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Mixed whey protein isolate-egg yolk or yolk plasma heat-set gels: Rheological and volatile compounds characterisation

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ABSTRACT

The influence of egg yolk (EY) or yolk plasma addition on the rheological properties and flavour characteristics of heat-set gels based on whey protein isolate (WPI) was the objective of this study. Large deformation compression tests and small deformation oscillatory measurements have shown that WPI gel structure-strengthening may take place following the incorporation of either EY or yolk plasma, indicating a possible synergistic effect between the yolk constituents on one hand and those of WPI on the other. Headspace solid-phase microextraction technique, coupled to gas chromatography–mass spectrometry (GC–MS) analysis, was applied to characterise the volatile compounds of the gels. A total of 29 compounds were identified. Furthermore, GC–Olfactometry analysis revealed that the major contributors to the overall odour quality of the mixed gels were 2,3-butanedione (buttery, caramel-like odour), hexanal (green), heptanal (fishy), 2-acetyl-1-pyrroline (cooked/milky), 1-hexanol (cooked) and methional (boiled potato-like odour), compounds originating either from WPI or EY.

1. Introduction

Whey protein isolate (WPI) is recovered from milk whey, a by-product of cheese and caseinate industry. It is a mixture of globular proteins, the main ones being β-lactoglobulin, α-lactalbumin and BSA (bovine serum albumin) (Fitzsimons, Mulvihill, & Morris, 2007; Fox & McSweeney, 1998). Because of the well established nutritional and functional properties, WPI is often exploited as ingredient in the preparation of foods such as meat, dairy, bakery or liquid diet products (Fox & McSweeney, 1998). Most of these foods are, however, of a mixed nature and their structure development is mainly based on the interaction of a number of proteins differing in structure and functionality. As a result, the incorporation of whey proteins into such systems may lead to final products that exhibit structural and sensory characteristics widely different from those not containing whey proteins. More importantly, whey protein interaction with other protein constituents (of meat, egg or flour) may be of a synergistic nature, resulting in the development of mixed gel network structures with enhanced rheological and mechanical characteristics and diversified functionalities (Arunepanlop, Morr, Karleskind, & Laye, 1996; Comfort & Howell, 2002; Ngarize, Adams, & Howell, 2004, 2005).

Egg proteins constitute the basis for the preparation of a number of food products such as creams, confectionery, pastry and cakes (Kiosseoglou, 1989). Whole liquid, frozen or dehydrated egg or their respective white and yolk fractions are used in the preparation of these products. The final product structure development depends on phenomena such as protein denaturation and interaction that take place during the heating/baking process leading to gel or solid foam formation. Protein interactions between the egg proteins are mainly hydrophobic in nature. In the case of both egg white and yolk fractions covalent disulphide bonds, as a result of sulphydryl group oxidation or disulfide–sulphydryl interchange reactions, may also play a role in the final product structure development (Kiosseoglou & Paraskevopoulou, 2005).

In addition to the complex nature of egg yolk proteins in terms of size, solubility and molecular flexibility, a further complication arises from the fact that most of these proteins are organised into small-sized particles, the micelles and the granules, where they offer physical protection to egg yolk lipids by mainly being positioned at the particle surface (Kiosseoglou, 1989). Therefore, gel structure formation in egg yolk dispersions is bound to involve both the water-soluble proteins as well as the surface proteins of the yolk particles, possibly leading to the development of a mixed protein gel matrix embedded with fully or partially disorganised yolk particles acting thus as fillers (Kiosseoglou, 2003). This structure may become even more complicated when egg yolk is used in admixture with globular proteins of a different origin and nature than those of egg, with the proteins of whey protein isolate constituting such an example. Howell and Lawrie (1984, 1985) suggested that synergistic effects in heat-set gels may take place in yolk plasma-egg albumen mixtures as well as in systems based on egg albumen–milk serum protein mixtures. The opposite effect, however, was observed when heating

Abbreviations: EY, egg yolk; WPI, whey protein isolate; HS-SPME, headspace solid-phase microextraction; LDL, low density lipoproteins; β-lb, β-lactoglobulin.

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mixtures of whey proteins with other proteins, where molecular interactions and phase separation phenomena resulted in weaker gel structures (Comfort & Howell, 2002).

Apart from the development of a mixed gel structure, heating of egg yolk–WPI mixtures is also expected to result in gel flavour characteristics widely different from those of the heated-treated single constituents. As aroma is unquestionably important to flavour perception, the analysis of volatile compounds, and particularly of those perceived by the human nose, contributes to the evaluation and/or prediction of product's potential appreciation by the consumers. To this aim, the development of different extraction methods, such as headspace solid-phase microextraction (HS-SPME), and analytical techniques (e.g. gas chromatography) in combination with olfactometry constitute valuable tools for the isolation and, following separation, identification and selection of aroma-active components from a complex mixture. In an effort to further exploit the relatively low cost whey protein products, such as concentrates and isolates, in the preparation of semi-liquid or solid food products containing whole egg or egg yolk, it would be of interest to understand the way the yolk constituents will behave in the presence of the highly functional globular whey proteins, especially during heating and gel preparation. More specifically, the study of possible synergistic interactions during gel formation between the protein constituents of the yolk, on one hand, and the whey, on the other, is needed in order to characterise the properties of products based on egg yolk–whey protein mixtures. In addition to the study of physicochemical characteristics, such an investigation should also include the characterisation of the gel volatile compounds as their profile and hence the flavour notes of the mixed gel are expected to influence the aroma of the final gel products. The composition of the volatile compounds was followed by means of a HS-SPME technique coupled to GC/MS analysis while the screening of the potent odorants was conducted by gas chromatography/olfactometry (GC–O).

