk. Skim milk samples were heated at temperatures between 75 and 100 °C for 0–60 min. The irreversible denaturation of β-LG and α-LA decreased with increasing lactose concentration. Lactose increases the ordering of the water structure around protein molecules and thereby stabilises the native protein conformation (Anema et al., 2006). However, for β-LG, the stabilising effect of lactose diminished at heat treatment temperatures >90 °C (e.g., 95 °C for 5 min) (Anema, 2000; Anema et al., 2006). When heating skim milk with lactose content varying from approximately 5 to 20% at 95 °C for 5 min, β-LG denaturation is extensive and varies from approximately 95 to 85% (Anema et al., 2006). Thus, the firmness of a yoghurt made from yoghurt milk bases subjected to a conventional heat treatment (95 °C for 5 min) would probably be mostly unaffected by varying the lactose content from 5 to 20%. If the heat treatment temperature was reduced (80 °C for 5 min) the stabilising effect of lactose was increased, and variation in the lactose content of the yoghurt milk base is expected to have a greater influence on the thermal denaturation degree of β-LG (Anema et al., 2006), and thereby on yoghurt firmness (McKenna & Anema, 1993).

Meletharayil, Patel, Metzger, and Huppertz (2016b) investigated the effect of lactose level (no added lactose, 5.6% or 11.2%) on acid gels (4% protein) of reconstituted MPCs heat-treated at 90 °C for 10 min and acidified with GDL. Increasing the lactose content of the MPC dispersions to 5.6 or 11.2% increased the final G' and water-holding capacity and decreased the microstructural porosity of the acid gels at pH 4.6. This observation was linked to increased levels of soluble κ-CN and whey protein aggregates of the heat-treated MPC dispersions with increasing lactose concentration. Higher amounts of soluble aggregates of κ-CN and whey protein have previously been reported to increase the number and density of gelling protein particles, thereby increasing the G' of acid gels due to increased points of attachment during acidification with GDL (Anema et al., 2004; Guyomarc'h et al., 2003b) or starter culture (Ozcan, Horne, & Lucey, 2015). However, for bacterially fermented yoghurt gels, a balance of both soluble and bound aggregates of κ-CN and whey proteins seem to contribute to the stiffness of the gels (Ozcan et al., 2015). Due to the reported different rheological and physical properties between acid gels made with GDL or bacterial fermentation (Lucey, Tamehana, Singh, & Munro, 1998), the effect of lactose on G' of acid gels prepared with GDL observed by Meletharayil et al. (2016b) should be investigated using bacterial cultures.

A high-protein yoghurt may, for instance, be obtained by fortifying milk with dairy powders such as MPCs or MCCs to reach the desired protein level (Agarwal et al., 2015; Bong & Moraru, 2014; Meletharayil, Patel, & Huppertz, 2015). Protein fortification with low-protein MPC or MCC significantly increases the lactose content of the yoghurt milk base. For instance, protein fortification of skim milk with MPC42 (42% protein, 46% lactose) to a protein content in the yoghurt milk base of approximately 9% would concurrently increase the lactose content to approximately 11%. Vinderola, Costa, Regenhardt, and Reinheimer (2002) investigated the effect of lactose concentration (5, 15, or 20%) on the growth of some strains of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*. A lactose concentration of 15% inhibited some strains of these lactic acid bacteria. Thus, the lactose content of a yoghurt milk base must be taken into consideration, because excessive lactose content may inhibit or decline the rate of acid production by the yoghurt culture due to increased osmotic pressure (Vedamuthu, 2006).

The influence of lactose on rheological properties of high-protein yoghurt seems to be a relevant focus for further research. Lactose has become a surplus milk component with the emerging use of membrane filtration technologies in the dairy industry. Application of lactose to manage texture and water-holding capacity of yoghurts could be an interesting option for reducing cost of yoghurt production.

3. Methods for increasing protein content of yoghurt

Protein content of yoghurt can be increased prior to fermentation by adding dairy powders, by evaporation, or by membrane filtration, alternatively after fermentation with the use of straining, mechanical separation, or membrane filtration. Concentrating the yoghurt after fermentation produces large volumes of acid whey. Acid whey has been a major concern in the dairy industry. By concentrating prior to fermentation, acid whey production is avoided. It is, however, also possible to combine concentration prior to and after fermentation. The main focus in the following text will be on methods for protein concentration prior to yoghurt fermentation, because these methods eliminate the production of acid whey. However, the various manufacturing possibilities for concentrating fermented yoghurt are briefly mentioned.

3.1. Concentration of yoghurt after fermentation

The rheology and microstructure of high-protein yoghurt (i.e., Labneh) concentrated after fermentation has been previously studied (Ozer, Stenning, Grandison, & Robinson, 1999a,b; Abu-Jdayil, Jumah, & Shaker, 2002; Ozer, Bell, Grandison, & Robinson, 1998; Tamime, Kalab, & Davies, 1991) and reviewed (Nsabimana et al., 2005; Özer, 2006).

Traditionally, strained yoghurt can be made by using cloth bags for whey drainage. Yoghurt is poured into cloth bags and strained to the desired total solids content. Depending on the pressure applied, the drainage time can be 6–18 h. Today, cloth bags are mostly replaced by nozzle or quarg separators in industrial productions. Fermented milk is vigorously stirred and optionally passed through a metal strainer or filter to break up any large clumps. The yoghurt is concentrated at 35–40 °C, cooled to ~15 °C, cream is optionally added, and the product is packaged. Fat standardisation can also be performed prior to fermentation if specially designed separators are used for the straining of whey (Nsabimana et al., 2005; Tamime et al., 2014). Membrane technologies, mainly UF, are other options for yoghurt concentration after fermentation. The fermented, warm (~40 °C) yoghurt is gently stirred and concentrated by a UF plant with 5–6 bar inlet pressure. The concentrated yoghurt is cooled to ~10–20 °C and packaged. A thermisation step can be added prior to concentration to improve the release of whey, inactivate most of the lactic acid microflora in the product, reduce the extent of proteolysis, and improve the keeping quality (Rukke, Sørhaug, & Stepaniek, 2014; Zakrzewski, Stepaniak, Abrahamsen, & Sørhaug, 1991; Özer & Tamime, 2013). The thermisation step may be undesirable if a content claim is made on the product label, referring to the presence of a specific live microorganism (Codex Alimentarius, 2011).

Manufacturing techniques influences the rheological and structural properties of the concentrated yoghurt, as investigated by Ozer et al. (1998), and Ozer et al. (1999a,b). Concentrated (strained) yoghurt (~9% protein) produced with the traditional cloth bag method has shown a more compact microstructure than yoghurt concentrated with UF after fermentation, which had a more discontinuous network with thicker casein clusters (Ozer et al., 1999a). Tamime et al. (1991) reported that the firmness of Labneh (~8% protein) was higher and had more complex micellar strains when UF took place at 55 °C than Labneh concentrated at lower temperatures as for instance 35 °C. The differences were explained by agglomeration of casein particles caused by the higher temperature. UF at 50 or 55 °C yielded Labneh with a similar firmness to Labneh concentrated (strained) by the cloth bag method (~9% protein). Transmission electron microscopy revealed, however, that the traditional Labneh had simpler and less complex protein chains than the UF-Labneh. The lower firmness of the UF Labneh concentrated at 35 °C was attributed to the different processing conditions during concentration; i.e., pressure-driven concentration versus gravitational concentration. Abu-Jdayil et al. (2002) observed a greater loss of apparent viscosity measured at a shear rate of 106 s^{-1} as a function of shearing time for commercial Labneh produced by the traditional method compared with commercial Labneh produced by centrifugation. They suggested that the different production methods produced products with different space occupancies in the structure.

Centrifugation in the production of high-protein yoghurt has been adapted from quarg manufacturing processes. The resemblance between processing technologies for high-protein yoghurts and quarg might underpin the need for a clearer distinction between products in the "concentrated fermented milk"-category and the "fresh cheese"-category, to close loopholes in the Codex standard (Codex Alimentarius, 2011).

