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To cite this version:

Marie-Hélène Famelart, Thomas Croguennec, Thomas Sevrin. Optimisation of microparticle formation by dry heating of whey proteins. Journal of Food Engineering, 2021, 291, pp.110221. 10.1016/j.jfoodeng.2020.110221 . hal-02905785

HAL Id: hal-02905785

https://hal.inrae.fr/hal-02905785v1

Submitted on 23 Jul 2020

Contents lists available at ScienceDirect

Journal of Food Engineering

journal homepage: http://www.elsevier.com/locate/jfoodeng

Optimisation of microparticle formation by dry heating of whey proteins

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ARTICLE INFO

Keywords:
Whey protein
Microparticle
Dry heating
Aggregation
Maillard reaction
Optimisation

ABSTRACT

A process of dry heating alkaline powder containing whey protein (WP) and lactose is used to produce microparticles that can trap large amounts of water and thus be used in many food applications as a thickening agent. The objective of this study was to optimise the yield of particles formed and the amount of water they trap as well as to minimise the heating duration. The parameters studied were the contents of WP and lactose, the pH, storage time at that pH, water activity (a_w) of the powder, and the temperature and duration of dry heating. The best parameters to reach the objective were WP at 100 g/kg with 0.5 g lactose/10 g protein, adjusted and stored at pH 10 for 3 h, and dry heating at 120 °C for ca. 30 min at $a_w = 0.33$. Approximately 70% of the WP converted into wet particles that contained 96% water. This result can help advance large-scale production of particles in a multi-stage spray-dryer that uses an in-line heating fluidised bed.

1. Introduction

Many studies have examined how to increase functional properties of whey proteins (WP) by applying heat treatments (El-Salam et al., 2009; Foegeding et al., 2002; Guyomarc'h et al., 2015; Nicolai, 2016). An increasing number of studies have considered effects of heating WP powders in a process called "dry heating" (DH) (Augustin and Udabage, 2007; O'Mahony et al., 2017; Schong and Famelart, 2017). DH at 100 °C for up to 36 h of a WP powder conditioned at pH 9.5 and with a water activity (a_w) close to 0.2 leads to crosslink formation between the WP inside the powder grains (Famelart et al., 2018; Schong and Famelart, 2018). As a result, WP becomes insoluble when the dry heated powder is dispersed in water and stable WP microparticles (WPM) are observed. These WPM have a size and shape similar to those of powder particles: When freeze-dried powder flakes or spray-dried powder grains are dry heated, angular flakes or spherical capsular hollow particles are observed after powder dispersion in water (Famelart et al., 2018; Schong and Famelart, 2018). Due to the porous structure of powders, WPM can trap large amounts of water (20–40 g water/g dry WPM), with a yield of formation of more than 0.5 g WPM/g powder (Schong and Famelart, 2018). Suspensions of spray-dried powder have been shown to have high viscosity after DH (Schong and Famelart, 2018); thus, this dry-heated powder could be used in many food applications as a thickening agent.

DH in the presence of a reducing sugar such as lactose (even in trace amounts) causes powders to brown and advanced glycation end-products to form via the Maillard reaction. The Maillard reaction is a cascade of reactions that begins with glycosylation of proteins, followed by complex rearrangements that lead to nitrogenous polymers responsible for browning (Ames, 1992; Hiller and Lorenzen, 2010; Martins et al., 2000). It also produces degradation products that can crosslink protein compounds to form large nitrogenous polymers (Gerrard, 2002; Hiller and Lorenzen, 2010; Miller et al., 2003; Wei et al., 2018; Zamora and Hidalgo, 2005). Increasing the lactose content of WP powder has been shown to increase the rate of WPM formation and the amount of WP converted into WPM, but also to decrease the amount of water that the WPM can trap (Schong and Famelart, 2019). Thus, producing functional WPM that traps water seems to be a compromise between a high conversion yield of WP into WPM and the potential to trap a large amount of water in the WPM. DH of WP at pH 3.5 and 6.5 also leads to powder browning, but without any WPM observed when the powder is dispersed in water (Famelart et al., 2018). The mechanism by which DH forms protein crosslinks remains under study.

As the utility of DH a WP powder in alkali conditions to produce functional microparticles has only recently been demonstrated (Schong and Famelart, 2018), this study is, to our knowledge, the first to focus on optimising the conditions for producing this functional powder. Its objective was thus to optimise the conditions for producing a functional dry-heated WP powder to be manufactured at an industrial scale with an in-line process. DH at 100 °C for 36 h is costly and incompatible with an in-line process. To shorten the DH duration, effects of several process

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https://doi.org/10.1016/j.jfoodeng.2020.110221
Received 9 March 2020; Received in revised form 16 June 2020; Accepted 19 June 2020

parameters were studied while attempting to increase the yield of particle production and maintaining their water-trapping potential. The parameters studied were the contents of WP and lactose, the pH, storage time at that pH, water activity (a_w) of the powder, and the temperature and duration of dry heating. As DH of either freeze or spray-dried powers has been shown to produce WPM with similar properties (Famelart et al., 2018; Schong and Famelart, 2018), powders in this study were produced by freeze-drying to assess many conditions that influence the composition of WP solutions before they are dried and to decrease the amount of WP used.

2. Materials and methods

2.1. Materials

The WP powder had a protein content of 888 ± 0.13 g/kg, as determined by the Kjeldahl method using 6.38 as the conversion factor for total nitrogen. Approximately 93.5% of this protein was WP and ca. 6.5% was casein (a mixture of αs1, αs2, β and κ caseins), as determined by polyacrylamide gel electrophoresis (Chevallier et al., 2018). One hundred g of this powder contained 6.31 ± 0.20 g moisture and 0.17, 0.02, 0.15 and 0.11 g Ca, Mg, Na and K, respectively, as measured by atomic absorption spectroscopy (Chevallier et al., 2018).

Nile blue, 37% hydrochloric acid (HCl), α-lactose monohydrate (4-*O*-β-D-galactopyranosyl-α-D-glucose) and sodium hydroxide were obtained from Sigma-Aldrich (Saint-Quentin-Fallavier, France). Saturated solutions of CH_3COOK (Sigma-Aldrich), $MgCl_2$ (Merck, Fontenay-sous-Bois, France) and $Mg(NO_3)_2$ (Merck) were used to adjust the a_w of powders to ca. 0.23, 0.30 and 0.52, respectively.

2.2. Methods

2.2.1. Preparation of WPM

The WP powder was dissolved for ca. 5 h at a concentration of 100–200 g/kg into water with lactose added at 0.2–1 g/10 g protein. Solutions were then adjusted to pH values between 7 and 10 using 5 to 1 M NaOH. The amount of lactose added was expressed by its R ratio (in g lactose/10 g protein) to maintain a constant ratio of moles of lactose per mole of protein as the protein concentration varied. Solutions were freeze-dried either immediately or after a time of storage (TS) at 4 °C for up to 16 h. Powders were then ground to obtain fine particles, first in a Lab Blender 400 (Stomacher, Humeau, La Chapelle-sur-Erdre, France) for 1 min and then in a coffee grinder (Prepline, SEB, Mayenne, France) for 40 s, and then adjusted to a final a_w by storing them in saturated salt solutions at 20 °C for 2–3 weeks. This duration was sufficient to reach equilibrium at the targeted a_w value, as verified by subsequent measurement. One batch of powder was prepared per condition (i.e. a combination of factor levels). Powders were then dry heated at 100 or 120 °C in sealed tubes (two per DH condition, i.e. per temperature and DH duration) for up to 12 h. To check for air leaks, the a_w of powders were measured after DH. As a_w increases during DH (Schong and Famelart, 2019), a decrease in a_w indicated a poorly sealed tube. In this case, the tube was removed from further analysis, and another tube was dry heated for analyses.

2.2.2. Experimental design

To identify optimal production conditions, the parameter space was explored in a series of 4 experiments:

Experiment 1: a WP solution at 150 g/kg with lactose added at R = 0.2 or 1 was adjusted to pH 9.5 and stored at 4 °C for 0, 3 or 16 h before freeze-drying. Powders were adjusted at $a_w = 0.23$ and dry heated at 100 °C for 0, 3 or 12 h.

Experiment 2: WP solutions at 100 or 200 g/kg and at R = 0.2 were adjusted to pH 7, 8.5 and 10 and stored at 4 °C for 0 or 3 h before freeze-drying. Powders were adjusted at $a_w = 0.23$ and dry heated for 0, 3 and 8 h.

Experiment 3: WP solutions at 100 or 200 g/kg at R = 0.2, 0.3 and 0.5 were adjusted to pH 10. They were stored at 4 °C for 3 h before drying, and powders were adjusted at $a_w = 0.23$ and dry heated for 0, 1.5 (only at 200 g/kg WP and R = 0.3 and 0.5), 3 and 8 h.

