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Interactions in casein micelle - Pea protein system (Part II): Mixture acid gelation with glucono-δ-lactone

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ABSTRACT

In our preceding study (Part I), the thermal denaturation and aggregation of enriched pea protein fractions, namely vicilin/convicilin 7S (Vic) and legumin 11S (Leg), were investigated in the absence or in presence of casein micelles (CM) at pH ≈ 7.1. The present report (Part II) focuses on the glucono-δ-lactone (GDL) acid-induced gelation of either co-heated proteins in admixture (namely route 1) or thermally-aggregated pea proteins mixed with unheated CM (route 2), while applying a pea protein (either non fractionated PP or Vic or Leg) - to - CM weight ratio of 1: 1 and total protein concentration of 3.6 wt%. The pea protein thermal aggregates obtained by route 1 were of lower size and less soluble than those yielded in isolation. For route 1, gelation of the heated CM − Vic mixture was triggered at a higher pH value and led to higher final storage modulus G′ than corresponding protein samples in isolation. In contrast, the presence of large and sedimentable aggregates in the case of the CM − PP and CM − Leg mixtures impaired gelation. Concerning route 2, either the PP or Vic aggregates mixed with unheated CM resulted in rapid gelation and higher final G' values than those measured for their single-protein sample counterparts. Viscoelastic properties of the mixed gel depended on the pea protein fraction used, thermal aggregation route, extent of physical interactions between pea proteins during acidification and further involvement of CM. Hence, route 2 would be more reliable than route 1 to produce a "mixed" dairy-like gelled product containing pea proteins with improved texture properties.

1. Introduction

Though health benefits of plant protein-based products are increasingly documented, the promotion of plant proteins requires the resolution of several issues concerning food policy, industrial processes, consumption habits and sensorial acceptance (Gonzalez, Frostell, & Carlsson-Kanyama, 2011). However in developed countries, the overconsumption of animal-based products coupled with saturated fat intake could be detrimental regarding public health problems, ecological footprint, as well as sustainability and equitability of human food production models (FAO, 2013). Hence, one current strategy investigated by food scientists is to develop animal protein-based product supplemented or partially-substituted with plant proteins. Owing to well-recognizable flavor and texture, high digestibility and other health claims, dairy yoghurts represent a reference product as compared to "mixed" acid milk gels containing soy protein concentrate or legume flour (Drake, Chen, Tamarapu, & Leenanon, 2000; Zare, Boye, Orsat, Champagne, & Simpson, 2011). The "mixed" gelled products were reported to display lower organoleptic properties than regular dairy yoghurt with increasing levels of plant proteins. In addition, acid-gelation of milk in admixture with plant proteins was possibly affected by interactions between dissimilar proteins (Roesch & Corredig, 2006). In this regard, reliable data are lacking concerning the influence of food processes on the physicochemical properties of dissimilar protein mixtures. Hence, increasing knowledge on the interactions between milk proteins and plant ones would allow better control the functional properties of these blended ingredients.

Numerous rheological studies have dealt with the acid-gelation of preheated milk for yoghurt manufacture (Guyomarc'h, Queguiner, Law, Horne, & Dalgleish, 2003). Heating of milk above 80 °C for several minutes is usually carried out for both sanitization and denaturation of whey proteins (WP), of which β-lactoglobulin (β-LG). When unfolded, β-LG interacted with κ-casein at the surface of casein micelles (CM) via sulfhydryl-disulfide bond (S^-/S–S)

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Abbreviation

α-LA	alpha-lactalbumin
β-LG	beta-lactoglobulin
CM	casein micelles
DLS	dynamic light scattering
DW	deionized water
GDL	glucono-δ-lactone
Gp	gel point
I	ionic strength
L_α	acidic legumin polypeptide
L_β	basic legumin polypeptide
Leg	enriched-pea legumin (11S) fraction
MP	micellar phase
Mw	molecular weight
PN	total protein nitrogen
PP	non-fractionated pea proteins
$S^-/S-S$	sulfhydryl/disulfide bond exchange
SP	soluble phase
SCM	suspension of casein micelles
SEC-HPLC	size-exclusion chromatography
SMUF	simulated milk ultrafiltrate
Vic	enriched-pea vicilin/convicilin (7S) fraction
WP	whey proteins
wt%	weight percent (%, w/w)

exchange reactions. Depending on pH of milk and WP-to-CM weight ratio, heat treatment led to the formation of serum (soluble) and micelle-bond (sedimentable) WP- κ-casein thermal co-aggregates, which were shown to interact to each other and/or with CM during further milk acidification procedure (Graveland-Bikker & Anema, 2003). Consequently, acid gels made up with preheated milk proteins displayed both higher pH value at gelation (>4.5, i.e. the isoelectric point of CM) and elasticity than those from unheated milk.

