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Review

Analysing cheese microstructure: A review of recent developments

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ABSTRACT

Cheese is a versatile nutrient-dense dairy product and it is of high importance to fully characterise its properties, e.g. texture and flavour, which contribute to the quality of this dairy food. These properties are substantially affected by the microstructure. This paper reviews recent developments in the study of microstructure of cheeses, using light microscopy (LM) and electron microscopy (EM) techniques, as well as the use of software to analyse microstructure images, i.e. micrographs, obtained. LM techniques used in the analyses of cheeses involve bright field, polarised, fluorescence and confocal scanning laser microscopy; whereas EM methods refer to scanning and transmission EM. Software used in microstructural image analyses is to quantify various components, such as protein and fat, and also to re-construct micrographs for further image processing. Finally, the use of microstructure techniques in the identification and localisation of bacterial cells within the cheese matrix is discussed.

Contents

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1. Introduction

Although cheese production is a very ancient and traditional craft, it enjoys a consistent and substantial growth rate, with a healthy and positive image (Fox and McSweeney, 2004; O'Brien and O'Connor, 2004). Cheese, a versatile nutrient-dense dairy product, could be regarded as the original convenience food which may be consumed as the main component of a meal, as a dessert, as a snack, as a condiment, or as a food ingredient. In addition, new varieties of cheese continue to be developed despite the fact that there are over 500 types. This dairy product is a good source of protein, vitamins and minerals particularly calcium and phosphorus, which are essential components in most highly consumed foods. Therefore, it is of high importance to assess the cheese properties, i.e. the end-product characteristics, which contribute to the quality of this dairy food.

The end-product characteristics; flavour, physicochemical and functional properties (texture and melting properties), and quality of cheeses are significantly affected by the microstructure (Hort and Le Grys, 2001; Joshi et al., 2004; El-Bakry et al., 2009). Accordingly, study of the cheese microstructure is of great importance to the cheese manufacturer and consumer. For instance, functional properties are particularly important for pizza-style cheese, as they include stretching, melting, browning, free oil development and expressible moisture (Awad et al., 2002; Noronha et al., 2008a,b). The prediction and/or control of the properties of cheese require an understanding of the location of the various components and their interactions, which are made possible through the study of the microstructure, during manufacture and subsequent storage.

Cheese microstructure, comprising of the casein matrix in which the fat globules are entrapped (Fox and McSweeney, 2004; Joshi et al., 2004), is routinely and most commonly examined using light microscopy (LM), confocal scanning laser microscopy (CSLM), electron microscopy such as scanning electron microscopy (SEM) and transmission electron microscopy (TEM), (Auty et al., 2001; Bowland and Foegeding, 2001; Kheader et al., 2002; El-Bakry et al., 2011a,b; Ong et al., 2013b). The main advantage of electron microscopy techniques is that they allow for a much higher resolution imaging of the components of the cheese, in comparison to LM and CSLM techniques. Comprehensive reviews on cheese microstructure are provided by Everett (2007) and Everett and Auty (2008) highlighting all historical developments and techniques, including not only microscopy but also rheology, light scattering, scanning calorimetry and magnetic resonance instruments. These techniques are used in the study of factors affecting cheese microstructure, such as physical/thermal or chemical treatments and ripening, and its impact on functional properties.

The current paper reviews the most recent/significant advances and approaches to study the microstructure of cheeses using microscopy techniques. Firstly, there is an overview of the methods and techniques used in the microstructure analyses. Secondly, an update on the use of recent software in the microstructure image analyses is provided. Finally, the use of microstructure techniques in the identification and localisation of bacteria within the cheese matrix is discussed.

2. Methods and techniques used for the microstructure analyses of cheese

2.1. Light microscopy (LM)

Light microscopy (LM) is regularly used to obtain rapid, inexpensive qualitative and quantitative information in cheese microstructural analyses (El-Bakry et al., 2009, 2011a,b). This is

Table 1
Summary of sample preparation of microscopy techniques used for the microstructural analyses of cheeses.

Microscopy techniques		Sample preparation	References
Light microscopy (LM)	Bright field and polarised LM	Sectioning of rigid samples (embedded or frozen) followed by staining for ingredients present in cheese, e.g. fast green and oil red O are used to stain protein and fat respectively. Used for the analyses of processed cheese products	Bowland and Foegeding (2001); Awad et al. (2002); Arimi et al. (2008); Colin-Cruz et al. (2012)
	Fluorescence LM	Same sample preparation as for Bright Field and polarised LM, but fluorescent dyes are used such as Nile red and Nile blue for fat and protein respectively Used for the analyses of processed cheese products	(Yiu, 1985; Shirashoji et al., 2010)
	CSLM (confocal scanning laser microscopy)	Samples are directly cut followed by staining using a fluorescent dyes. Used for the analyses of all cheeses	Auty et al. (2001); Panouille et al. (2011); Ong et al. (2012)
Electron microscopy (EM)	Conventional scanning EM (SEM)	Cheese strips are fixed, dehydrated and defatted (if fat is not of interest) followed by freeze fracturing, thawing and drying. The dried samples are mounted on aluminium stubs coated with gold–palladium in a sputter coater in argon medium. Used mainly for the analyses of semi-hard and hard cheeses	Floury et al. (2009); Karami et al. (2009); Romeih et al. (2012)
	Cryo-scanning EM (cryo-SEM)	Samples are mounted on a specimen holder followed by cryo-fixation by plunging into nitrogen slush and specimens are then transferred to the cryo-chamber (−180 °C) where the apex of the specimen is sliced off. This is followed by sublimation and sputter-coating with gold. Used for the analyses of cheddar and processed cheese products	El-Bakry et al. (2011a,b); Ong et al. (2013a,b)
	Environmental scanning EM (ESEM)	Cheese samples are directly cut and mounted on the cooling stage (5 °C), where no excessive sample preparation like in other SEM techniques is needed. Used for the analyses of white cheese and processed cheese products	Noronha et al. (2008a); Ayyash and Shah (2011)
	Transmission EM (TEM)	Samples are chemically fixed, embedded by a resin and sectioned to ultra thin sections that are shed in a copper grid and stained with uranyl acetate and lead citrate. Used for the analyses of semi-hard cheese and processed cheese products	Lee et al., 2003; Geng et al., 2011