Experiment 4: a WP solution at 100 g/kg and R = 0.5 was adjusted to pH 10 and stored at 4 °C for 3 h before freeze-drying. Powders were crushed and adjusted at 3 a_w values (0.23, 0.33 or 0.52) and dry heated at 100 or 120 °C. The DH durations tested were 0, 2 and 4 h at 100 °C and 0, 0.5 and 1 h at 120 °C, as approximatively equivalent heat treatments.

2.2.3. Analyses

2.2.3.1. Powders. The a_w of powders were measured using an a_w meter at 25 °C after 15 min of equilibrium (Novasina aw-center RTD-200, Lachen, Switzerland). The intensity of the Maillard reaction was estimated by changes in the browning index (BI) of powders. Briefly, the BI was deduced from L*a*b* measurements using a CR-300 Chromameter (Minolta, Roissy, France) following the equation of Famelart et al. (2018). Three measurements were performed per tube.

2.2.3.2. Suspensions. Dry-heated powders were reconstituted in distilled water with 0.2 g/kg NaN_3 and stirred overnight at room temperature. The resulting powder suspensions had their pH adjusted to 6.50 ± 0.03 with HCl and their final concentration adjusted to 10 g powder/kg. Powder suspensions (ca. 2 g) were centrifuged at 10 000 g for 15 min to recover pellets that contained sedimented WPM. The mass of pellets was measured and converted into an equivalent per 100 g of suspension (i.e. per 1 g of powder), called the "wet yield" (WY). Pellets were then dried using a Savant Speed Vac Concentrator SVC-100H (Hicksville, New York, USA) at 45 °C for 13 h and weighed, and this mass was also converted into an equivalent per 100 g of suspension, called the "dry yield" (DY). DY and WY represented the conversion of powder into dry and wet WPM (in g/g of powder), respectively.

The water content (WC, in g water/g of dry WPM) in pellets was calculated on a dry basis as $(WY - DY)/DY$. WY, which included both the particles and the amount of water they trapped in 1 g of powder, was thus the most critical parameter for a thickening agent. DY, WY and WC were measured 4 times for each powder suspension. The WPM formed in suspensions were observed using an inverted confocal laser scanning microscope 30 μm above the lamella using Nile blue and a 20 × objective, following Schong and Famelart (2019). Four micrographs were taken per DH condition.

2.2.4. Statistics

The main effects and their interactions were assessed using analysis of variance (ANOVA) (tables of results not shown). For experiment 4, the ANOVA was performed using a single heat-treatment factor with 5 levels (no treatment, 100 °C short duration (2 h), 100 °C long duration (4 h), 120 °C short duration (0.5 h) and 120 °C long duration (1 h)), instead of using 2 factors (temperature and duration), as the DH durations at 100 °C differed from those at 120 °C. Only significant effects ($P < 0.05$) were selected in each final model, and when significant interactions were found, interaction plots (not shown) were examined to help understand their complex effects. Tukey's multiple comparison tests were used for paired comparisons of means, with differences considered significant at $P < 0.05$. Statistical analyses were performed using R studio, version 1.1.456 (The R Foundation for Statistical Computing).

3. Results and discussion

3.1. Experiment 1: effects of TS, DH duration and R at 150 g/kg WP

In experiment 1, with a WP solution at 150 g/kg and different levels of DH duration at 100 °C (0, 3 or 12 h), TS (0, 3 or 16 h) and R (0.2 or 1),

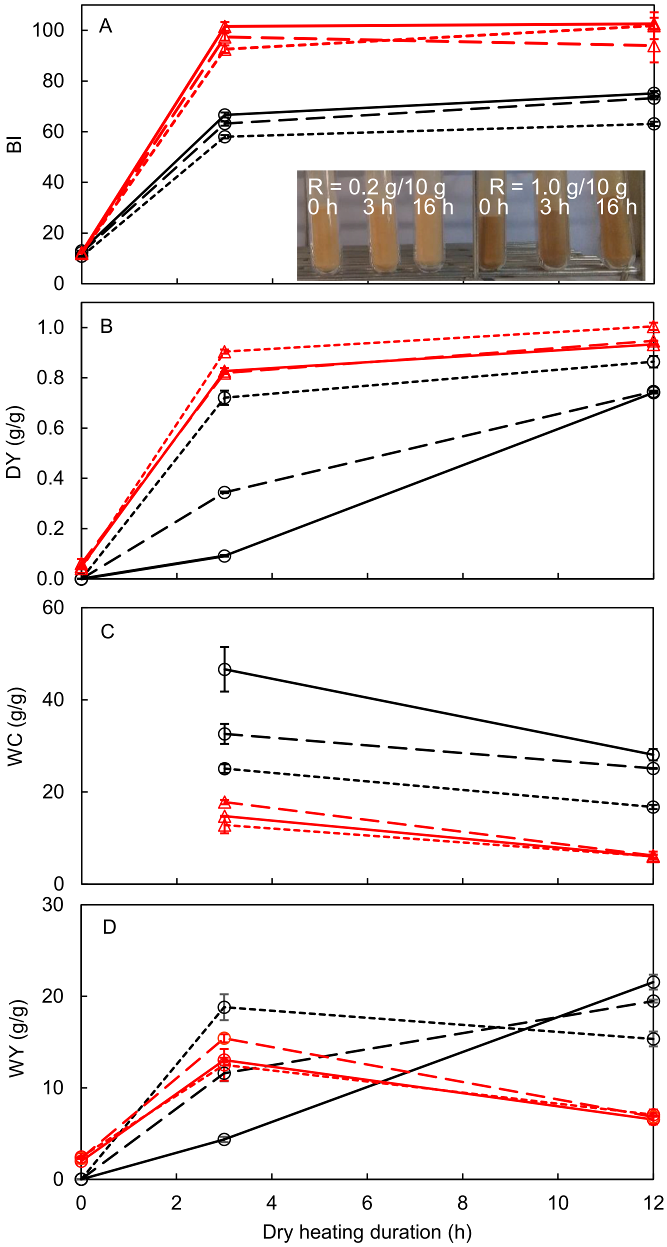


Fig. 1. Changes during dry heating (DH) at 100 °C of a whey protein (WP) powder in experiment 1. A: browning index (BI) of powder; B: dry yield (DY) of conversion of powder into microparticles; C: water content (WC) trapped in microparticles; D: wet yield (WY) of conversion of powder into microparticles. Powders were prepared by freeze-drying a 150 g/kg WP solution adjusted to pH 9.5 with lactose added at R = 0.2 g/10 g protein (◯ black) or 1.0 g/10 g protein (△ red) and stored at 4 °C for 0 h (solid), 3 h (dashed) or 16 h (dotted) before drying. Values are mean ± standard deviation. Plotted lines connect the measured points for readability. Inset: Photographs of powders at R = 0.2 g/10 g and 1.0 g/10 g after for 0, 3 and 16 h of storage and 12 h of DH. (To interpret references to colour in this figure, see the electronic version of this article).

browning of powders was almost complete after 3 h of DH and increased little from 3 to 12 h of DH (Fig. 1A). The kinetics of powder browning could level off before 3 h, but the present study did not examine shorter DH durations. Increasing R from R = 0.2 to 1 led to 40% increase of the BI after 12 h of DH (Fig. 1A and inset). This result is consistent with previous studies (Norwood et al., 2017; Schong and Famelart, 2019) and suggests that the lactose content of the powder is a limiting factor for the Maillard reaction to occur. However, high browning was also observed after the DH of WP powder at pH 3.5 or 6.5, but under these conditions, no WPM was observed after the dry heated powder was dispersed in water (Famelart et al., 2018).

DY increased as the DH duration increased (Fig. 1B). This result is consistent with previous reports (Famelart et al., 2018; Gulzar et al., 2012, 2011; Schong and Famelart, 2019, 2018). The rate of conversion was lower at R = 0.2 than at R = 1. At R = 1, the DY increased only slightly from 3 to 12 h of DH. These results are in accord with our previous study (Schong and Famelart, 2019) where DY increases as R increases from 0 to 0.8 and the reaction is complete after 3 h of DH at R = 0.8. One notable result is that extending the TS increased DY greatly at R = 0.2, so that DY at R = 0.2 and at the longest TS (16 h) was nearly as high as the DY at R = 1 without storage. As the influence of TS on the formation of microparticles has never been studied, its ability to increase DY raises questions. At pH from 7 to 9, β-lactoglobulin (β-Lg) or WP have been reported to denature, with exposure of carboxyl groups and hydrophobic residues, along with partial dissociation of β-Lg dimer (Mckenzie and Sawyer, 1967; Monahan et al., 1995; Pessen et al., 1985; Roels et al., 1971). Then, at pH 9–12, protein polymerisation has been reported (Mercadé-Prieto and Chen, 2005; Mleko, 2001; Monahan et al., 1995; Pessen et al., 1985; Roels et al., 1971) involving a decrease in sulfhydryl contents of proteins, probably due to their oxidation (Monahan et al., 1995; Roels et al., 1971). Denaturation and/or polymerisation of WP were likely involved in the increased DY after longer storage of the WP solution in alkaline conditions.

