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Usefulness of some Maillard reaction indicators for monitoring the heat damage of whey powder under conditions applicable to spray drying

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ABSTRACT

The Maillard reaction (MR) rate was observed according to available lysine loss, furosine, hydroxymethylfurfural (HMF), furfural, and brown colour during the heating of freeze-dried nano-filtered whey at 60, 75, and 90 °C and water activities of 0.11, 0.33, 0.43, and 0.73. The physical state of lactose was measured and associated with MR rate. The values obtained for available lysine, furosine, HMF and browning index ranged between, respectively, 11.3 and 1.63 (g 100 g^{-1} protein), 0.44 and 11.1 (g 100 g^{-1} protein), not detected and 57.7 (mg 100 g^{-1} protein) and 0.0104 and 0.1707. The greatest heat damage occurred with low moisture content and high temperature. The MR rate was influenced by the physical state of lactose, heating temperature and the moisture content of the whey. This occurred to a greater extent during the initial and intermediate stages of the MR as opposed to during the formation of coloured compounds.

1. Introduction

Whey powder is widely used in the food industry as an ingredient (Contreras-Calderón, Guerra-Hernández, & García-Villanova, 2008; Díaz, Pereira, & Cobos, 2004; Ibrahim, Babiker, Yousif, & Eltinay, 2005) because of the high nutritional value of its proteins (Parra, 2009). Whey powder is usually obtained through membrane filtration and subsequent spray drying (Tunick, 2008).

During the spray drying process, each droplet undergoes mass and energy transfer during its residence time in the drying chamber. The specific drying kinetics of each droplet lead to its final characteristics. When the droplets come into contact with hot air (160–220 °C) (Schmitz, Gianfrancesco, Kulozik, & Foerst, 2011a), they rapidly increase their temperature until they reach the wet bulb temperature, where rapid water evaporation occurs. Eventually a shell of solid material is formed on the surface of the droplet. This shell limits water diffusion from the interior to the surface, meaning that it is no longer possible to maintain the solid–gas interface of the droplet as it is saturated with water. As a result the droplet increases in temperature (Woo et al., 2008). However, at this point, the surrounding air is no longer at the inlet temperature, but rather close to the outlet temperature, which is usually between 60 and 90 °C (Schmitz-Schug, Kulozik, & Foerst, 2016). As a result the whey particle ends its drying at a temperature close to the air outlet temperature of the drying chamber (Schmitz, Gianfrancesco, Kulozik, & Foerst, 2011b). Depending on the distribution of residence times in the drying chamber, the whey particles can experience high temperatures for either a few seconds or up to several minutes (Jeantet, Ducept, Dolivet, Méjean, & Schuck, 2008; Kieviet & Kerkhof, 1995).

The nutritional quality of whey powder can be affected by the combination of the moisture content and temperature during the spray drying process due to the Maillard reaction (MR) (Contreras-Calderón, Guerra-Hernández, & García-Villanova, 2009). For instance, the high proportion of lactose and protein in sweet whey is a result of controlling this reaction (Parra, 2009).

MR occurs rapidly at water activities between 0.3 and 0.7 (Eskin, Ho, & Shahidi, 2013; Malec, Pereyra Gonzales, Naranjo, & Vigo, 2002; Schmitz-Schug, Kulozik, & Foerst, 2014), which correspond

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to a comparatively dry sample state (Eskin et al., 2013). If the lactose is not crystallised before the spray drying process, the whey will be more susceptible to a high MR rate. This is because lactose is more reactive when it is in an amorphous or rubbery state (Roos, 2002; Schmitz et al., 2011a). This implies that the whey particles are subjected to more severe conditions at the end of drying, when the moisture content is low and the lactose is more concentrated, and possibly in the amorphous or rubbery state, thus favouring the MR.

Several indicators have been proposed to evaluate heat damage in dairy products. Available lysine and furosine are indicators of the initial stages of the MR (Mehta & Deeth, 2016; Rufián-Henares, Delgado-Andrade, Jiménez-Pérez, & Morales, 2007). The loss of available lysine has been evaluated in infant formulas under storage conditions (Malec et al., 2002) and under conditions similar to those of a spray drying process (Schmitz et al., 2011b). Likewise, furosine has shown to be a good indicator of heat damage in dairy products (Rufian-Henares, García-Villanova, & Guerra-Hernández, 2004; Thao, Bhandari, Holland, & Deeth, 2011).

Alternatively, 5-hydroxymethyl-2-furfuraldehyde (HMF) and 2-furfuraldehyde (furfural) are indicators of the intermediate stages of the MR. They have been evaluated in several dairy products (Dattatreya & Rankin, 2006; Mesías-García, Guerra-Hernández, & García-Villanova, 2010). Similarly, the advanced stages of the MR can be monitored using the browning index (BI) (Ju-Woon et al., 2006). This is calculated by subtracting the absorbance measurement taken at 420 nm from that taken at 600 nm, and considering the colorimetric measurements taken using reflectance (colour parameters L*, a*, b* in the CIELab system) (Dattatreya & Rankin, 2006; Rufian-Henares et al., 2004).

Heat damage during spray drying has been evaluated in dairy products (Contreras-Calderón et al., 2009; Schmitz-Schug, Foerst, & Kulozik, 2013). However, few studies have evaluated simulations of heat damage in whey, which is produced via spray drying under conditions that favour the MR to a greater extent (Schmitz et al., 2011a; Schmitz-Schug et al., 2014). Additionally, no study has evaluated the different stages of the MR in a raw material such as whey under these conditions.

