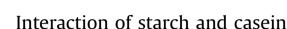
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ABSTRACT

Starch was commonly used to improve the stability of set yogurt. In this research, casein and modified starches were placed in a simulated yoghurt system giving the advantage of a low variation in the environment parameters compared to a natural yoghurt system. The interactions between modified starches (phosphate starch, hydroxypropyl starch and starch ester of octenyl succinic) and casein were investigated with several independed types of measurements methods. The combination of zeta potential and fluorescence micrograph were used to research the interactions between modified starches and casein. And the results proved that modified starches not only absorbed with casein via electrostatic forces, but also had steric stabilization. Besides this, fourier transform infrared spectroscopy (FTIR) and differential scanning calorimetry (DSC) were used to investigate the role of hydrogen bonds between modified starches and casein. It could be deduced that the interactions of modified starches and casein included electrostatic adhesion, steric stabilization and hydrogen bond. At the same time, electrostatic adhesion was the main interaction between phosphate starch and casein. The main role of the interactions between starch ester of octenyl succinic and casein should be steric stabilization.

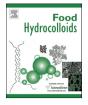
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1. Introduction

The development of set yogurt is very rapid in the domestic market because of its unique flavor and healthy function. Milk protein is one of the main nutrients of set yogurt. In the system of yoghurt, casein, which is about 65% of the total proteins, is made up of four proteins: α s1, β , α s2, and κ -casein (De Kruif & Tuinier, 2001). A network of three-dimensional casein strands is contained in the milk. Casein exists as colloidal particles in milk, which are stabilized by steric repulsion due to the extended portion of the κ -casein present mainly on the surface of micelles. During acidification, the extended portion of the κ -casein collapses, there is a decrease in charge repulsion, and the micelles aggregate (Everett & McLeod, 2005). The change of micellar conformation and precipitation of milk protein are the critical problem that effect the production and development of yogurt. In order to keep the stability of set yoghurt, stabilizers are used in yoghurt, such as gelatin, starch, pectin, alginate and derivatives of methylcellulose. Matia-Merino et al. studied the dynamics of the formation of the acid gel network for the mixtures of milk protein and pectin (Matia-Merino & Singh, 2007). The gelation profiles of the mixture showed a gradual decrease in the shear modulus with the incorporation of pectin, in which the casein micelles had a synergistic effect of pectin added to the systems. The gelation of low methoxyl pectin is due to the interactions between calcium ions and blocks of galacturonic acid (Braccini & Pérez, 2001). The milk protein can be also stabilized by carrageenan. It has concluded that both carrageenan adsorption to casein micelles and carrageenan helix aggregation were required to prevent casein micelles phase separation from polysaccharides using dynamic images scattering and scanning electron micrograph (Spagnuolo, Dalgleish, Goff, & Morris, 2005). However, the stable systems remain phase separated in microstructure (Thaiudom & Goff, 2003).

Starch is becoming a popular additive for set yoghurt as a thickener and colloidal stabilizer because of its relatively low cost and availability (Considine et al., 2011). Modified starches are added to yoghurt in order to increase the gel strength and viscosity (Angkana Noisuwan, Hemar, Wilkinson, & Bronlund, 2009). In order to provide maximum benefit to yoghurt product, it is important to understand the mechanisms, interactions and synergistic effects between modified starches and milk protein (A Noisuwan, Hemar, Wilkinson, & Bronlund, 2011). In fact, the structure, gelatinization, pasting, rheological properties and other physic–chemical properties of the milk protein–starch mixtures have been carried out. The cross-linked acetylated starch is similar to block copolymer which not only keeps them attached to the particles to form a thick







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adsorption layer, but also retains sufficient conformational freedom to avoid aggregation (Dickinson, 2003; Kiani, Mousavi, Razavi, & Morris, 2010; Sandoval-Castilla, Lobato-Calleros, Aguirre-Mandujano, & Vernon-Carter, 2004). Angkana et al. investigated the adsorption of sodium caseinate (NaCAS) and whey protein isolate (WPI) to both normal and waxy rice starch granules using SDS-PAGE. It exhibited the proteins present in NaCAS and WPI adsorbed to normal and waxy rice starch granules. In the case of NaCAS, both normal and waxy rice starch, α s-casein adsorbed preferentially and in higher amounts than β -casein. The changing of the swelling and solubility for acetylation may be ascribed to the introduction of hydrophilic substituting groups, which retained water molecules to form hydrogen bonds in the starch granules (Angkana Noisuwan, Bronlund, Wilkinson, & Hemar, 2008; A Noisuwan et al., 2011).