According to the ANOVA, all main factors and first-order interactions had a significant effect on DY ($P < 10^{-14}$), with mean DY differing significantly between R = 0.2 and 1, among the 3 DH durations and among the 3 TS ($P < 10^{-6}$). The interaction TS × DH duration reflected that increasing TS increased the DY of dry-heated powder, but not of unheated powder. The interaction TS × R reflected that increasing TS at the low lactose content (0.2) always increased the DY, whereas increasing TS from 0 to 3 h at the high lactose content (1) did not (Fig. 1B). In addition, increasing the lactose content when powders were dry heated for 3 or 12 h increased the DY but had no significant effect when powders were not dry heated.

As no pellet formed without DH, only the WC after DH were considered. The WC of WPM decreased from 3 to 12 h of DH, and from R = 0.2 to R = 1, a pattern opposite to that of DY: the higher the DY, the lower the WC, and vice-versa. This relationship, which was observed in a previous study (Schong and Famelart, 2019), was likely related to the increased number of protein crosslinks formed after prolonged DH and a higher lactose content. Dry-heated powder grains have been shown to swell when suspended in the water phase, probably due to residual negative charges on the WP at pH 6.5 (Schong and Famelart, 2018). The increasing number of crosslinks observed with increasing DY probably hindered this swelling and led to WPM with lower WC and higher density.

Under confocal laser scanning microscopy, no WPM were present without DH, but WPM appeared after DH for 3 h s angular flakes, as expected if the size and shape of the WPM were similar to those of the freeze-dried powder flakes usually obtained, in agreement with previous studies (Famelart et al., 2018; Schong and Famelart, 2019) (Fig. 2). Increasing the DH duration to 12 h seemed to yield smaller particles along with the larger ones (i.e. increased polydispersity). The sizes of particles also seemed more polydisperse at R = 1 than at R = 0.2. However, it was not possible to assess the size distribution of particles from the 4 micrographs obtained per condition, because of the few particles on them. We can discuss only the trends in particle size observed in micrographs. The size distribution of hydrated particles is determined by the initial size of powder grains, which results from the grinding of the freeze-dried powder and their ability to swell when placed in water (Famelart et al., 2018; Schong and Famelart, 2018). Prolonged DH produces more crosslinks, less ability of particles to swell and a decrease in the amount of water trapped in particles of higher density (Schong and Famelart, 2019). The fact that smaller particles appeared after longer DH duration could have been due to a decreased

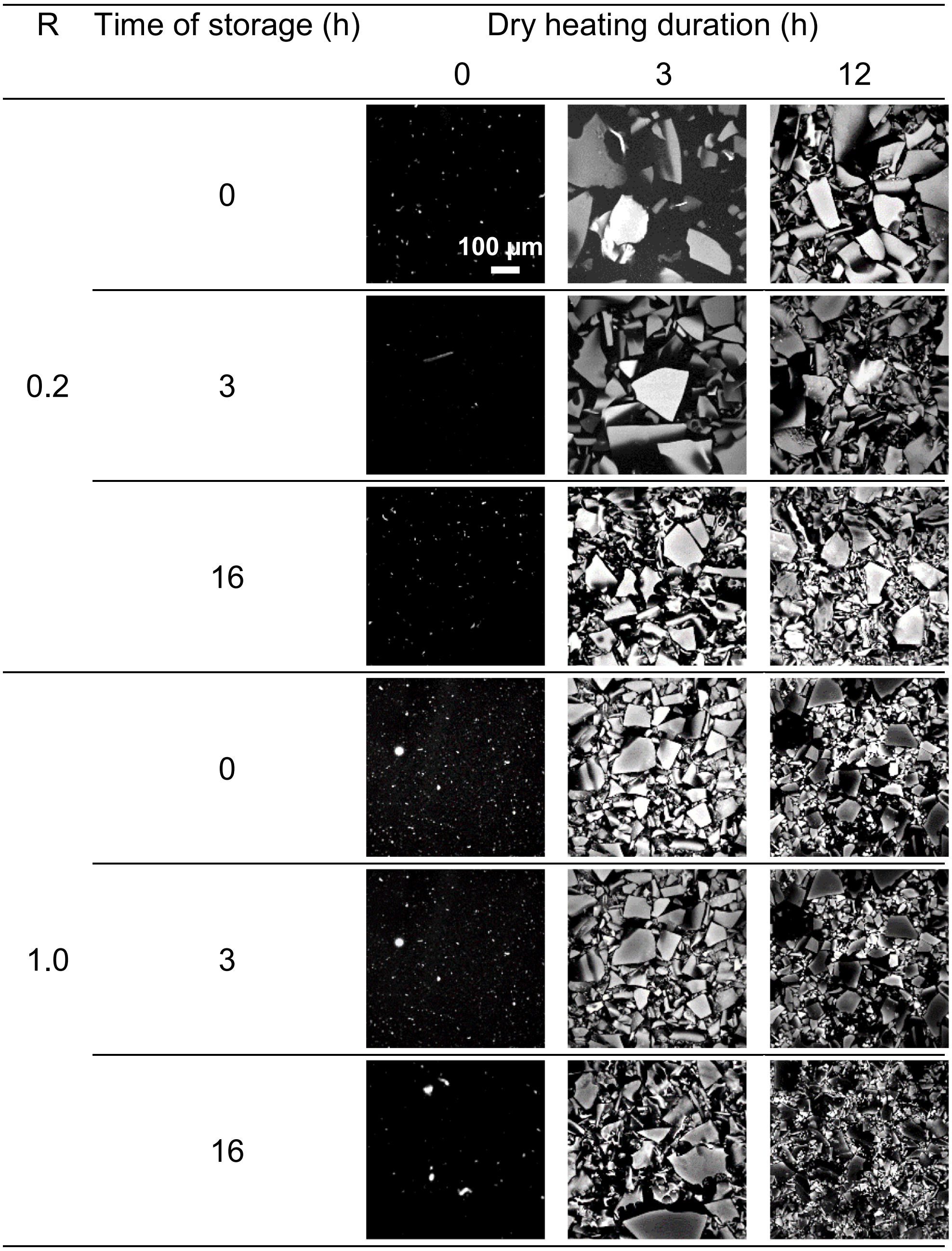


Fig. 2. Confocal images of dry-heated powder suspensions. Solutions of whey proteins contained lactose added at R = 0.2 or 1.0 g/10 g protein and were stored for 0, 3 or 16 h before freeze-drying, and powders were dry heated at 100 °C for 0, 3 or 12 h.

ability of particles to swell, but this could not explain the increased polydispersity of sizes (Fig. 2). Grinding freeze-dried powder produces grains with a wide size distribution (Famelart et al., 2018). The question arises why fewer small particles were visible after a short DH duration or at low lactose content. As the intensity of DH-induced crosslinking was likely influenced more by the local environment of protein residues in the grains than by their size, we doubt that only large grains would be crosslinked by a short DH duration or at low lactose content. The most probable hypothesis is that when the amount of crosslinking increased (i.e.at longer DH duration or higher lactose content), the amount of water trapped in particles decreased, thus increasing their density. This could have caused smaller particles to settle toward the focal plan located 30 µm from the bottom lamella, where larger particles of 100–200 µm were present. This possible sedimentation of smaller particles rendered interpretation more difficult, and micrographs likely allow one to draw conclusions only about the presence of particles.

The highest WY were obtained at R = 0.2, with TS = 0 or 3 h and 12 h of DH (21.6 ± 0.8 and 19.5 ± 0.2 g/g powder, respectively) and with TS = 16 h and 3 h of DH (18.8 ± 1.4 g/g; Fig. 1D). In other words, 1 g of powder yielded ca. 20 g of hydrated particles, of which ca. 96% of the mass was water. Thus, either short TS/long DH duration or long TS/short DH duration resulted in the highest WY. Adding lactose at R = 1 led to maximum WY after 3 h of DH of only 15.4 g/g and 13.0 g/g of powder at TS = 3 h and TS = 0 h, respectively.

