Image of microstructure of Exopolysaccharides in low fat yoghurt

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Introduction

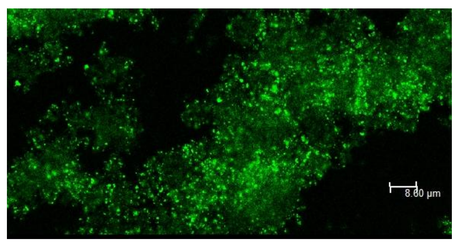
The overall objective of this project is to understand how microstructure is generated by developing more robust methods for investigating and analyzing specifically the microstruture of fermented milks. The hypotheses investigated in the project are the following

•The structures of exopolysaccharides (EPS) and milk proteins can be visualized in complex food systems by using confocal laser scanning microscopy in conjunction with application of antibodies and/or lectins for staining.
•Microstructure can be related to other product characteristics, primarily rheological properties such as texture of yoghurt.
•By using well characterized starter cultures it will be possible to produce a set of fermented milks with varying properties that can be used to elucidate the interplay between starter culture and process.
•In-line methodology for monitoring viscosity and particle size in fermented milk as a consequence of mechanical treatment can be established and will complement microstructural information

Results

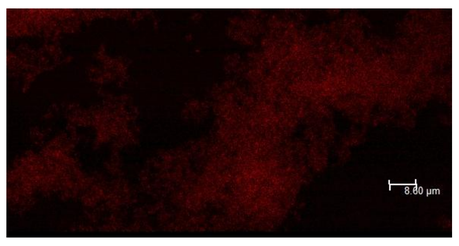
Image of EPS and milk protein by using Confocal Laser Scanning Microscopy

A



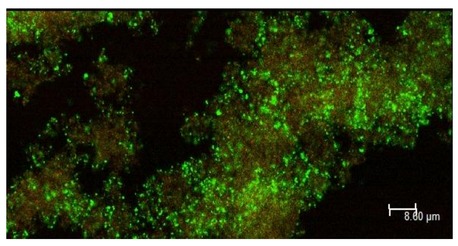
Con.A and EPS within milk protein network of commercial low fat yoghurt, visuallized at excitation wavelength 488 nm

B



Milk protein network stained by Nile Red, visuallized at excitation wavelength 550 nm

C



Overlaid image of A and B

Exopolysaccharide (EPS)

Exopolysaccharides (EPS)

EPS is polysaccharides excreted by a variety of Lactic acid bacteria (LAB) during fermentation and are divided into two types of heteropolysaccharides and homopolysaccharides (Cerning, 1995). Molecular weights of EPS are in the range from $1x10^4$ to $6 x10^6$, which are built up of repeated subunits of various monosaccharides such as galactose, glucose, mamnose, rhamnose, fructose, fucose, glucosamine and galactosamine (De Vuyst et al. 2001).

EPS-production

EPS was found to have major effects on yoghurt/cheese texture by improving certain physical properties of yoghurt/cheese such as mouth thickness, creaminess, ropiness and decreasing gel formness and syneresis of milk protein network. These effects are highly caused by the interactions of EPS with milk proteins depending on EPS molecular propertis of glycosidic linkages, branching, charges, etc. (Folkenberg et al. 2005).

Protein network

Protein network is formed due to decrease in pH by LAB during yoghurt fermentation and is ultimately resulted from the interactions between denatured whey proteins and caseins with small and evenly distributed pores. Stirring results in a denser network and larger areas of separated whey, causing syneresis (Hassan et ql. 2003b).

EPS-milk protein interactions

The interactions between EPS and milk proteins can be either repulsive or attractive forces, which depend on a number of factors, such as size and charges of the proteins and EPS, their concentrations, the ratio between them, pH, ionic strength etc. (Goh et al. 2009) . Normally in yoghurt EPS is located in the pores within protein network or associated with protein network

Methods for visulization of microstructure

A variety of methods can be used to study EPS in milk or model systems. The microscopy techniques: Scanning Electron Microscopy (SEM), Cryo-scanning Electron Microscopy (cryo-SEM) and Confocal Laser Scanning Microscopy (CLSM) are often used for visualization of the protein network and the EPS distribution.

For SEM visualization, the preparation includes steps of fixation, preservation of fat globules, dehydration by alcohol, defatting and drying. This allows the EPS to obtain a filamentous appearance that is different from the actual structure in non-manipulated samples (Kalab 1993). Some of these problem are solved by the use of cry-SEM, as wet samples are rapidly frozen and the sample is only partly dehydrated (Hassan et al. 2003a). This visualizes the image together with protein, fat and water.

In CLSM, not only the formation of the protien network, but also the distribution of the EPS in relation to the protein network can be studied by staining EPS with an EPS-specific probe, which is labelled with a fluorecent dye that is visible in the CLSM image.

The first part of the project

Fermentation for EPS production

Four single strains are selected from Chr Hansen Culture Collection Bank for producing EPS in specific culture mediums. Three out of four strains worked during the fermentation.

EPS purification

Three different EPS of 3 strains have been purified after the fermentation. The monosaccharides composition analysis is in progress currently. The purified EPS will be the material for image visualization of Confocal Laser Scanning Microscopy, EPS-milk protein interactions by Surface Plasmon Resonance and immuno Western blotting.

Confocal Laser Scanning Microscopy

Development and establishment of a method to localize EPS by using specific probes, for example lectins/antibodies with fluorophore attached, in a model system similar to what is found in fermented milk products of variated pH, proteins, sugar, salt, ionic strength etc., and to visualize the EPS distribution within protein networks.

Application of the method for visualizing the structure of EPS and protein
If the method could be established successfully, it will be used industrially to control and improve the quality of the products to meet the growing demands of today's customers.

Materials and methods

Strain, media and growth condition

Four single strains and culture media were kindly provided by Chr Hansen. The strains within the culture media were grown in 2 L fermentation containers under anaerobic conditions separately. The head spaces of containers were kept under anaerobic conditions by a nitrogen flow of 0.5 L/min. and the cultures were kept homogenous by egitation at 300 rpm. Two strains' fermentations were carried out at 38°C and kept at pH 5.4, and the other two were at 40°C and kept at pH 6 by automatic titration with 24% ammonia.

Purification of EPS

The fermentation product was centrifuged to remove cells at 5000 g at 4°C for 10 min. The following step was to precipitate EPS in ice-cold alcohol overnight and harvest EPS by centrifugation at 10.000 g at 4°C for 1 hour. The precipitated pellets were dissolved in MilliQ water and dialyzed in Slde-A-Lyzer Cassette (Thermo Scientific) overnight at 4°C to obtain purified EPS.

Monosaccharides composition of EPS analysis

The samples of hydrolyzed EPS by 4 M trifluoroacetic acid at 100°C for 2 hours and unhydrolyzed samples of EPS will be analyzed by HPLC.

Image capture by Confocal Laser Scanning Microscopy

Two mixtures were prepared in advance. Nile Red solution (SIGMA-ALDRICH) for staining milk protein mixed with low fat stirred yoghurt (Arla Food) and Concanavalin A (conjugated with Alexa Fluor green fluorescence dye, Invitrogen) for staining EPS mixed with purified EPS. The specimen was prepared just before CLSM visualization by combining the two mixtures.

Conclusions

The EPS distributed in the protein network were visualized clearly by CLSM with the use of concanavalin A as a probe. It indicates that concanavalin A conjugate was successful, when coupled with CLSM, in terms of observing lactic acid bacterial EPS in fully hydrated stirred low fat yoghurt. This technique showed that EPS seemed to associate with the skeletal structure of the protein network rather than inside the the pores.

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