3.2. Concentration of yoghurt milk base prior to fermentation

3.2.1. Technical aspects of membrane filtration for the concentration of yoghurt milk base

Ultrafiltration. UF of milk gives a protein-rich retentate, and a protein-free permeate (Codex standard 207–1999, Codex Alimentarius (2011)). It is common industrial practice to concentrate milk with UF to increase the protein content of the yoghurt milk base before fermentation into yoghurt with less than 5.6% protein (Rattray & Jelen, 1996). Evaporation or addition of SMP are other common industrial methods. However, these two methods also significantly increase the lactose content of the yoghurt milk base, which influences the nutritional quality of the product, and its textural characteristics (Abrahamsen & Holmen, 1980).

UF membranes are offered in a variety of module configurations, including hollow fibers, tubular, plate and frame, and spiral wound (Pouliot, 2008). For producing milk retentates, the spiral wound configuration is typically used (Gésan-Guiziou, 2013). Milk UF can be performed at around 50 °C or around 10 °C. Permeation fluxes are higher at 50 °C, but the process duration must be reduced due to precipitation of calcium phosphate in the membrane pores and due to possible bacterial growth in the retentate. UF at around 10 °C results in little bacterial growth, and the process duration can be doubled depending on process parameters (Gésan-Guiziou, 2013). However, UF at 10 °C and with a membrane cutoff greater than ~20 kDa potentially increases the permeation of β-casein (β-CN), as β-CN leaks out from casein micelles during low temperatures (Farrell et al., 2004; Liu, Weeks, Dunstan, & Martin, 2013; Rose, 1968; Schmitt, Saulnier, Malhautier, & Linden, 1993; van Hekken & Holsinger, 2000). Milk retentate produced by UF of milk with a membrane cutoff of ~10 kDa, or smaller, has a whey protein to casein ratio which is unchanged from that of the original milk (~20:80).

Microfiltration. MF of milk with membrane pore sizes in the range from 0.05 to 0.20 μm produces a casein-rich retentate ("casein concentrate") and a permeate with native whey proteins. The content of whey proteins in the MF permeate cannot exceed the content of whey proteins in the feed. However, with UF of the MF permeate with membranes with a cutoff ~10 kDa, the whey proteins can be concentrated into a NWPC (Maubois, 2002). The casein concentrate has increased contents of casein and colloidal-calcium-phosphate (CCP) compared with the original milk (Brandsma & Rizvi, 1999; Jørgensen et al., 2016; Neocleous, Barbano, & Rudan, 2002). The casein concentrate can be used in cheese production, especially of hard cheese varieties (Daufin et al., 2001; Kumar et al., 2013), and will give an improved rennet coagulation of cheese milk with a moderate increase in casein content (~30–40 g kg^{-1}) (Heino, Uusi-Rauva, & Outinen, 2009, 2010; Maubois, 2002). However, in yoghurt production, the presence of whey proteins in the yoghurt milk base is essential. Addition of NWPC (Jørgensen et al., 2015) or whey powders, like WPC or WPI, makes the casein concentrate from MF suitable as a milk base for yoghurt manufacture.

A major concern in protein fractionation of skim milk by MF is to minimise and control fouling. Fouling means the deposition of milk components, such as proteins and calcium phosphate, on the membrane surface or in the pores of the membrane (Koh, Ashokkumar, & Kentish, 2013; Saxena, Tripathi, Kumar, & Shahi, 2009). Fouling appears as a flux decline with filtration time at a constant transmembrane pressure (TMP), or as a TMP increase at a constant flux. Flux is the amount of permeate (mass or volume) removed from the feed stream per unit of membrane area and time (Hausmann, Duke, & Demmer, 2013). The term “critical flux” describes the flux at which fouling begins to occur (Field, Wu, Howell, & Gupta, 1995; Howell, 1995). Below the critical flux, there is a linear relationship between flux and TMP, where the selectivity of the MF process is controlled entirely by the membrane (Bacchin, Aimar, & Field, 2006) (Fig. 2). Operation in this region is termed subcritical (Howell, 1995) and is advised for optimal separation of casein and whey proteins (Brans, Schroën, van der Sman, & Boom, 2004). MF for protein fractionation is often operated above, but close to the critical flux, where the relationship between flux and TMP is no longer linear (Brans et al., 2004). The critical flux is reached when fouling occurs locally on the membrane (Bacchin, 2004). Above the critical flux, the deposit layer (fouling) acts as a secondary membrane, which leads to an alteration of the selectivity of the MF process and a decrease in whey protein permeation (Koh et al., 2013). The term “limiting flux” describes the highest flux that can be achieved by increasing TMP at specific hydrodynamic conditions (Bacchin et al., 2006). The limiting flux is reached when the whole membrane surface is controlled by the deposit layer (Bacchin, 2004). Further increases in TMP cause compaction of the deposited layer, and ultimately flux decline (Brans et al., 2004). Bacchin et al. (2006) introduced the term “sustainable flux”, meaning the flux that the system can operate at for extended periods of time. The sustainable flux refers to operational and economic sustainability of the MF process, and is somewhere between the critical and limiting flux, where the fouling rate is low.

MF of milk became industrially feasible with the hydraulic concept proposed by Sandblom (1974). The pressure-driven cross-flow of milk through the filter channels, tangential to the filter area, creates a pressure drop along the module. The pressure drop is relatively large because of the high cross-flow velocity required to obtain high permeation flux and accurate membrane selectivity (Saboya & Maubois, 2000; Smith, 2013b). The pressure drop on the retentate side causes heterogeneous fouling (Saboya & Maubois, 2000). To obtain a constant TMP over the length of the module, a permeate pump is installed, causing the permeate to recirculate co-currently with the feed/retentate stream in a separate loop, creating a pressure drop on the permeate side similar to the pressure drop on the feed/retentate side (Sandblom, 1974). The uniform transmembrane pressure (UTP) obtained, results in better control of the fouling and consequently in a more acceptable MF performance (Gésan-Guiziou, 2013).

The membranes used can be formed by combining metals such as aluminum, titanium, or zirconium with support materials, and are commonly referred to as ceramic membranes. Ceramic membranes can tolerate a wide range of pH values (0–14) and temperatures, high pressures (up to ~300 bar), and high TMPs (>170 bar). Some drawbacks of ceramic systems include high capital costs, sensitivity of the membranes to fast temperature changes, and labor-intensive membrane replacement (Smith, 2013a). The increased operational costs caused by the energy demand of the permeate pump in the UTP system can be reduced by filling the permeate compartment with plastic beads (Saboya & Maubois, 2000).

Another hydraulic concept that ensures a stable MF regime along the membrane is the so-called inhomogeneous ceramic membranes, with a higher hydraulic resistance at the membrane inlet where the TMP is high and a lower resistance at the membrane outlet (Gésan-Guiziou, 2013). The longitudinal permeability gradient can be built into the support structure, often referred to as ceramic graded permeability membranes (Garcera & Toujas, 1997), or can be obtained by modifying the thickness of the separating layer (Skrzypek & Burger, 2010). Both these commercially available concepts avoid the need for a permeate pump, thus reducing the

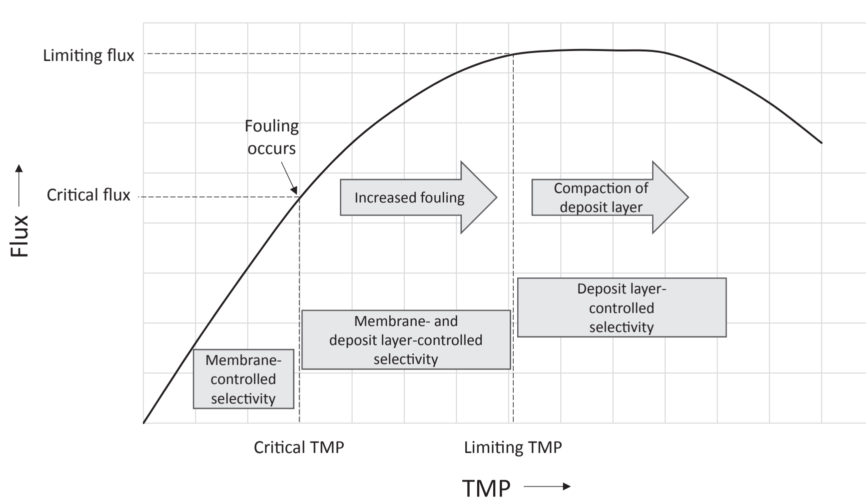

Fig. 2. Effect of fouling on flux as a function of transmembrane pressure (TMP) during microfiltration (MF) of skim milk. This figure is based on information from Bacchin et al. (2006), Brans et al. (2004), Field et al. (1995), Howell (1995) and Piry (2010).

investment and running costs compared with the UTP system. Skrzypek and Burger (2010) reported that industrial plants using 0.14 μm Isoflux membranes (TAMI Industries) were established in Poland and the Czech Republic for casein standardisation of skim milk for quarg production. MF of skim milk to a casein concentration factor of 1.6–2.0 reduced the amount of acid whey by 40–60%.

