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Food Chemistry 333 (2020) 127514

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

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ARTICLE INFO

Keywords:
Camel whey proteins
X-ray Photoelectron spectroscopy
LC–MS
Glass transition temperature

ABSTRACT

This study investigated the effect of pH on the denaturation extent, the surface chemical composition, the water sorption isotherm and the glass transition temperature of camel and bovine whey protein's powders. The LC-MS analysis indicated that the β-Lactoglobulin was the most denatured protein in bovine whey powders regardless the pH value, while this protein was totally absent in camel whey. The α-Lactalbumin was relatively heat stable after drying and predominated the powder surface (X-ray photoelectron spectroscopy results) in both camel and bovine whey powders regardless the pH (neutral (6.7) or acidic (4.3 and 4.6)). Analysis of the water sorption isotherms indicated that decreasing the pH induced the increase of the water activity of lactose crystallization for camel and bovine whey powders. Finally, decreasing the pH led to the decrease of the glass transition temperature of camel and bovine whey powder (at 0.13, 0.23, and 0.33 of water activity).

1. Introduction

Whey proteins are important hydrocolloids that offer interesting techno-functional properties in food formulations such as emulsifying, foaming, and gelling properties. Whey proteins are often obtained by membrane filtration, chemical separation or enzymatic process. Most of them are spray-dried to produce stable whey protein powders. The spray drying is a rapid dehydration technique that allows an effective preservation of the biological products, such as proteins (Roos, 2002a; Schuck, Dolivet, Méjean, & Jeantet, 2008).

The physical stability of whey powders is one of the most challenging step during storage. Indeed, these powders contain residual lactose which is in a thermodynamically instable amorphous state (i.e. non-equilibrium glassy state) (Roos, 2002a; Schuck et al., 2005). The exposure of whey powders to high relative humidity enhanced water adsorption from the ambient storage environment. This can induce glass transition mechanisms and amorphous lactose crystallization (Jouppila & Roos, 1994; Kelly et al., 2016; Roos, 2002b). Since lactose crystallization is followed by the release of the adsorbed water, the sorption isotherms are important tools for characterizing changes in whey powders during and after the glass transition (Foster, Bronlund, & Paterson, 2005; Jouppila & Roos, 1994; Shrestha, Howes, Adhikari, Wood, & Bhandari, 2007). Several other studies have also considered the glass transition temperature (T_g) as a useful criterion to prevent physicochemical, nutritional and techno-functional deteriorations (e.g. stickiness, caking, non-enzymatic browning) of whey powders during storage (Foster et al., 2005; Jouppila & Roos, 1994). It was extensively demonstrated that the glass transition temperature and the sorption adsorption isotherm depend on the surface composition of whey powders, which was extensively studied using the X-ray photoelectron spectroscopy (XPS) (Gaiani et al., 2011). The XPS allows the measurement of the distribution of relative atomic elemental composition (carbon, oxygen, and nitrogen) at the studied surface (a layer of 5 to 10 nm). In case of whey powders, these elements are used to quantify the surface composition in terms of proteins, fats, and lactose (Fäldt & Bergenståhl, 1994). Actually, two mechanisms are known to describe the transport of these previous compounds to the surface of whey powder. These mechanisms include the air/liquid interface interaction (through active substances) (Fäldt & Bergenståhl, 1994) and the solid/solute segregation system (Kim, Esther, Dong Chen, & Pearce, 2003). In both systems, proteins start to accumulate preferentially at the surface (in the first 5 nm) of the whey powder particles (Kim et al., 2003; Shrestha et al., 2007).

On the other hand, it was acknowledged that during drying the

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https://doi.org/10.1016/j.foodchem.2020.127514
Received 17 February 2020; Received in revised form 4 July 2020; Accepted 5 July 2020
Available online 10 July 2020
0308-8146/

temperature of the sprayed droplets increased to reach asymptotically the air outlet drying temperature (Woo, 2013). Several researchers demonstrated that the thermal denaturation of whey proteins depends on time, temperature, free thiol group (SH), and heat treatment intensity (Manzo, Nicolai, & Pizzano, 2015). The most denatured protein in bovine milk whey is the β-lactoglobulin (Roefs & De Kruif, 1994). The particularity of camel milk whey is the lack of β-Lactoglobulin and the overexpression of α-lactalbumin (Lajnaf, Picart-Palmade, Attia, Marchesseau, & Ayadi, 2017). Recently, Felfoul, Jardin, Gaucheron, Attia, and Ayadi (2017) indicated that the thermal degradation of camel milk proteins results in the denaturation of the camel serum albumin (CSA) followed by the peptidoglycan recognition protein (PGRP) and the α-lactalbumin.

Lajnaf et al. (2018) demonstrated that camel whey proteins exhibited higher foaming properties as compared to bovine whey proteins', which can be an important foaming agent in food formulations. In addition, as far as the authors are aware of, the influence of pH on the denaturation extent, the surface chemical composition, the water sorption isotherm, and the glass transition temperature of camel whey protein's powders, is not yet studied. Thus, this study aimed at producing and characterizing camel whey's powders at neutral (=6.7) and acidic (=4.3) pH. As a first approach, the denaturation extent of spray drying on camel whey protein was analyzed using the LC-MS. Then, the influence of the pH on the sorption isotherms and the glass transition temperature was evaluated. The surface composition of the produced powders was investigated using the X-ray Photoelectron Spectroscopy (XPS). To create a well-based comparative study, we also assessed the bovine whey's powders (pH = 6.7 and 4.6 for neutral and acidic whey protein powders, respectively) produced under the same drying conditions and analyzed following the same evaluation techniques.

