Cheese is a classical dairy product, which is strongly judged by its appearance and texture; hence, a renewed interest in its microstructure has been on the rise, as sophisticated techniques of analysis become more and more informative and widely available. Processing parameters that affect microstructure play a dominant role upon the features exhibited by the final product as perceived by the consumer; rational relationships between microstructure (which includes biochemical and microbiological indicators), and quality and safety of the products are accordingly required. Subsequent to that extra fundamental knowledge, technological innovations may eventually improve current cheesemaking processes, and permit mechanistic design of novel ones. This review thus focuses on recent advances pertaining to the microstructure of cheese, and discusses them in a logical and critical manner.

**Introduction**

Cheese is a highly regarded food in most human cultures, and has accordingly been present throughout ages in mankind daily life. The variety of cheeses currently available is large, because a myriad of topical advances — encompassing manufacture and ripening, have cumulatively taken place over time. In addition to the somewhat intrinsic (and unpredictable) variability within each cheese type, tailor-made cheese matrices have been proposed based on specific microstructures — which have emerged side by side with introduction of alternative (or improved) methodologies in cheesemaking.

Microstructure is not a static concept; it evolves instead along the food processing chain, and eventually leads to major transformations relative to the original microstructure of the milk feedstock itself. This realization thus encompasses specific molecular compositions and spatial arrangements. On the other hand, it should be emphasized that, besides the supporting proteinaceous/fatty matrix, microorganisms are also an integral part of cheese. Microbial activity produces indeed major transformations of the cheese matrix, which will affect the final microstructure as well. Hence, it is also crucial to elucidate microorganism—matrix interactions, in attempts to understand the whole picture.

Consumer acceptance of a cheese product depends directly on its appearance, flavour and texture — which are in turn originated by a thorough combination of microbiological, biochemical and technological parameters, that affect microstructure directly or indirectly. Note that the ultimate success of any food product relies on consumers’ reactions: in fact, human perception of organoleptic characteristics is closer to the consumer status at the moment of decision than data generated by any type of analytical instrumentation (Adhikari, Heymann, & Huff, 2003) — despite its constraints in repeatability and objectiveness.

Texture is intrinsically related to the arrangement of various chemical components within distinct micro- and macrostructure levels — e.g. proteic network or fat fraction; it is the external manifestation of such structures that eventually determines the uniqueness and distinctive character of a cheese product. However, cheeses are particularly complex systems, so full and meaningful assessment of the effects of microstructure (and texture) upon flavour and appearance is still incipient.

This paper discusses a number of fundamental aspects pertaining to microstructure of cheese — and specifically focuses on issues associated with microstructural effects arising from technological and processing approaches.

**Manipulation of physical properties of milk**

A huge variety of dairy products exists at present, depending on the deliberate alterations of the original
pressurization of cheesemaking milk at 300—400 MPa for 30 min caused an increase of 14—20% in curd weight, and a decrease of 7.5—15% in protein loss in whey. Huppertz, Fox, de Kruif, and Kelly (2006), Lopez-Fandiño (2006) and Considine, Patel, Anema, Singh, and Creamer (2007) have comprehensively reviewed the molecular changes induced by HP in milk proteins, whereas Trujillo, Capellas, Saldo, Gervilla, and Guamis (2002) highlighted the principles of HP and their implications on the final properties of dairy products. However, a thorough description of the aforementioned chemical and molecular mechanisms, and of the underlying theoretical issues is not within the scope of this review — but rather the implications of such a type of process on the structural features of cheese.

It is widely accepted that HP treatments alter milk coagulation characteristics — either by reducing or increasing the gelation time (Lopez-Fandiño, 2006), or by inducing proteolytic and lipolytic activities that promote acceleration of cheese ripening (Guerzoni et al., 1999). Exposure to HP

Table 1. Processing applications in cheesemaking milk, and effects thereof upon microstructure of cheese

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Pressure treatments

High pressure (HP) has been proposed as a suitable technology for milk treatment to substitute, or in addition to thermal treatment. Its relatively recent development has been associated mainly with the possibility of inactivating undesired microorganisms and/or enzymes in milk; however, one cannot disregard the effect of such a practice upon milk constituents themselves, and consequently on the final characteristics of ripened cheese — beyond improvement of microbial safety and extension of shelf-life.