The combined effect of temperature and the moisture content, when maintained within the range of greater reactivity for the MR, is useful to estimate heat damage of whey during the spray drying process. Thus, the present study aimed to establish the usefulness of various indicators for monitoring the progress of the MR under conditions applicable to the spray drying of whey.

2. Materials and methods

2.1. Chemicals

All reagents were analytical grade. Acetonitrile (HPLC grade), methanol (HPLC grade), hydrochloric acid, glacial acetic acid, and chloramine T were obtained from Panreac (Barcelona, Spain). Sep-Pack (C_{18}) cartridges were obtained from Millipore Waters (Milford, MA, USA). DNP-lysine standard was purchased from Sigma. Furosine standard was purchased from PolyPeptide Laboratories (San Diego, USA). HMF and furfural standards were obtained from Merck (Darmstadt, Germany).

2.2. Sample preparation

Liquid sweet whey was supplied by a local cheese producer. Whey was centrifuged to remove fat and casein residues. Pasteurisation and nanofiltration were then used as concentration processes to produce lactose and protein, with salts also being partially removed. The pasteurised and nanofiltered whey (83.07% moisture, 0.95% ash, 1.87% protein, 13.14% lactose) was freeze-dried to obtain a powder (2.91% moisture, 5.62% ash, 11.07% protein, 77.78% lactose). This avoided additional heat damage. Portions of the freeze-dried whey were stored at 4 °C with saturated salt solutions (LiCl, $MgCl_2$, K_2CO_3, KI) for a period of 6 weeks. This was done to adjust water activity (a_w) of the salt solutions to 0.11, 0.33, 0.43, and 0.73, respectively. These a_w are within the range in which the MR is affected to a greater extent (Eskin et al., 2013). As the MR can occur even at room temperature (Nursten, 2005), moisture conditioning was performed at 4 °C to avoid non-enzymatic browning.

2.3. Heating experiments

The conditioned samples were packed into vacuum-sealed metal bags, forming a thin layer of the sample inside the bag. The packed samples were heated in a water bath at temperatures of 60, 75, and 90 °C for periods of 2, 5, 10, 20, 30, and 40 min. After heating, the samples were cooled down and stored in freezing conditions until analysis. Heating curves were developed by measuring the temperature at the centre of the packed sample. Data were measured using a LM35 sensor (Texas Instruments, 1999) and a microcontroller board (Arduino UNO), and recorded in a spreadsheet. Induction time, which describes the elapsed time before the working temperature is reached, was 90 s. The temperatures used in the heating experiments are similar to those usually experienced by the droplets in a spray drying process at the outlet of the chamber (Schmitz et al., 2011b). Even though the residence times of the particles are short in the spray drying chamber, it was necessary to use longer heating times to clearly observe the kinetics of the MR indicators. However, it should be noted that some industrial dryers can achieve fractioning of particles within the same time scale used in the present study (Jeantet et al., 2008).

2.4. Methods

2.4.1. Delay of crystallisation

Lactose crystallisation was determined by differential scanning calorimetry (DSC; Q100, TA Instruments, Eschborn, Germany) using the method described by Schmitz et al. (2011a). The samples previously conditioned at different a_w were transferred to aluminium pans, which were subsequently hermetically sealed. The samples were heated at 10 °C min^{-1}, starting at 20 °C until the working temperatures were reached (60, 75, and 90 °C) and kept at this temperature until the crystallisation peak was observed. Measurement was stopped if no crystallisation peak was observed after 60 min. The time at which fastest crystallisation occurred was taken as crystallisation time.

2.4.2. Available lysine determination

ε-NDP-lysine was determined by HPLC following the method described by Contreras-Calderón et al. (2009). A sample of whey powder containing approximately 4 mg of protein was transformed through derivatisation by adding 1-fluoro-2,4-dinitrobenzene (FDNB). Hydrolysis of FDNB derivative was conducted using HCl 7.95 N. Analysis was performed using HPLC Shimadzu equipment, which had a quaternary gradient pump (LC-20AD), a diode array detector (SPD-M20A) and an autosampler (SIL-20A HT). 20 μL of filtered solution was separated in a LiChospher column 100 RP-8 (5 μm) in LiChroCART 250-4, operating at room temperature. Duplicate analyses were carried out (n = 2).

2.4.3. Furosine determination

Furosine (ε-N-furoylmethyl-L-lysine) content was determined using the method described by Rufian-Henares et al. (2004) with some modifications. A sample of whey powder, containing approximately 8 mg of protein, was hydrolysed with 7.95 N HCl, then 50 μL

of the sample was analysed with the HPLC Shimadzu equipment (previously described) using a LiChospher column 100 RP-8 (5 μm) in LiChroCART 250-4. Duplicate analyses were carried out (n = 2).

2.4.4. HMF and furfural determination

Furanic compounds were determined using the method described by Contreras-Calderón et al. (2008) with some modifications. Approximately 0.75 g of the powdered sample was clarified with Carrez-I and Carrez-II solutions. The supernatant was passed through a filter with a pore size of 0.2 μm and 50 μL of the filtered sample was analysed in the HPLC Shimadzu equipment previously described. Duplicate analyses were carried out (n = 2).

2.4.5. Browning index

The absorbance of the filtrate, whose obtainment was previously described in section 2.4.4, was measured at 420 and 600 nm according to the method reported by Contreras-Calderón et al. (2016). BI represents the measurement of absorbance at 420 nm minus the measurement of absorbance at 600 nm (the measurement of turbidity in water). Duplicate analyses were carried out (n = 2).