2. Materials and methods

2.1. Whey proteins preparation

Fresh skimmed camel (*Camelus dromedarius*) and bovine (*Bos Taurus*) milks were acidified with HCL 1 M to produce acidic whey proteins (isoelectric pH = 4.6 and 4.3 for skim bovine milk and skim camel milk, respectively), followed by centrifugation at 4000*g* for 15 min at 20 °C. Acidic camel and bovine milk's whey proteins were then collected and stored in sterile plastic bottles at −20 °C.

Sweet camel and bovine whey proteins (pH = 6.7) were obtained after a rennet enzyme coagulation (*M. miehei*, strength = 1:10,000, Laboratories Arrazi, Parachimic, Sfax, Tunisia) at 40 °C as described by Felfoul, Lopez, Gaucheron, Attia, & Ayadi, 2015.

2.2. Powder production

Acidic and sweet whey samples were spray-dried using a Bücchi mini spray dryer B-290 (Büchi Labortechnik AG, Flawil, Switzerland). The inlet and outlet drying temperatures were set up to 200 ± 2 °C and 80 ± 2 °C, respectively. During all experiments, the absolute humidity of air was equal to 5 g of water per kg of dry air; and the average droplets residence time in the drying chamber was equal to 1 s. The obtained powders are: ACWP: acidic camel whey powder, ABWP: acidic bovine whey powder, SCWP: sweet camel whey powder, and SBWP: sweet bovine whey powder. These powders were immediately stored at 4 °C in sterilized glass vials. The Size distribution of the produced powder was performed as detailed by Zouari et al. (2020). The d_{50} (diameter of 50% of the particles) was chosen as a size distribution indicator as recommended by Nikolova et al. (2014).

2.3. Bulk and surface composition

The bulk composition of acidic and sweet camel and bovine whey powders was performed according to the AOAC (Horwitz & Latimer, 2000). The surface composition of acidic and sweet camel and bovine whey powders were estimated using X-ray photoelectron spectroscopy. The XPS analyses were conducted using a Kratos Axis Ultra spectrometer (Kratos Analytical, Manchester, UK) working with a monochromatic AlKα X-ray source (E = 1486.6 eV; P = 90 W) as described by Gaiani et al. (2011). The analyzed powder area was about 300 × 600 μm with a depth of 6 to 10 nm. The relative atomic concentration of carbon, oxygen and nitrogen in the powder surface was quantified and integrated in an equation system proposed by Fäldt and Bergenståhl (1994). This equation system allows the quantification of the powder surface content in relation to lactose, proteins and fat.

2.4. LC–MS analysis

Before proceeding to the LC–MS analysis, the acidic and sweet whey powders were reconstituted in MiliQ water to reach the same solid content of unprocessed whey (6.5 w/w for acidic whey and 7.2 w/w for sweet whey). The mixtures were stirred (580 rpm) for 30 min at 25 °C. The reconstituted milks were stored at 4 °C overnight and then warmed up to the room temperature (25 °C). The total solids and the protein content of the reconstituted powders were then determined according to the AOAC (Horwitz & Latimer, 2000). Afterwards, 100 μL of unprocessed whey and their corresponding reconstituted powders were diluted in 450 μL of buffer solution (Urea 4 M/Tris 25 mM pH8). Diluted samples were filtered through Millipore® Millex® filters HV PVDF membrane (0.45 μm Pore Size) and were subjected to a second dilution (½) in 5% of TFA solution. Then, 40 μL of each sample were injected in the LC-MS system.

The LC-MS analysis was performed using an Agilent-1100 Rp-HPLC system (Agilent Technologies, Waldbronn, Germany) coupled with a Q-Exactive™ Hybrid Quadrupole-Orbitrap™ (Thermo-Fisher scientific, Waltham, MA, USA) mass spectrometer (Felfoul et al., 2017). The HPLC system was equipped with a column C4 (VYDAC, reference 214TP5215, length 150 mm, inner diameter 2.1 mm, pore size 300 Å, Grace™, Fisher-scientific, USA). A gradient from 37% to 90% of a solvent (acetonitrile: 80% (v/v) and TFA: 0.1% (v/v) in deionized water) was applied during 50 min for the elution of the proteins at a flow rate of 0.25 mL min^{-1}. Eluted proteins were then, electro-sprayed in a mass spectrometer Q-Exactive. The mass spectra acquisition speed was set up to a resolving power of 140,000 and an *m/z* ranging from 800 to 3000. Mass spectra were then analyzed using BioPharma Finder™ software (version 2.0.66.12, Thermo-Fisher scientific, Waltham, MA, USA). The Uniprot database (http://www.uniprot.org/) was used to identify the camel and bovine whey proteins (*Camelus dromdarus*, Taxon identifier: 9838 and *Bos taurus*, Taxon identifier 9913). The quantification of camel and bovine whey protein fractions was estimated based on the integrated peak areas of HPLC chromatographs (see Supplementary data, Fig. S1) and protein content of unprocessed whey and their corresponding reconstituted powders. The denaturation extent was evaluated as follow:

$$\text{Denaturation extent} = \frac{\text{Concentration before drying - Concentration after drying}}{\text{Concentration before drying}} \times 100$$

2.5. Dynamic vapor sorption

Dynamic vapor sorption was used to assess the water sorption isotherms at 25 °C. About 20 mg of each produced powder were loaded into a clean and a dry aluminum pan. The filled pans were subjected to water adsorption cycle using surface measurement DVS advantage (Surface Measurement Systems Ltd., London, UK) equipped with a Cahn microbalance. The DVS program was setup as described by Carpin et al. (2017). The lactose crystallization characteristics [water activity and necessary water for lactose crystallization (X_c)] were identified.