Protein fractionation of milk by MF can also be performed with polymeric spiral wound membranes. Polymeric membranes have, in general, a wider pore size distribution than ceramic membranes, and a shorter membrane life (Brans et al., 2004; Pouliot, 2008). Zulewska, Newbold, and Barbano (2009) reported that ceramic membranes (0.1 μm pore size) in a UTP system gave significantly better whey protein removal from skim milk than ceramic graded permeability membranes (0.1 μm) and polymeric spiral-wound membranes (0.3 μm). Under the operational MF conditions used in their study, the highest flux value was observed for the ceramic graded permeability membrane, followed by the ceramic UTP system. Fractionation of caseins and whey proteins with the polymeric spiral wound membrane was, however, only effective after the formation of a boundary layer of milk proteins on the surface of the membrane. Before formation of the boundary layer, significant amounts of caseins were detected in the permeate. Lawrence, Kentish, O'Connor, Barber, and Stevens (2008) also observed that the effect of MF with polymeric spiral wound membranes (0.3 and 0.5 μm) was dictated by a protein layer that rapidly formed on the membrane surface. Formation of a protein layer increased the rejection of caseins; however, rejection of β-LG also increased. Today, a significant portion of dairy plants (~80%) performing milk protein fractionation use polymeric spiral wound systems (Tetra Pak Filtration Solutions, personal communication).

Protein fractionation with ceramic membranes is a strategic choice for optimal separation of caseins and whey proteins. However, membrane designs and systems are not the only factors influencing protein fractionation of skim milk by MF, other factors influencing the composition of retentates and permeates are: Composition and pretreatment of the skim milk (Brandsma & Rizvi, 1999; Svanborg, Johansen, Abrahamsen, & Skeie, 2014), membrane pore size (Jørgensen et al., 2016; Punidadas & Rizvi, 1998), membrane channel diameter (Hurt, Adams, & Barbano, 2015b,c), membrane length (Piry et al., 2008), filtration temperature (Hurt, Adams, & Barbano, 2015a; Jørgensen et al., 2016; Kersten, 2001), volume concentration factor (VCF) (Kersten, 2001; Punidadas & Rizvi, 1998), ratio of permeation flux to wall shear stress (Gésan-Guiziou, Boyaval, & Daufin, 1999; Le Berre & Daufin, 1996), and fouling (Gésan-Guiziou et al., 1999; Jimenez-Lopez et al., 2008). Table 1 summarises some experimental approaches and reported effects from some previous studies.

To obtain maximal separation of caseins and whey proteins by MF of skim milk, the skim milk should preferably be unpasteurised (Svanborg et al., 2014) (Table 1). Prior to protein fractionation by MF, the microbial load of the unpasteurised skim milk can be reduced by MF with pore sizes in the range 0.8–1.4 μm (Maubois, 2002). Casein micelles are polydisperse in size (Fox & Kelly, 2004), and research supports that the mean diameter of casein micelles varies between individual cows (Devold et al., 2000; de Kruif & Huppertz, 2012), feeding regimes (Devold et al., 2000), and seasons (Glantz et al., 2010). Thus, retention of casein micelles with a specific membrane pore size depends on the casein micelle size distribution of the original milk.

There is no standard to define the separation ability of ceramic membranes, thus the given pore size of a membrane should be considered as an indication of its separating ability, not as a precise definition. The volume concentration factor (VCF) during MF affects the flux and permeation of whey proteins, and a moderate VCF is advantageous for high specific mass flux of whey proteins (Kersten, 2001) (Table 1). The filtration temperature should preferably be high enough to limit microbial growth and promote high flux values (≥50 °C), but low enough to avoid heat denaturation of whey proteins and to limit possible interactions between whey proteins and caseins deposited on the membrane surface (<60 °C) (Hurt et al., 2015a; Jimenez-Lopez et al., 2008; Jørgensen et al., 2016; Kersten, 2001). A critical value of the ratio of flux to wall shear stress has been reported (~1.0 L m^{-2} h^{-1} Pa^{-1}), and parameters should be chosen to ensure MF operation below this value, thereby limiting fouling while maintaining high whey protein transmission (Jimenez-Lopez et al., 2008; Le Berre & Daufin, 1996).

Microsieves and dynamic filtration devices. Other membranes, like microsieves and dynamic filtration devices, have been developed to exceed performances of conventional MF membranes (Jaffrin, 2008; Saxena et al., 2009). Microsieves have well-defined, uniform pores and low flow resistance due to a very thin selective silicon-nitride layer. The main advantage of microsieves is the high permeate flux obtainable (Saxena et al., 2009). So far, research on applications of microsieves in milk processing seems to be limited to bacteria removal (Brito-de la Fuente, Torrestiana-Sánchez, Martínez-González, & Mainou-Sierra, 2010; Verwijst, Baggerman, Liebermann, & van Rijn, 2015). In dynamic or shear-enhanced filtration systems, high shear rates are created to limit deposit formation, resulting in higher permeate fluxes and increased membrane selectivity (Jaffrin, 2008). Dynamic filtration systems for processing of milk have been investigated for protein concentration by UF (Akoum, Jaffrin, & Ding, 2005; Ding, Zhang, Ould-Dris, Jaffrin, & Tang, 2016; Meyer, Mayer, & Kulozik, 2015) and protein fractionation by MF (Al-Akoum, Ding, Chotard-Ghodsnia, Jaffrin, & Gésan-Guiziou, 2002; Espina, Jaffrin, Frappart, & Ding, 2008). Meyer et al. (2015) and Meyer, Hartinger, Sigler, and Kulozik (2017) suggested that dynamic filtration systems can be operated in cascade mode as a supplement to conventional cross-flow filtration processes to reach higher volume reduction ratios during UF of skim milk (e.g., 30% protein in the final retentate). The investment costs per membrane area of microsieves and dynamic filtration systems are relatively high compared with conventional cross-flow systems with ceramic membranes or polymeric spiral wound membranes (Jaffrin, 2008; Meyer et al., 2015; Saxena et al., 2009; Verwijst et al., 2015). Jaffrin (2008) concluded that the sales of industrial dynamic systems seemed to be limited in volume, but predicted that further development of dynamic systems could make these filtration devices more popular in the future.

3.2.2. Addition of membrane-manufactured powders to the yoghurt milk base

Traditionally, SMP has been used to enrich protein and total solids of yoghurt milk bases (<5.6% protein). Tamime et al. (2014) reported that the carbohydrate content of commercial strained fermented milks ranged from 1 to 12%. Yoghurts produced by the addition of SMP or whey powders had higher lactose content and lower protein content, and thus, lower firmness than yoghurts produced by concentration after fermentation, or from UF retentate. The use of membrane-manufactured powders containing less lactose and more protein than SMP, could enhance the composition and textural characteristics of high-protein yoghurts.