2.4.6. Colour measurement

Colour was determined according to the method described by Contreras-Calderón et al. (2016) with some modifications. One gram of the powdered sample was diluted in a volumetric flask to produce a 10.0 mL solution. Additionally, the yellowness index (YI) was calculated as 142.86b*/L* (Francis & Clydesdale, 1975), whilst ΔE was calculated as $((L\text{-}L_0)^2 + (a\text{-}a_0)^2 + (b\text{-}b_0)^2)^{1/2}$. Within these calculations L_0, a_0, and b_0 refer to colour parameters of the sample at time zero. The measurements were made in triplicate.

2.4.7. Complementary analysis

Protein content was determined through estimation of the nitrogen content, applying the AOAC no. 920 105 Kjeldahl method (AOAC International, 1990). The percentage of lactose monohydrate was determined according to the method described in the standard FIL-28: 1964 (IDF, 1964). With regards to pH determination, 1.0 g of the sample was dissolved in 10.0 mL of distilled water. The pH was then determined using a pH meter inoLab® pH 7200, which had previously been calibrated with buffer solutions at 20 °C. Moisture content was determined using an infrared lamp (RADWAG MA 210.X2.IC.A) at 60 °C until weight changes of less than 1 mg were observed within a 120 s period. This followed the stipulations set out in the instrument's user manual. The a_w was determined at 25 °C (room temperature) using a previously-calibrated water activity measurement instrument (NOVASINA). Analyses were made in duplicate.

2.5. Statistical analysis

The effects of a_w, temperature and time over other chemical indicators were evaluated using multifactor ANOVA. Significant differences were established using the LSD test with confidence intervals of 95%. Correlations between variables were assessed according to Pearson's correlations with confidence being set at 95%. All statistical analyses were performed using Statgraphics Centurion XVI®. Left-censored data for HMF was assumed to be zero for the purpose of the statistical analysis.

Table 1
The moisture content of the whey after equilibration with saturated salts at 4 °C.[a]

Sample	Salt	a_w at 4 °C	a_w at 25 °C	Moisture content
1	LiCl	0.11	0.14	2.91 ± 0.11
2	Mg_2Cl	0.33	0.35	3.65 ± 0.06
3	K_2CO_3	0.43	0.45	6.74 ± 0.35
4	KI	0.73	0.71	9.74 ± 0.25

[a] Values (g 100 g of sample^{-1}) are reported as the mean ± standard deviation. Salts are those used to condition the sample of lyophilised whey; a_w at 4 °C is the theoretical a_w of the saturated salt solution at 4 °C, while a_w at 25 °C is the measured a_w of the samples at room temperature.

3. Results and discussion

3.1. Delay of crystallisation

Table 1 shows the moisture content and the a_w of the samples used in the heating experiments and provides the values recorded for a_w at 4 °C within saturated salt solutions (Largo, Cortés, & Ciro, 2014) and those recorded for a_w at 25 °C within whey samples after the conditioning period. The a_w can change during heating experiments due to temperature changes, water release during lactose crystallisation (Roos, 2009), and water production during the non-enzymatic browning reaction (Eskin et al., 2013). The present study seeks to establish the progress of non-enzymatic browning in samples with different moisture contents and temperatures, with this not being affected by water generation during the heating process.

According to Roos (2002), the glass transition temperatures (T_g) observed in milk solids correspond closely to those of pure lactose. Roos (2002) reported that values of T_g relative to water content could be used to estimate the T_g of conditioned samples. This was utilised in the present study since whey samples are also composed of milk solids. The data reported by Roos (2002) were fitted to the Gordon-Taylor equation to estimate the T_g of conditioned samples. The T_g was estimated at 60, 52, 24, and 3 °C for samples 1, 2, 3 and 4, respectively, using the moisture content of Table 1. The results obtained suggest that sample 4 crystallised during the conditioning period, which was performed at 4 °C. This hypothesis was reinforced by the results obtained from the crystallisation delay assays. The crystallisation peak was not reached in sample 4 (after 60 min at all temperatures), suggesting that the lactose in this sample was already crystalline. Schmitz et al. (2011b) conducted similar conditioning of an infant formula model system, using saturated salt solutions at 25 °C. They reported that the lactose crystallised during equilibration of the model system at a_w of 0.53 and 0.75.

Fig. 1a shows the crystallisation delay of lactose as a function of the difference between the isothermal heating temperature (T) and the T_g. The crystallisation delay was seen to be reduced when the difference between the two temperatures ($T-T_g$) was greater. Similar results have been reported in several studies (Ibach & Kind, 2007; Kedward, Macnaughtan, & Mitchell, 2000; Schmitz et al., 2011a). During heating at a constant temperature, lactose crystallisation occurs above a critical water content and at a rate defined by the corresponding $T-T_g$ (Ibach & Kind, 2007). Values pertaining to critical water content describe the glass transition of lactose which occurs at the storage temperature (Roos, 2009). Higher temperatures and moisture contents resulted in shorter crystallisation times as both variables increased the temperature difference ($T-T_g$). No crystallisation peak was found in sample 1 at 60 °C. However, this peak was observed in the same sample at higher temperatures. This may be because more time is required to observe the crystallisation peak, or because lactose does not crystallise under the examined conditions. On the other hand, the lactose possibly crystallised during heating in sample 3 at 90 °C. This is because the