The experimental data (up to 0.40 of a_w) were fitted to the GAB (Guggenheim, Andersen and de Boer) model using the OriginPro 8 software (OriginLab Corporation, Northampton, USA). The GAB model

constants X_m, K, and C were, then, calculated and used to interpret the obtained experimental data following the equation below:

$$X = \frac{X\text{m}.\ C.\ k.\ a\text{w}}{(1 - k.\ a\text{w}).\ (1 - k.\ a\ \text{w} + C.\ k.\ a\text{w})}$$

where, X_m is the monolayer moisture capacity (g 100 g^{-1}); the C constant is the water binding energy by the first layer; K constant describes the multilayer sorption ability; and a_w is the studied water activity. The K constant was not considered in our study, since it described the multilayer sorption binding energy, up to 0.95 of a_w.

2.6. Modulated dynamic scanning calorimetry (MDSC)

The MDSC was used to evaluate the glass transition temperature (T_g) of the produced whey powders at three a_w (0.13, 0.23, and 0.33). Six milligrams of each samples were sealed in a hermitic aluminum pan and placed in a differential scanning calorimetry (DSC) Q1000 (TA Instruments, Eschborn, Germany). The 'heat mode only' (−30 to +200 °C) was used to assess the MDSC analysis against an empty aluminum pan as presented by Syll et al. (2012). The T_g was identified as the midpoint temperature when change in heat capacity in the reversed MDSC profile was observed.

2.7. Statistical analysis

The whole experiment was carried out three times for statistical accuracy. All analyses and measurements in this work were conducted in triplicate. The statistical differences ($p < 0.05$) were examined using SPSS 19 software (IBM SPSS statistics, Version 19, USA) following descriptive tests including the student's *t*-test, the one-way ANOVA, and the *Tukey* post-hoc test with a confidence level of 95%.

3. Results and discussion

3.1. Bulk composition and size distribution

Table 1 shows the bulk composition of ACWP, ABWP, SCWP, and SBWP. All produced powders presented statistically the same water content, fat and lactose contents ($p > 0.05$, Table 1). The protein content of SCWP was slightly but significantly higher than SBWP's (14.5 ± 0.1 g 100 g^{-1} and 13.6 ± 0.1 g 100 g^{-1}, respectively, $p < 0.05$, Table 1). Lower protein content was observed in both ACWP and ABWP ($p < 0.05$, Table1) which was linked to the absence of the glucomacropetide. The latter existed in sweet whey as results of rennet coagulation (El-Salam, El-Shibiny, & Salem, 2009). Nevertheless, the acidic whey powders exhibited higher ash content than SCWP and SBWP ($p < 0.05$, Table 1). This observation was related to the additional quantity of colloidal minerals of casein micelles released into whey while acidifying.

The composition of acidic and sweet camel whey powders (ACWP and SCWP) has not been reported elsewhere. However, the bulk composition of acidic and sweet bovine whey powders (ABWP and SBWP) has been extensively studied (Deeth & Hartanto, 2009; El-Salam et al., 2009). According to these previous studies, the composition of bovine whey protein powders ranged from 9% to 93% of protein, 0.1% to 85% of lactose, 0.1% to 7% of fat, and 1.5% to 27% of ash. In this current study, the composition of ABWP, ACWP, SBWP, and SCWP was in line with the reported values in the literature (Deeth & Hartanto, 2009; El-Salam et al., 2009).

Besides, the size distribution of the produced powder was analyzed (Table 1). The results indicated that ACWP and ABWP presented similar d_{50} diameter (6.3 ± 0.4 and 6.0 ± 0.8, $p > 0.05$, Table 1). However, the sweet camel whey powder (SCWP) showed significantly higher d_{50} than that of sweet bovine whey powder (SBWP) (25.4 ± 1.7 and 13.1 ± 1.0, $p < 0.05$, Table 1). Interestingly, sweet whey powders had higher d_{50} than those of acidic ones. Several studies have reported that the d_{50} mainly depends on the feed characteristics (e.g. composition and concentration) and the spray-dryer configuration (Fitzpatrick et al., 2007; Nijdam & Langrish, 2006). Indeed, in the current study the same drying conditions and the same dryer configuration were used to produce acidic and sweet camel or bovine whey powders. This fact indicated that the variation of whey composition (depending on the pH and the species) before drying was the main determining factor for the size distribution. Actually, it was demonstrated that a significant decrease in the particle size distribution of milk powder could be observed as a result of the decrease of the protein content and the increase of the ash quantity (McSweeney, Maidannyk, Montgomery, O'Mahony, & McCarthy, 2020). This fact may explain the variation in the size distribution between acidic or sweet camel and bovine whey powders. Indeed, our findings indicated that decreasing the pH resulted in lower protein content and higher ash quantity for both camel and bovine whey powders (Table 1). These observations may explain the lower size distribution of acidic camel and bovine whey powders as compared to those of sweet ones (Table 1).

3.2. LC/MS of camel and bovine whey proteins before and after drying

The protein denaturation extent of acidic and sweet camel or bovine whey during drying was studied using LC–MS. The chromatograms profiles of unprocessed whey and reconstituted whey powders are shown in Supplementary data. By comparing these profiles, a slight and significant thermal denaturation was observed during the drying step. The proteins concentrations before and after reconstitution as well as their denaturation extent are presented in Table 2.

Analysis of the LC-MS profiles of SBWP and RSBWP (Supplementary data, Fig. S1) indicated that the bovine milk serum albumin protein (BSA) was co-eluted with α-lactalbumin (Peak 1, Table 2). These proteins were identified by comparing their molecular weight (results of mass spectroscopy) with the existing data in Uniprot database (http://www.uniprot.org/). The serum albumin protein (BSA) and α-lactalbumin denaturation extent was equal to 13% (Peak 1, Table 2) in sweet bovine whey, meanwhile, these proteins were denatured to an extent of

Table 1
Bulk composition and size distrubition of sweet and acidic camel and bovine milk whey powders.