In conclusion, lactose added at 1 g/10 g protein was likely too much to obtain high WY, but R can be optimised further between 0.2 and 1. A shorter DH duration (1–2 h) might have increased WY values at R = 1. Longer TS increased the rate of WPM formation, especially when the kinetics were slow, such as at the lowest value of R. Nonetheless, a TS as long as 16 h was ruled out because it would be too long to apply in

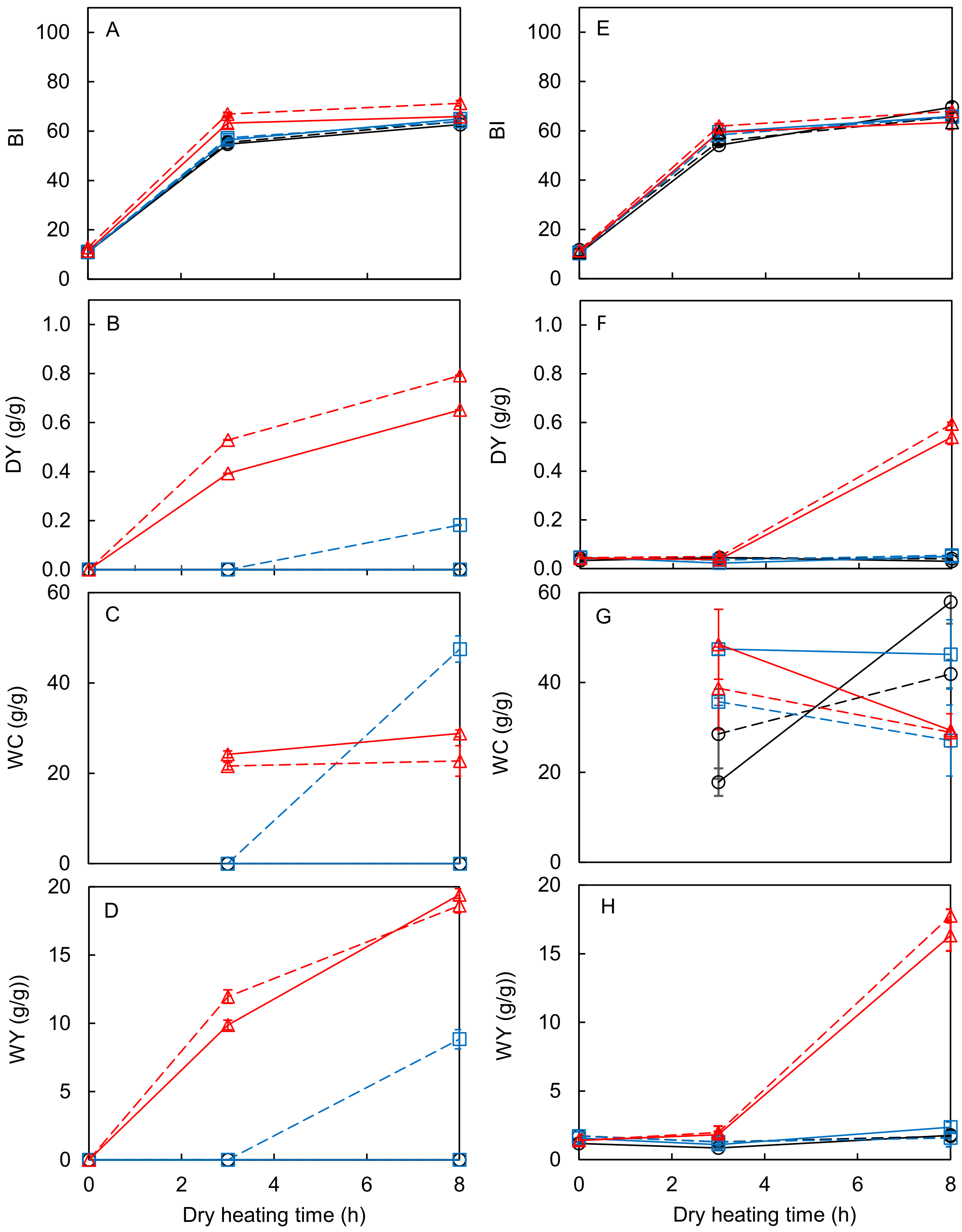


Fig. 3. Changes during dry heating at 100 °C of a whey protein (WP) powder in experiment 2. A and E: browning index (BI) of powder; B and F: dry yield (DY) of conversion of powder into microparticles; C and G: water content (WC) trapped in microparticles; D and H: wet yield (WY) of conversion of powder into microparticles. Powders were prepared by freeze-drying a WP solution at 100 g/kg (A–C) or 200 g/kg (D–F), with lactose added at R = 0.2 g/10 g protein, adjusted to pH 7 (◯ black), 8.5 (□ blue) or 10 (△ red) and stored at 4 °C for 0 h (solid) or 3 h (dashed) before drying. Values are mean ± standard deviation. Plotted lines connect the measured points for readability. (To interpret references to colour in this figure, see the electronic version of this article).

industrial processes. In the next experiment, the effect of TS (0 or 3 h) was tested with WP solution concentration and pH.

3.2. *Experiment 2: effects of WP concentration, TS and pH of the WP solution at R = 0.2*

In experiment 2, with R = 0.2 and different levels of WP solution concentration (100 or 200 g/kg), pH (7, 8.5 or 10), TS (0 or 3 h) and DH

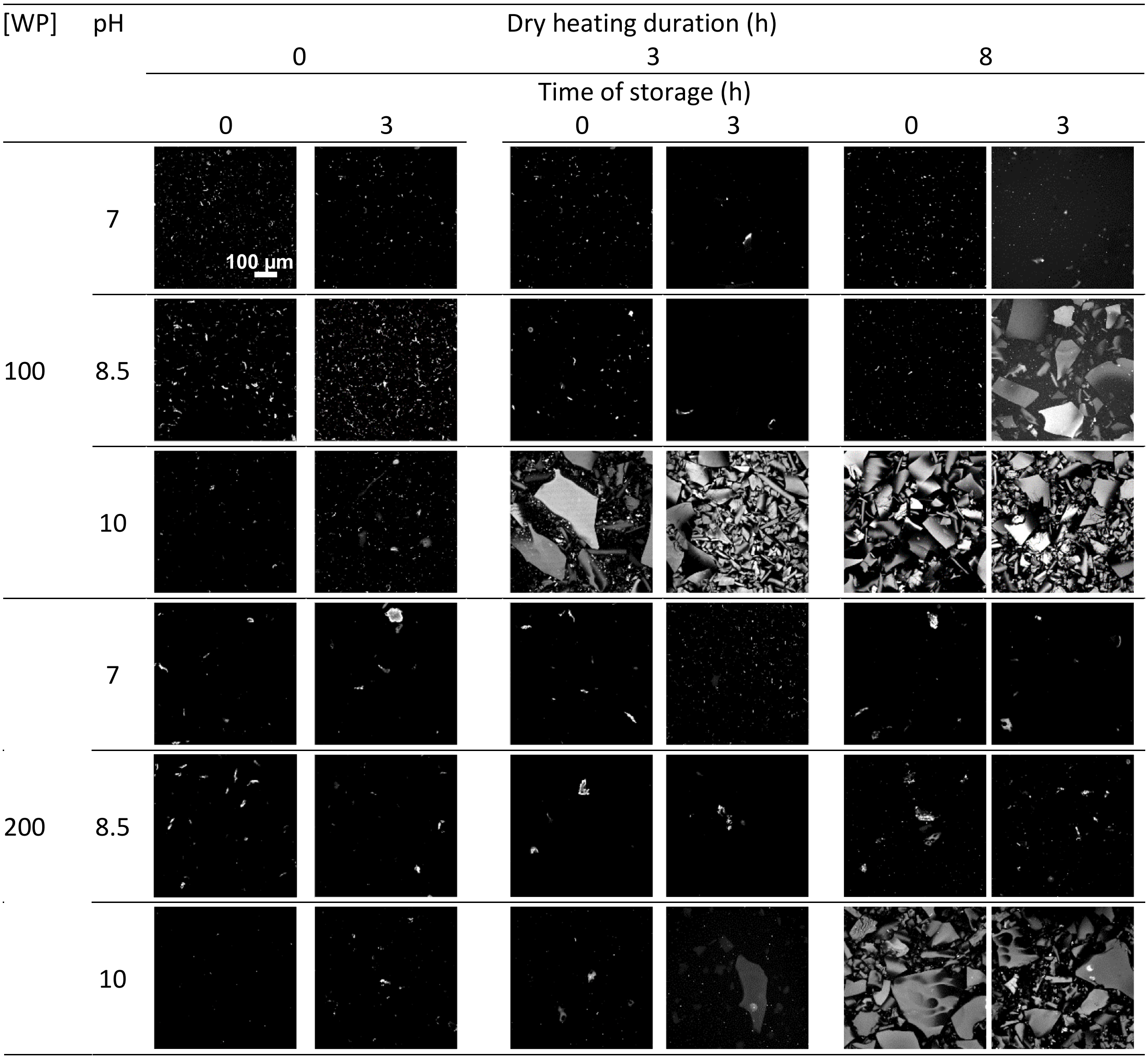


Fig. 4. Confocal micrographs of dry-heated powder suspensions. Solutions of whey proteins ([WP]) at 100 or 200 g/kg were adjusted to pH 7, 8.5 or 10, stored for 0 or 3 h before freeze-drying, and powders were dry heated at 100 °C for 0, 3 or 8 h.

duration (0, 3 or 8 h), browning of powders was also almost complete after 3 h of DH (Fig. 3). The mean effect of WP concentration on the BI was not significant regardless of the levels of the other factors, although at pH 10 the BI increased faster with WP at 100 g/kg than at 200 g/kg, as shown by a significant interaction between WP concentration and pH ($P < 10^{-15}$) (Fig. 3A and D). The pH had a significant positive effect on the BI ($P < 10^{-10}$), but only the BI at pH 10 was significantly higher than that at pH 7 or 8.5. As suspected, two interactions were significant: WP concentration × pH ($P < 10^{-15}$) and pH × DH duration ($P < 10^{-4}$). The latter interaction reflected that pH had an effect only after 3 h of DH, whereas it had hardly any effect after 8 h of DH and no effect at all at 0 h of DH.