So far, the most important concern in terms of HP-induced changes pertains to the physicochemical properties of casein micelles and whey proteins — as HP affects intramolecular bonds, either reinforcing or weakening them. Lopez-Fandiño, Carrascosa, and Olano (1996) reported that pressurization of cheesemaking milk at 300—400 MPa for 30 min caused an increase of 14—20% in curd weight, and a decrease of 7.5—15% in protein loss in whey. Huppertz, Fox, de Kruif, and Kelly (2006), Lopez-Fandiño (2006) and Considine, Patel, Anema, Singh, and Creamer (2007) have comprehensively reviewed the molecular changes induced by HP in milk proteins, whereas Trujillo, Capellas, Saldo, Gervilla, and Guamis (2002) highlighted the principles of HP and their implications on the final properties of dairy products. However, a thorough description of the aforementioned chemical and molecular mechanisms, and of the underlying theoretical issues is not within the scope of this review — but rather the implications of such a type of process on the structural features of cheese.

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increases cell membrane permeability (Malone, Shellhammer, & Courtney, 2002), thus favouring release of intracellular enzymes and their access to substrates. This technique can also be used to attenuate the viability of starter bacteria throughout ripening, and thus enhance the release of metabolic compounds upon lysis (Upadhay, Huppertz, Kelly, & McSweeney, 2007). Furthermore, HP aids in release of Ca, P and $\alpha_{s1}$- and $\alpha_{s2}$-caseins — depending on the operating conditions, hence leading to destabilization of micelles (Huppertz, Fox, & Kelly, 2004; Regnault, Dumay, & Chefiel, 2006); this renders caseins more susceptible to eventual action by proteolytic enzymes.

From a microscopic point of view, HP treatments of milk produce a closer-packed structure in cheese, due to the reduction in average size of casein micelles (Needs, Stenning, Gill, Ferragut, & Rich, 2000); the finer structure strengthens the cheese matrix, *viz.* establishment of casein—casein and/or casein—fat complexes (Kheadr, Vachon, Paquin, & Fliss, 2002), thus producing firmer curds (López-Fandiño et al., 1996). These findings are well documented *via* microscopy, both electronic (Capellas, Mor-Mur, Sendra, & Guamis, 2001; Kheadr et al., 2002) and optical one (Capellas et al., 2001; Wendin, Langton, Caous, & Hall, 2000).

Such a technology has been as well applied to cheese after manufacture (Garde, Arqué, Gaya, Medina, & Nuñez, 2007; Juan, Ferragut, Guamis, & Trujillo, 2008). Several authors (O’Reilly et al., 2003; O’Reilly, Murphy, et al., 2002; O’Reilly, O’Connor, Murphy, Kelly, & Beresford, 2000; O’Reilly, O’Connor, Murphy, Kelly, & Beresford, 2002) have studied in depth the effects of a number of cheese HP treatments on key ripening characteristics of both Cheddar and Mozzarella cheeses. In general, high pressures (ca. 200–400 MPa) for relatively short times (ca. 20 min) brought about changes in the protein structure itself — which in turn improved the functional features of the final cheese matrix, *viz.* an increase in the fluidity and fluidity, and a reduction in the melt time upon heating. On the other hand, lower pressures (ca. 50–200 MPa) combined with longer processing times (up to 82 h) did not seem to affect structural features, as indicated by confocal laser scanning microscopy.

Membrane treatments

Membrane technology is more and more widely used in the dairy industry, because of its versatility and high energetic efficiency. It basically consists on a pressure-driven operation through a porous solid medium, aimed at separation and/or concentration; it ranges from plain filtration to reverse osmosis — also known as hyperfiltration (Mistry, 2004), depending on the percolating pore diameter and consequent particle size partition capacity, from ca. 100 µm down to ca. 0.1 nm (Kelly, 2004).

