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Crucial role of remaining lactose in whey protein isolate powders during storage

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ABSTRACT

This study aimed to investigate the effect of lactose on the ageing-induced structural changes through a varying lactose content in whey protein powders. In this scope, four powders differing in their lactose content (0.1–16 w/w %) were stored at 40 °C and 60 °C for up to 3 months and sampled periodically. A small variation in lactose content of WPI powders was found to affect the level of structural and functional changes during powders ageing. At a higher lactose content (2.6 w/w %), proteins were more rapidly lactosylated and aggregated, with a higher rate than the control WPI powder; whereas at a lower content (0.1 w/w %) the rate of lactosylation and aggregation was considerably limited. These results highlighted the great contribution of the Maillard reaction during ageing of WPI powders. Reducing lactose content in the WPI powders is a promising way to extend their stability among storage.

1. Introduction

To date, several works have shown that storage conditions affect the structural and functional properties of high protein dairy powders (Anema et al., 2006; Gazi and Huppertz, 2015). However, the link between these two properties still remains relatively indirect: namely, it would be inherent to the initial state of the powder, including composition and physical properties, and on reactions and migrations occurring upon storage. As an example, Schokker et al. (2011) demonstrated that increasing the concentration of non-micellar caseins in micellar caseins concentrate before drying reduced the solubility loss after storage. On the other hand, Gaiani et al. (2009) showed that lipids were released through pores onto the surface of native micellar caseins powder particles upon storage, which is believed to have repercussions on powder functional properties such as the wetting properties. Together with proteins and lipids, lactose is also a component known to modify the properties of dairy powders during long-term storage (Guyomarc'h et al., 2000; Yazdanpanah and Langrish, 2013). In one of our previous work based on WPI powder storage (Norwood et al., 2016a), it has been demonstrated that the level of lactosylated proteins increased from the first months of storage reaching a maximum, then decreased with storage time. The decrease was concomitant with an increase in the level of aggregated proteins and powder browning. The hypothesis was that proteins were extensively modified by lactosylation and the degradation of the Maillard reaction products contributed to the formation of aggregated proteins in the dry state and the browning of the powder. In addition, these changes were shown to affect whey proteins functionalities such as heat-induced aggregation.

The presence of the lactose, albeit at low concentration (<2%) in WPI powders, is believed to have a strong effect on this ingredient stability during storage through interaction with proteins (Norwood et al., 2016a, 2016b). Therefore, the role of remaining lactose in the WPI powder was studied so as to relate its presence to the changes observed in the previous studies. For this purpose, four different whey protein powders with a high protein content, namely whey protein concentrate (WPC) and three whey protein isolate (WPI) powders were aged in controlled storage conditions. Obtained from milk microfiltration/ultrafiltration, and then spray dried, WPC and WPI powders are thus essentially composed of proteins (in the range of 70% and 95% of the dry matter,

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respectively), with a reduced lactose content and almost free from fat (Gulzar et al., 2011; Vignolles et al., 2007). More particularly, this study would give a better understanding of the role played by lactosylated proteins on protein aggregation in the dry state, whether or not it is essential to the aggregation process, and if controlling its content is decisive for controlling powders ageing.

From an industrial point of view, it seems crucial to study the influence of the initial composition of WPI powder, namely the lactose content, on the powder physico-chemical changes and the ageing mechanisms at the molecular level. Indeed, WPI powder is a widely used ingredient, with a high added value, that experiences change upon storage which lead to non-negligible losses for industrials on the market. This study aims at giving a lever so as to improve the WPI powder stability upon storage.

2. Materials and methods

2.1. Powders production

The WPI powders with different lactose content were all obtained from a protein concentrate (ultrafiltration/diafiltration of a skim milk microfiltrate) with a dry matter of ≥24% (w/w). Four powders differing from their lactose content (16, 2.6, 1.2 and 0.1% for WPC, WPI $^{[+]}$, WPI (control) and WPI $^{[-]}$, respectively; Table 1) were obtained by spray drying. The WPI $^{[+]}$ and WPI $^{[-]}$ were obtained by diafiltration of the WPC and WPI liquid protein concentrates, respectively, in order to remove the lactose surplus. For this purpose, an ultrafiltration pilot (Carbosep TECH-SEP, Rhône-Poulenc, France) equipped with dual ceramic membranes (Tami Industries, Nyons, France) with a total membrane surface area of 13.3 m^2 and a molecular weight cut off of 10 kg mol^{-1} was used. The flow rate of the retentate was set at 40 L h^{-1}. A diafiltration at 1.9 and 3 vol was carried out on WPC and WPI liquid concentrate, respectively, with a salt solution to keep the soluble fraction of minerals constant. The composition of the salt solution was determined by quantifying the soluble fraction of minerals of a WPI (Control) reconstituted at the protein concentration of the retentate subjected to diafiltration. The WPI $^{[+]}$ and WPI $^{[-]}$ diafiltered retentates had a dry matter of 16.2 and 20.9% (w/w). The four powders were then spray dried and packed under air in airtight tins of 400 g capacity.