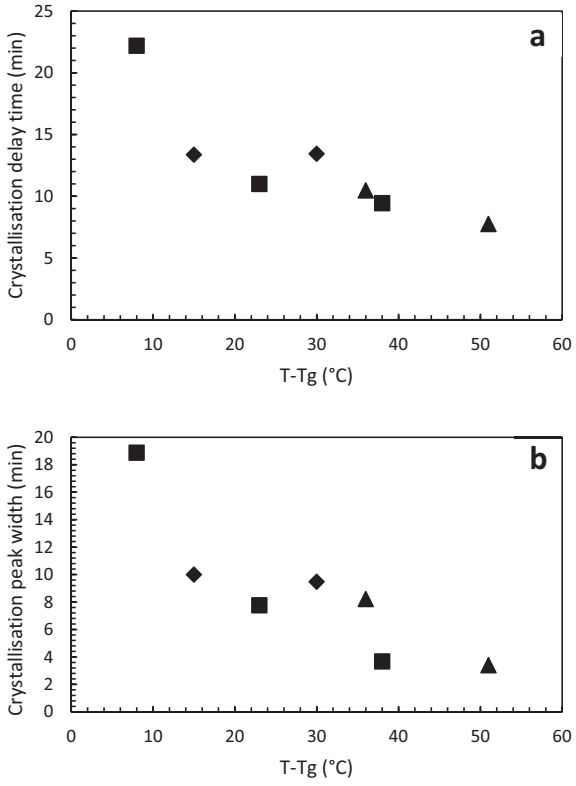

Fig. 1. Crystallisation delay (a) and crystallisation peak width (b) versus the temperature distance from glass transition temperature (◆, a_w = 0.11; ■, a_w = 0.33; ▲, a_w = 0.43).

crystallisation peak was not observed at this temperature but it was observed at lower temperatures in the same sample.

Fig. 1b presents the crystallisation peak width as a function of T-T_g. The crystallisation peak width refers to the time required from the beginning of nucleation and the initial growth of crystals, until all lactose is crystallised (Ibach & Kind, 2007). The lactose in the sample passes through multiple states during this period where it is gradually converted from a rubbery state to a crystalline state (Kedward et al., 2000). The crystallisation peak width was smaller the higher the temperature difference (T−T_g), exhibiting a similar behaviour to that of the crystallisation delay (Fig. 1b). The crystallisation peak width is reduced by increasing moisture content. This is due to the plasticisation effect of water which produces a decrease in the T_g of the sample and a consequent increase in the T−T_g difference. Likewise, a higher isothermal heating temperature (T) increases the T−T_g difference. The lactose state is an important factor in the present discussion since non-enzymatic reactions exhibit higher reaction rates when lactose is in its amorphous or rubbery state (Schmitz et al., 2011b).

3.2. Available lysine and furosine

Table 2 exhibits the available lysine content of the whey samples at different temperatures and times. Maximum lysine loss was 84%, this being seen in sample 1 after 40 min of heating at 90 °C. Lysine loss was higher at higher temperatures. The loss of available lysine was rapid at the beginning of the heating process but then decreased after some minutes of heating.

The greatest rate of lysine loss occurred when moisture content was at its lowest (Table 2). This is because the lactose remains in an amorphous or rubbery state for a longer time (higher crystallisation delay time). Schmitz et al. (2011a) found similar results when they

Table 2
Available lysine content during heating at different a_w and temperatures.[a]

Time (min)	Temperature		
	60 °C	75 °C	90 °C
Sample 1			
0	10.6 ± 0.47	10.6 ± 0.47	10.6 ± 0.47
2	11.0 ± 0.29	9.89 ± 0.22	7.15 ± 0.18
5	9.51 ± 0.05	8.22 ± 0.13	4.79 ± 0.26
10	9.88 ± 0.15	7.69 ± 0.16	3.63 ± 0.04
20	10.0 ± 0.45	7.18 ± 0.08	2.22 ± 0.03
30	8.94 ± 0.22	6.29 ± 0.17	1.76 ± 0.05
40	8.42 ± 0.28	5.68 ± 0.02	1.63 ± 0.01
Sample 2			
0	9.70 ± 0.33	9.70 ± 0.33	9.70 ± 0.33
2	8.82 ± 0.30	9.11 ± 0.54	7.91 ± 0.60
5	9.03 ± 0.08	8.46 ± 0.61	5.32 ± 0.32
10	9.30 ± 0.20	7.61 ± 0.01	4.08 ± 0.06
20	8.06 ± 0.34	6.68 ± 0.14	2.65 ± 0.10
30	8.47 ± 0.21	5.67 ± 0.04	2.43 ± 0.34
40	9.05 ± 0.81	5.05 ± 0.23	2.12 ± 0.01
Sample 3			
0	9.52 ± 0.33	9.52 ± 0.33	9.52 ± 0.33
2	9.13 ± 0.44	8.35 ± 0.45	7.90 ± 0.30
5	9.45 ± 0.38	7.98 ± 0.41	6.65 ± 0.16
10	8.74 ± 0.52	8.20 ± 0.16	4.99 ± 0.32
20	7.92 ± 0.29	7.52 ± 0.06	4.03 ± 0.39
30	8.40 ± 0.47	6.29 ± 0.74	2.91 ± 0.26
40	7.66 ± 0.37	6.12 ± 0.08	2.32 ± 0.10
Sample 4			
0	11.3 ± 0.57	11.3 ± 0.57	11.3 ± 0.57
2	10.6 ± 0.06	11.2 ± 0.26	8.74 ± 0.65
5	11.2 ± 0.42	10.7 ± 0.23	7.64 ± 0.30
10	11.9 ± 0.18	10.8 ± 0.19	6.50 ± 0.16
20	10.8 ± 0.46	7.85 ± 0.05	4.51 ± 0.23
30	10.9 ± 0.41	6.78 ± 0.41	3.54 ± 0.10
40	10.2 ± 0.19	6.99 ± 0.63	3.03 ± 0.14

