Assessment of Food Ingredient Functionality using Laser Microscopy

(The Use of Confocal Laser Microscopy in Food Ingredient Evaluation)

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Summary and Conclusions

The microscopic structure of food significantly affects its processing characteristics, flavour release and texture. Food microstructure studies thus provide a key to understanding, and therefore controlling, food behaviour. Confocal laser scanning microscopy (CLSM) is one of the most useful microscopy techniques for studying the microstructure of a wide variety of foods, in particular dairy products. A primary feature of the CLSM is its ability to "optically section" through samples. This gives a 3-dimensional view of a food product with minimal sample disturbance. This is of great benefit when examining shear-sensitive samples such as dairy spreads and soft cheeses, where other microscopy techniques would involve physical disruption of the sample. CLSM is also an effective problem solving tool and can be used to identify food contaminants. Ireland does not possess a specialised food microscopy facility. Hence, there exists a need in Ireland to develop expertise in microstructural analysis in order to maintain a competitive edge and provide a service to both research and commercial institutions.

Hence the objectives of this project were, to establish a confocal microscopy facility at Moorepark, to develop suitable methodology for the examination of food products and ingredients, to apply confocal microscopy techniques to food research projects and to use the above technological expertise for commercial applications in the Irish Food Industry.

The main conclusions were as follows:

- A new Zeiss LSM310 confocal laser scanning microscope (CLSM) was purchased and installed in a purpose-built laboratory. An experienced microscopist was employed to set up and run the new facility. Features of the microscope include: multiple lasers for maximum dye flexibility, sophisticated image analysis software for quantifying images and heating/ cooling stages to observe microstructural changes over time.

- Confocal microscopy methods were developed and applied to a wide range of food products and ingredients. These included: milk powders, dairy spreads, chocolate, protein gels, starches and a variety of cheese types. The structures of a wide variety of foods could thus be rapidly determined by CLSM.
- CLSM can be used to solve product problems, for example contaminant analysis. Several commercial clients have already used the confocal microscopy service to help identify foreign bodies.
- The development of microstructure in Mozzarella cheese, milk powders and milk chocolate could be monitored during processing by CLSM. This will enable food manufacturers to optimise processing conditions for these products.
- The texture and flavour of smear ripened cheeses depend on the types of micro-organisms (bacteria and yeasts) growing on the surface of the cheese.
- The CLSM proved the ideal technique to study these micro-organisms directly and rapidly.
- The gelation properties of protein ingredients affects product texture and flavour release. CLSM was used to visualise the gelation of milk proteins, whey protein concentrates and b-lactoglobulin. Knowing precisely how gels develop will help food technologists to create novel textures.
- Image analysis techniques were used to quantify confocal microscopy images. For example, measuring droplet size in dairy spreads helps topredict product stability and shelf life.
- Industrial interest in the confocal microscopy service is growing. Over 25 individual requests for confocal analysis were received from Irish food companies in 1997.

In summary, The confocal laser scanning microscopy (CLSM) facility is now established and is fully integrated into the Teagasc research program at Moorepark. The new Confocal Microscopy Service has attracted significant commercial interest and client work is expanding. Results show that confocal laser scanning microscopy is a valuable technique for assessing the functionality of food ingredients in a wide range of food products, as well as being a powerful problem-solving tool. Work is ongoing to develop further specific ingredient localisation techniques, and to promote commercial awareness of the service. Confocal laser scanning microscopy offers a unique contribution to product research and development in the Irish food industry.

Research and Results:

Powdered Ingredients and Chocolate

Milk powders and powders micro-encapsulated with fish oil were examined using a newly developed dual-staining method for examination of fat and protein components. Both the fat and protein components could be identified simultaneously within the powders using mixtures of the stains Nile Blue, Nile Red and Rhodamine B. The distribution of fat in low- and high-free fat milk powders could be visualised (see Fig. 1). Results indicated that powders with high free fat contents (as determined by solvent extraction) did not necessarily have high levels of surface fat but had large fat droplets within the powder particle. Powders with high surface fat dispersed poorly in water but were ideal for chocolate manufacture, where fat is the continuous phase.

Milk chocolates made with milk powders containing varying levels of free-fat were examined by CLSM. Chocolates made with low free-fat powders had a high viscosity and CLSM showed discrete fat droplets surrounded by protein. High free-fat powders resulted in a more homogeneous distribution of fat as seen by CLSM. The major components of milk chocolate (sugar, fat, protein and cocoa solids) could be simultaneously visualised using the dual staining technique (Fig. 2).