2.2. Determination of powder composition

2.2.1. Dry matter

The dry matter (DM) was determined as described by Schuck et al. (2012). It was calculated by weight loss after drying 1 g of sample mixed with sand in a forced air oven at 105 °C for 5 h.

2.2.2. Nitrogen contents

The nitrogen content (TN) and non-protein nitrogen (NPN) of powders were determined by the Kjeldahl method according to the IDF Standards (2001a,b), respectively. The protein content was calculated by TN-NPN. Each powder was analysed in duplicate.

2.2.3. Lactose

The amount of free lactose in the WPI powder was determined using a High Performance Liquid Chromatograph (HPLC) chain (Dionex, Germering, Germany) linked to a 6.5 mm diameter and 300 mm length column “house-packed” with an ion exchange resin Aminex A-6 (Biorad, St Louis Mo., USA). The oven was kept at 60 °C and the elution flow was 0.4 mL.min-1 using a 5 mM H2SO4 buffer. Free lactose content was detected through a differential refractometer (model RI 2031 plus, Jasco).

2.2.4. Powder composition

Table 1 provides the chemical composition of the four powders differing in their lactose content.

2.3. Storage conditions

Whey protein powders were packed under air in airtight tins, which were stored in heat chambers at 4 (control powder), 40 and 60 °C for periods of up to 3 months. 1 tin per temperature condition was opened in order to analyse the structural and functional properties of the powders.

2.4. Chemical analysis

2.4.1. Reverse-phase chromatography

Denaturation profile of β-lactoglobulin (β-LG) and α-lactalbumin (α-LA) in stored powders were observed by reverse phase (RP) chromatography using a 300 A 8 μM 150 × 2.1 MM PLRP-S column (Polymer Laboratories, Amherst, MA, USA) after precipitation at pH 4.6 (solubility criteria). The latter was connected to a high performance liquid chromatography (HPLC) made of a separation system Waters 2695, a double wavelength detector Waters 2487 and an acquisition and Empower data processing software (Milford, USA). The elution flow was 0.2 mL min^{-1} using a gradient of acetonitrile obtained by an appropriate combination of buffer solution A (0.1% trifluoroacetic acid (TFA)) and buffer solution B (80% acetonitrile and 0.1% TFA). The column was first equilibrated with 35% buffer solution B and then linear gradients of buffer solution B moving from 35 to 44% in 3 min, from 44 to 48% in 11 min, from 48 to 53% in 12 min, and finally from 53 to 62% in 14 min were used to elute the proteins. Proteins were detected at 214 nm.

2.4.2. Gel permeation chromatography

Native proteins (Gulzar et al., 2013) were quantified during powder storage after gel permeation chromatography (GPC) using a Yarra SEC 3000 column (Phenomenex, Le Pecq, France) connected to a HPLC apparatus comprising a Waters 2695 separation system, a Waters 2489 double wavelength detector and Empower (Milford, USA) acquisition and data processing software. The protein elution flow applied was 0.8 mL min^{-1} using a 0.05 M phosphate buffer at pH 7 containing 0.1 M NaCl. Proteins were detected at 214 nm.

2.4.3. Fourier Transform Infra-Red

The protein secondary structure in the dry state was monitored

Table 1
Composition of the powders with different lactose content.

		WPC	WPI $^{[+]}$	WPI	WPI $^{[-]}$
Dry matter (w/w %)	DM	94.7	92.4	92.9	92.7
Water activity	aw	0.33	0.36	0.25	0.27
Nitrogen composition (% N × 6.38)	TN	74.4	86.9	88.6	89.5
	NPN	0.85	0.28	0.19	0.21
	NCN	69.6	80.7	84.6	84.7
Carbohydrates (w/w %)	Lactose	16.2	2.6	1.2	0.1