[a] Values (g 100 g of protein^{-1}) are reported as mean ± standard deviation.

evaluated lysine loss within an infant formula model system under similar a_w and temperature conditions. Likewise, Malec et al. (2002) studied the influence of a_w and temperature on lysine availability in a milk-like system. They found that lysine loss was greater at higher temperatures. They also found maximum lysine loss to occur at intermediate a_w (0.52) when temperature was set in the range 37−50 °C. The present study examined temperatures that ranged from 60 to 90 °C and found maximum lysine loss to occur at a lower a_w (0.11−0.33). This is explained by the fact that at high temperatures a lower a_w allows lactose to be in a rubbery state for a longer period of time, what favours the MR. The reaction rate decreased at high a_w possibly due to the fast transition of lactose from the amorphous to crystalline state. Crystallised lactose is less available for reactions with proteins (Schmitz et al., 2011b).

Furosine content ranged between 0.44 and 11.1 g 100 g^{-1} of protein. Maximum furosine content was observed after heating sample 1 for 40 min at 90 °C (Table 3). High temperatures increased the furosine formation rate. Furosine content increased during heating at 75 and 90 °C. However, no clear trend was observed at 60 °C.

Furosine formation occurred faster when a_w was lower, at all temperatures evaluated. However, this trend became clearer at higher temperatures (Table 3). The reaction rate of furosine formation was slower when a_w was higher. Two possible explanations are that the lactose was crystallised faster, or that it was already crystalline at the beginning of the heating process. The effect of a_w on furosine formation was similar to that observed in the available lysine loss.

Thomsen, Lauridsen, Skibsted, and Risbo (2005) studied the effect of temperature (37, 45, and 55 °C) on the MR in whole milk powder (a_w 0.23 at 25 °C). They found that furosine development was not affected by lactose crystallisation. This is contrary to the

Table 3
Furosine content during heating at different a_w and temperatures.[a]

Time (min)	Temperature		
	60 °C	75 °C	90 °C
Sample 1			
0	1.26 ± 0.61	1.26 ± 0.61	1.26 ± 0.61
2	2.12 ± 0.12	2.01 ± 0.04	2.48 ± 0.21
5	1.44 ± 0.43	2.57 ± 0.02	5.92 ± 0.11
10	1.33 ± 0.56	3.48 ± 0.19	7.75 ± 0.08
20	0.69 ± 0.07	4.88 ± 0.15	11.0 ± 0.15
30	1.94 ± 0.30	5.02 ± 0.41	10.9 ± 0.20
40	2.10 ± 0.10	5.54 ± 0.62	11.1 ± 0.41
Sample 2			
0	2.17 ± 0.03	2.17 ± 0.03	2.17 ± 0.03
2	1.64 ± 0.08	1.26 ± 0.07	2.26 ± 0.32
5	1.01 ± 0.06	1.44 ± 0.03	3.72 ± 0.13
10	0.69 ± 0.05	2.99 ± 0.18	3.93 ± 0.18
20	0.67 ± 0.04	3.68 ± 0.41	4.68 ± 0.25
30	1.20 ± 0.11	4.16 ± 0.31	5.01 ± 0.14
40	2.16 ± 0.15	3.57 ± 0.21	5.25 ± 0.21
Sample 3			
0	1.55 ± 0.06	1.55 ± 0.06	1.55 ± 0.06
2	1.53 ± 0.27	1.26 ± 0.16	2.05 ± 0.40
5	1.59 ± 0.26	1.76 ± 0.07	2.27 ± 0.25
10	1.86 ± 0.20	1.53 ± 0.38	2.90 ± 0.20
20	2.09 ± 0.13	2.37 ± 0.04	4.32 ± 0.28
30	1.75 ± 0.25	2.70 ± 0.02	4.61 ± 0.26
40	2.04 ± 0.03	3.51 ± 0.26	5.52 ± 0.40
Sample 4			
0	0.44 ± 0.10	0.44 ± 0.10	0.44 ± 0.10
2	0.49 ± 0.11	0.72 ± 0.09	0.94 ± 0.24
5	0.33 ± 0.01	0.51 ± 0.04	1.18 ± 0.30
10	0.40 ± 0.06	0.72 ± 0.06	1.90 ± 0.30
20	0.40 ± 0.01	0.95 ± 0.03	2.95 ± 0.20
30	0.55 ± 0.01	1.63 ± 0.35	3.62 ± 0.25
40	0.68 ± 0.11	1.99 ± 0.04	3.99 ± 0.32

[a] Values (g 100 g of protein^{-1}) are reported as mean ± standard deviation.

Table 4
Hydroxymethylfurfural (HMF) content and browning index during heating at different times, a_w and temperature.[a]

