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Using fractal image analysis to characterize microstructure of low-fat stirred yoghurt manufactured with microparticulated whey protein

Isabel Celigueta Torres [a], José Manuel Amigo Rubio [b], Richard Ipsen [a,*]

[a] Department of Food Science, Dairy Technology, Faculty of Life Sciences, University of Copenhagen, Rolighedsvej 30, DK-1958 Frederiksberg C, Denmark
[b] Department of Food Science, Quality and Technology, Faculty of Life Sciences, University of Copenhagen, Rolighedsvej 30, DK-1958 Frederiksberg C, Denmark

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ABSTRACT

Differences in the microstructure of low fat yoghurt manufactured with microparticulated whey proteins used as fat replacer were investigated. Images were obtained by confocal laser scanning microscopy and studied using a technique for image analysis that combines an initial 2D-wavelet compression followed by fractal analysis and inspection of the fractal curves by principal components analysis (PCA). One commercial and three experimental microparticulated ingredients with different chemical characteristics were used in the yoghurt formulations and compared to both full and low fat yoghurts without fat replacer. The results showed that the amount of native and soluble whey proteins present in the microparticles had a positive influence on the structure of the formed gel. The created structure, dominated by dense aggregates and low amount of serum, had an increased degree of self similarity or fractality with yoghurts in which fat was present.

1. Introduction

Stirred yoghurt can be considered as a concentrated dispersion of protein particle aggregates in serum where homogenized fat globules act as linking agents between protein clusters (van Marle, 1998). The effectiveness of this linkage will depend both on the nature of the fat globule surface material and the processing steps applied during yoghurt manufacture (Sandoval-Castilla et al., 2004; Lucey et al., 1998; Cho et al., 1999).

Reduction or elimination of fat in yoghurt can be achieved by introducing changes in formulation, e.g. selection of raw materials, use of fat replacers; and/or by controlling process parameters such as heating temperature, smoothing pressure applied to the final gel, etc. These changes result in modification of the gel microstructure and are finally reflected in the texture and sensory perception of yoghurt.

For better understanding of the functionality of the ingredients involved in fat replacement, yoghurt microstructure has been extensively studied. Common techniques are light microscopy (LM), e.g. confocal laser scanning microscope (CLSM), as well as electron microscopy (EM), i.e. transmission electron microscopy (TEM) or scanning electron microscopy (SEM) (Kalab, 1979, 1981; Skriver, 1995; Folkenberg et al., 2005). Nonetheless, only few studies exist on the microstructure of yoghurt or gel systems in which microparticulated whey proteins (MWP) have been incorporated as fat substitute (Sandoval-Castilla et al., 2004; Renard et al., 2002). It has been shown that whey microparticles are able to become an integral part of the protein matrix limiting casein micelle aggregation (Sandoval-Castilla et al., 2004). However, these conclusions are restricted to the use of Simplesse 100® (Monsanto SNC, Kelco Nutrasweet Division), the first microparticulated product on the market for use as fat substitute.

The evaluation of microstructural differences in yoghurt due to addition of fat replacers has been focused so far on mere visual analyses. However, in order to extract the maximum amount of information and detect minimal changes within the yoghurt microstructure, it is necessary to use mathematical approaches for image analysis rather than subjective and less accurate methodology. In recent years, fractal analysis techniques have become prevalent tools for characterization of the morphology in food systems with complex and highly irregular structure (Hibbert, 1991). The fractal concept studies the degree of symmetry or self-similarity found in a structure at all scales of observation (Tominaga and Fujiwara, 1997; Mandelbrot, 1982). The fractal dimension (D) usually produces a single numeric value that summarizes the irregularity or "roughness" of the feature boundary (Russ, 2007). Therefore, the comparison between different images becomes a subjective qualitative way for classifying or comparing features. One way of using fractals as a quantitative technique is to merge the use of the box counting technique and further principal component analysis (PCA) of the box counting curve.

* Corresponding author. Tel.: +45 35333225; fax: +45 35333190.
E-mail address: ri@life.ku.dk (R. Ipsen).

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Calculation of the fractal dimension has been successfully applied to examine the aggregation behavior of proteins and the final structure in gel systems (Hagiwara et al., 1998; Pugnaloni et al., 2005; Kuhn et al., 2010; Davila et al., 2007). Therefore, the study of the fractal structure of these protein gels was used to establish a link between changes in formulation and final mechanical properties.

The objective of the present work was to investigate the effect of whey protein microparticles upon the microstructure of low fat stirred yoghurt by using fractal image analysis as a tool for detecting and quantitating structural changes. It is known that changes in the processing steps involved in the manufacture of MWP influence the chemical and physical characteristics of the final proteins and thus, their functionality. Therefore, one commercial and three experimental microparticulated ingredients with different chemical characteristics were examined and compared to both full and low fat yoghurts without fat replacer. To gain understanding on the structure of the yoghurt gels, a novel 2D-wavelet compression of CLSM images was carried out followed by fractal image analysis and inspection of the curves by principal components analysis (PCA).

