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Spectrophotometric analysis of polysaccharide/milk protein interactions with methylene blue using Independent Components Analysis

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ABSTRACT

Interactions between xanthan gum, carrageenan, guar gum and milk protein were studied under various conditions of ionic strengths, temperature and pH. The proposed methodology was to examine the associative interactions by using a methylene blue (MB) spectrophotometric method combined with data analysis by a chemometric method. Independent Components Analysis (ICA) simplified the interpretation of the spectrophotometric results by decomposing absorbance spectral data into "pure signals" that could be related to chemical compounds. Addition of milk protein to MB/xanthan gum or carrageenan solution gave rise to spectral changes indicating electrostatic interactions between positively charged regions of milk protein and anionic polysaccharides at neutral pH and low ionic strength. Thus, it has been shown that negative polysaccharides are able to interact with milk proteins only in absence of NaCl. On the other hand, it was shown that no attractive interactions were established in neutral guar gum/milk protein systems, which highlighted the contribution of the charge density to the interactions. Through acidification, associative interactions between xanthan gum and milk proteins were strongly enhanced as shown by changes in the IC proportions. Therefore, ICA proved to be an efficient tool to facilitate interpretation of spectrophotometric data and identify associative interactions.

1. Introduction

The control of interactions between the different ingredients involved in formulated foods is a key problem in regard to the overall texture and stability of the products. When proteins and polysaccharides are mixed, the normal tendency for the two polymers is to demix. Depending on the affinity between the different biopolymers and the solvent, the interactions can be segregative (the biopolymers repel each other) or associative (the biopolymers attract one another) (Corredig, Sharafbafi, & Kristo, 2011; De Kruif & Tuinier, 2001; Doublier, Garnier, Renard, & Sanchez, 2000; Syrbe, Bauer, & Klostermeyer, 1998; Tolstoguzov, 1995). Generally, associative interactions are the result of net electrostatic interactions between the polymers carrying opposite charges and thus are strongly affected by the charge density of the two biopolymers, the pH and the ionic strength (De Kruif, Weinbreck, & De Vries, 2004; Schmitt & Turgeon, 2007).

At neutral pH, repulsive interactions between the globally negatively charged casein micelles and negatively charged polysaccharides would be expected. However, associative interactions could exist between a "positive patch", situated between residues 97 and 112 of the κ-casein and the negative sulphated groups of carrageenan (Garnier, Michon, Durand, Cuvelier, & Doublier, 2003; Snoeren, 1976; Snoeren, Payens, Jeunink, & Both, 1975). In contrast, the mechanism classically described in the literature for low charged or neutral polysaccharides/casein micelles mixtures is a segregative phase separation, thus involving no electrostatic interactions (Aichinger, Dillmann, Rami-Shojaei, Michel, & Horne, 2007; Bhat, Tuinier, & Schurtenberger, 2006; Bourriot, Garnier, & Doublier, 1999; Hemar, Tamehana, Munro, & Singh, 2001; Tuinier, Grotenhuis, & de Kruif, 2000).

Upon lowering the pH, the electrostatic repulsion of the less negatively charged casein micelles is modified and their point of zero charge is reached close to pH 4.6. Therefore, adsorption of

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anionic polysaccharides on casein micelles strongly depends on pH, as has been shown in the case of pectin (Maroziene & de Kruif, 2000; Tuinier, Rolin, & de Kruif, 2002), carboxymethylcellulose (Du, Jing, Hongbin, Ping, & Long, 2007) or xanthan gum (Kobori, Matsumoto, & Sugiyama, 2009). Recent studies have shown the formation of soluble complexes between whey proteins and anionic polysaccharides even when the pH is close to neutral (Benichou, Aserin, Lutz, & Garti, 2007; Koupantsis & Kiosseoglou, 2009). However, electrostatic interactions between casein micelles and carboxylated polysaccharides such as xanthan gum above the isoelectric point of casein micelles remain undetermined.

The interactions of proteins with polysaccharides in solution have been previously investigated using visible absorption spectroscopy in a methylene blue (MB) solution (Michon, Konaté, Cuvelier, & Launay, 2002; Snoeren, 1976). In dilute aqueous solution, the absorption spectrum of planar cationic MB is characterized by a sharp peak at 664 nm and a slight secondary shoulder at 610 nm (Gurov, Nuss, Gurova, & Dotdaev, 1988; Tafulo, Queiros, & Gonzalez-Aguilar, 2009). When an anionic polysaccharide is added to the MB solution, the peak at 664 nm decreases and a new absorption band appears, which corresponds to metachromatic complexes (Nandini & Vishalakshi, 2011; Schoenberg & Moore, 1964; Shirai, Nagaika, & Tanaka, 1977). It should be noted that the spectral changes are a consequence of a precise orientation of neighbouring dye molecules and reflect the steric arrangement of the polar residues on the macromolecule that are available for interaction with the dye. Therefore, the binding process depends on polysaccharide conformation and charge density as well as environmental conditions such as ionic strength or temperature (Shirai et al., 1977). When increasing the temperature, and thus the thermal agitation, the lifetime of the long-range force interactions with water molecules is higher, and statistically, a higher number of methylene blue molecules are aligned and form metachromatic complexes (Michon et al., 2002).