	ACWP	ABWP	SCWP	SBWP
Bulk composition				
Water content (g 100 g^{-1})	4.8 ± 0.1 ^{a}	5.0 ± 0.1 ^{a}	4.9 ± 0.1 ^{a}	4.8 ± 0.1 ^{a}
Proteins (g 100 g^{-1})	12.1 ± 0.1 ^{a}	11.6 ± 0.1^{b}	14.5 ± 0.1^{c}	13.6 ± 0.1 ^{d}
Fats (g 100 g^{-1})	0.1 ± 0.02 ^{a}	0.1 ± 0.02 ^{a}	0.1 ± 0.02 ^{a}	0.1 ± 0.02 ^{a}
Lactose (g 100 g^{-1})	72.1 ± 0.6 ^{a}	72.6 ± 0.5 ^{a}	71.7 ± 0.5 ^{a}	71.9 ± 0.5 ^{a}
Ash (g 100 g^{-1})	11.4 ± 0.1 ^{a}	10.5 ± 0.1^{b}	9.1 ± 0.1^{b}	7.6 ± 0.1^{c}
Calcium (g L^{-1})	1.2 ± 0.01 ^{a}	1.2 ± 0.01 ^{a}	0.58 ± 0.04^{b}	0.57 ± 0.01^{b}
Lactate (g L^{-1})	6.3 ± 0.8 ^{a}	5.7 ± 0.5 ^{a}	0.97 ± 0.02^{b}	0.99 ± 0.02^{b}
Size distrubition: d_{50} (μm)	6.3 ± 0.4 ^{a}	6.0 ± 0.8 ^{a}	25.4 ± 1.7^{b}	13.1 ± 1.0^{c}

Same letters in the same row represent the statistical data significance ($p > 0.05$).

Table 2
Protein characterization from LC-MS peak integration for sweet and acidic camel and bovine milk whey protein before and after powders reconstitution.

Peaks			Before reconstitution			Reconstituted powders			DE (%)
Number	Identification	MW (Da)	Area (AU × s)	Proportion (%)	Concentration (μg μL^{-1})	Area (AU × s)	Proportion (%)	Concentration (μg μL^{-1})	
Acidic bovine whey									
1	α-La, V-B	14 176	8.8	26.4	2.112 ± 0.02 [a]	6.9	28.5	1.955 ± 0.02[b]	7.4
2	BSA	66 000	1.6	4.9	0.392 ± 0.01 [a]	1.2	4.9	0.343 ± 0.01[b]	12.5
3	Mixture of β-Lg variants	n.id	7.1	21.3	1.704 ± 0.02 [a]	5.1	21.1	1.477 ± 0.01[b]	13.3
4	β-Lg, V-B + 1 lactose β-Lg, V-B	18 60118 277	3.1	9.3	0.744 ± 0.02 [a]	1.5	6.1	0.427 ± 0.01[b]	42.6
5	β-Lg, V-A + 1 lactose β-Lg, variant A	18 36318 687	11.2	33.6	2.688 ± 0.02 [a]	8	32.7	2.289 ± 0.02[b]	14.8
Acidic camel whey									
1	α-La	14 421	9.7	80.2	9.624 ± 0.1 [a]	9.1	69.9	7.191 ± 0.07[b]	25.3
2	PGRP	19 137	0.5	4.2	0.504 ± 0.02 [a]	0.6	4.8	0.432 ± 0.01[b]	14.3
3	CSA	66 600	1.1	9.2	1.104 ± 0.02 [a]	1.2	9.4	0.846 ± 0.01[b]	23.3
Sweet bovine whey									
1	α-La, V-B BSA	14 176 66 000	9.3	39.2	4.3 ± 0.05 [a]	5.3	41.7	3.7 ± 0.02[b]	13
2	Mixture of β-Lg variants	n.id	4.5	28.3	2.6 ± 0.02 [a]	1.6	12.6	1.1 ± 0.01[b]	56
3	β-Lg, V-B + 1 lactose β-Lg, V-B β-Lg, V-A + 1 lactose β-Lg, V-A	18 601 18 277 18 363 18 687	9.1	28.5	2.2 ± 0.02 [a]	3.3	25.7	1.8 ± 0.02[b]	18
Sweet camel whey									
1	α-La	14 421	13.9	60.2	7.224 ± 0.07 [a]	11.6	59.4	4.94 ± 0.05[b]	31.6
2	PGRP	19 137	1.4	6.2	0.744 ± 0.01 [a]	4.5	2.3	0.341 ± 0.01[b]	54.2
3	CSA	66 600	3.1	13.6	1.632 ± 0.02 [a]	1.5	7.9	0.711 ± 0.01[b]	56.4

n.id: non-identified; La: lactalbumin; Lg: Lactoglobulin; V: variant; BSA and CSA: Bovine and Camel Serum Albumin, respectively; PGRP: peptidoglycan recognition protein; DE: denaturation extent; MW: Molecular weight; Same letters in the same row represent the statistical data significance ($p > 0.05$).

12.5% and 7.4% during the drying of the acidic one (Peak 1, Peak 2, Table 2). The most denatured sweet bovine whey protein was β-Lactoglobulin (variant A or B) with a total denaturation extent of 74% (Peaks 2 and 3, Table 2). As such, this protein was denatured to an extent of 14.8% for variant A (Peak 5, Table 2) and 42.6% for variant B (Peak 4, Table 2) in acidic bovine whey, respectively.

On the other hand, the analysis highlighted that the α-lactalbumin was the most denatured in acidic camel whey powders with a denaturation extent of 25.3%, respectively (Peak 1, Table 2). However, the peptidoglycan recognition protein (PGRP) and the camel milk serum albumin protein (CSA) presented the highest denaturation extent up to 54.2% and 56.4% for (Peak 2 and Peak 3, Table 2) in sweet camel whey, respectively. Lower denaturation extent was observed for these proteins in acidic camel whey (Peak 2, Peak 3, Table 2). These results are in agreement with Felfoul et al. (2017), which showed that CSA (one free thiol group) is the most heat sensitive protein in camel whey followed by PGRP and α-Lactalbumin.