Thus, confocal microscopy revealed the distributions of fat and protein in powdered ingredients. This information could be used to control the physical properties of food powders, for example free-fat, and hence determine the quality of the final product.

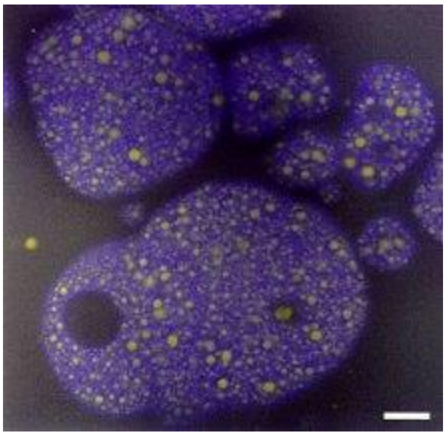

Fig 1. Whole milk powder showing fat droplets (yellow) and protein (blue) Bar=10 mm

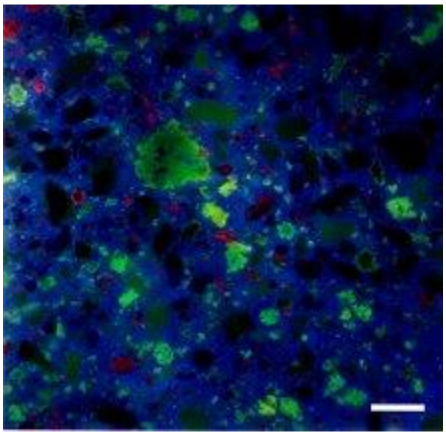

Fig 2. Confocal micrograph of milk showing milk protein (green), fat (blue), sugar crystals (black) and cocoa solids (red). Bar = 25 mm

Cheeses

Cheddar, cream cheese, analogue Mozzarella and Mozzarella cheeses were examined using the new dual staining technique developed for powders. The distribution of protein and fat in each of the cheeses varied depending on the variety. Cheddar cheese consisted of irregular fat droplets surrounded by a protein matrix. Cream cheese consisted of aggregates of small fat droplets and protein. Analogue Mozzarella was an emulsion of rounded fat droplets in a homogeneous protein matrix containing crystals of emulsifying salts. Mozzarella consisted of elongated protein fibres containing small fat droplets with some free fat in larger pools. These observations are illustrated in Figs. 3a - d.

The microstructural development of Mozzarella cheese was followed from the addition of rennet through to 76 days storage. CLSM showed changes in both the fat and protein components. Following plasticisation there was a gradual expansion of the protein fibres until a protein continuum formed, gradually entrapping the fat droplets (Figs.4a - c). These observations were related to other physico-chemical measurements such as curd firmness and expressible fat and moisture.

Hence, the development of cheese microstructure could be followed at each stage of processing using the confocal microscope. This will give food manufacturers greater awareness of how processing affects food structures, thereby controlling the final product texture.

Dynamic Confocal Microscopy

Dynamic confocal microscopy enables direct examination of samples as they are affected by temperature and time. A temperature controlled stage was fitted to the microscope and the aggregation of milk protein following rennet addition was observed. A time-lapse animation of protein gelation was produced and the visible onset of aggregation was related to gel formation as determined by low amplitude oscillation rheometry. The gelling point, as determined by oscillation rheometry, coin-cided with an observed aggregation of protein into a three-dimensional network.