In the literature, the dried milk retentate from UF of milk (MPC) is often termed "milk protein concentrate", but is also referred to as "retentate powder", "native milk protein concentrate", "milk powder from ultrafiltered skim milk", "skim milk retentate powder", and "high-protein lactose-free milk powder". The dried retentate from protein fractionation of skim milk by MF (MCC) is often referred to as "micellar casein concentrate", "native phosphocaseinate", "micellar casein powder", or "micellar casein isolate" (Carr & Golding, 2016). MPCs are manufactured to contain

Table 1
Summary of studies of effects of various factors on composition of permeates and retentates from protein fractionation by ceramic microfiltration.[a]

Processing factors and experimental approach	Results	Reference
Pretreatment of skim milk		
Ceramic UTP MF, 0.2 μm (Jiuwu); feed: unpasteurised or pasteurised (73 °C/15 s) skim milk; average filtration temperature 56.3 °C; average VCF 2.47.	Permeate from MF of unpasteurised skim milk contained higher amounts of calcium, phosphorus, and native whey proteins, and fewer casein fragments than permeate from MF of pasteurised skim milk.	Svanborg et al. (2014)
Ceramic UTP MF, 0.2 μm (Membralox P19-40, US Filter Corp.); feed – pasteurised skim milk (HTST); filtration temperature 50 °C; cross-flow velocity 7.5 m s^{-1}; VCF 8–9 (to ~20% total protein in retentate); GDL added to retentate during filtration to reduce pH from 6.6 to 6.3 and 6.0.	A reduction in retentate pH from 6.6 to 6.0 decreased retentate calcium content by 20.1%, increased whey protein retention by 12.6%, and reduced permeate flux.	Brandsma and Rizvi (1999)
Membrane pore size		
Ceramic MF with asymmetric membranes (Ceramem), 0.05- or 0.20-μm pore size; feed – pasteurised skim milk; filtration temperature 50 °C; cross-flow velocity 5.4 m s^{-1}; mean pressure on retentate side 138 kPa; concentration factor 2.5.	MF using a membrane with 0.05-μm pore size retained all caseins and allowed whey proteins to permeate. Permeate from MF using a membrane with 0.2-μm pore size contained a significant amount of casein. Higher cross-flow velocity gave a higher flux for the membrane with 0.05-μm pore size.	Punidadas and Rizvi (1998)
Ceramic UTP MF, 0.05- and 0.10-μm (Orelis), 0.20-μm (Atech) pore sizes; feed – pasteurised skim milk (73 °C/15s); filtration temperature 50 °C; cross-flow velocity 6.9 m s^{-1}; constant flux 44 L m^{-2} h^{-1} for 0.05 μm, ~58 L m^{-2} h^{-1} for 0.10 and 0.20 μm; VCF 2.5.	MF membranes with 0.10- and 0.05-μm pore size retained all caseins. Transmission of native whey proteins increased with increasing pore size. Significant amounts of small casein micelles permeated the membrane with 0.20-μm pore size resulting in a permeate with 1.4% casein and a casein distribution (α_{s2}-CN: α_{s1}-CN: κ-CN: β-CN) similar to that of skim milk.	Jørgensen et al. (2016)
Membrane length		
Ceramic MF, 0.1-μm pore size (Atech); feed – pasteurised skim milk (71 °C/20s); filtration temperature 55 °C; wall shear stress 115 Pa; cross-flow velocity ~6 m s^{-1}; permeate and retentate were recirculated (no concentration). A special module design consisting of 1.2-m long membrane with four sections. Average TMPs in section 1–4: 82 kPa–17 kPa.	Flux was independent of TMP in the first three sections; filtration was deposit layer-controlled. Section 4 had pressure-dependent flux. Permeation of β-LG increased from 38% in section 1–87% in section 4. The deposit layer was responsible for the retention of whey proteins in the first sections. An optimum exists for the relationship of protein permeation and flux.	Piry et al. (2008)
Filtration temperature		
Ceramic UTP MF, 0.10-μm pore size (Orelis); feed – pasteurised skim milk (73 °C/15s); filtration temperature 50 or 60 °C; cross-flow velocity 6.9 m s^{-1}; constant flux ~59 L m^{-2} h^{-1}, VCF 2.5, no recirculation.	Permeate from MF at 50 °C contained significantly more native whey proteins and calcium than MF at 60 °C. Retentate from MF at 60 °C had less caseins, probably due to deposition of caseins on the membrane surface. MF at 60 °C had a more rapid increase in TMP.	Jørgensen et al. (2016)
Ceramic UTP MF, 0.10-μm pore size (Membralox, Pall Corp.); feed - pasteurised skim milk (72 °C/16s); VCF 3.0; total recirculation mode; filtration temperature sequentially increased from 50 to 55 to 60 –65 °C, MF operated for 1 h at each temperature.	Content of calcium and native whey proteins in permeate decreased as the filtration temperature was increased to 65 °C, and at the same time casein contamination in the permeate decreased. Increasing the MF temperature from 50 to 65 °C decreased the TMP required to maintain a flux of 54 kg m^{-2} h^{-1}.	Hurt et al. (2015a)
Volume concentration factor		
Ceramic UTP MF, 0.10-μm pore size (Société des Céramiques Techniques); feed – pasteurised skim milk; filtration temperature 55 °C; wall shear stress 150 Pa; constant difference in pressure loss on the retentate side to the permeate side 40 kPa; concentration factor from 1 to 5, expressed as the ratio of casein content in retentate to skim milk, which is close to VCF during 0.1 μm MF.	Flux decreased with increasing casein concentration factor. Permeation of β-LG and α-LA increased with increasing concentration factor. The specific mass flux of β-LG increased with increasing concentration factor and reached a maximum at concentration factor ~2.5. A further increase in concentration factor to 5 decreased the specific mass flux. The protein fractionation was optimal at concentration factor ~2.5.	Kersten (2001)
Wall shear stress		
Ceramic UTP MF, 0.10-μm pore size (Membralox, Société des Céramiques Techniques); feed – skim milk heated at 63 °C/15 s; filtration temperature 50 °C; VRR 2.0; wall shear stress from 40 to 110 Pa; various constant flux from 30 to 110 L m^{-2} h^{-1}.	Micellar casein retention was >0.99 in all MF runs. MF at flux 90 L m^{-2} h^{-1} and wall shear stress 110 Pa gave steady MF for 400 min with 70–80% whey protein transmission and almost total casein rejection. Higher flux and/or lower wall shear stress gave divergent runs with a sudden increase in fouling and sharp decrease of transmission. A critical ratio of flux to effective wall shear stress (convection towards the membrane/erosion) was found: ~1.0 L m^{-2} h^{-1} Pa^{-1}. MF below this value gave slow increase of fouling resistance and high whey protein transmission with slow decrease.	Le Berre and Daufin (1996)
Fouling		
Ceramic UTP MF, 0.1-μm pore size (Orelis); feed – skim milk heated at 63 °C/15 s; filtration temperature 50 °C; VRR 2.0; wall shear stress 100 Pa; retentate pressure 400 kPa; gradual increase in TMP from 0 kPa to 100 kPa.	TMP <10 kPa: Linear relationship of flux as function of TMP, permeate turbidity low, transmission of whey proteins high, thickness of deposit low. TMP ~10–30 kPa: Increased deposit thickness, decreased transmission of whey proteins, increased permeate turbidity. TMP ~30–100 kPa: Flux stabilized at a limiting permeation flux (~75 L m^{-2} h^{-1}), continued decrease in whey protein transmission, deposit thickness remained constant.	Gésan-Guiziou et al. (1999)
Ceramic UTP MF, 0.1 μm pore size (Kerasep, Novasep); different feed solutions – skimmed milks (thermised (68 ± 1 °C/25s) and pasteurised (78 ± 1 °C/20s)) suspensions of native casein micelle powder, and aqueous phases of milk; filtration temperature 48 °C; constant flux of 50 L m^{-2} h^{-1}; VRR 2.0; wall shear stress decreased step-by-step from 120 Pa to the critical wall shear stress (55 Pa), after 1 h wall shear stress was increased step-by-step to 120 Pa.	MF of thermised skim milk: Before the formation of deposit layer (wall shear stress 120 to 80 Pa), TMP was stable (~12 kPa), soluble protein (whey) transmission was constant (~65%). Decrease in wall shear stress to 55 Pa gave quick and sharp increase in TMP and soluble protein transmission was decreased down to 25%. Increase in wall shear stress to 120 Pa yielded 67% increase in TMP, soluble protein transmission was 55%.	Jimenez-Lopez et al. (2008)

Table 1 (*continued*)

Processing factors and experimental approach	Results	Reference
	Aqueous phases of milk (no casein): Decrease in wall shear stress to 55 Pa gave no sharp increase in TMP; however, TMP increased at the end of the filtration. Soluble protein transmission (~80%) was higher than for thermised skim milk. A critical ratio of flux to wall shear stress was found for MF of milk: ~0.91 L m^{-2} h^{-1} Pa^{-1}. Change in transmission of soluble proteins at critical wall shear stress was due to fouling. Casein micelles were the major milk constituent responsible for fouling. Minerals enabled easier set-up of the fouling layer (membrane–micelle interaction and micelle–micelle interaction). Soluble proteins had no effect on the set-up of the fouling layer, but their presence in milk increased irreversible fouling by 20%. Soluble proteins could be rejected or entrapped (e.g., electrostatic interactions) in the micelle deposit, limiting their permeation.	

