# Use of image analysis on cheese sections for microstructural characterization

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## Introduction

**T**he perceived texture of Serra da Estrela cheese is an important quality parameter for typical consumers. As the microstructure of cheese is closed related to its texture, light microscopy has been used for a long time for structural studies. Microscopy provides visual qualitative information about the structures and changes in the samples, but this type of information is difficult to handle if one wants to make comparisons between a number of samples. Therefore, methods to quantify the visual information have been applied to microscopic data. Optical microscopy techniques are often employed to reveal the internal geometrical structure of a product in detail; stereology and image analysis can be employed as auxiliary tools for these purposes (Langton, 1993; Schoonman, 2001). The present study was carried out so as to investigate the structural differences in the gel network of the cheese throughout maturation via 2D image analysis, and to correlate microstructural with textural parameters.

## Materials and methods

**Sample preparation.** Serra da Estrela cheese was examined for its microstructure using light microscopy. The cheese was produced using raw ewe's milk as described by Macedo *et al*. (1993). Blocks of cheese (10 x 10 x 20 mm) were taken vertically through the top of the cheeses, completely submerged in 4%(v/v) formalin and fixed for at least 1 mo before cutting. These blocks were cut into approximately 2 x 2 x 1 mm sections (perpendicular to the base) and dehydrated in an ethanol series (10 - 100%). The dry samples were embedded in Epon 812, sectioned (~0.25 μm) and stained using a mixture of 1% toluidine blue and 1% sodium borax (1:1).

**Light Microscopy.** Stained sections were observed at x400 magnification with an Olympus CX-31 microscope, and the images were digitised using an Olympus camera.

**Image analysis.** Images were processed on a PC using the UTHSCSA Image Tool IT V2.0 (University of Texas Health Science Center, San António). Images were binarized by simple thresholding in order to extract numeric information. The threshold grey-level was selected after examination of the histogram of the grey-level values of the image. Geometrical parameters such as porosity, area, perimeter, elongation and roundness were measured using the object analysis option of the software. For each cheese, 2 blocks were analysed, 6 sections were obtained and 2 images from each section were produced. Images were digitised on a matrix of 2272 x 1704 pixels, with 32 bit resolution.

## Results and discussion

A typical LM micrograph of the inner structure of Serra da Estrela cheese is shown in Fig. 1-a. From section images, three characteristic sizes have been determined: (a) "porosity"; (b) pore area distribution; and (c) roundness (or shape) factor, which describes the deviation of an object from a true circle. In the following image-processing step, utilizing binarized images, "porosity" measurements were performed. In Fig. 1-b, the black pixels represent solid matrix and the white pixels represent voids (pores).

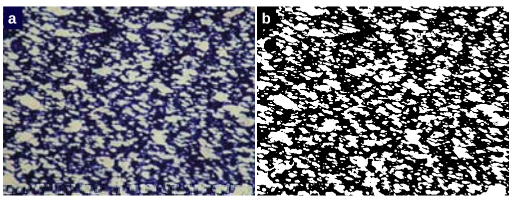


**Figure 1.** Light micrograph of the inner structure of 14-d-old Serra da Estrela cheese at x400 original magnification (a). Image binarized by thresholding to extract the pores (b).

The software used counts the black and white pixels and evaluates porosity as the ratio between white pixels and total pixels in the image. The Porosity so calculated (under stereological assumptions) is equal to the pore volume proportion. Results obtained for different ripening times show that the porosity is highest in cheeses by 14 d of ripening (Table 1); however, statistically significant differences were not found between ripening days ($p > 0,05$). In contrast, the ANOVA indicated that porosity was significantly affected by cheese block ($p = 0,002$). This observation reveals the high heterogeneity present amount distinct regions in cheese.

The frequency of pores sorted by area is depicted in Fig. 2. While the most probable particle size is rather small, it is the few larger pores that contribute most of the volume. The importance of the larger particles is apparent in Fig. 3, which shows the area-weighted distribution of pore area.

| Ripening time (day) | Porosity (%) |
|---|---|
| 1 | 45,12 ± 2,35 |
| 7 | 44,80 ± 3,47 |
| 14 | 46,02 ± 3,31 |
| 21 | 44,65 ± 3,10 |
| 70 | 42,25 ± 2,38 |

**Table 1.** Porosity evaluated using image analysis on micrographs of cheese throughout ripening time (Mean ± Standard Deviation).

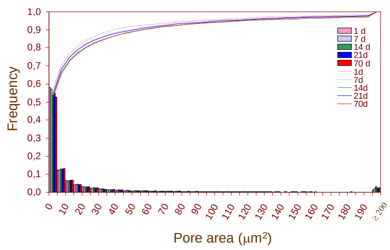


**Figure 2.** Histogram of "number-weighted" distributions of pore area, and corresponding cumulative curves at various ripening times.

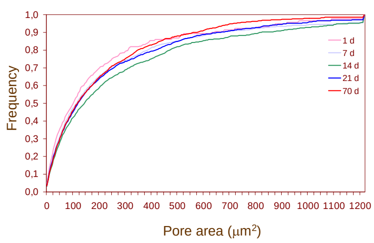


**Figure 3.** Cumulative curves of "area-weighted" distributions of pore area at various ripening times.

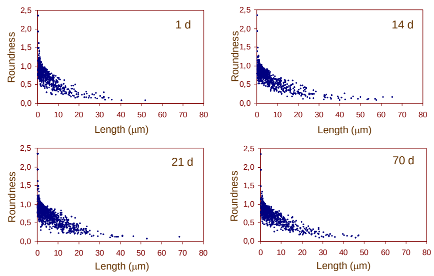


**Figure 4.** Roundness *vs.* length (major axis) of the pores at different ripenning times.

The roundness of the pores is plotted versus pore size in Fig. 4. Independently of the ripening time, the roundness of the pores decreases with increasing size. This fact could suggest that the small irregular particles can be produced by fragmentation of the large smooth ones.

Furthermore, the void space can be regarded as a network of pores connected by smaller void channels, so the measurement of size are not a simpler matter. By image analysis, voids do appear as patches that vary in size and shape. Regarding the morphological complexity in the plane of the image (Figure 1), such patches can represent single pores or a network consisting of connected pores.

Regarding textural properties, the cheeses were creamy and exhibited a decrease of firmness over the ripening time (data not shown). A relationship between microstructural and textural parameters in cheese seems to exist, although further studies have still to be conducted in this area.

## Literature

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Schoonman, A. et al. (2001). *Food Res. Int.* 34: 913-929.

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