If a protein, interacting associatively with polysaccharides, is introduced into an MB/anionic polysaccharide solution, it will compete with the cationic MB molecules and bind preferably to anionic polysaccharides. As a consequence, the release of the free MB molecules increases the peak at 664 nm while the metachromatic peak decreases. Using this method, interactions between gelatin or casein micelles and carrageenan (Garnier et al., 2003; Michon et al., 2002; Michon, Vigouroux, Boulenguer, Cuvelier, & Launay, 2000), or whey protein/xanthan gum, carboxymethylcellulose or λ-carrageenan (Benichou et al., 2007; Koupantsis & Kiosseoglou, 2009; Perez, Carrara, Sanchez, Patino, & Santiago, 2009) have well been demonstrated.

The interpretation of spectrophotometric data is sometimes complex and is often more qualitative than quantitative. Several chemometric tools can be used for the treatment of chemical data such as Principal Components Analysis (PCA) or Independent Components Analysis (ICA). Similarly to PCA, ICA is based on the construction of latent variables, called Independent Components (ICs), which are linear combinations of the original variables. The main advantage of this method compared with PCA is that it aims to extract "pure signals" and their proportions from mixtures which means that the resulting latent variables are easier to interpret (Hyvärinen & Oja, 2000; Rutledge & Jouan-Rimbaud Bouveresse, 2013; Stone, 2004).

The objective of the current study was to use a chemometric tool to examine the interactions between milk protein and different polysaccharides under various conditions on the basis of spectrophotometric analysis. The first part of this paper focuses on the detection of interactions depending on the type of polysaccharide. In the second part, ICA was performed to understand the effect of various factors on interactions such as type of milk protein, ionic strength, temperature or pH.

2. Materials and methods

2.1. Materials

Xanthan gum (Satiaxane), guar gum (Viscogum MP) and iota-carrageenan (HMR) were supplied by Cargill (Baupte, France). The samples were used without purification. Protein content of xanthan gum, guar gum and iota-carrageenan was about 7, 5 and 4 wt%, respectively, and moisture content about 11, 7 and 4 wt%, respectively. Iota-carrageenan chains were composed of a mix of 97% iota-carrageenan and 4% kappa-carrageenan with a charge density of 2 sulphate groups per dimer. Intrinsic viscosity of xanthan gum, guar gum and iota-carrageenan in 0.1 M NaCl were 70 ± 10 at 43 °C, 11 ± 2 at 43 °C and 7.4 ± 0.4 dL g^{-1} at 60 °C, respectively. Mineral content of polysaccharides was not significant compared to ions introduced by the milk protein or NaCl addition.

Methylene blue was purchased from Merck (Darmstadt, Germany) and the glucono-δ-lactone (GDL) from Sigma (St Louis, MO, USA). A methylene blue (MB) solution with a concentration of about 0.0008% was prepared by dissolving the correct amount in Milli-Q water (made by reverse osmosis followed by filtration through a Milli-Q apparatus) and stirring for 1 h at room temperature. Its concentration was adjusted so that its optical density at 664 nm was below 2. The same methylene blue solution was used for all the results described in this paper.

Casein micelles (CM) were purified by tangential ultrafiltration followed by purification through water diafiltration, and then freeze-dried (INRA Rennes, France). CM (1 wt%) was dispersed in 0.1 M NaCl at 20 °C and pH 7 under stirring for 5 min and then sonicated for 8 min at 50 W (Bioblock Scientific, Illkirch, France).

Low-heat skim milk powder was kindly provided by Ingredia (Arras, France). Skim milk (3.3 wt% proteins) was prepared by dissolving skim milk powder in Milli-Q water using a magnetic stirrer for 1 h at room temperature. The reconstituted skim milk was then left to fully hydrate overnight in a refrigerator.

2.2. Methods

2.2.1. Preparation of biopolymer mixtures

Methylene blue (MB)/polysaccharide (xanthan gum (X), guar gum (G) or iota-carrageenan (C)) solutions were prepared by adding the polysaccharide (0.002 wt%) to MB solutions under stirring at 70 °C for 30 min. The same thermal treatment was applied to the MB solution without polysaccharide. Then, the pH value was adjusted to 7 using a 1 M NaOH solution. Reproducibility of MB/polysaccharides spectra was checked by carrying out two separate studies.

Methylene blue (MB)/polysaccharide/casein micelles (CM) or milk protein (MP) were prepared by adding casein micelles or milk protein to the MB/polysaccharide solutions at a final protein concentration of 0.005 wt%. A unique MB/polysaccharide solution was used for MB/polysaccharide/casein micelles or milk protein preparation in order to avoid any variability in polysaccharide concentration. Ionic strength of all the mixtures was adjusted at 25 °C in the range of 0–0.1 M by addition of the required amount of NaCl under stirring for 5 min. Table 1 shows the experimental domain tested for each factor.

Glucono-δ-lactone (GDL) was added to some mixtures containing xanthan gum at 25 °C to lower the pH gradually, so as to mimic the fermentation process. During the acidification process, the sample was transferred into a spectrophotometric cuvette and the pH of the sample was measured during the corresponding spectrophotometric measurement.