[a] Abbreviations are: MF, microfiltration; UTP, uniform transmembrane pressure; TMP, transmembrane pressure; VCF, volume concentration factor; VRR, volume reduction ratio; GDL, glucono-δ-lactone; CN, casein; β-LG, β-lactoglobulin; α-LA, α-lactalbumin.

protein on a dry basis from 42% (MPC42) to 85% (MPC85). The content of lactose of these powders is typically in the range from 46% (MPC42) to 4% (MPC85). Fat and ash contents are around 1.5 and 7%, respectively.

The composition of MCC powders with respect to ratio of whey protein to casein, and content of protein, lactose, and ash depends on the membrane processing (Hurt & Barbano, 2015; Hurt, Zulewska, Newbold, & Barbano, 2010). While MPCs provide casein and whey protein in the same ratio as milk, MCCs in general have an increased casein to whey protein ratio or can be almost devoid of whey proteins. MPCs are used to fortify yoghurt (Agarwal et al., 2015), although MCCs or MPCs with the addition of WPCs or NWPCs could provide a yoghurt milk base with beneficial protein composition.

Solubility, wettability, flavour, browning behavior, gelation abilities, water binding, and viscosity are important functional attributes of MPCs and MCCs used to fortify yoghurt milk bases for manufacturing high-protein yoghurts. Because the dissolution of powder is necessary for the expression of the various functional attributes, solubility was regarded by Agarwal et al. (2015) as a critical property of MPC. The mechanisms of the development of insolubility of MPCs, key factors responsible for their insolubility and approaches to increase the solubility of these powders has recently been comprehensively reviewed by Meena, Singh, Panjagari, and Arora (2017).

Nasser et al. (2017a) and Nasser, Moreau, Jeantet, Hédoux, and Delaplace (2017b) investigated the effect of storage time and temperature on the rehydration time of a MCC. When the MCC was stored at 20 °C, the rehydration time was doubled after 6 months of storage. At 40 °C, the rehydration time doubled after 15 days. And at 60 °C, the rehydration time more than doubled after only 1 day. MCC stored at 4 °C was only slightly affected by ageing. An increased browning index and decreased solubility of the powders were also obvious results of storage time and temperature.

MPCs with high protein contents (e.g., MPC80) have relatively poor solubility upon reconstitution in water at 20 °C. However, the solubility is increased at elevated reconstitution temperatures (e.g., 37 °C) and if the reconstituted solution is homogenised (e.g., 138 bar) (Sikand, Tong, Vink, & Walker, 2012). The solubility of MPCs is higher when reconstituted in milk permeate (Sikand et al., 2012) or in milk (Udabage, Puvanenthiran, Yoo, Versteeg, & Augustin, 2012), than in water. Sikand, Tong, Roy, Rodriguez-Saona, and Murray (2011) observed that the solubility of commercial MPC80s and milk protein isolate (~90% protein) depended on their mineral composition. Solubility was correlated with increased sodium content and reduced calcium, magnesium, and phosphorus content. Mao, Tong, Gualco, and Vink (2012) reported that the solubility of MPC80 could be enhanced by adding NaCl during the DF stage. Gazi and Huppertz (2015) observed that MPC35–MPC90 powders were fully soluble immediately after their production. However, the solubility of MPCs with protein contents ≥60% changed during storage, and depended on the storage temperature. Solubility of MPC80 and MPC90 remained high upon storage at 20 °C for 60 d, but decreased strongly to ~50% solubility upon storage at 37 °C for 60 d. Smith, Campbell, Jo, and Drake (2016) found that the solubility of MPC80 stored at 3 °C was reduced by 15% after 12 months. The solubility of MPC80 stored at 25 °C was reduced by 51% and at 40 °C by 88%.

The reduced solubility of MPCs has been linked to reduced solubility of caseins, suggesting that the insoluble caseins primarily were in the micellar form. Recently Silva and O'Mahony (2017) compared the flowability and wetting behavior of milk protein ingredients used for instance in the milk base for production of yoghurt. A comparison of SMP, whole milk powder, buttermilk powder and MPC showed that the wetting properties of MPC was less good than the values obtained for SMP and buttermilk powder. These observations were explained by the comparatively high protein content of the MPC and in particular the high casein content. It is known from other works as well that powders high in protein and casein content are somewhat difficult to reconstitute sufficiently. A certain inhibition of the transfer of water into the powder particles is considered as the prime reason for reconstitution difficulties (Crowley et al., 2015; Vos et al., 2016).

According to Udabage et al. (2012), high pressure treatment (200 MPa at 40 °C) applied to the concentrate before spray drying improved MPC solubility. This increased solubility was linked to an increased concentration of non-micellar casein. Reducing colloidal calcium content by carbon dioxide treatment of the milk before and during UF (Marella, Salunke, Biswas, Kommineni, & Metzger, 2015; Meletharayil, Metzger, & Patel, 2016), or calcium removal by ion exchange (Bhaskar, Singh, & Blazey, 1999), are other options to tailor the solubility of MPCs for their application in high-protein yoghurts. Further research seems to be needed to determine the possible effect of increased content of non-micellar casein in a yoghurt milk base prepared from MPC on the various properties of high-protein yoghurt. Increased knowledge of the effect of processing conditions and powder composition on the functionality of MPCs could ease the selection of MPC for high-protein yoghurt manufacturers. Low solubility of MPC and MCC differentiates the application of these powders from the application of liquid concentrates. The reasons for the insolubility is not fully understood. Microstructural analyses have, however, shown that casein micelles interact with each other on the surface of the powder particle, giving a "skin formation". The skin slows down the release of casein micelles from the dispersed powder particles (Schokker et al., 2011; Sikand et al., 2011).

The flavour profiles of commercial MPCs differ and depend on several factors. Drake, Miracle, and Wright (2009) found that commercial MPCs with lower protein contents (56 or 70% protein on dry basis) had similar flavour profiles as fluid milk. The flavour of these MPCs was characterised as cooked/milky, sweet aromatic, sweet taste and cereal. As the protein content increased, the flavour profile changed. Some of the MPC70 and MPC80 were characterised by tortilla, brothy, cardboard, and animal flavours and higher astringency. However, Drake et al. (2009) did not provide information on the age of the powders. It is important to be aware of the possible changes in flavour profiles of MPCs with increasing protein contents.

4. Challenges and possibilities in producing high-protein yoghurt

4.1. By-products

Concentrating fermented yoghurt produces large volumes of acid whey, which became a major concern to the dairy industry with the increased production of high-protein yoghurts. Nishanthi, Vasiljevic, and Chandrapala (2017) reported that the composition of acid whey from commercial production of "Greek-style yoghurt" differed from sweet whey from commercial hard rennet cheese production. The acid whey contained ~3.2% lactose and had a low pH (4.55) due to relatively high amounts of lactic acid (0.55%). It contained low amounts of total protein (0.24%) and high amounts of calcium (0.13%) and total phosphate (0.18%) compared with the sweet whey with 1.04%, 0.06%, and 0.07%, respectively. Only limited amounts of whey proteins are lost to the whey stream during yoghurt concentration, because most whey proteins are heat-denatured and retained in the product.