Roesch and Corredig (2006) investigated the acid gelation of heated milk − soy protein mixtures, using soluble soy proteins. The acid gelation of heated CM − soy protein mixtures (90 °C) was reported to occur more slowly and final elasticity of the "mixed" gel was lower than that measured for either heated CM − WP or CM − WP − soy protein mixtures. Therefore heat-denatured soy proteins may contribute to the acid gel network formation by interacting with CM, though a higher affinity between WP and CM was strongly expected.

Following a similar experimental approach as those in the above mentioned studies, our preceding work (Part I) aimed at characterizing the heat-induced protein interactions between pea proteins and CM (Mession, Roustel, & Saurel, 2015). The fractionated pea globulins, namely legumin 11S (Leg) and vicilin/convicilin 7S (Vic)-enriched fractions, were heated in the presence of suspended CM (SCM) at single weight protein ratio of 1:1 and total protein concentration of 3.6 wt%, pH 7.1. The thermal denaturation and aggregation (85 °C for 60 min incubation) of each globulin fraction was altered in presence of CM. In the heated SCM − Leg mixture, the denatured legumin molecules produced in comparable amounts soluble and insoluble disulfide-bonded aggregates, which involved predominantly the acidic and basic legumin polypeptides L_α and L_β, respectively. The possibility of a covalent bonding between sulfur-containing pea legumin molecules and κ-cas. via $S^-/S-S$ exchange reactions was not observed under experimental conditions applied. Regarding the heated SCM − Vic mixture, the denatured vicilin molecules yielded more likely soluble (≈70% of total vicilin) and exclusively non-covalent aggregates. The removal of WP during the preparation of SCM was performed to restrict the formation of denatured WP - κ-casein thermal co-aggregates, with the aim to evidence any interaction between caseins and denatured pea proteins. Previous data would indicate the formation of denatured pea protein self-aggregates, with no peculiar involvement of caseins.

In the present study (Part II), glucono-δ-lactone (GDL) acid-induced gelation at 20 °C of the SCM - pea protein mixtures was performed. Two heating routes of the mixtures (3.6 wt% total protein, CM-to-pea protein weight ratio of 1: 1) were utilized. Route 1 consisted of co-heating SCM and pea proteins together. Concerning route 2, pea proteins (3.6 wt%) were heated in isolation to obtain soluble aggregates that were further mixed with unheated SCM. Additionally, unfractionated pea proteins (PP) were also investigated here to yield multicomponent gels that mimic a more complex food system. Changes in viscoelastic properties of the mixtures upon acidification was considered in relation to particle size, apparent solubility and composition of the pea protein thermal aggregates prepared by either route 1 or route 2.

2. Material and method

2.1. Materials

The starting CM and pea protein samples were identical to those prepared in previous works (Mession, Roustel, et al., 2015). The stock suspension SCM_0 (5.4 wt% in simulated milk ultrafiltrate [SMUF: 10 mM Tris−HCl, 7.5 mM $CaCl_2$ and 20 mM NaCl, ($I \approx 0.045$)]) and at pH 6.95 ± 0.05 was obtained from microfiltered, low-heat skim milk. On a dry basis, protein, lactose and salts composition of SCM_0 were 85.8 ± 1.1, 5.9 ± 0.3 and 6.8 ± 0.6 wt%, respectively. As evaluated by electrophoresis (SDS-PAGE), total caseins ($\alpha_{S1\text{-}2}$-, β- and κ-casein) and major WP (β-LG and α-lactalbumin, α-LA) represented 86.0 ± 2.5% and 6.1 ± 1.5% of total proteins in SCM_0, respectively.

Pea protein samples were extracted using a low-denaturing process, yielding a soluble protein isolate at pH 7 (Mession, Chihi, Sok, & Saurel, 2015). The stock solutions of non-fractionated pea proteins (P_0), legumin- (L_0) and vicilin- (V_0) enriched fractions in 10 mM Na_2HPO_4 buffer, pH 7.2 ± 0.1, were prepared at 4, 3.6 and 4 wt% protein concentration, respectively. Samples were centrifuged (10,000 *g*, 30 min, 20 °C). While PN of the freeze-dried PP and Vic samples was completely soluble, 10−15% of PN of the Leg sample failed to resolubilize.

The GDL powder was obtained from Prolabo (99.5% Fontenay-sous-Bois, France). All other reagents and chemicals purchased from Sigma-Aldrich (St Louis, USA) were on analytical grade.

2.2. Methods

2.2.1. Chemical analyses

Protein nitrogen (PN) was determined according to EN ISO 20483:2006. Crude protein content was calculated using a (protein) nitrogen-to-protein conversion factor of 6.38 and 6.25 for milk and pea proteins, respectively.