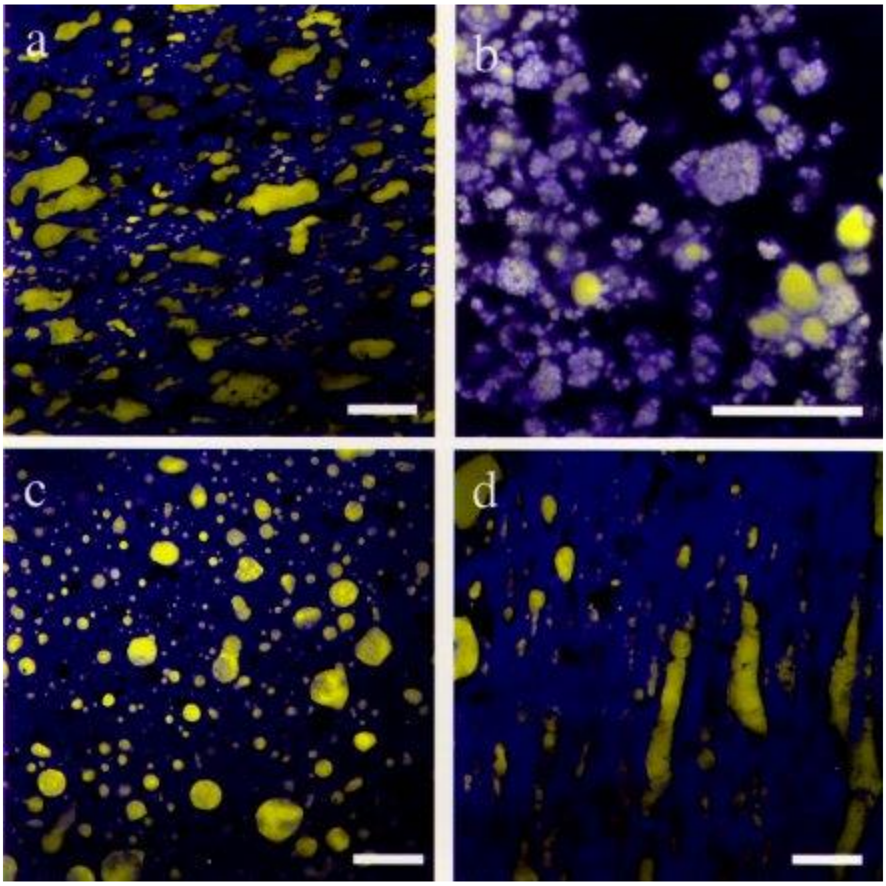

Fig 3. CLSM of various cheese types. a) Cheddar, b) Cream cheese, c) Analogue Mozzarella, Mozzarella. Protein = blue, Fat = yellow. Bar = 25 mm

The formation of acid-induced gels (skim milk) and heat-induced gels (b-lactoglobulin) were also studied using CLSM fitted with the temperature-controlled stage. This technique has since been used by one commercial client to study gelation of several milk protein ingredients.

Thus, it is now possible to "see", for the first time in real time, how food structures develop and how these structures are affected by temperature. CLSM will therefore help food manufacturers assess how their particular ingredient will perform in a given food.

Microbiology Applications

The microbial populations on the cheese surface changes as smear-ripened cheeses mature. The distribution of micro-organisms and their population development on the surface of smear ripened cheese (Gubbeen) were studied by CLSM. Bacteria (*Brevibacterium spp.*) and yeast cells (*Geotrichum sp.*) could be differentiated and changes in the relative proportions of these two organisms were related to cheese maturity.

CLSM offers a rapid, direct assessment of microbial populations on food surfaces. This would be of benefit to food microbiologists and quality control personnel.

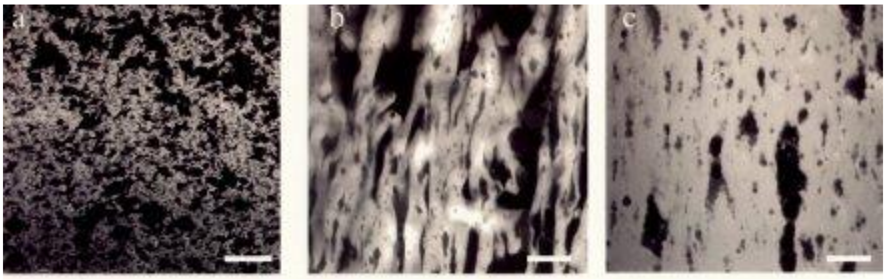

Fig 4. Confocal micrographs of Mozzarella cheese showing protein which appears as bright areas against a dark background, a) at drainage, b) plasticised (stretched), c) 76 days storage. Bar = 25 mm

Image Analysis

The size of droplets in food emulsions, for example dairy spreads, determines product stability; if water droplets are too large, then microbial spoilage may occur. Measuring droplet sizes in shear-sensitive emulsions is difficult, for example using laser scattering instruments. CLSM was used to visualise the undisturbed internal structure of dairy spreads and image analysis was carried out on the confocal images. The water droplet size distribution of water-in-oil dairy spreads was then calculated using the image analysis software. The whole measurement process took approximately 10 minutes.

Image analysis was also used to determine air vacuole volume in spray dried powders intended as ingredients for milk chocolate. Increased air in powders results in an increase in chocolate viscosity, making chocolate processing difficult. Air vacuole volumes (expressed as % air) for milk powders could be determined within 5 minutes using confocal microscopy and image analysis.

Image analysis is a rapid technique allowing measurement of size and shape from confocal images. As a result of the above work, droplet sizing of emulsions by confocal microscopy and image analysis is now regularly requested by several industrial clients.

The new Zeiss Confocal Laser Scanning Microscope (CLSM)