According to Wijayasinghe, Vasiljevic, and Chandrapala (2015), the presence of lactic acid hindered the removal of water during evaporation of acid whey and limited lactose crystallisation in freeze-dried powders. The presence of calcium was reported by Chandrapala and Vasiljevic (2017) to impair lactose crystallisation in spray dried lactose powders at certain ratios (0.12% and 1%) of lactic acid to calcium. Because of the challenge with lactose crystallisation in spray-drying of acid whey, many manufacturers of high-protein yoghurt are faced with two options: distribution of acid whey as animal feed, or disposal of acid whey, both options creating additional costs. Thus, a reduction in the volume of acid whey from production of high-protein yoghurts could be beneficial for the dairy industry.

The production of membrane-manufactured liquid concentrates or powders produces milk permeate as a by-product. Milk permeate is, as opposed to acid whey, free from lactic acid, galactose from lactose catabolism, and metabolites from yoghurt fermentation, and has a neutral pH. The composition of milk permeate makes it more suitable for downstream processing than acid whey. Milk permeate could for instance be used for "down-standardisation" of SMP to a minimum protein content of 34% (Codex Alimentarius, 2011; Rattray & Jelen, 1996; Williams, D'Ath, & Zisu, 2008). Concentration of the yoghurt milk base prior to fermentation can also be combined with subsequent concentration of the yoghurt after fermentation. Fig. 3 gives an example of the volume distribution of by-products from the production of a high-protein yoghurt (8% protein) as influenced by the sequence of the concentration step in the production line. Concentration of the yoghurt after fermentation produces ~1.3 kg of acid whey per kg yoghurt. Concentration of the yoghurt milk base to 5% crude protein by UF, and subsequent concentration of the fermented yoghurt by nozzle separator approximately halves the volume of acid whey. In the latter examples, the volume reduction of acid whey is balanced by a corresponding increase in volume of milk permeate.

4.2. Yoghurt structure and rheology

When manufacturing yoghurts with <5.6% protein, textural defects such as a weak body and wheying-off may appear. Also insufficient whey protein denaturation during heat treatment,

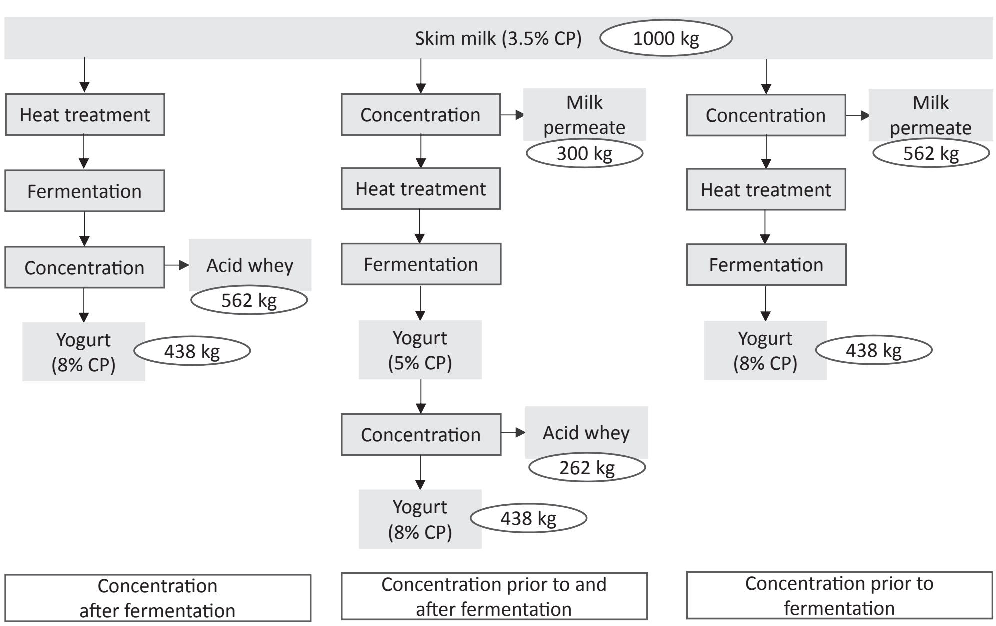

Fig. 3. Illustration of volume distribution of by-products from production of high-protein yoghurt (8% protein) influenced by the sequence of the concentration steps: concentration after fermentation (nozzle separator); combination of concentration prior to and after fermentation (ultrafiltration and nozzle separator); concentration prior to fermentation (ultrafiltration). Ellipses indicate volumes. Abbreviation: CP, crude protein.

excessive mechanical shearing during pumping and stirring, and/or physical mishandling during distribution may give textural defects (Lucey, 2004). Set yoghurts fortified with MPC to a protein content above 8% have been reported to possess a grainy texture (Mistry & Hassan, 1992). Stirred yoghurts made from yoghurt milk bases of liquid protein concentrates with 8% protein and heat-treated at 95 °C for 5 min were perceived as grainy and coarse (Jørgensen et al., 2015). The firmness and G' values of these yoghurts tended to increase with increasing ratio of whey protein:casein. Sensory roughness, coarseness, and graininess of stirred yoghurts correlates well with the coagulum particle size of the yoghurt (Jørgensen et al., 2015; Krzeminski et al., 2013, 2011; Kücükcetin, 2008b; Laiho et al., 2017; Tomaschunas et al., 2012). Thus, very firm gels with high interconnectivity in the gel network, makes it more difficult to break the yoghurt gel into smaller coagulum particles during stirring.

If the protein content of the yoghurt is increased after fermentation, the concentration step will both increase the protein content and provide mechanical shearing. Concentration of Labneh with UF at 35 °C (Tamime et al., 1991) and at 42 °C (Ozer, Stenning, Grandison, & Robinson, 1999b) gave Labneh with decreased firmness compared with Labneh concentrated (strained) by gravitation (cloth bag). Thus, the forces applied to the yoghurt during concentration and the parameters chosen during processing (i.e., temperature) influence the physical properties of the end product.

The coagulum particles of stirred high-protein yoghurt should be small enough to ensure a smooth texture (Cayot et al., 2008; Jørgensen et al., 2015; Krzeminski et al., 2013, 2011; Kücükcetin, 2008b; Laiho et al., 2017; Tomaschunas et al., 2012). Cayot et al. (2008) reported that it was impossible to perceive creaminess of stirred yoghurts when the coagulum particles were larger than 150 μm. For stirred yoghurts with particle sizes below 100 μm, the perception of creaminess was related with the visual and oral consistency of the product, and creaminess increased with increased sensory thickness. Thus, small coagulum particles contribute to smoothness and creaminess (Desai et al., 2013; Frøst & Janhøj, 2007). However, in the production of high-protein yoghurts, processing conditions ensuring small coagulum particles would, to some degree, sacrifice firmness and high viscosity. Because thickness is important for the perceived creaminess of yoghurts, a challenge would be to establish processing conditions that yield optimal coagulum particle size, without extensive losses in firmness and viscosity.

Adjustment of the heat treatment load applied to the yoghurt milk base or implementation of shearing devices in the process line after fermentation seem to be useful unit operations for tailoring structural, rheological, and sensory properties of high-protein yoghurts. As reported by Jørgensen et al. (2015), leaving a considerable amount of the whey proteins in undenatured state (~40–50%) significantly reduced the coagulum particle size and increased the smoothness and shininess of high-protein yoghurts (8% protein) containing whey protein:casein ratios of 25:75–35:65. Reducing the heat treatment of the yoghurt milk base from 95 °C for 5 min to 75 °C for 5 min also reduced the firmness and G' of the stirred yoghurts. This was necessary to obtain a viscous, smooth, and shiny high quality yoghurt.