The main goal of ultrafiltration (UF) is to concentrate bulk milk *via* retention of whey proteins; this enhances yield of cheese, which also contains lower concentrations of lactose and minerals (Hinrichs, 2001). A few physicochemical properties of milk are critical in cheesemaking *via* UF — *viz.* viscosity, buffering capacity and rennet-driven coagulation. Upon performance of UF on milk, proteins and salts are simultaneously concentrated, thus leading to an increased buffering capacity; this influences metabolism of lactic acid bacteria favourably, whereas coagulation time is reduced and firmness of the resulting coagulum is increased (Mistry, 2004).

Furthermore, membrane treatments have been used for recovery of milk components and their incorporation as new ingredients in cheese manufacture formulations — *viz.* UF retentates (Govindasamy-Lucey, Jaeggi, Bostley, Johnson, & Lucey, 2004), milk protein concentrates and phosphocasein (Guinee, O’Kennedy, & Kelly, 2006). Therefore, membrane technology can be regarded as an efficient tool for development and continuous production of cheese, *via* intermediate production of highly concentrated matrices — that can be coagulated with little to no acid whey production at all (Nelson & Barbano, 2005).

Despite its intrinsic advantages, UF has not yet been fully adopted for manufacture of hard- and semi-hard cheeses — because of the harder texture in the final product, when compared with conventional soft and semi-soft cheeses. Furthermore, the high water binding capacity of whey proteins — coupled with retarded proteolysis, influences the texture of cheese, which becomes progressively firmer (i.e. more resistant to fracturing), more cohesive, grainier and drier, whereas the structure of the protein matrix becomes coarser and more compact, thus resulting in slower softening during ripening (Fox, McSweeney, Cogan, & Guinee, 2000). Finally, Erdem (2000) suggested that casein micelles decrease in size, and possibly undergo rearrangement *via* establishment of hydrophobic bonds — thus producing a more compact structure and an increase in elasticity; Karami, Ehsani, Mousavi, Rezaei, and Safari (2009) provided evidence for this fact *via* scanning electron microscopy. However, fundamental knowledge on how the changes in milk proteins and fat during UF affect cheese manufacturing is still limited.

Enzymatic treatments

Milk coagulation can be influenced by various factors, including enzyme-mediated cross-linking of casein micelles *via* microbial transglutaminase (TGase) — a transferase that forms bonds both within and between several proteins, *via* glutamine and lysine residues (Kashiwagi et al., 2002). Caseins are particularly suitable substrates for this activity, owing to their low level of tertiary structure (Huppertz & de Kruif, 2007).

Cross-linking of food proteins brought about by TGase affects their gelling, rheological, emulsifying and renneting properties; Bönisch, Heidebach, and Kulozik (2008) showed that such cross-linking affects both the primary and the secondary stages of rennet coagulation. Hinz et al. (2007) reported, in turn, that TGase-induced cross-linking of milk proteins affects considerably their emulsifying features, *via* increasing the stability of fat globules against coalescence. Finally, Cozzolino et al. (2003) suggested use of
TGase as a means to incorporate whey proteins into cheese-making milk, in a way somewhat similar to UF. These features seem promising in cheese manufacture, as they increase yield and gel strength — thus favouring microstructure.

Characterization of cheese

The whole cheesemaking process contributes to develop a distinct and more complex matrix than the departing feedstock milk. During coagulation — which, in most cases, is enzyme-driven, rennet enzymes bring about breakdown of κ-casein, that is present especially on the surface of casein micelles. After such a chemical step, physical agglomerations take place — which gives rise to a more uniform protein mass, that spontaneously expresses whey; this process of compactionation (termed syneresis) may be taken one step further, by externally applying pressure onto the curd. On the other hand, fat globules normally retain their membranes — and are thus observed as single entities, which may form clusters that are entrapped within the so formed proteinaceous, three-dimensional matrix.