DY were higher at 100 g/kg of WP than at 200 g/kg. At 100 g/kg and pH 7, no particles formed during DH (DY = 0). At pH 8.5, particles appeared only after a TS of 3 h and after 8 h of DH, with DY < 0.2 g/g and high WC (ca. 50 g/g), which resulted in a relatively low WY (ca. 8.9 g/g of powder). At pH 10, particles formed, with DY increasing as DH duration increased, to reach DY > 0.65 g/g and WC of ca. 25 g/g after 8 h of DH. This configuration resulted in maximum WY of 19.42 ± 0.44 and 18.62 ± 0.54 g/g of powder at TS = 0 h and 3 h, respectively. These results are likely to be related to the increase in β-Lg denaturation and polymerisation as pH increases (Monahan et al., 1995; Roels et al., 1971) and agree with the aggregation of WP by DH as pH increases (Gulzar et al., 2013, 2012; 2011; Liu et al., 1991; Zhou et al., 2008). The ANOVA of DY confirmed these results. The 2 main factors with a positive effect on DY were DH duration and pH ($P < 10^{-6}$). Protein concentration had no significant effect alone, but did in interaction with pH ($P < 10^{-5}$): increasing the pH at 100 g/kg increased the DY (especially from pH 8.5–10), whereas increasing it at 200 g/kg had less effect. There was also a significant interaction between pH and DH duration ($P < 0.0004$): increasing the DH duration at pH 7 and 8.5 increased the DY slightly from 3 to 8 h of DH, whereas it increased DY greatly from 0 to 8 h of DH at pH 10). This interaction reflects that particles were formed mainly by DH at pH 10 and not or only slightly at pH 7 or 8.5.

Fewer WPM seemed to form when the WP were solubilised and dried at 200 g/kg than at 100 g/kg: WPM formed at 200 g/kg but not at 100 g/kg at pH 10 and after 8 h of DH. The WC (Fig. 3G) was high and non-reproducible (i.e. with large standard deviations) after 3 h of DH for all samples and even after 8 h of DH at pH 7 and 8.5, because hardly any WPM were formed. For these samples, almost no pellets were collected,

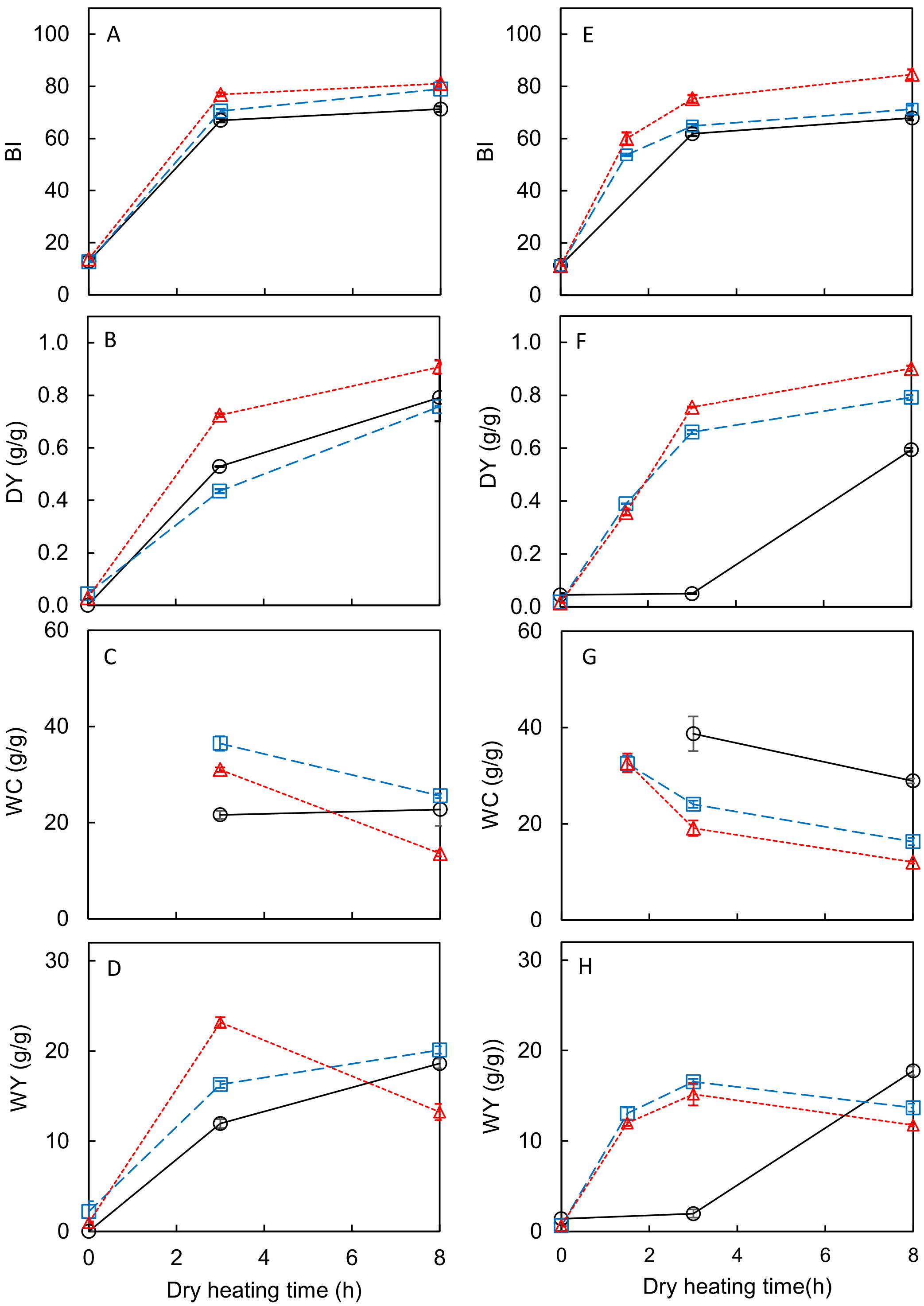


Fig. 5. Changes during dry heating at 100 °C of a whey protein (WP) powder in experiment 3. A and E: browning index (BI) of powder; B and F: dry yield (DY) of conversion of powder into microparticles; C and G: water content (WC) trapped in microparticles; D and H: wet yield (WY) of conversion of powder into microparticles. Powders were prepared by freeze-drying a WP solution at 100 g/kg (A–C) or 200 g/kg (D–F), with lactose added at R = 0.2 (black solid), 0.3 (blue dashed) or 0.5 (red dotted) g/10 protein, adjusted to pH 10 and stored at 4 °C for 3 h before drying. Values are mean ± standard deviation. Plotted lines connect the measured points for readability. (To interpret references to colour in this figure, see the electronic version of this article).

and their WC varied greatly depending on whether the tube had retained a drop of water above the pellet or not. Only WC after 8 h of DH at pH 10 can be discussed with confidence, as DY were larger. In conclusion, at 200 g/kg WP, particles formed only at pH 10 after 8 h of DH, with DY of ca. 0.56 g/g and WC of ca. 29 g/g, which resulted in WY of 16.3 ± 1.1 and 17.7 ± 0.5 g/g of powder at a TS of 0 and 3 h, respectively (i.e. slightly lower than those at 100 g/kg WP).