In a co-current drying configuration (system used in this study), several authors stated that the temperature of the particles increases until reaching asymptotically the air outlet drying temperature (Schuck et al., 2008; Wu et al., 2014). At relatively low heat intensity, the denaturation of whey proteins was relatively low and mainly involved their initial free 'SH' groups. The free thiol function of β-Lactoglobulin (the main protein in bovine's whey) may explain its sensitivity to denaturation during drying (Roefs & De Kruif, 1994). Felfoul et al. (2015) indicated that the denaturation temperatures of β-Lactoglobulin was equal to 79.6 °C and 83.4 °C in sweet and acidic bovine whey, respectively, which were close to the outlet drying temperature in this study (80 °C). Recently, Lajnaf et al. (2018) demonstrated that acidic whey presented significantly lower free SH group after heating as compared to the sweet one. Therefore, both the denaturation temperature of β-Lactoglobulin (close to 80 °C) and the low free SH groups of bovine whey protein at acidic pH (=4.6) may explain its higher heat stability as compared to the neutral pH (=6.7) during drying.

Besides, results of this study highlighted the total absence of β-lactoglobulin in both acidic and sweet camel whey, whereas the α-Lactalbumin was their main major protein. It is important to note that according to the literature, the camel and bovine α-Lactalbumin have no free thiol 'SH' function (Lajnaf et al., 2017; Relkin & Mulvihill, 1996) and presented similar secondary structure (Atri et al., 2010). This explains the low denaturation extent of α-Lactalbumin during drying of both camel and bovine whey at neutral (=6.7) or acidic (=4.3 or 4.6) pH.

3.3. Surface elementary composition

The relative elemental composition of ACWP, ABWP, SCWP, and SBWP is presented in Table 3. Analysis of XPS survey of ACWP and ABWP indicated the existence of three peaks corresponding to Carbone 'C' (67.3 ± 3.4% and 65.5 ± 3.3%, $p > 0.05$), Azote 'N' (9.8 ± 0.5% and 9.1 ± 0.4%, $p > 0.05$), and Oxygen 'O' (21.3 ± 1.1% and 23.1 ± 1.1%, $p > 0.05$), respectively. The XPS analysis showed that SCWP and SBWP spectra are composed of C (68.9 ± 3.4% and 69.3 ± 3.5%, $p > 0.05$), N (8.5 ± 0.4% and 6.7 ± 0.5%, $p < 0.05$), and O (20.9 ± 1.1% and 22.6 ± 1.1, $p > 0.05$), respectively. These findings indicated that there were no statistical differences in Carbone and Oxygen amounts, while lower azote content ($p < 0.05$) was observed especially for camel whey powders at neutral pH (6.7). The calculated values for C/O were equal to 3.2 ± 0.2, 2.8 ± 0.2, 3.3 ± 0.2, and 3.1 ± 0.2 for ACWP, ABWP, SCWP, and SBWP, respectively (Table 3). The C/O ratio reflected the surface hydrophobicity of the studied powders. All whey powders displayed the same C/O ratio, indicating that their surfaces had the same hydrophobicity (Table 3), regardless the pH.

The deconvolution of C_{1s} spectra indicated the presence of four distinct sub-peaks (C—C, C—COOH, C—O, C═O and O—C═O) in all

Table 3
Complete elemental composition and surface compotion of camel and bovine milk whey powders.

	ACWP	ABWP	SCWP	SBWP
Elemental composition				
O_{1s} (%)	21.3 ± 1.1 [a]	23.1 ± 1.1 [a]	20.9 ± 1.1 [a]	22.6 ± 1.1 [a]
N_{1s} (%)	9.8 ± 0.5 [a]	9.1 ± 0.4 [a,b]	8.5 ± 0.4[b]	6.7 ± 0.5[c]
C_{1s} (%)	67.3 ± 3.4 [a]	65.5 ± 3.3 [a]	68.9 ± 3.4 [a]	69.3 ± 3.5 [a]
C−(C,H)	47.1 ± 2.4 [a,b]	46.1 ± 2.3 [a]	48.7 ± 2.4 [a,b]	51.4 ± 2.6[b]
C−O	33.6 ± 1.7 [a]	35.3 ± 1.7 [a]	33.4 ± 1.7 [a]	31.8 ± 1.6 [a]
C═O	13.9 ± 0.7 [a]	13.7 ± 0.7 [a]	13.2 ± 0.7 [a]	13.7 ± 0.7 [a]
O−C═O	5.4 ± 0.3 [a]	4.9 ± 0.2 [a,b]	4.7 ± 0.2[b]	3.1 ± 0.1[c]
Stoichiometry: C\O	3.2 ± 0.2 [a ,b]	2.8 ± 0.2[b]	3.3 ± 0.2 [a]	3.1 ± 0.2 [a]
Surface Composition (%)				
Proteins	65.5 ± 3.3 [a]	60.8 ± 3.0 [a,b]	56.6 ± 2.8[b]	44.8 ± 2.2[c]
Fats	21.8 ± 1.1 [a]	20.2 ± 1.0 [a]	29.4 ± 1.5[b]	34.5 ± 1.7[c]
Lactose	11.7 ± 0.6 [a]	17.6 ± 0.9[b]	12.7 ± 0.6 [a]	19.7 ± 1.0[b]
Ash	1.0 ± 0.2 [a,b]	1.4 ± 0.2 [a]	1.3 ± 0.1 [a]	0.9 ± 0.1[b]

Same letters in the same row represent the statistical data significance ($p > 0.05$).