Time (min)	HMF (mg 100 g of protein^{-1})			Browning index		
	60 °C	75 °C	90 °C	60 °C	75 °C	90 °C
Sample 1						
0	1.14 ± 0.04	1.14 ± 0.04	1.14 ± 0.04	0.0162	0.0162	0.0162
2	0.84 ± 0.06	1.81 ± 0.01	1.71 ± 0.04	0.0175	0.0186	0.0176
5	1.11 ± 0.18	1.68 ± 0.24	1.82 ± 0.04	0.0155	0.0187	0.0180
10	1.76 ± 0.23	1.82 ± 0.33	2.66 ± 0.16	0.0170	0.0210	0.0215
20	2.07 ± 0.13	1.89 ± 0.38	12.2 ± 0.62	0.0179	0.0224	0.0305
30	1.93 ± 0.06	2.02 ± 0.10	35.3 ± 1.17	0.0198	0.0245	0.0463
40	1.26 ± 0.49	1.86 ± 0.01	57.7 ± 0.04	0.0194	0.0190	0.1177
Sample 2						
0	ND	ND	ND	0.0192	0.0192	0.0192
2	ND	0.98 ± 0.08	2.10 ± 0.09	0.0196	0.0191	0.0142
5	ND	1.45 ± 0.15	2.53 ± 0.29	0.0180	0.0189	0.0177
10	ND	1.45 ± 0.08	3.99 ± 0.13	0.0189	0.0225	0.0235
20	ND	1.78 ± 0.06	16.3 ± 0.34	0.0249	0.0182	0.0545
30	ND	1.88 ± 0.11	20.6 ± 3.08	0.0176	0.0207	0.1065
40	ND	1.98 ± 0.04	46.3 ± 3.56	0.0222	0.0242	0.1707
Sample 3						
0	ND	ND	ND	0.0105	0.0105	0.0105
2	ND	ND	ND	0.0104	0.0133	0.0164
5	ND	ND	ND	0.0108	0.0149	0.0166
10	ND	ND	0.53 ± 0.01	0.0110	0.0141	0.0214
20	ND	ND	3.37 ± 0.38	0.0127	0.0171	0.0369
30	ND	ND	13.0 ± 1.01	0.0144	0.0169	0.0650
40	ND	ND	26.5 ± 0.52	0.0155	0.0221	0.0961
Sample 4						
0	ND	ND	ND	0.0127	0.0127	0.0127
2	ND	ND	ND	0.0160	0.0109	0.0152
5	ND	ND	ND	0.0110	0.0183	0.0130
10	ND	ND	ND	0.0155	0.0148	0.0191
20	ND	ND	0.91 ± 0.12	0.0135	0.0150	0.0337
30	ND	ND	4.04 ± 0.43	0.0135	0.0160	0.0534
40	ND	ND	8.74 ± 0.37	0.0120	0.0209	0.0729

[a] Values are reported as mean ± standard deviation. ND: not detected.

findings in the present study, possibly due to the different temperature range used which directly impacts lactose crystallisation kinetics. The aforementioned authors reported that lactose crystallised gradually during storage (126 days). This implies that the lactose remained in a mixture that was somewhere between a rubbery and crystalline state for a long time. On the other hand, the lactose crystallised faster in the present study because of the higher temperatures used, or it was already crystalline before the heating experiments were initiated (sample 4).

Likewise, Cheng et al. (2017) evaluated the effect of temperature (25, 40, 55, and 70 °C) on furosine formation in an infant milk powder formula. These authors found that furosine content was higher at higher storage temperatures, which is in agreement with the results of the present study. The only exception was at 70 °C, where the furosine content initially increased and then rapidly decreased at the end of the storage period. This was possibly due to the lactose crystallising at this temperature, reducing the rate of furosine formation. The same outcome may well have occurred in the present study, if longer times would have been evaluated.

A significant ($P < 0.05$) correlation ($r = -0.8270$) was found between furosine and available lysine. Lysine blockage through aldol condensation and posterior Amadori rearrangement forms lactulosyllysine (Eskin et al., 2013). The correlation found can be explained considering that furosine is an indirect measurement of the lactosylated lysine linked to the protein (Thomsen et al., 2005).

3.3. Hydroxymethylfurfural and furfural

The HMF contents of the heating experiments are shown in Table 4. Maximum HMF content was 57.7 mg 100 g^{-1} of protein, and was found in sample 1 after 40 min of heating at 90 °C. HMF was promoted quicker at low a_w and also occurred faster at higher temperatures. The effect of temperature was more evident at low a_w. Furfural was not detected, possibly due to the degradation of lactose through the MR that favours the formation of HMF (Eskin et al., 2013). Other authors have previously found similar results, with furfural not being found in dairy products (Ferrer, Alegría, Courtois, & Farré, 2000; Gómez-Narváez, Medina-Pineda, & Contreras-Calderón, 2017). As furfural was therefore not a useful indicator, it was not included either in the statistical analyses or in the general discussion. HMF content exhibited the same behaviour as available lysine and furosine. This therefore implies that lactose crystallisation also affects the intermediate stages of the MR. Since HMF is formed via 1,2-enolisation from the Amadori compound (Eskin et al., 2013), a reduction in the reaction rate of lysine loss and furosine formation leads to a reduction in the HMF formation rate. Additionally, significant ($P < 0.05$) correlations were found for HMF with lysine ($r = -0.6327$) and furosine ($r = 0.7016$).

Dattatreya and Rankin (2006) evaluated the evolution of HMF in whey powder heated at different temperatures (40, 60, and 80 °C, $a_w = 0.18$), during a 72 h period. They found that HMF increased initially, before subsequently decreasing. In the present study, HMF increased steadily throughout the entire duration of the experiment. This may be because the time range used in the experiment was shorter relative to that used by Dattatreya and Rankin (2006). It was therefore not possible to observe the exact time interval at which HMF decreased. Dattatreya and Rankin (2006) also evaluated the effect of pH fluctuations and found that a lower pH value (4.3) favoured the formation of HMF in comparison with higher pH values (6.3). In the present study, pH was measured during heating (data not shown) and was shown to decrease slightly from 6.8 to 6.2.

Chávez-Servín, Castellote, and López-Sabater (2006) measured the evolution of HMF during conservation of infant formulas over a 12 month period, at 25 and 37 °C. No significant increase in HMF was found under the studied conditions. These results support the idea that an elevated temperature is required to form HMF in dairy products. This can be seen in Table 4, where an increase in HMF content is only observed at elevated temperatures.