In addition, freeze-drying a WP solution at 200 g/kg with R = 0.2 caused a small amount of material to sediment without DH, regardless of the TS or pH, whereas this did not occur at 100 g/kg WP. At 200 g/kg

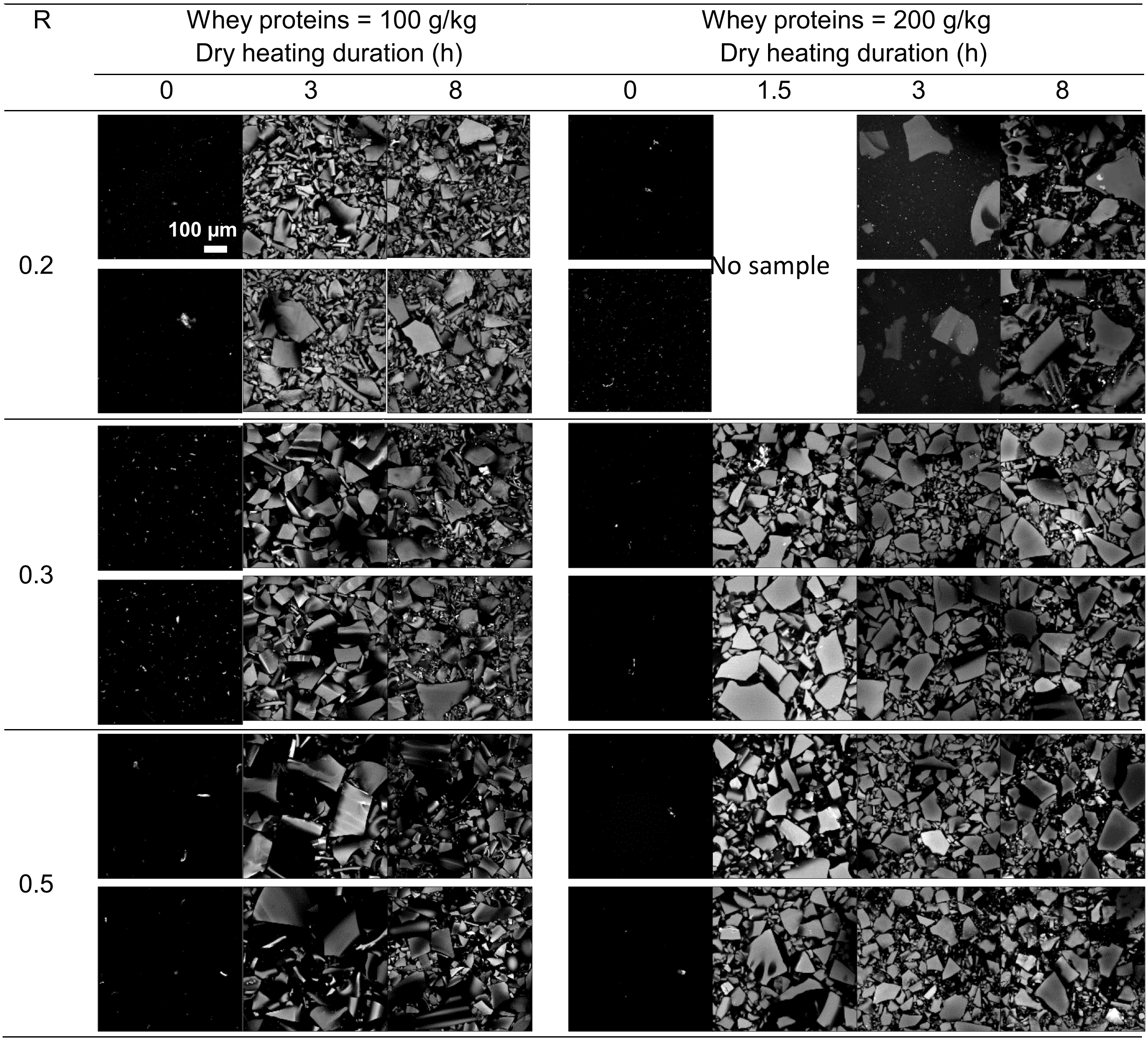


Fig. 6. Confocal micrographs of dry-heated powder suspensions. Solution of whey proteins at 100 or 200 g/kg with added lactose at R = 0.2, 0.3 or 0.5 g/10 protein were adjusted to pH 10, stored for 3 h before freeze-drying, and powders were dry heated at 100 °C for 0, 1.5, 3 or 8 h.

without DH, DY was ca. 0.04 ± 0.005 g/g. WP at 200 g/kg and pH 7–10 were likely able to interact and form some aggregated material that was recovered in the pellet.

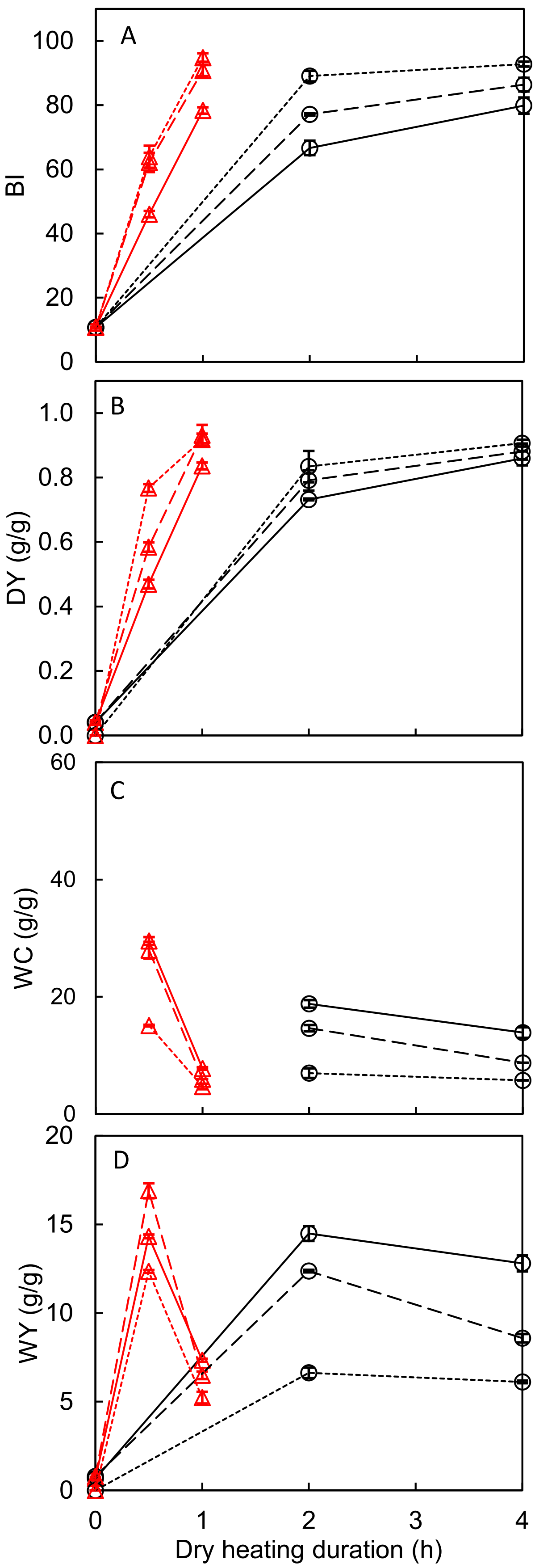
The confocal laser scanning microscopy (Fig. 4) confirmed that WPM formed at pH 10 after 3 or 8 h of DH (regardless of the TS) and at pH 8.5 after 8 h of DH and TS of 3 h at 100 g/kg WP in agreement with the DY values (Fig. 3B and E). When WPM were present, their mean size appeared more polydisperse with a TS of 3 h than one of 0 h. The presence of a small pellet without DH, as observed at 200 g/kg WP with a DY of 0.04 g/g (Fig. 3E), was not due to the presence of large particles such as WPM, but could have been due to the presence of polymers <1 µm in size recovered in the pellet but not visible under confocal laser scanning microscopy.

In conclusion, WPM formed with the highest WY at pH 10 after DH for 8 h (Fig. 3D and H). Mean WY were ca. 19.0 g/g at 100 g/kg WP and 16 g/g at 200 g/kg. In the next experiment, we tested the effect of different R values at these 2 WP concentrations at pH 10 and after a TS of 3 h.

3.3. *Experiment 3: effect of R at 100 and 200 g/kg WP*

In experiment 3, with pH 10 and TS = 3 h and different levels of WP concentration (100 or 200 g/kg), R (0.2, 0.3 or 0.5) and DH (0, 1.5 (only at 200 g/kg WP and R = 0.3 and 0.5), 3, or 8 h), we confirmed that the more lactose that was added, the lower the WP concentration, and the higher the BI after DH. Nevertheless, WP concentration had relatively little effect (Fig. 5). An increase in powder browning with a higher lactose content has been reported (Guyomarc'h et al., 2015; Norwood et al., 2017; Schong and Famelart, 2019). The BI increased much faster at the beginning of DH than for prolonged DH, as more than 66% of the total increase in the BI was reached within 1.5 h of DH. However, the BI continued to increase at longer DH durations, as shown in previous studies (Famelart et al., 2018; Liu and Zhong, 2014; Martinez-Alvarenga et al., 2014; Renn and Sathe, 1997; Schong and Famelart, 2018).

DY increased as the DH duration increased. An increase in the oligomerisation of WP dry-heated in the presence of lactose has been reported (Famelart et al., 2018; Gulzar et al., 2012, 2011; Hiller and Lorenzen, 2010; Schong and Famelart, 2019, 2018). According to the



(caption on next column)

Fig. 7. Changes during dry heating at 100 °C (◯ black) or 120 °C (△ red) of a whey protein (WP) powder at a water activity (a_w) = 0.23 (solid), 0.33 (dashed) or 0.52 (dotted) in experiment 4. A: browning index (BI) of powder; B: dry yield (DY) of conversion of powder into microparticles; C: water content (WC) trapped in microparticles; D: wet yield (WY) of conversion of powder into microparticles. Powders were prepared by freeze-drying a WP solution at 100 g/kg, with lactose added at 0.5 g/10 protein, adjusted to pH 10 and stored at 4 °C for 3 h before drying. Values are mean ± standard deviation. Plotted lines connect the measured points for readability. (To interpret references to colour in this figure, see the electronic version of this article).