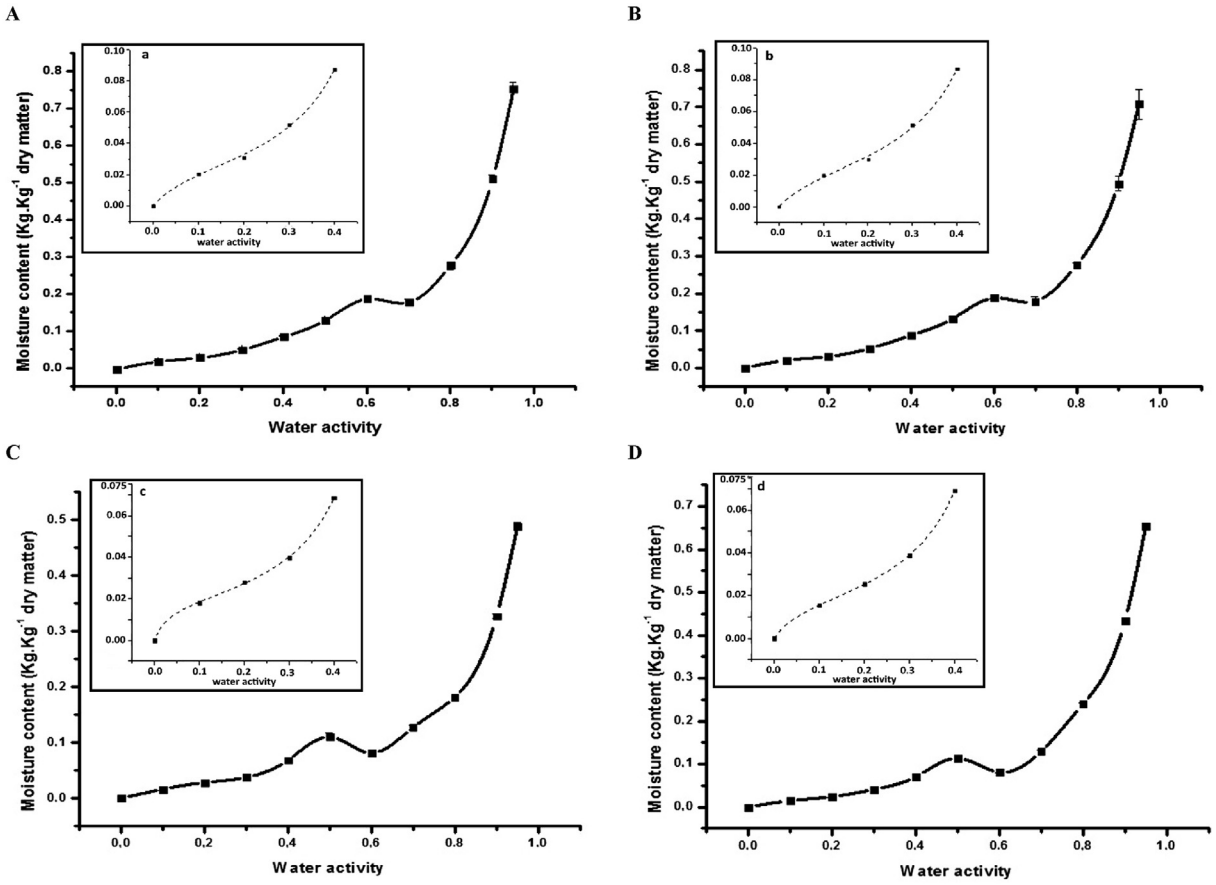

Fig. 1. Representative adsorption isotherms of ABWP (A), ACWP (B), SBWP (C) and SCWP (D) determined at 25 °C. The experimental data from 0 to 0.40 of water activity were adjusted to the GAB model ($R^2 > 0.98$): ABWP (a), ACWP (b), SBWP (c), and SCWP (d).

samples. These sub-peaks corresponded to the different function of the macromolecules (proteins, fats, and lactose). Analysis of the surface composition showed that acidic whey protein powders of both camel and bovine milks statistically had the same composition regarding fats, proteins, and minerals ($p > 0.05$, Table 3). However, a significantly low surface lactose content was measured for ACWP as compared to ABWP ($p < 0.05$, Table 3). On the other hand, our findings highlighted that the surface of SCWP has a significantly higher protein and mineral content than SBWP's ($p < 0.05$, Table 3). The latter had higher lactose and fat content than SCWP's ($p < 0.05$, Table 3).

It was acknowledged that during drying, adsorption mechanisms at the water-air interface are undergone by proteins (interfacial properties) as the water is evaporated (Fäldt & Bergenståhl, 1994). The difference in the interfacial properties of whey proteins of both camel and bovine milks can be related to the difference in protein composition as well as the modification of their flexibility as function of pH. Suttiprasit, Krisdhasima, and McGuire (1992) proved that at neutral pH, the α-Lactalbumin is more efficient to reduce the surface tension than the β-Lactoglobulin, as it shows smaller size and more flexible molecular structure. Lajnaf et al. (2018) reported that the α-Lactalbumin dominates the air water interface of acidic bovine whey, whereas β-Lactoglobulin covers rapidly the interface at neutral pH. At acidic pH, camel

and bovine whey proteins (especially α-lactalbumin) have higher interfacial properties than neutral pH due to the reduction of negative charges and electrostatic repulsions (Marinova et al., 2009). In bovine's whey, β-Lactoglobulin and α-Lactalbumin act in synergy at the water/air interface with a predominance of α-Lactalbumin due to its high flexibility at acid pH (Marinova et al., 2009). This suggests that the surface of ABWP is covered by the α-Lactalbumin, whereas this protein coexisted with the residual β-Lactoglobulin (highly denatured, Table 2) in SBWP.