Although the research to understand the interactions of modified starches and milk proteins have made significant progresses, several aspects to get a complete picture are still not answered completely. Depending on starch type and modification as well as on pH-depended casein conformations different interaction mechanisms can be expected. For milk systems near neutral pH, Azim et al. reported the influence of cross-linked waxy maize starch on the aggregation behavior of casein micelles (Azim, Alexander, Koxholt, & Corredig, 2010). The results suggested that starch granules were not attached to milk casein micelles, but only embedded in protein gel matrix. However, Angkana et al. suggested that adsorption of certain fractions of milk proteins to the starch granule through hydrophobic interactions might restrict the diffusion of water into the starch granules, which could result in a delay to the swelling of the starch granule. In addition, the interactions of modified starches and casein is based on a combination of certain types of intermolecular forces (Angkana Noisuwan, Hemar, Bronlund, Wilkinson, & Williams, 2007). Therefore, the main role of different intermolecular forces will be researched in this investigation using different starch modifications and an adequate set of characterization methods.

Cui et al. deduced that starch adsorbed onto the surface of casein micelle and this phenomenon prevented flocculation of casein owing to electrostatic adhesion, steric stabilization and osmotic effect (Mi, Liang, Lu, Tan, & Cui, 2014). Cui et al. studied the interaction between hydroxypropyl distarch phosphate and casein. The result showed that protein particle adsorpted on the surface of starch via electrostatic forces (Cui, Tan, Lu, Liu, & Li, 2014). And the stability of the system of starch and casein was the result of electrostatic repulsion and steric stabilization. The objective of this work was to study the interaction of starches (phosphate starch, hydroxypropyl starch, starch ester of octenyl succinate) and casein in a simulation yoghurt environment. Casein, as the main composition of the milk, was stabilized by steric repulsion due to the extended portion of the κ -casein present mainly on the surface of micelles. During acidification, the net electrostatic charge and repulsive steric interactions resulted in the aggregation of the casein and the stability of set yoghurt was damaged (Kruif, 1998). By studying and comparing their interactions with casein, the interactions between modified starches and casein were determined. At the same time, the main role of the reactions was established.

2. Materials and methods

2.1. Materials

Modified starches were obtained from Roquette (France). Modified starches contain 0.05% protein, 0.3% ash. Casein was supplied by Sigma (Sigma–Aldrich Chemine, Steinheim, Germany). The solution of casein was made by mixing casein in MilliQ water using a magnetic stirrer at room temperature. The pH of the solution was adjusted to 6.7 using 1.0 M NaOH and was kept overnight at 4 °C to ensure complete hydration. In order to simulate the yoghurt environment, the protein/starch gels were made by mixing at room temperature. Prior to starch and casein heating (95 °C for 20 min), the pH of the samples was adjusted to 4.0 using 1.0 M hydrochloric acid (Oh, Wong, Pinder, Hemar, & Anema, 2007) while stirring the samples.

All chemicals used were analytical pure and were purchased from either Sigma Chemical Co. (Sigma–Aldrich Chemine, Steinheim, Germany) or other Chemicals company.

2.2. Zeta potential

The calculated zeta potentials, were determined by electrophoresis and phase analysis light-scattering (PALS) using a Zeta-PALS instrument. It was a disadvantage that the zeta potential measurements must be made on dilute dispersions, which altered the structural properties of the sample. Tholstrup Sejersen et al. observed that the type of diluent greatly influenced the obtained results. In preliminary experiments, filtered supernatant of the corresponding AMD was tested as a diluent. However, a zeta potential of zero was observed, presumably due to an excessively high ion/particle ratio. It was assumed that the particles could not move in the electric field due to the high concentrations of counter-ions in the double laver, which neutralized the electronegative charges, and due to screening ions in the bulk solution. For these reasons, deionized water was chosen as diluent rather than the supernatant (Seiersen et al., 2007). Casein, starch and the gels of casein/starch were diluted 100-fold with distilled water before the measurement. The samples were measured in triplicate. Measurement of Zeta potential was performed with a Zeta Potential Analyzer (Brookhaven Instruments, USA). The zeta potential was calculated from the electrophoretic mobility using the Smoluchowski equation (Anema & Klostermeyer, 1996).

2.3. Fluorescence micrograph

The microstructure of the gels was observed using fluorescence micrograph. A fluorescent protein dye fluorescein isothiocyanate (FITC, Sigma Chemical, Oakville, ON, Canada) was dissolved in distilled water to a concentration of 0.5% (w/w). The pH of FITC was adjusted to 8.0 using 0.005 M NaOH. FITC with 40 μ L was added to 10 mL of the samples and stirred for 1 min. Few drops of the sample were placed to a slide and a cover slip was placed over it. The lenses used were 400 \times objective.

2.4. Fourier transform infrared spectroscopy (FTIR spectroscopy)

The preparation of the samples was freeze dried in liquid nitrogen conditions to ensure that no water existed in the samples. The samples were examined in KBr discs containing 3 mg of either complex or its components in 300 mg of KBr. The mixtures were compressed into slices by equipment. The spectra was obtained with a Mattson 3000 FTIR (Pye- Unicam, Cambridge, UK) spectrophotometer.

2.5. Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) measurements of the samples were performed with a Pyris-1 Thermal Analytical System (Perkin–Elmer, Norwalk, CT). An amount of 3 ± 0.5 mg of each sample was weighed in steel pans, and then heated from 35 to 150 °C at a heating rate of 2 °C/min. DSC experiments were carried out in triple.

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