2. Materials and methods

2.1. Materials

Fresh hens' eggs were obtained from the local market while WPI (91% w/w in protein) was a product of Davisco (BIPRO JE 153-6-420) (USA). The liquid yolks obtained from a number of eggs, by separating the albumen by hand, were pooled. The plasma fraction of yolk was fractionated by first diluting with three volumes of 0.17 M NaCl and stirring for 45 min at 4 °C (Anton et al., 2003). The resulting yolk dispersion was subjected to centrifugation at 10,000 ×*g* and the granules were precipitated and discarded. The plasma was centrifuged two more times to remove traces of granules and sodium azide was added to act as preservative. The total protein of yolk (16.10% w/w) and its plasma fraction (4.55% w/w) were determined by the Kjeldahl method. The solids content (50.5% and 15.5% w/w for the yolk and plasma, respectively) was determined gravimetrically by drying at 103 °C (AOAC, 2006).

2.2. Preparation of mixed protein solutions

A WPI stock solution 20% (w/v) in protein was first prepared by dissolving under continuous agitation for 20 h with the aid of a magnetic stirrer the appropriate amount of isolate in a phosphate buffer of pH 7.0 (containing 0.17 M NaCl). A mixed WPI–yolk plasma (1:1) stock dispersion (M1), 10% (w/w) in whey protein was then prepared. This dispersion, therefore, contained 7.75% w/w (2.3% protein) plasma solids. A similar mixed WPI–egg yolk stock dispersion (M2) containing 10% (w/w) whey protein and the same yolk total solids as M1, that is 7.75% w/w (2.5% protein), was also prepared. In addition, stock WPI solutions were suitably diluted with the phosphate buffer to obtain whey protein solutions with the same total protein contents as those of the WPI–yolk plasma or yolk mixtures.

2.3. Application of dynamic rheometry to heated protein solutions

Stock mixed WPI–plasma and WPI–egg yolk solutions were diluted with the appropriate volumes of phosphate buffer (pH 7.0) to obtain a series of dilute whey-egg plasma or yolk protein mixtures of varying protein concentration. Diluted whey protein, yolk plasma and egg yolk solution samples were also prepared, to act as controls, following mixing of their respective stock solutions with suitable volumes of the phosphate buffer. A rotational Physica MCR 300 rheometer (Physica Messtechnic GmbH, Stuttgart, Germany) was then employed to conduct dynamic oscillatory measurements using concentric cylinder geometry (inner and outer cylinder diameter 4.2 and 4.7 mm). A quantity of around 4 mL of each liquid protein sample was poured into the rheometer cup and following the lowering of the inner cylinder the sample surface was covered with silicon oil to prevent evaporation of water during heating. Each sample was heated from 20 to 92 °C at a scan rate of 3 °C/min, held for 15 min at 92 °C and then cooled to 20 °C at the same rate. The G' and G'' (storage and loss modulus, respectively) were continuously recorded at a strain level set at 0.1%, which according to preliminary experiments was well within the linear viscoelastic region for all the samples, and a frequency of 1 Hz during the thermal scan periods. The gelation temperature was defined as the temperature at which $G' = G''$ ($\tan\delta = 1$) during heating of the sample. All the measurements were performed in triplicate.

2.4. Gel preparation and measurement of liquid losses and gel fracture properties

The solutions prepared as described above were poured into cylindrical glass tubes (1 cm diameter) with inner surfaces covered with silicon oil. The tube openings were sealed with the aid of a plastic film, the tubes were placed in a water bath (90 °C), heated for 30 min and then removed from the bath and left for 24 h at room temperature (~22 °C). Following their removal from the tubes and drying their surface with the aid of a filter paper, the gels were weighted and the liquid losses that took place during the course of gelation, expressed as a percentage of the initial solution weight, were determined. To determine the liquid losses upon compression, gel cylindrical samples of a thickness of 2 mm were placed between filter tissue pieces and compressed for 30 min to 10% of their width with the aid of a Texture Analyzer (Stable Micro Systems Ltd., Surrey, UK). The compressions were carried out by using a cylindrical aluminium probe (2 cm diameter). The liquid lost from the gel sample was measured from the weight difference before and after the application of compression and expressed as a percentage of the initial liquid weight (Funami, Yada, & Nakao, 1998).

The measurement of gel fracture properties was conducted by subjecting cylindrical gel samples of a height of 1 cm to a compression of 80% at a speed of 0.5 mm/s with the aid of the Texture Analyzer (Bourne, 1978). The resulting force–time curves were converted into compression stress–Hencky strain ones to determine the gel yielding and fracture behaviour. The Hencky strain, ε_H, was calculated from Van Vliet (1999):

$$\varepsilon_H = \ln\frac{L(t)}{L_0}$$

where L_0 is the original sample height and $L(t)$ the respective height after a compression time t. The stress, σ, is given by equation:

$$\sigma = \frac{F(t)}{A(t)}$$