ANOVA (excluding DH duration = 1.5 h), the 3 main factors (WP concentration, R and DH duration) influenced the DY significantly, less so for WP concentration ($P < 0.04$) but greatly for the 2 others ($P < 10^{-4}$), and 2 interactions were also significant: WP concentration × R ($P < 0.003$) and R × DH duration ($P < 10^{-4}$). DY differed significantly among the 3 DH durations (0, 3 and 8 h) ($P < 10^{-16}$) and increased as DH duration increased from 0 to 8 h. DY also differed significantly between R = 0.2 and 0.5 ($P < 10^{-3}$), between R = 0.3 and 0.5 ($P < 0.03$), but not between R = 0.2 and 0.3. The WP concentration × R interaction reflected that increasing WP concentration led to a large decrease in DY at R = 0.2, a small increase at R = 0.3 and no effect at R = 0.5. In comparison, the R × DH duration interaction reflected that increasing R led to no effect on DY after 0 h of DH, a strong increase after 3 h of DH and a smaller increase after 8 h of DH.

However, even though after 8 h of DH the DY still increased as R increased, WC tended to decrease as R increased. WY was highest for a WP solution at 100 g/kg conditioned at pH 10, stored for 3 h, with R = 0.5 after 3 h of DH (23.17 ± 0.57 g/g; Fig. 5D). The 3 h of DH needed for this highest WY was much shorter than the DH duration in previous studies (36 h at 100 °C) (Famelart et al., 2018; Schong and Famelart, 2018). DH duration had a positive effect on WY ($P < 10^{-15}$), but WP concentration had a negative effect on it ($P < 10^{-5}$). R also had a significant effect ($P < 0.03$), which was positive after 3 h of DH and negative after 8 h of DH. The interaction between DH duration and WP concentration also had a significant effect ($P < 10^{-3}$), as increasing the WP concentration after 3 h of DH decreased the WY more than after 8 h of DH, but in both cases, increasing the WP concentration led to a decrease in WY. The WY after 0 h of DH differed significantly from that after 3 or 8 h of DH ($P < 10^{-12}$), but WY after 3 and 8 h did not differ. Only WY at R = 0.2 and 0.3 differed significantly ($P < 0.03$).

The confocal laser scanning microscopy confirmed that WPM formed when the WP solution was adjusted to pH 10 after 3 h of DH, and even after 1.5 h (Fig. 6). Particle polydispersity again increased after longer DH duration, and WPM formed at 100 or 200 g/kg WP did not appear to differ.

In conclusion, the optimum condition for producing WPM that led to the highest WY (23.17 ± 0.57 g/g) was a WP solution at 100 g/kg, with lactose added at 0.5 g/10 g protein, adjusted to pH 10, stored at 4 °C for 3 h before drying, and subsequent DH of powder adjusted at $a_w = 0.23$ at 100 °C for 3 h. In the next experiment, parameters in the heating step were examined to determine whether they influenced the production of WPM.

3.4. *Experiment 4: effects of a_w and temperature of DH*

In experiment 4, with a WP solution of 100 g/kg, R = 0.5, pH 10 and TS = 3 h, different levels of a_w (0.23, 0.33 or 0.52), temperatures of DH (100 or 120 °C) and DH durations (0, 2 and 4 h at 100 °C and 0, 0.5 and 1 h at 120 °C) were studied. Increasing a_w from 0.23 to 0.52 and the temperature of DH from 100 to 120 °C increased the rate of browning and particle formation (Fig. 7), as observed in other studies (Dattatreya and Rankin, 2006; Martinez-Alvarenga et al., 2014; E. A. Norwood et al., 2016a, 2016b; E.A. Norwood et al., 2016a, 2016b; O'Mahony et al., 2017; Renn and Sathe, 1997). In contrast, Liu and Zhong (2014) observed that a high-temperature/short-duration treatment (130

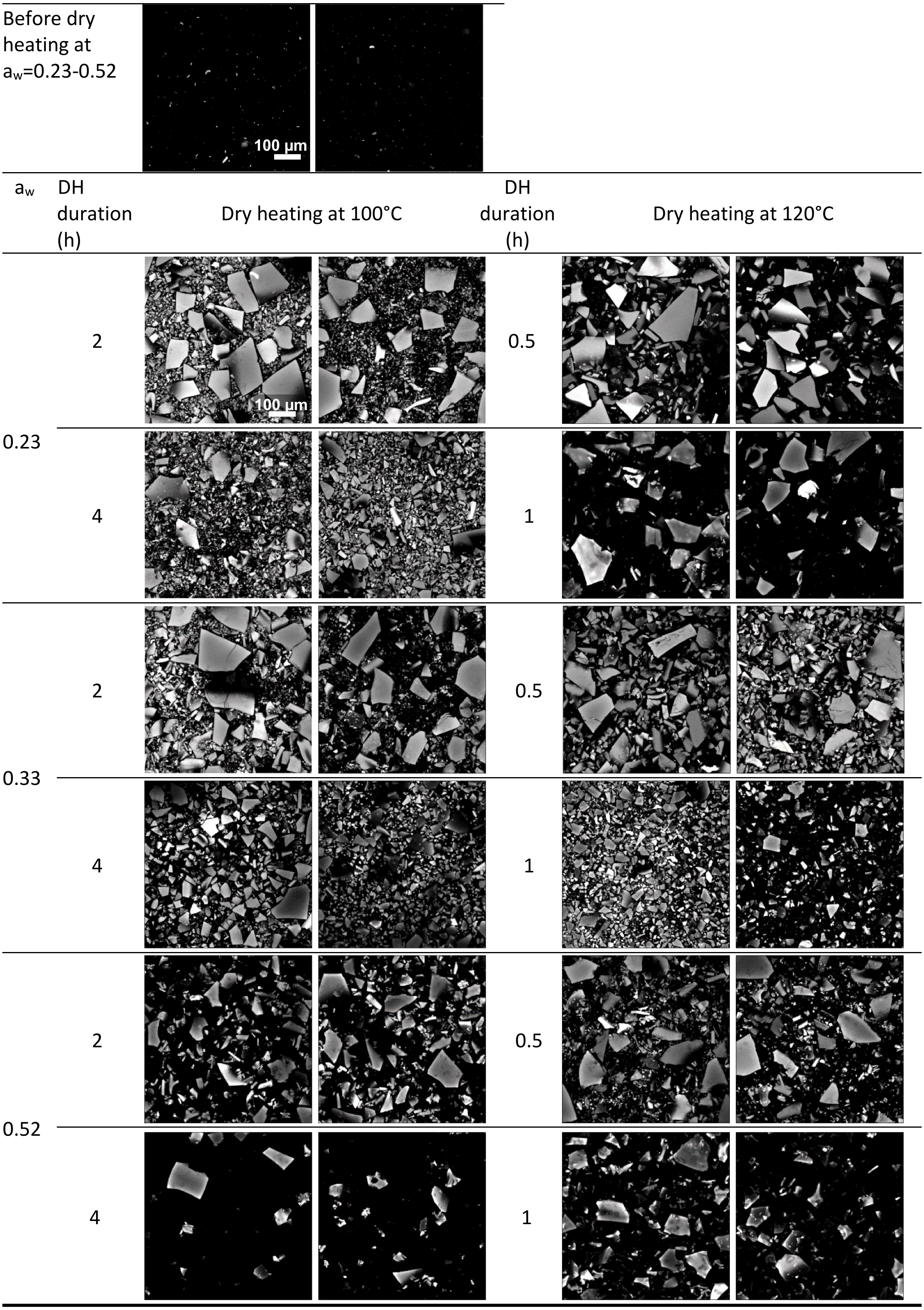


Fig. 8. Confocal micrographs of dry-heated powder suspensions. Solutions of whey proteins at 100 g/kg with lactose added at 0.5 g/10 g protein were adjusted to pH 10, stored for 3 h before spray-drying, and powders were conditioned at 3 a_w (0.23, 0.33 and 0.52) and then dry heated for 0, 2 or 4 h at 100 °C or for 0, 0.5 or 1 h at 120 °C. Micrographs at the top show the powder before dry heating (DH) (i.e. dry heated for 0 h), which appeared the same regardless of a_w.

°C/0.5 h) causes less colour formation than a low-temperature/long-duration treatment (60 °C/48 h). Increasing a_w to 0.6–0.8 is known to increase powder browning and aggregation of WP during DH, due to increased mobility of proteins (Zhou and Labuza, 2007), but higher a_w values reduce the Maillard reaction, because of a dilution effect (Ge Pan and Melton, 2007; Martinez-Alvarenga et al., 2014; Zhou et al., 2008) and because water inhibits this reaction (O'Mahony et al., 2017).