2. Methods

2.1. Microparticulated whey proteins

Four microparticulated whey protein ingredients (Arla Food Ingredients, Nr. Vium, Videbæk, Denmark) were included in the study. The first type (MC) was a commercial product available in the market whereas the other three types (M1, M2 and M3) were experimental proteins manufactured at pilot plant scale. The amount of native whey protein content in the proteins was analyzed in duplicate by size exclusion HPLC as described by Torres et al. (2011).

2.2. Particle size distributions of microparticulated protein particles

10% (w/v) MWP protein solutions were prepared in MilliQ water (18.2 Ω) and left to hydrate for 1 h under stirring at 22 °C. Particle size distributions of the MWP solutions were measured by static light scattering (Malvern Mastersizer Micro Particle Sizer, Malvern Instruments Ltd., Worcestershire, UK). Particle refractive index 1.52 (real part), 0.1 (imaginary part) and dispersant refractive index 1.33 were used. The data was fitted using the Mie scattering model (residuals <2%). Each sample was measured in triplicate.

2.3. Preparation of yoghurt milks

Low-heat skimmed milk powder (Arla Foods Vimmerby, Sweden), was reconstituted to 9% (w/w) milk solids non-fat (MSNF). This milk was used as base for the manufacture of all the yoghurt milk mixes. The fat content was standardized with cream (38% fat, Arla Foods, Slagelse, Denmark) and the protein content was adjusted either with skimmed milk powder (SMP), e.g. reference batches, or with MWP e.g. experimental batches. Two control yoghurt batches were manufactured using skimmed milk powder (SMP) as the only protein source: full-fat reference yoghurt (FFY) with 3.5% w/w fat and 3.5% w/w protein, and low-fat (0.5% w/w fat) reference yoghurt (LFY) with 5.0% w/w protein. The four low-fat experimental yoghurts (0.5% w/w fat) were manufactured using first, SMP for the reconstituted milk base and second, MWP for the adjustment of the protein level to 5.0% w/w. The experiment was repeated five times, thus 30 batches of yoghurt were made in total. Table 1 shows an overview of the experimental design. The reconstituted milk base was stored overnight at 4 °C to allow hydration of the powders. Yoghurts were manufactured maintaining constant casein to whey protein ratio of 70:30 in order to avoid this factor affecting the gel microstructure.

2.4. Stirred yoghurt manufacture

The several yoghurt milk formulations were preheated to 63 °C, homogenized (14.7 MPa), pasteurized (85 °C for 15 min), cooled to fermentation temperature (43 °C) and inoculated with a yoghurt starter culture (F-DVS YF-3331; Chr. Hansen A/S, Hørsholm, Denmark). The pH of the milk was monitored until it had decreased to 4.60. Subsequently, the yoghurt gel was mechanically stirred for 1 min using a Silverson L4R mixer (Silverson Machines Ltd., Waterside, UK). The yoghurt was then cooled to 22C in a plate heat exchanger and smoothed by passing through a pipe system (Ø12 mm) at a constant counterpressure of 0.1 MPa (FH Scandinox A/S, Tarm, Denmark). Finally, the yoghurt samples were packaged in plastic containers and kept at 4 °C for 7 days before analyses were performed.

2.5. Confocal laser scanning microscopy

A drop of yoghurt was gently filled into a microscope slide with a conical depression. Protein was stained by adding 1 μL of a 0.02% FITC (fluorescein-5-isothiocyanate) solution in acetone to the cover slip. When the acetone was fully evaporated, the yoghurt samples were covered with the slip and the specimens were left to rest for 30 min at room temperature before micrographs were recorded. The samples were examined using a 63× water immersion objective (63×/1.2 HCX PL APO CS) on a Leica TCS SP2 confocal scanning laser microscope (Leica Microsystems, Heidelberg, Germany) fitted with an Ar/Kr laser. FITC was excited at 488 nm, and the emitted signal was collected from 500 to 547 nm. For each yoghurt batch, three slides were prepared and 20 images of 1024 × 1024 pixels were recorded at 10 μm below the cover glass. 100 micrographs for each yoghurt type were recorded, making a total of 600 images.

2.6. Image analysis

Image analysis of the CLSM micrographs encompassed three stages: compression of the information stored in the images by using 2D-wavelets; fractal analysis for each image and a principal component analysis (PCA) model of all the fractal curves obtained.

2.6.1. 2D-Wavelet transformation for compression

The size of the original CLSM images was minimized by using wavelet transformation (WT). WT is widely used in signal processing for its ability of de-noising and compression. It implies decomposing of a signal using a set of scaled and shifted versions of a particular wavelet function as a basis. WT in the context of image analysis has been used to underline edges and contours of different orientations, to distinguish objects with various sizes and structures and for image compression (Kucheryavski, 2010). In this work, WT has been used for compression purposes of the digital images obtained. That compression was done using Haar wavelet transformation. It consisted of the application of filters successively to the image's rows and columns. As shown in Fig. 1, this transformation produces three types of details: horizontal (d_1^1), vertical (d_1^3) and diagonal (d_1^2). These details did not contain any relevant information in our images. Therefore, the final compressed image (top left side in Fig. 1) shows only the features that retain important information whereas markedly reducing the size of the original picture (the image size was reduced from 2 MB to 200 KB). Wavelets compression was performed using the wavelets toolbox in MATLAB (The Mathworks Inc.).