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Microstructure Analysis of Process Cheese by Cryo-Scanning Electron Microscopy and Light Microscopy

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Introduction

The process cheese in this study is a mozzarella-like cheese made by blending water, milk protein (Rennet casein) and fat into a homogeneous mass in the presence of the necessary emulsifying salts (ES), heat, and mechanical shear. The ES fulfil a major role in protein hydration, solubilization and subsequent fat emulsification. Changing the ES concentration could thus affect the microstructure, i.e. fat globule size and distribution, which in turn influences important functional properties like texture and meltability.

Objective

To use microstructural analysis to explain and predict the effects of reducing ES and NaCl on functional properties of process cheese.

Methods

Manufacture of Process cheese (50% moisture)

Water, vegetable oil, rennet casein and salts were mixed in a temperature controlled mixer (Farinograph) at 50°C, heated to 80°C until a homogeneous mass was formed; citric acid was then added. The standard cheese formulation is shown in table 1.

Table 1. Standard formulation of 50% moisture process cheese

Ingredients	% w/w in Cheese
Rennet Casein	22.5
Vegetable Oil	24
ES	1.4
NaCl	1.5
Citric Acid	0.5
Sorbic Acid	0.1

Light Microscopy (LM)

Cryosections (thickness = 8µm) were sequentially stained for fat (red) and protein (green) by immersion in oil red 0 (30 seconds) and fast green (1 min). Farrant was used as mountant prior to examination by 10×, 20× and 40× lens.

Cryo-Scanning Electron Microscopy (cryo-SEM)

Cryofixed cheese blocks were sublimated (-85°C/5min) and gold coated (-180°C/2min). Examination was done at an accelerating voltage of 10 kV and a magnification of 500-7500×. ImageJ image analysis software was used for the determination of the mean fat globule diameter.

Cheese Functional Properties

Hardness measured with an Instron Testing Machine.
Meltability empirically determined after heating (180°C/10min).

Results

Micrographs showed smooth surfaced spherical fat globules (F) uniformly distributed in a continuous protein matrix (P), (**Fig 1**).

In comparison to the standard formulation, the average fat globule diameter systematically decreased on reducing ES and increased on decreasing NaCl (**Fig 2**). The changes in fat globule size could be related to the processing time, which increased or decreased, relative to that of the standard formulation, on reducing ES or NaCl, respectively. Cheese hardness increased from 272 to 545 N on reducing ES and decreased to 221 N at 0% NaCl. The meltability decreased from 105 to 55 mm on reducing ES by 40%,.

Quadratic relationships were observed between fat globule diameter and cheese hardness and meltability, with $R^2 \geq 0.90$ in both cases (**Fig 3**). This suggested that the microstructure analysis could be used to predict cheese functionality.