Either at neutral or acidic, the interface water/air in camel whey is suggested to be mainly covered by the α-Lactalbumin in the absence of β-lactoglobulin (Lajnaf et al., 2017). The efficiency of the camel α-Lactalbumin to reduce the surface tension depends on both the pH value and the presence/absence of calcium fixation. Indeed, Lajnaf et al. (2017) indicated that at acidic pH (=4.3) a partially denaturation of α-Lactalbumin occurred with the release of its chelated calcium, which enhanced its interfacial properties. The release of bounded calcium by α-Lactalbumin at acidic pH (=4.3) enhanced its presence at the surface of the ACWP ($p < 0.05$, Table 3). Furthermore, it was reported that the absence of β-Lactoglobulin in this serum reduces the rigidity properties of the protein film created at the air water interface (Lajnaf et al., 2018). This could promote the presence of the α-Lactalbumin at the surface of sweet and acidic camel whey powders.

3.4. Water sorption isotherms

The water adsorption isotherms of SCWP, SBWP, ACWP, and ABWP are shown in Fig. 1. These isotherms exhibited typically sigmoid curves showing a breaking point, which corresponded to the total crystallization of amorphous lactose. Similar observations were reported in several other studies (Foster et al., 2005; Jouppila & Roos, 1994).

Our findings showed that, in both ACWP (Fig. 1A) and ABWP (Fig. 1B), lactose crystallized at 0.60 of water activity. Nevertheless, ABWP needed significantly higher water for total lactose crystallization (X_c) than ACWP's (19.0 ± 0.1 g 100 g^{-1} against 17.4 ± 0.2 g 100 g^{-1}, $p < 0.05$, Table 4). The analysis of adsorption isotherms for SCWP (Fig. 1C) and SBWP (Fig. 1D) indicated that both powders presented the same water activity (0.50, $p > 0.05$, Table 4) and the same amount of water to induce lactose crystallization (11.4 ± 0.2 and 11.1 ± 0.7, $p > 0.05$, Table 4).

In this study, SCWP and SBWP have the same a_w and X_c for lactose crystallization (Table 4). The main difference between SCWP and SBWP is the presence of β-lactoglobulin. At neutral pH, it was shown that the presence of β-lactoglobulin caused a_w increase of the total lactose crystallization (Ibach & Kind, 2007; Thomas, Scher, & Desobry, 2004). Indeed, non-covalent interactions between lactose and β-lactoglobulin will occur during drying and storage indicating the lactosylation phenomenon during the Maillard reaction. These interactions have stabilized whey protein powders against lactose crystallization (Ibach & Kind, 2007; Thomas et al., 2004). However, camel whey is free of β-lactoglobulin and has a high content of α-lactalbumin. In the absence of β-lactoglobulin, lactose should be less stable in sweet whey (Ibach & Kind, 2007; Thomas et al., 2004). This was not the case for sweet camel whey powder, which has the same water activity for lactose crystallization as for sweet bovine whey's powder. The explanation could be that proteins (mainly α-lactalbumin) dominated the surface of SCWP and encapsulated most of the amorphous lactose (Table 3). This could limit the water availability to induce lactose crystallization. Furthermore, it was demonstrated that the α-lactalbumin from camel milk was more hydrophobic than its bovine's milk counterpart (Atri et al., 2010; Lajnaf et al., 2017). This protein could constitute a hydrophobic barrier to water uptake and limits its contact with lactose. Hence, it caused the increase in lactose stability in sweet camel whey, even in the absence of β-Lactoglobulin. This hypothesis was checked by the analysis of the surface composition of SCWP. Indeed, as discussed in the Section 3.1, SCWP presented a protein content of 4.5 times higher that of lactose. However, this was only equal to 2.2 in SBWP. Thus, the presence of high protein content at the surface of SCWP could stabilize lactose against crystallization.

At acidic pH, lactose in ACWP (pH = 4.3) and ABWP (pH = 4.6) crystallized at the same water activity (a_w = 0.60) and with the same water quantity. Moreover, in acidic whey powders, lactose was more stable than in sweet ones (a_w = 0.50, $p < 0.05$, Table 4). The acidification process releases hydro-colloidal calcium (from casein micelles and from α-lactalbumin) and increases lactate content. The calcium and lactate contents were statically the same for ACWP and ABWP (Table 1) and were significantly higher than those of sweet ones. This could explain the increase in crystallization water activity (from 0.50 to 0.60, Table 4). Furthermore, as in the case of neutral pH, we assume that α-lactalbumin adsorbs at the water-air interface during drying and saturates the surface of acidic camel and bovine whey powder particles, enhancing the lactose stabilization against crystallization.

On the other hand, the obtained sorption isotherms were adjusted to the Guggenheim-Anderson-de Boer (GAB) model (up to 0.40 of a_w, Table 4, Fig. 1). In this study, ABWP (Fig. 1a) and ACWP (Fig. 1b) had statistically the same water monolayer content (X_m = 2.6 ± 0.1 g 100 g^{-1}, $p > 0.05$) and the same water binding energy (7.3 ± 1.1, 7.5 ± 0.5, $p > 0.05$, Table 4). This could be explained by their similar d_{50} diameter (Table 1). Likewise, SBWP (Fig. 1c) and SCWP (Fig. 1d) also showed similar water monolayer content (X_m = 2.3 ± 0.3 g 100 g^{-1} and 1.9 ± 0.1 g 100 g^{-1} g 100 g^{-1}, $p > 0.05$, Table 4). However, SCWP had a significantly lower water binding energy (6.0 ± 2.7) than SBWP (14.9 ± 1.1, $p < 0.05$, Table 4). This could be explained by the highest lactose content at the surface SBWP as compared to SCWP ($p < 0.05$, Table 2).