3.4. Browning index

BI is a qualitative measurement of melanoidins concentration (Rufián-Henares, Guerra-Hernandez, & García-Villanova, 2006). Table 4 shows the development of BI during the heating experiments. BI ranged between 0.0105 and 0.1707. The maximum BI occurred at a_w 0.33, 90 °C and 40 min. BI was higher at greater temperatures (Fig. 2a). Moreover, higher values were exhibited at lower a_w (0.11–0.33), showing a similar behaviour to that of furosine and HMF, which are both precursors of the formation of melanoidins. BI was significantly ($P < 0.05$) correlated with available lysine ($r = -0.6668$), HMF ($r = 0.8778$), and furosine ($r = 0.5488$). The correlations found can be explained by considering the MR pathways. Brown colour pigments are formed during the final stages of the MR through a polymerisation process (Eskin et al., 2013). In this manner, a higher concentration of initial and intermediate compounds of the MR, such as furosine and HMF, leads to a higher formation of coloured compounds and, likewise, invokes lysine loss as a reactant.

Liu, Yang, Jin, Hsu, and Chen (2008) evaluated the effects of temperature (60, 75, and 90 °C) on the formation of melanoidins (absorbance at 420 nm) in a model system composed of galactose and glycine during heating (50 h). They found that BI development was greater at higher temperatures, with these findings being corroborated in the present study. De Block et al. (2003) observed that BI increased in milk powder when it was stored at 25 °C and a_w 0.65 for 77 days. In both studies, the authors required a long time to observe the development of brown colour. In the present study, heating times of up to 40 min were used when attempting to observe the development of BI. Non-significant changes in BI were observed at low temperatures and high a_w (Table 4). This shows that the MR takes place slowly towards its final stages.

3.5. Colour

The colour parameters L*, a*, and b* represent luminosity, with colours ranging from green (−) to red (+), and from blue (−) to yellow (+), respectively. A low L* value is usually associated with greater heat damage in dairy products (Dattatreya & Rankin, 2006). Moreover, a* and b* tend to increase during the heating of dairy products (Rufián-Henares et al., 2006). Fig. 2 exhibits the colour formation found in sample 1 using CIELab colour space measurements (Fig. 2b–d). Similar results were obtained for other samples. Coloured compounds tended to increase during heating experiments, with this being associated with the advanced stages of the MR (Eskin et al., 2013). An increase in temperature promoted greater colour formation. Fig. 3 shows the values of ΔL*, Δa*, Δb*, and ΔE, which were calculated as the difference between measurements at 40 and 0 min of heating.

Dattatreya, Etzel, and Rankin (2007) tracked colour development in sweet whey powder subjected to temperatures between 40 and 80 °C, using the parameter L*. They found parameter L* to be lower the higher the temperature. Likewise, Rufian-Henares et al. (2004) assessed colour development in a liquid model system of whey protein and lactose, during heating at temperatures between 100 and 140 °C. They found a higher decrease to the L* parameter at higher temperatures. They reported that parameter b* was the most sensitive parameter, while the parameter a* only showed significant changes at the highest temperature of the experiment. However, in the present study, a* and b* exhibited similar temperature dependence.

On the other hand, the effect of a_w on colour development was not so clear (Fig. 3). For instance, at lower a_w, ΔL* was significantly ($P < 0.05$) lower at 60 and 75 °C than at 90 °C. Nonetheless, the opposite occurred at higher a_w (Fig. 3b). Δa* was higher in samples 1 and 4 at all of the temperatures evaluated (Fig. 3b). Δb* was higher at 60 and 75 °C when at a higher a_w, but was correspondingly lower when a_w was lower (Fig. 3c). Thomsen et al. (2005) evaluated the development of colour in whole milk powder stored at different temperatures. They also monitored the changes in a_w during storage. They observed that a_w of the milk powder depended on the crystallisation of lactose during storage, since crystallisation released bound water. Likewise, the authors

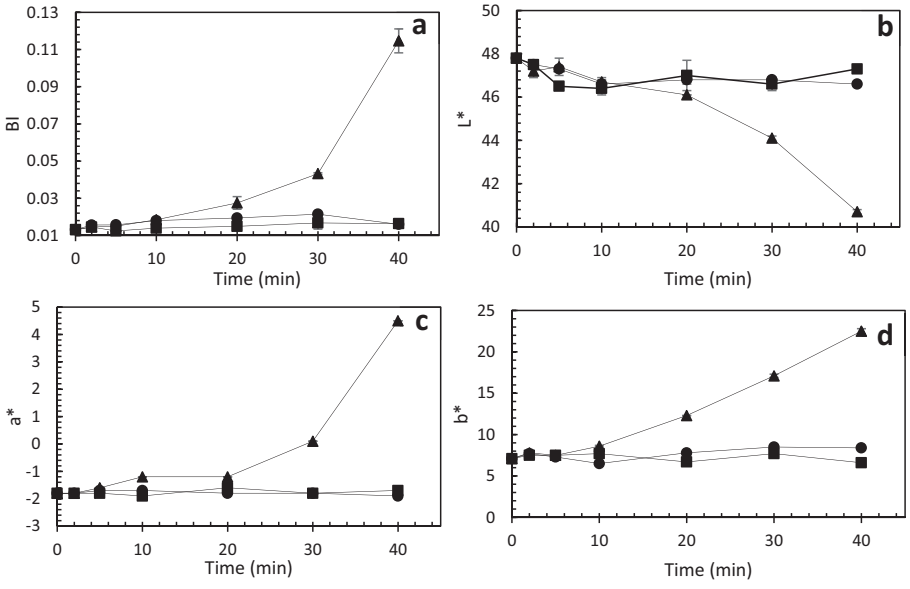

Fig. 2. Kinetics of colour formation at (■) 60 °C, (●) 75 °C and (▲) 90 °C for (a) browning index (BI), (b) L*, (c) a* and (d) b* in sample 1.