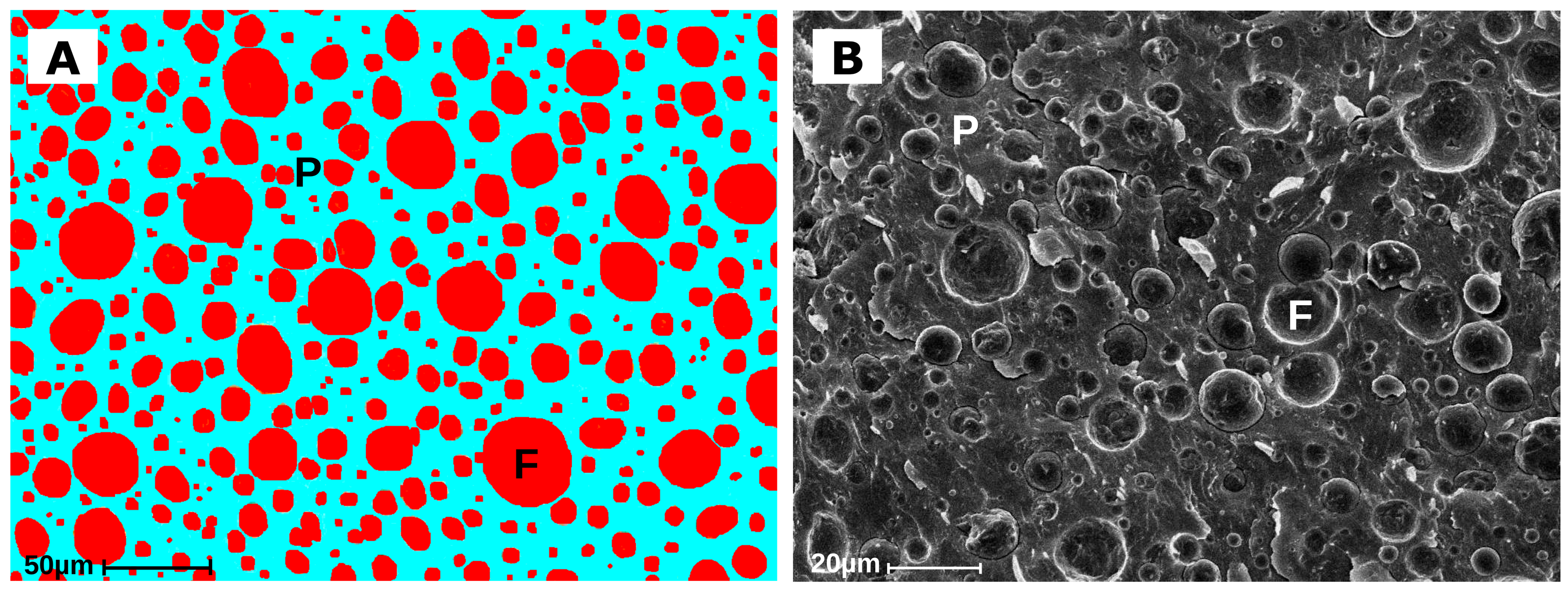

Fig 1. (A) LM and (B) cryo-SEM micrographs of standard formulation cheese

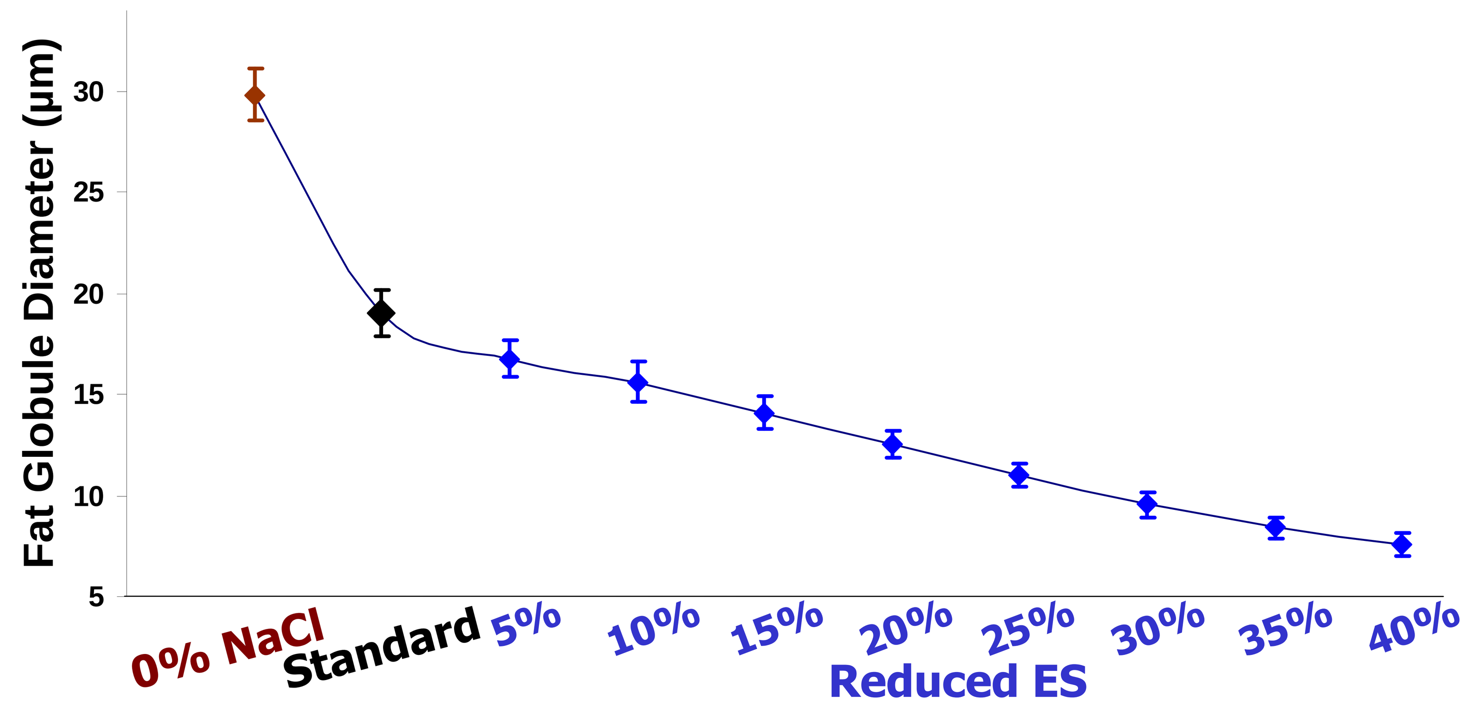

Fig 2. Mean fat globule diameter at standard formulation, 0% NaCl and reduced ES process cheese