Hahn et al. (2012) investigated the effect of post-processing fresh cheese (8.4% protein) with a rotor/stator-device at rotational speeds 300, 1500, and 3000 min^{-1}. Increasing the rotational speed at post-processing reduced the coagulum particle size and G' of the fresh cheese, and at the same time, the product was perceived as more smooth. Similarly, Weidendorfer, Bienias, and Hinrichs (2008), reported that the G' of yoghurt with 3.9% protein was reduced upon increased mean peripheral velocities during post-processing of yoghurt with a colloidal mill. Below a certain G' (170 Pa), syneresis was observed on the yoghurt surface after 3 weeks of storage. This observation underpins the importance of determining the effect of processing conditions on the amount of surface-whey, which is considered a quality defect in yoghurt (Lucey, 2001). However, high-protein yoghurts may have a better resistance against whey separation caused by post-processing than traditional yoghurts (<5.6% protein), as high-protein yoghurts have higher G' and higher firmness. Meletharayil, Metzger, and Patel (2016a) studied the effect of hydrodynamic cavitation at different rotor speeds on rheological and structural properties and serum-holding capacity of "Greek yoghurt" (~9% protein). Increasing the extent of cavitation (rotor speed) increased the structural breakdown, and thereby reduced the firmness and number of grains in the yoghurt. Cavitated yoghurt obtained better serum-holding capacity than a non-cavitated yoghurt and a commercial "Greek yoghurt". They suggested that cavitation led to the incorporation of moisture as finely dispersed molecules into the protein matrix. Application of post-processing unit operations, like rotor/stator devices or shock wave reactors (hydrodynamic cavitation), seems promising in terms of reducing coagulum particle sizes of high-protein yoghurts. The effect of these treatments on the yoghurt bacteria due to elevated temperatures caused by cavitation (Milly, Toledo, Kerr, & Armstead, 2008; Moholkar & Pandit, 2001) might require investigation. Concentration prior to fermentation could potentially yield a very firm yoghurt gel, challenging the pumping capacity and downstream processing.

The structure of high-protein yoghurt could also be improved by adding fat to the yoghurt milk base. An increased fat content reduces the coagulum particle size of the yoghurt and increases the viscosity and G' (Brauss et al., 1999; Krzeminski et al., 2011). Cream can be added to the yoghurt milk base or to the fermented yoghurt. The addition of cream to the fermented yoghurt gives the opportunity to produce yoghurts with various fat and protein compositions from the same yoghurt milk base. However, as observed by Schkoda et al. (2001b), the added fat only contributed to the gel network as pseudocasein particles and increased the viscosity of the fermented milk when the cream was added to the milk prior to fermentation and homogenised (150 bar at 62 °C) together with the other milk constituents. When homogenised cream was added to the fermented milk, a slight increase in serum-binding capacity was observed; however, the viscosity of the fermented milk decreased as the amount of added cream increased.

For yoghurts with reduced fat content, microparticulated whey proteins with a high ratio of native-to-denatured whey proteins can be added to the yoghurt milk base to increase the G' and creaminess of the yoghurt (4.25 or 5% protein, 0.5% fat) (Torres, Janhøj, Mikkelsen, & Ipsen, 2011). However, creaminess is, besides smoothness and thickness/viscosity, correlated with fatty afterward mouth-feel and creamy/milk fat flavour. Thus, high-protein yoghurt with a high intensity of creaminess is only obtainable if milk fat is also present (Desai et al., 2013; Folkenberg & Martens, 2003a; Frøst & Janhøj, 2007; Tomaschunas et al., 2012).

Yoghurt milk bases with high protein contents have high buffering capacities, resulting in increased fermentation times to obtain a predetermined pH-value (Salaün, Mietton, & Gaucheron, 2005). Peng, Horne, and Lucey (2009) studied the effect of fermentation time by varying the amount of yoghurt culture added to yoghurt milk bases of recombined SMP. Longer fermentation time yielded yoghurts with lower G' at pH 4.60, increased whey separation, and microstructures with large strands with fewer apparent interconnections in the strands and larger pores in the gel. Jørgensen et al. (2015)) observed that stirred high-protein yoghurt (~8% protein) produced from casein concentrate made by MF of skim milk, had microstructures with large protein clusters and large pores, and the yoghurt was perceived as granular and coarse by sensory evaluation. The increased fermentation time of this yoghurt, compared with yoghurts with increased whey protein to casein ratios, was linked to the increased amount of buffering compounds such as caseins and CCP. Peng et al. (2009) explained that increased fermentation time increased the time needed for rearrangements, allowing strands and protein clusters to aggregate further, resulting in denser clusters and larger pores. Shorter fermentation time gave yoghurts with finer structures and more branching. Thus, it is possible that reducing the CCP of liquid or dried concentrates from membrane filtration could improve structural and rheological properties (finer microstructures, smoother texture) of high-protein yoghurts produced by concentration prior to fermentation. The calcium and ash content of casein concentrates (MF) or milk retentates (UF) can be reduced by pre-acidification of the milk prior to filtration (Brandsma & Rizvi, 1999; Marella et al., 2015). Meletharayil et al. (2016a) observed that a high-protein yoghurt (~9% protein) produced from MPC with reduced CCP had shorter fermentation time than a yoghurt produced from MPC where no CCP had been removed. The use of MPC with reduced CCP in combination with hydrodynamic cavitation of the fermented yoghurt yielded a high-protein yoghurt with similar physical characteristics as a commercial strained high-protein yoghurt. On the other hand, Peng et al. (2009) reported that lower pre-acidification pH of recombined SMP, gave yoghurt gels (~4% protein) with lower G' and more whey separation. However, high-protein yoghurts may possibly respond differently than yoghurts with lower protein content (4%) to yoghurt milk bases where the CCP has been reduced prior to fermentation.

4.3. *Flavour of high-protein yoghurts*

Some reported flavour defects in high-protein yoghurts are burnt/beefy, too acidic, bitter, and astringent mouthfeel. Desai et al. (2013) reported that burnt/beefy flavour, caused by aromatics associated with sulphurous compounds, was a consistent driver of dislike of commercial "Greek yoghurts". A burnt/beefy flavour was detected in some fortified "Greek yoghurts" containing WPC or MPC, but not in the strained yoghurts. Sulphurous compounds may originate from the WPC powder (Carunchia Whetstine, Croissant, & Drake, 2005; Carunchia Whetstine, Parker, Drake, & Larick, 2003; Lee, Laye, Kim, & Morr, 1996; Wright, Zevchak, Wright, & Drake, 2009) or the MPC powder used for fortification (Drake et al., 2009; Smith et al., 2016), or may be produced during heat treatment of the yoghurt milk base (White, Fox, Jervis, & Drake, 2013). According to Drake et al. (2009), rehydrated NWPC (34% protein) has a superior flavour to WPC from Cheddar whey (34% protein), due to its bland taste and low flavour intensity. Evans, Zulewska, Newbold, Drake, and Barbano (2009) reported that sulphur-containing aroma compounds were more prevalent in spray-dried than in freeze-dried WPC and NWPC. This was explained by the higher denaturation degree of whey proteins during spray-drying, and consequently higher amount of degradation products like dimethyldisulphide from sulphur-containing amino acids. The aroma intensity of dimethyldisulphide measured by gas chromatography-olfactometry was higher in WPC than in NWPC regardless of drying technique, yet the overall aroma intensities of these powders were low as evaluated by a sensory panel. Evans et al. (2009) also compared WPC and NWPC produced experimentally with six commercial WPCs with similar moisture and protein contents. The commercial WPCs generally had higher sensory intensities of the attributes like cardboard, diacetyl, and astringent. Higher concentrations of volatile compounds related to lipid oxidation (hexanal, heptanal, pentanal), fermentation (diacetyl), and degradation of sulphur-containing amino acids (dimethyldisulphide) are also reported. Thus, careful selection of dairy protein powders for fortification of yoghurt milk bases in the production of high-protein yoghurts seems important.

Sensory analysis of high-protein yoghurts produced from yoghurt milk bases of liquid NWPC and casein concentrates showed no off-flavours or bitter flavour (Jørgensen et al., 2015). The use of liquid dairy protein concentrates in the production of high-protein yoghurts may exclude potential undesirable flavour compounds produced during powder manufacture and storage. However, further research is needed to evaluate the influence of the origin (source, processing, storage) of whey protein and casein on the physical and sensory properties of high-protein yoghurts.