With temperature and DH duration merged into a heat-treatment factor, ANOVA results showed that the 2 factors and their interaction (a_w, heat treatment and the a_w × heat treatment) significantly influenced the BI. The BI after a long DH duration at 100 and 120 °C did not differ ($P > 0.2$). DH at 120 °C for 1 h resulted in the same BI as DH at 100 °C for 4 h, which may also mean that the maximum BI was reached in these two heat treatments because of limited lactose content. Also, increasing the a_w from 0.33 to 0.52 did not increase the BI at 120 °C significantly, regardless of the duration of the heat treatment ($P > 0.99$ for short heat treatment and $P > 0.22$ for long heat treatment), whereas doing so did increase the BI at 100 °C.

For the conversion of WP into dry WPM, ANOVA results were similar to those for the BI, with the 2 factors and their interaction also having significant effects. DY for long duration at 100 and 120 °C did not differ significantly ($P > 0.80$); thus, heating the powder for 1 h at 120 °C or for 4 h at 100 °C resulted in equivalent DY. Gulzar et al. (2012) reported that increased temperatures and a_w during DH produce more insoluble aggregates from WP, and that these 2 factors interact. In the current study, increasing a_w influenced DY more with a shorter duration than with a longer duration.

For the heated powders, increasing the a_w and the intensity of the heat treatment (either the temperature or duration) led to a decrease in the WC of particles. Once again, a_w had a stronger negative effect at shorter DH duration than at longer DH duration. At 100 °C, WC changed little from the short to long DH duration, as did DY, but at 120 °C, WC decreased greatly from the short to long DH duration, while DY increased greatly. WC were highest after DH at 120 °C for 0.5 h at a_w of 0.23 or 0.33 (i.e. 29.52 ± 0.66 and 27.93 ± 1.44 g/g, respectively).

Finally, WY, which represents how efficiently DH can produce hydrated particles, varied as a function of a_w, temperature and duration (Fig. 7D). WY achieved a local maximum due to increasing DY and decreasing WC during DH. The 2 factors and their interaction again had significant effects ($P < 10^{-11}$), with WY differing among the 3 levels of a_w ($P < 10^{-5}$) and 5 levels of heat treatment ($P < 10^{-16}$). WY was highest for the powder conditioned at $a_w = 0.33$ after a DH at 120 °C for 0.5 h. In this condition, DY was 0.58 ± 0.01 g, and WY was 16.90 ± 0.42 g, of which 96.5% of the weight was water. This WY of 16.90 g/g was lower than the highest WY in the entire study, but it was reached after only 0.5 h of DH, a value compatible with in-line development in a multi-stage spray-dryer that uses a fluidised bed.

Under confocal laser scanning microscopy, some samples (aw = 0.23 and DH at 120 °C, aw = 0.52 and DH at 100 and 120 °C), appeared to have fewer of the smallest particles after prolonged DH (Fig. 8). The limited swelling of particles associated with high-intensity DH could have resulted in particles smaller than the microscope's resolution that were thus invisible on the micrographs. Moreover, WPM after long DH at the highest a_w appeared slightly different, with higher fluorescence intensity on their surface than in their core. This could be due to more intense crosslinking of proteins on the surface of powder flakes due to a higher available humidity on their surface than in their core, because of a residual gradient of available humidity in the powder, even after 2–3 weeks of exchange. This effect requires further investigation, but in a future development of the process, the targeted a_w of powders would be reached during spray-drying by using specific process parameters instead of equilibrating freeze-dried powder with a saturated salt solution. Optimising in-line treatment on a spray-dryer would allow for production of a large amount of functional powder and verification of its thickening properties.

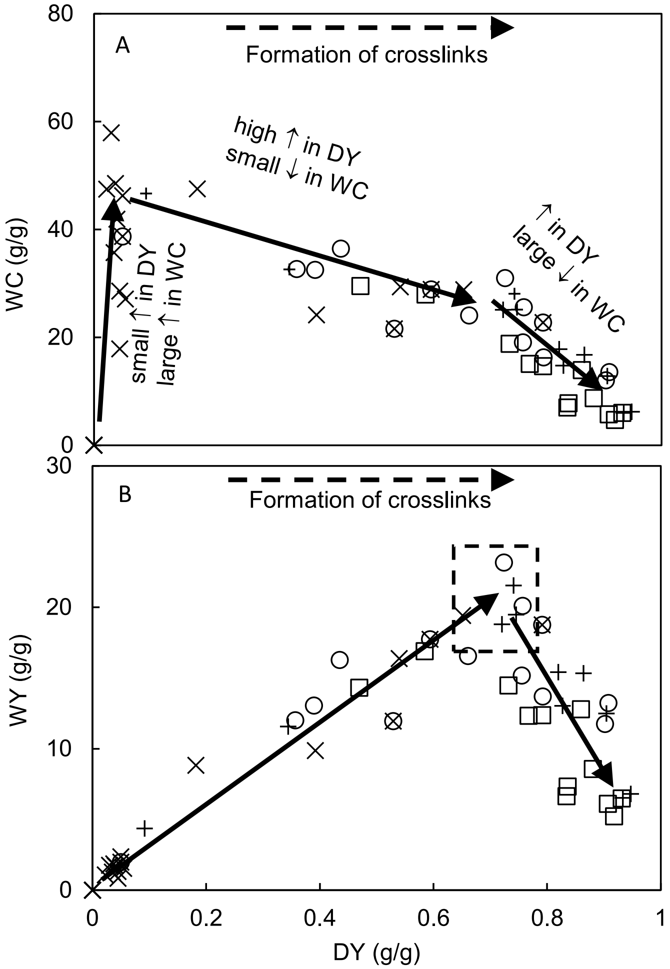


Fig. 9. Relationship between (A) water content (WC) trapped in microparticles and dry yield (DY) and (B) wet yield (WY) and DY of microparticles produced by dry heating in experiments 1 (+), 2 (x), 3 (◯) and 4 (☐). Arrows indicate the direction in which particles were produced.

3.5. WY and WC as a function of DY

Pooling results of all experiments allowed us to examine the WC and WY of WPM as function of DY, which was likely correlated with the formation of crosslinks between WP (Fig. 9). For limited crosslinking that resulted in DY < 0.1 g/g, WC increased significantly up to ca. 50 g water/g (Fig. 9A). For increasing crosslinking that resulted in DY of 0.1–0.7 g/g, WC decreased moderately to ca. 30 g water/g, and for the highest crosslinking in this study (DY > 0.7 g/g), WC decreased sharply toward 5 g water/g. Examining WY as a function of DY (Fig. 9B) revealed 2 distinct dynamics: a linear increase in WY up to ca. 20 g/g at a DY of ca. 0.7 g/g, followed by a linear decrease in WY for DY > 0.7. At maximum WY, WPM contained ca. 96% water. Thus, WP crosslinks were essential for forming WPM, but too many crosslinks limited WPM swelling and the WC. In experiment 4, one condition with a_w at 0.33, a DH temperature of 120 °C and a DH duration of 0.5 h resulted in a WY (16.90 g/g) that was closest to the maximum WY (ca. 20 g/g) and located before maximum WY in the DY-WY plane (Fig. 9B), whereas a longer DH duration of 1 h resulted in a lower WY (14.49 g/g) located after maximum WY. The optimum for producing functional WPM could thus be reached by DH at 120 °C for a duration slightly longer than 0.5 h.

4. Conclusions

The optimal production condition used a WP powder produced by freeze-drying a solution of WP at 100 g/kg with lactose added at 0.5 g/10 g protein (e.g. ca. 0.48 g/kg of α-lactose monohydrate), adjusted to pH 10 and stored at 4 °C for 3 h. Functional powder with a high water-binding capacity was then obtained by adjusting the powder at $a_w = 0.33$, and heating at 120 °C for slightly longer than 0.5 h. These conditions produced WPM with a yield of ca. 20 g of hydrated WPM per g of WP, which can be used as a thickening agent in food matrices. Further studies are needed to transfer this technology to a multi-stage spray-dryer that uses an in-line heating fluidised bed.

Declaration of competing interest

None.

CRediT authorship contribution statement

Marie-Hélène Famelart: Funding acquisition, Supervision, Conceptualization, Writing - original draft. **Thomas Croguennec:** Writing - review & editing. **Thomas Sevrin:** Investigation, Visualization, Validation, Writing - original draft, Writing - review & editing, Formal analysis.

Acknowledgments

The authors are grateful to Regional councils of Brittany, France (grant no. 13008651) and Pays de la Loire, France (grant no. 2014–07081) for financial support and INRAE for scientific coordination (J. Leonil) through the interregional project PROFIL, managed by the Bba industrial association.

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