3.5. Glass transition temperature

The glass transition temperature (T_g) of acidic and sweet whey powders of camel and bovine's milk is presented in Table 5. The results indicated that the T_g of all studied whey powders decreased with increasing the a_w and the water content (Table 5). At 0.13 water activity, camel and bovine whey powders had the same T_g value ($p > 0.05$, Table 5), regardless the pH. At 0.23 and 0.33 of a_w, both camel and bovine whey powders' T_g was significantly lower at acidic pH ($p < 0.05$, Table 5), although they adsorbed the same water amounts as measured at neutral pH ($p > 0.05$, Table 5).

Actually, it was demonstrated that the glass transition temperature (T_g) of dairy powders was relatively dominated by the physicochemical properties of lactose (Shrestha et al., 2007). Fan and Roos (2017) indicated that the glass transition of pure lactose was equal to 65.0 ± 4.0 °C, 40.0 ± 2.0 °C, and 30.0 ± 3.0 °C at 0.13, 0.23, and 0.33 of water activity, respectively. Results of this current study highlighted that the measured T_g for sweet or acidic camel and bovine whey powders were mostly different from those of pure lactose (Table 5). Indeed, it has been reported that, while increasing water activity, lactose mobility is enhanced which induce the glass transition (Haque,

Table 4
Water adsorption properties of sweet and acidic camel and bovine milk whey powders derived from the obtained sorption isotherms at 25 °C.

	Total lactose crystallization		GAB model constants (up to 0.40 of a_w)	
	a_w	X_c	X_m	C
SCWP	0.50 ^{a}	11.4 ± 0.2 ^{a}	2.3 ± 0.3 ^{a,b}	6.0 ± 2.7 ^{a}
SBWP	0.50 ^{a}	11.1 ± 0.7 ^{a}	1.9 ± 0.1^{b}	14.9 ± 1.1^{b}
ACWP	0.60^{b}	19.0 ± 0.1^{b}	2.6 ± 0.1 ^{a}	7.3 ± 1.1 ^{a}
ABWP	0.60^{b}	17.4 ± 0.2^{c}	2.6 ± 0.1 ^{a}	7.5 ± 0.5 ^{a}

X_c: Total water of lactose crystallization (g 100 g^{-1}); X_m: The monolayer water capacity (g 100 g^{-1}); C: Constant related to the water binding energy. Same letter in the same column represent the statistical data significance ($p > 0.05$).

Table 5
Glass transition temperatures of sweet and acidic camel and bovine milk whey powders derived from the obtained MDSC curves.

	Water activity					
	0.13		0.23		0.33	
	Moisture*	T_g	Moisture*	T_g	Moisture*	T_g
SCWP	3.7 ± 0.4 [a]	58.3 ± 2.5 [a]	5.4 ± 0.9 [a]	47.6 ± 0.9 [a]	7.6 ± 0.9 [a,b]	29.7 ± 1.1 [a]
SBWP	3.6 ± 0.5 [a]	55.0 ± 2.4 [a]	5.0 ± 0.5 [a]	45.8 ± 2.1 [a]	6.5 ± 0.7 [a]	31.5 ± 0.8 [a]
ACWP	3.2 ± 0.3 [a]	56.5 ± 2.0 [a]	5.1 ± 0.7 [a]	41.2 ± 0.6[b]	7.9 ± 0.2[b]	24.6 ± 1.5[b]
ABWP	3.9 ± 0.4 [a]	55.0 ± 1.0 [a]	5.1 ± 0.6 [a]	41.5 ± 0.7[b]	6.6 ± 0.6 [a]	23.1 ± 0.9[b]

*: moisture content in g.100 g^{-1}; T_g: glass transition temperature (°C). Same letter in the same column represent the statistical data significance ($p > 0.05$).

Kawai, & Suzuki, 2006). This mobility depends on the presence of other components in the powdered dairy matrix such as whey proteins. Indeed, whey proteins interact with water thanks to their polar and ionizable residues, which are likely to produce hydrogen bonds (Foster et al., 2005; Hardy, Scher, & Banon, 2002; Jouppila & Roos, 1994). By competing for the available water, whey proteins are likely to change the equilibrium balance between the amorphous and crystalline state of lactose. Therefore, depending on their quantity, hydrophobicity, and pH, whey protein could affect the thermal properties of lactose, its structural strength (α-relaxations) (Fan, Mou, Nurhadi, & Roos, 2017; Silalai & Roos, 2010) and its mobility (Fan & Roos, 2016; Maidannyk & Roos, 2017). This may explain the observed differences in the glass transition temperatures of acidic or sweet camel and bovine whey powders as compared to pure lactose.

4. Conclusion

Whey proteins are important byproduct with highly valuable techno-functional properties. These proteins are often dried and used in food formulations in powders. The present study aimed at studying the effect of pH on the thermal denaturation extent, the surface composition, and the water sorption isotherms and the glass transition temperature of camel and bovine whey powders. Analysis of camel and bovine whey powders before and after drying indicated that decreasing the pH led to the stabilization of β-Lactoglobulin against thermal denaturation. The α-Lactalbumin in camel or bovine whey powder was relatively heat stable regardless the pH, thanks to the absence of initial SH group. Analysis of the surface chemical composition of camel and bovine whey powders showed that regardless the pH, the α-Lactalbumin predominated the surface. This was related to its flexible structure and its capacity to adsorb to the interface air/water during water evaporation. Besides, analysis of the sorption isotherms indicated that decreasing the pH induced the stabilization of lactose against crystallization. Finally, the pH did not influence the glass transition of both whey powders at 0.13 of water activity. However, at acidic pH, the glass transition temperature of both camel and bovine whey powders was significantly lower than that at neutral pH at 0.23 and 0.33 of water activity.

This study provides valuable knowledge for better understanding of the physical and biochemical properties of camel and bovine whey powders as affected by pH. It will also provide some assistance in the development of whey protein powders application in whey-based food products.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

The authors would like to thank Mr. Julien JARDIN (STLO-INRA, France) for helping in performing the proteomic analyses.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2020.127514.

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