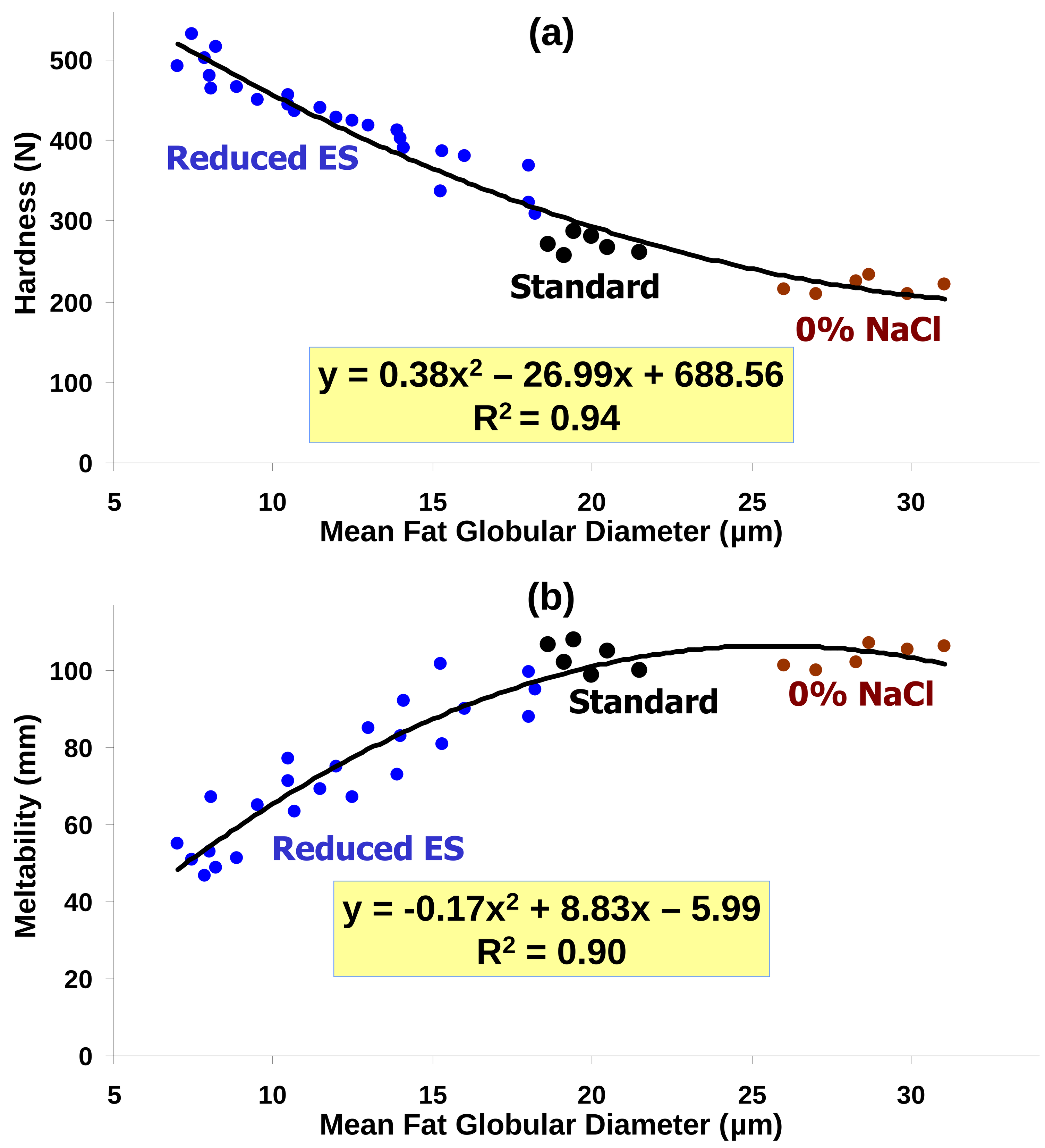

Fig 3. Relationship between mean fat globule diameter and (a) hardness and (b) meltability of process cheese

Conclusion

Cryo-SEM and LM (a very easy and inexpensive method) were very useful techniques for assessing the process cheese microstructure.

Fat globule diameter is a good predictor for cheese functional properties over a broad range of formulations.

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