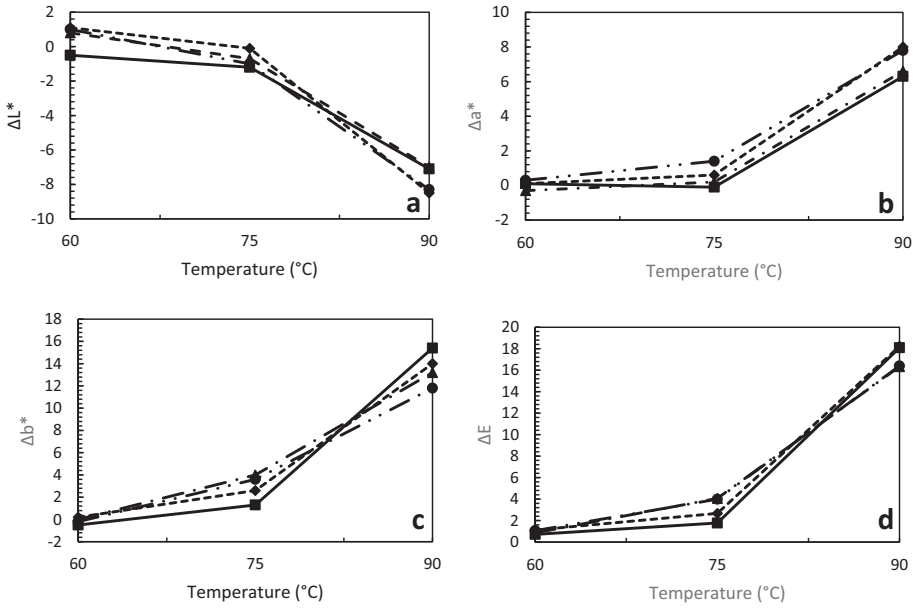

Fig. 3. Values of (a) ΔL*, (b) Δa*, (c) Δb* and (d) ΔE calculated as the difference between measurements at 40 and 0 min of heating at different temperatures (—■—, – ◆ –, – ▲ · ·, and —● –: Samples 1, 2, 3 and 4, respectively).

observed that browning simultaneously occurred with an increase in a_w, even at low temperatures. These results differ from those found in the present study. Thomsen et al. (2005) did not modify the water content of the samples, but they measured the changes produced during storage due to crystallisation. On the contrary, a_w was not measured during the heating experiments in the present study, but the water content was instead adjusted beforehand. Thus, it was expected to exhibit a differentiated type of behaviour with regards to colour formation in solutions with different moisture contents. However, no clear relationship was found between the moisture content and colour formation in the samples analysed (Fig. 3), despite the greater a_w range used in the present study (0.11–0.73), in comparison to that reported by Thomsen et al. (2005) (0.23–0.46). The results suggest that colour formation does not depend on the state of lactose in the samples analysed.

Table 5 shows the correlations between colour parameters and chemical indicators. All correlations were significant ($P < 0.05$). These correlations show that a higher value of L* is associated with a high available lysine content and a low value of furosine, HMF, and coloured compounds (BI). Likewise, high values for a* and b* parameters are related to low values for available lysine and high values for furosine, HMF, and coloured compounds. These results are in agreement with those previously reported by other authors (Dattatreya & Rankin, 2006; Rufian-Henares et al., 2004).

Table 5
Correlations found between indicators of non-enzymatic browning with colorimetric measurements.[a]

Parameter	Lysine	Furosine	HMF	BI
L*	0.7618	−0.6471	−0.7666	−0.8766
a*	−0.7563	0.5664	0.7684	0.9150
b*	−0.8090	0.6493	0.8072	0.8834
YI	−0.7969	0.6420	0.8261	0.9116
ΔE	−0.7783	0.6011	0.7999	0.9000

[a] Abbreviations are: HMF, hydroxymethylfurfural; BI, browning index. All correlations in the table are significant ($P < 0.05$).

Browning occurs during the advanced and final stages of the MR through polymerisation of initial and intermediate compounds (Eskin et al., 2013). The formation of colour compounds is characterised by a reduction in luminosity (L*), and the development of yellow and reddish tones. YI (data not shown) and ΔE (Fig. 3d) were calculated because they have been previously reported as good heat damage indicators in dairy products (Rufian-Henares et al., 2004). However, neither indicator exhibited better correlations (Table 5) than those seen with the original colour parameters (L*, a*, and b*). Thus, these parameters were not further analysed.

4. Conclusions

The physical state of lactose influenced the MR rate in the whey samples analysed. In turn, the physical state of lactose depended on temperature and moisture content. The MR occurred at a greater rate in lactate when it was in an amorphous or rubbery state. Greatest heat damage occurred at low a_w and high temperatures because lactose remained in an amorphous or rubbery state for a longer period of time. The results suggest that available lysine and furosine are sensitive indicators for tracking the MR in whey under conditions applicable to spray drying. However, more research is needed to confirm and validate these findings. HMF was not sensitive to high a_w and low temperatures which means it is only useful as an indicator in cases of severe heat treatment. BI and colour parameters were shown to significantly correlate with heat damage indicators despite them not being sensitive to low temperatures. The advanced stages of the MR could be monitored according to colour determination when samples are heated at a high temperature.

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Appendix A. Supplementary data

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