According to Desai et al. (2013), consumers of "Greek yoghurt" differed in liking of sour taste, defined as the basic taste associated with acid. Fortified yoghurts were scored as "too sour" by 50% or more of the consumers ($n = 155$). The rate of pH reduction during fermentation is controlled by the buffering properties of the yoghurt milk base. Jørgensen et al. (2015) reported that the rate of pH reduction during fermentation of yoghurt milk bases with true protein contents of ~8% to pH 4.60 significantly decreased with decreasing whey protein to casein ratios. The yoghurts with the longest fermentation times (highest casein, calcium, and phosphorus content) also had the highest content of lactic acid in the final yoghurts. Similar observations were reported by Amatayakul, Halmos, Sherkat, and Shah (2006) for yoghurts with ~3% total protein. Jørgensen et al. (2015), however, found no differences in perceived intensities of acid taste of high-protein yoghurts with different pH reduction rates. A higher final fermentation pH (4.8) may positively influence the characteristics of the high-protein yoghurt, in terms of less acidic taste and smoother yoghurt structure (Kücükcetin, 2008a; Martin, Skokanova, Latrille, Beal, & Corrieu, 1999). A higher final fermentation pH has, however, been reported to give higher degree of syneresis, lower G', and lower apparent viscosity of final yoghurts with protein content <5.6% (Kücükcetin, 2008a; Martin et al., 1999). Stopping the fermentation of high-protein yoghurts at a higher pH could be an interesting approach to reduce challenges related to excessively firm yoghurt gel, graininess, and acidic taste.

Another important aspect of producing high-protein yoghurts with the use of liquid or dried protein concentrates is the possible presence of plasmin. Because plasmin is concentrated with the caseins during MF (Aaltonen & Ollikainen, 2011), the use of casein concentrates could give a yoghurt milk base with increased plasmin activity. Plasmin and plasminogen-derived activity has also been observed in commercial MPCs and micellar casein isolates (Gazi, Vilalva, & Huppertz, 2014). The optimum activity of plasmin is at pH 7.5 and 37 °C (Bastian & Brown, 1996; Ismail & Nielsen, 2010); however, proteolysis by plasmin during fermentation (42 °C) and storage (7 °C) of yoghurt (pH ~4.25) has been reported (Gassem & Frank, 1991). Plasmin can cause hydrolysis of caseins in yoghurt, leading to the formation of bitter peptides (Lemieux & Simard, 1991, 1992). Bitter taste in yoghurt has been reported to positively correlate with astringent mouthfeel, described as a puckering or tingling sensation on oral tissues (Brown & Chambers, 2015). Astringency in milk products can be caused by different compounds (Lemieux & Simard, 1994), including γ-casein from plasmin-induced degradation of β-CN (Harwalkar, Cholette, McKellar, & Emmons, 1993). Desai et al. (2013) reported that fortified commercial "Greek yoghurts" in general were described as astringent, while strained yoghurts were not. Mistry and Hassan (1992) suggested that the bitterness of high-protein yoghurt produced after fortification with MPC could be linked to proteolytic activity of the yoghurt starter bacteria in the absence of lactose.

Whether astringency and bitterness of high-protein yoghurts can be linked to possible casein degradation by plasmin or proteolytic activity of the yoghurt bacteria, or presence of other compounds, like calcium salts (Lemieux & Simard, 1991; Tordoff, 1996; Yang & Lawless, 2005), remains to be investigated. The plasmin system in milk is a complex system influenced by the presence of activators, inactivators and processing conditions such as heat treatment (Ismail & Nielsen, 2010). Further research could reveal the effect of yoghurt milk base composition (whey protein to casein ratio, calcium salts) and heat treatment on plasmin activity and potential development of bitter taste and astringency, especially in high-protein yoghurts produced by concentration prior to fermentation.

Use of membrane-manufactured powders or liquid concentrates for preparation of yoghurt milk bases, changes the milk substrate and affects the growth of the yoghurt starter culture. Özer and Robinson (1999) investigated the behavior of a yoghurt culture with a 1:1 ratio of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* in concentrated yoghurts produced by concentration prior to or after fermentation. In the yoghurt milk bases with 160 g kg^{-1} total solids content, *S. thermophilus* had an exponential growth phase ending at around 180 min of incubation (pH 5.2–5.6). After this, *S. thermophilus* entered a stationary phase, while *L. delbrueckii* subsp. *bulgaricus* grew more rapidly until the end of fermentation at pH 4.3. Yoghurt concentrated by UF after fermentation had significantly more acetaldehyde and had lower acidity than the yoghurt produced from UF milk, reflecting the favourable growth pattern of yoghurt bacteria in the yoghurt concentrated after fermentation. Interestingly, this difference was not noted when the sensory panel evaluated aroma/flavour intensity. The results of Özer and Robinson (1999) support the idea that manufacturers should take into consideration the production method when selecting yoghurt bacteria. Yoghurt starters that allow the development of acetaldehyde with restricted post-acidification and post-proteolytic activity are favourable.

5. Conclusions and future perspectives

Research supports that firmness and G' of acid milk gels and yoghurts increases with increasing protein content and increasing amount of denatured whey proteins. Additionally, the firmness and G' of high-protein yoghurts have shown to increase with the use of yoghurt milk bases with smaller casein micelle size distributions.

Very firm yoghurt gels were associated with increased sensory roughness, coarseness, lumpiness, and graininess of the stirred high-protein yoghurt. Because consumer liking of high-protein yoghurts is driven by smoothness, high viscosity, thickness, and creaminess, manufacturers should strive to obtain high-protein yoghurts with small coagulum particles without excessive losses of firmness and viscosity.

High-protein yoghurts with high sensory qualities can be obtained by concentrating the yoghurt milk base prior to fermentation. High-protein, non-fat yoghurts (8% protein) can be obtained by adding NWPC to casein concentrate from MF of milk. A reduction of the heat load to the yoghurt milk base is necessary to reduce whey protein denaturation, and thus reduce the firmness of the yoghurt gel and the coagulum particle size of the stirred yoghurt to a sensorial acceptable level. The combination of a high protein content and remaining undenatured whey proteins (approximately 50% of the available whey proteins) in the heat-treated yoghurt milk base ensures a smooth and viscous stirred high-protein yoghurt.

High-protein yoghurts can also be produced by fortifying the yoghurt milk base with dairy protein powders, such as MPC. Selection of a dairy protein powder with a bland flavour could provide a high-protein, non-fat yoghurt with good sensory properties. The use of CCP-reduced MPC in combination with post-processing treatment with shearing devices like rotor/stator or shock wave reactors, could reduce the coagulum particle size and provide a smooth high-protein yoghurt.

Research-based knowledge about the impact of processing conditions on rheology, structure, and particularly sensory properties, of high-protein yoghurt, is still limited. Further research is required to obtain a fundamental understanding of:

- How the composition (whey protein:casein ratio, casein micelle size, CCP, lactose) and heat treatment of the yoghurt milk base influence the formation of micelle-bound and soluble aggregates of whey proteins and κ-CN. Furthermore, it is not clear how this affects the mechanisms of gel formation, and gel structure, rheology, and sensory properties;
- The impact of a higher final fermentation pH;
- The impact of reduced CCP content of the MPC or casein concentrate;
- Strategies to avoid development of bitterness and astringent mouthfeel;
- The effects of the source of whey protein and casein ingredients on the physical and sensory properties;
- The effects of concentration prior to fermentation by using liquid or dried protein concentrates, in combination with concentration after fermentation.

Furthermore, there seems to be a lack of consistency in the Codex standards (Codex Alimentarius, 2011) with respect to distinguishing high-protein yoghurts and fresh cheeses like quargs. Closing these loopholes could ensure fair practices in the trade of fermented dairy products. However, efforts should be made to ensure that the Codex standards allow for innovative ways of processing high-protein yoghurt. Legislative provisions covering the composition of fermented dairy products could clarify the distinction between traditional and high-protein yoghurts.

There is no simple and straight forward answer to what is the best approach to produce high-protein yoghurts. However, MF is a technology with a high potential for optimal utilisation of the milk proteins, and provides superior ingredients for further processing into high-protein yoghurts with high sensory qualities.

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