Physical matrix

Cream cheese is a spreadable soft cheese — which structurally differs from other cheeses because of its lack of a compact protein matrix, coupled with a relatively high moisture content. Its major structural component is fat (ca. 33%), in the form of clusters of globules interspersed within milk proteins (Kalab, 1993); its microstructure has been defined as corpuscular, or composed of compact fat-casein aggregates with large spaces filled with whey (Kalab, 1985). The moisture content thereof is a factor that positively contributes to spreadability: if protein particles and fat globules are packed less densely, a high-moisture product will result.

An increasing demand for reduced fat cheese has arisen among more educated, health-aware consumers. However, decreasing the level of (or even totally removing) fat often leads to textural drawbacks, viz. a hard body with poor meltability and stretchability (Merrill, Oberg, McManus, Kalab, & McMahon, 1996), as well as a rubbery texture (Mistry, 2001). The flavour, colour and mouthfeel of cheese will also be adversely affected, since a few compounds dissolve preferentially in fat (Li, Marshall, Heymann, & Fernando, 1997) — although a higher smoothness may also be imparted to the cheese matrix (Mistry, 2001).

Fat substitutes — based on proteins, polysaccharides and synthetic chemical molecules (or even alternative fats), have been tested in attempts to convey at least as satisfactory sensory attributes; among others, Wendin et al. (2000) proved that it is possible to create a cream cheese with a lower fat content, but possessing an organoleptic profile similar to that of normal cream cheeses — simply by criterious modification of processing parameters. A more recent study (Sahan, Yasar, Hayaloglu, Karaca, & Kaya, 2008) showed that low-fat cheeses containing fat replacers exhibit a higher level of total free fatty acids than low-fat control cheeses, with a significant increase in their texture attributes and meltability; however, the full-fat cheese was awarded the best sensory scores, at all stages of ripening. Scanning electron micrographs helped elucidate how total (or partial) substitution of regular milk fat produces cheeses with different microstructures and inherently distinct textual characteristics (Lobato-Calleros et al., 2007).

Another good example of a matrix for which the interrelationship between microstructure and final quality is apparent is processed cheese. Cheese processing was originally proposed as a means to upgrade cheese of lower quality. Processed cheeses are typically prepared by heating a mixture of comminuted cheeses in the presence of salts (Piska & Štětina, 2004) — which usually include sodium citrate or phosphate, the main role of which is sequestering calcium from the calcium—caseinate complex and restoring the emulsifying properties of cheese proteins. The influence of emulsifying salts on the final processed cheese structure has been discussed by Kalab, Modler, Caríc, and Milanović (1991) and Awad, Abdel-Hamid, El-Shabrawy, and Singh (2002), among others; those salts also aid in controlling pH and stability of disperse proteins, thus contributing to the development of an appropriate microstructure.

Several reports are available pertaining to manufacture of processed cheeses, using lower grade White, Cheddar, Gruyère, Gouda, Kashkaval, Emmental and Feta cheeses (and other ingredients) as feedstock. For instance, Tamime, Kalab, Davies, and Younis (1990) studied the processing of Cheddar cheese with skim milk powder, and its effect upon the microstructure of the final product. Kalab et al. (1991) found, in turn, that White cheese is suitable to manufacture processed cheese, as the original matrix is harder even at high levels of cheese incorporation. Finally, Piska and Štětina (2004) focused on cheese ripening and rate of cooling of the blend.

Microbiological factors

In microstructural studies pertaining to Serra da Estrela cheese, Parker, Gunning, Macedo, Malcata, and Brocklehurst (1998) found bacteria embedded in the protein matrix, and especially lining the curd junctions; they also came across with protein that appears as strands and free fat in the smear — likely as a result of proteolytic and lipolytic activities. Furthermore, cells were either directly in contact with the fat globule membrane (as happened with most bacteria) or located at the casein—fat interface. On the other hand, Lopez, Maillard, Briard-Bion, Camier, and Hannon (2006) found bacteria organized as colonies in the matrix of Emmental cheese — such colonies were preferentially localized at the fat/protein interface, and both fat and bacteria were entrapped in the casein network; similar results were previously obtained during characterization of Mozzarella cheese (Oberg, McManus, & McMahon, 1993).

