White Paper

Advancing the Understanding of Emulsions in Food Products Using State of the Art Microscopy

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Introduction

The importance of understanding food microstructure should not be underestimated. Microstructure is of fundamental importance to the texture, stability and sensorial perception of every food product.

This is particularly true in the case of emulsions, such as margarine, mayonnaise, ice cream, milk, cream, soups, cake, batters, sauces, desserts, fruit beverages and salad dressings. Fully characterising and monitoring the emulsion system can bring many benefits to the food manufacturer: it will assist in the formulation of high quality products, help to optimise the manufacturing process, reduce costs, and ultimately result in increased profit.

This paper demonstrates how the use of microscopy techniques: Cryo-Scanning Electron Microscopy (Cryo-SEM), X-ray Micro-computed Tomography (µCT) and Confocal Laser Scanning Microscopy (CLSM) can be used to characterise, understand and monitor the stability of emulsions.

Background

The demand for more sophisticated food products with engineered (tailored) functionalities means that emulsions are becoming increasingly complex, albeit that the essential definition of an emulsion remains the same. Emulsions consist of an oil phase containing hydrophobic compounds and an aqueous phase comprising water-soluble components. These two phases are interspersed, with either oil droplets dispersed in a continuous water phase (O/W emulsion) or water droplets dispersed in a continuous oil phase (W/O emulsion). However, it is the precise nature of the dispersion that gives each product its own unique characteristics. Hence, the critical need to understand the microstructure and measure the droplet size and size distribution of the oil and water phases.

Determination of Ice Cream Microstructure Using Cryo-Scanning Electron Microscopy

Starting as a liquid, ice cream is a solidified foamed emulsion containing air as the primary dispersed phase. It has four main components: ice crystals, air bubbles, solidified fat globules and sugar. The microstructure of ice cream is very sensitive to temperature change, therefore it is essential that this fragile structure is preserved as the precise nature of the structure gives the sensory attributes experienced by the consumer.

Cryo-SEM is a quick, reliable and effective way to characterise the microstructures in ice cream, as it can inform how ingredients and the manufacturing process determine its texture and stability. In particular, attributes including creaminess, smoothness, the cold sensation when consumed and the way it melts in the mouth can be determined with the aid of Cryo-SEM. Using this technique the size of the air bubbles and their
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stability, ice crystal size and connectivity, fat distribution, and the nature of the interfaces between the various components can be determined.

The amount of air in ice cream is variable, depending on the product, but is generally around 50%. At low magnification, air bubbles of a variety of diameters are visibly dispersed amongst a frozen emulsion (Figure 1). Ice crystals range between about 5 and 50 µm in size (Figure 1). At high magnifications (Figure 2), fat globules between 1 - 10 µm are visible at the air interface and in the material trapped between the ice crystals.

![Figure 1](image1.png)

**Figure 1**
Cryo-SEM images of ice cream microstructure
A. Control ice cream sample (conventional processing)
B. Temperature abused ice cream sample

![Figure 2](image2.png)

**Figure 2**
Cryo-SEM image of ice cream microstructure at a high magnification showing fat droplets
Microstructure of Mousse by X-ray Micro-computed Tomography

Bubbles play a key role in foamed food products, including confectionery mousses because they create fine texture with a light, smooth and creamy mouth feel. The quality of such products is directly impacted by the formulation and processing conditions. µCT is the only known suitable technique to directly analyse such delicate structures non-destructively and in three dimensions.

µCT is able to distinguish between materials of slightly differing X-ray absorbance. Therefore, subtle differences in volume between bubbles with diameters ranging from a few millimeters down to a few micrometers can be resolved in foamed materials. Along with the percentage aeration of the mousse, the size and spatial distribution of the bubbles can be visualised using this powerful analytical tool. Figure 3 shows 3D µCT images of mousses with different fat formulations (formulation A and B) demonstrating the resulting different bubble size distributions and degrees of aeration.

This technique has been particularly effective in shelf life studies. For example, it can monitor aeration changes and emulsion stability over time (non-destructively). Figure 4 below shows cross section images obtained using µCT of an aerated mousse, showing the bubbles merging together with the resultant collapse of the structure.
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Figure 4
Comparison of µCT images of mousse over different storage time

Determination of Emulsion Stability by Confocal Laser Scanning Microscopy in Low Fat Spreads and Mayonnaise

Knowledge of the droplet size distribution is essential when developing and producing high quality, microbiologically safe, visually appealing, delicious emulsion based food products. In the case of low fat spreads that are water in oil emulsion systems, the size of the water droplets determines their microbial stability and sensorial properties.

Water droplet size and distribution can be determined by CLSM. When combined with a stage that allows heating, cooling or mixing of the sample, food processing stages can be monitored under the microscope. Dual staining a low fat spread with a fat and water soluble dye enables visualisation of both the continuous fat phase and the water droplets (Figure 5). The low fat spread emulsion is temperature sensitive. Figure 5 shows the CLSM images of a low fat spread sample; as the temperature is increased above 40 ºC, the emulsion destabilises and the water droplets coalesce.
Figure 5
CLSM images of a low fat spread under different temperatures
Water droplets stained by FITC as red; fat matrix stained by Nile red as green

Mayonnaise is an oil-in-water emulsion where egg proteins (including lipoproteins) act as emulsifiers stabilising the emulsion. To make a stable mayonnaise it is essential to form small oil droplets in a continuous water phase that is viscous enough to prevent the oil droplets combining.

Figure 6 shows CLSM images illustrating that a globally branded mayonnaise product is stable across a wide temperature range (up to 80°C: well above ambient temperature in most countries).
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Figure 6
CLSM images of mayonnaise under different temperatures
Protein stained by rhodamine B as red; non-fluorescent oil droplets as black

Summary
Cryo-SEM, X-ray micro-computed tomography (µCT) and confocal laser scanning microscopy (CLSM) can be used to visualise the microstructure of emulsion-based food products, ranging from the nanoscale in the case of Cryo-SEM, to the macroscale in the case of µCT. When these techniques are used in combination by a team of experienced food microscopists, they provide a deep understanding of the structure of emulsion systems, opening the gateway to better products, longer shelf lives and more efficient manufacturing processes.

RSSL’s Microscopy Team
The microscopy team comprises of 20+ scientists from a variety of disciplines and industries, spanning from conventional food scientists to the more esoteric areas of academia such as archaeometallurgy. This breadth and depth of knowledge within the group, combined with industry leading analytical capability, allows for a unique and powerful service offering for microstructure understanding. Be it NPD, product improvement or general problem solving such as foreign body identification, we are well placed to support all your needs.

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