Lactic acid bacteria have been claimed to contribute to cheese microstructure, viz. Lactococcus lactis — via their
role in proteolysis (Centeno, Tomillo, Fernández-García, Gaya, & Nuñez, 2002), and Lactobacillus casei — via its capacity to improve cheese body and texture (Merrill et al., 1996). Strains of Lactobacillus bulgaricus and Streptococcus thermophilus have also been currently used in cheese manufacture to increase moisture content and improve melting properties (Low et al., 1998; Peterson, Dave, McMahon, Oberg, & Broadbent, 2000).

The hyphae of Penicillium camemberti, Penicillium roqueforti, Penicillium glaucum or Mucor rasmussen penetrate the protein matrix, but their sporangia develop only on the surface (Kaláb, 1993); e.g. in Camembert cheese, P. camemberti spores germinate on the cheese surface and penetrate the curd, with a thick white mat of sporangia growing on the cheese surface (Rousseau, 1984). Brooker (1987) found that deamination of amino acids by moulds on the surface increases pH, and consequently leads to precipitation of calcium phosphate from the aqueous phase — which, in turn, produces a gradient between the surface and the bulk of the cheese; this promotes a sandy mouthfeel, which is directly related to microstructure. Furthermore, such an increase in pH facilitates the action of plasmin, thus contributing further to cheese softening (Everett & Auty, 2008).

On the other hand, yeasts in cheese have been claimed (Jakobsen & Narhus, 1996) to assist in growth of P. roqueforti by producing gas — which leads to curd openness; it thus affects microstructure, besides supporting said growth — via secretion of suitable nutrients thereinto.

As a vector to protect bacteria from the putative unfriendly conditions found in cheese environments, immobilization of cells — via e.g. microencapsulation, appears to be a promising technique. According to Özer, Kirmaci, Şenel, Atamer, and Hayaloglu (2009), microencapsulation does not adversely affect appearance or texture of experimental cheeses with immobilized bacteria, yet it impacts significantly upon aroma and flavour attributes.

Conclusions
At present, several food trends encompass creating desirable, distinctive or novel textures, framed by attractive appearance and appealing odour and taste. In particular, innovation in the dairy field relies nowadays mainly on: i) incorporation of nonconventional ingredients; ii) reduction in content, or avoidance of harmful-like ingredients; iii) incorporation of pre- or/and probiotic vectors; iv) inclusion of components with nutraceutical value; and v) enhancement of existing, or development of alternative flavours (Fig. 1). Consumers are indeed calling for improvements in the secondary (or organoleptic, including texture perception) role of foods, but are simultaneously becoming more and more aware of the health-promotion and safety constraints associated with (the tertiary role of) foods. In all cases, microstructural development and mastering is crucial — as the way texture is perceived depends on the actual components of the cheese matrix, and on their chemical and spatial interactions. The physically heterogeneous continuum of cheese affects, in turn, development of microbial populations — which are implicated in flavour development or may even be vectors of intoxications. If microstructure issues are not properly addressed, then technological improvement of foods will be commercially hampered by inadequate sensory and safety features. Furthermore, incorporation in cheese of bacteria that have proven in vitro to be beneficial to health requires adequate microenvironments — which are strongly dependent on the mutual effects of the proteinaceous network and the milk fat globule distribution.

A new era in dairy food processing appears to be about to begin; the chief concern of industry — which is obviously driven by consumer preference, will hereafter be coupled with fundamental knowledge made available via sophisticated analytical apparatus and imaging techniques; this will eventually lead to rational design of novel cheeses and rational improvement of existing ones. Further complementary studies on microstructure subjects are thus fully warranted.

Acknowledgments
Financial support for author C. I. P. — provided via a PhD fellowship (ref. SFRH/BD/18258/2004), issued by program POCI 2010, supervised by author F. X. M. and administered by Fundação para a Ciência e a Tecnologia (Portugal), is hereby gratefully acknowledged.

References

Fig. 1. Schematic formulation path of a novel dairy product.


