JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Effect of Moisture Content during Dry-Heating on Selected Physicochemical and Functional Properties of Dried Egg White

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This article addresses the effect of moisture content (0.8−9.9%) during dry-heating (80 °C) on selected physicochemical (solubility, turbidity, residual denaturation enthalpy, aggregation, surface hydrophobicity, and sulfhydryl content) and functional (foaming ability, foam density, and stability) properties of freeze-dried egg white (FDEW). Moisture content during dry-heating proved to be a parameter determining the functionality of the resulting egg white powder. The degree of conformational changes induced in the egg white proteins by dry-heating was strongly dependent on the amount of water present. Preferentially, dry-heating at 80 °C should be performed on egg white powder with a moisture content below 6.8%, as the loss of protein solubility above this value is extensive. In addition to insoluble aggregates, soluble, strongly stabilized aggregates were also formed, especially at higher moisture contents. The decrease in denaturation enthalpy, increase in surface hydrophobicity, and exposure of SH groups previously hidden in the protein core and their subsequent oxidation were more pronounced at prolonged dry-heating times and at higher moisture contents. These conformational changes resulted in improved foaming ability and foams with lower density. No effect of dry-heating on the foam stability was observed.

KEYWORDS: Egg white; dry-heating; foam; physicochemical properties; moisture content

INTRODUCTION

Dehydration is an interesting method of preserving egg components for several reasons: Transport and storage of dried egg products is less costly and requires less space; at low moisture content, egg products are less susceptible to microbial growth; and the uniformity and easy dosage of dried egg products make them an ideal ingredient in the food industry (*1*). The microbiological quality of unpasteurized dried egg white (DEW) can be improved, without damaging the functional quality, by storage at high temperature (70−80 °C) for several days (*2*). In contrast, beneficial effects of dry-heating on the gelling, foaming, and emulsifying properties of egg white powders have been observed (*2*−*8*). Dry-heating induces mild conformational changes (increased surface hydrophobicity, exposure of SH groups, etc.) in egg white proteins, resulting in the formation of water-soluble aggregates. These aggregates inhibit the formation of turbid coagula not only when the reconstituted egg white powder is heated, but also when they are added to fresh egg white before heating (*9*−*11*).

The aforementioned studies were performed under different dry-heating conditions (temperature, time, and DEW moisture content), depending on the research group. The denaturation temperature of proteins strongly depends on their moisture content. Therefore, it can be expected that the moisture content of the DEW will affect the conformational changes occurring during dry-heating and, thus, the functional properties of the DEW after reconstitution. The objective of this study was to investigate the effects of moisture content (0.8−9.9%) and dry-heating time on selected physicochemical (residual denaturation enthalpy, solubility, turbidity, surface hydrophobicity, sulfhydryl content, and aggregation) and functional (foaming) properties of freeze-dried egg white after dry-heating.

MATERIALS AND METHODS

Preparation of Samples. Fresh eggs (168) were obtained from the Zootechnological Center (K.U. Leuven, Heverlee, Belgium). The egg white was separated from the egg yolk, and the chalazae were removed. The egg white (4.6 L) was dialyzed against distilled water at 4 °C. Washing out of glucose and fructose was monitored using a D-glucose/D-fructose test kit (catalog no. 10 139 106 035, Boehringer-Mannheim/R-Biopharm, Darmstadt, Germany). The sugar-free egg white was freeze-dried (Christ Loc 1-m Alpha 2-4, Martin Christ, Osterode am Harz, Germany), pulverized, and subsequently stored above P_2O_5 (a highly hygroscopic compound) until use. Samples (FDEW) were handled under N_2 atmosphere in an airtight glove box (Captair pyramid, Erlab, Val de Reuil, France) to avoid resorption of water. Throughout the period of study, no conversion of ovalbumin into the more heat-stable *S*-ovalbumin was observed, as demonstrated by differential scanning calorimetry (data not shown) (*12*). The pH of a 10% solution of FDEW in demineralized water amounted to 7.65.

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10.1021/jf062370y CCC: $37.00
Published on Web 12/16/2006

Table 1. Overview of Sets of Moisture Content (g of H_2O/100 g of FDEW) Conditions during Dry-heating of FDEW (500-mg Samples)

set	average (g of H_2O/100 g of FDEW)	standard deviation
1	0.8[a]	0.30
2	2.4[a]	0.35
3	3.7[a]	0.26
4	4.9[a]	0.38
5	6.8[a]	0.63
6	8.2[a]	0.23
7	9.7[a]	0.30
8	2.9[b]	0.31
9	4.7[b]	0.35
10	6.1[b]	0.40
11	7.3[b]	0.41

[a] Ten individual determinations. [b] Twenty individual determinations; 4 determinations per dry-heating time.

Equilibrium Relative Humidity (ERH) Control. Constant ERH, and thus FDEW moisture content, was guaranteed using 0.3 L of a concentrated NaOH solution in hermetically sealed 1-L glass containers (Weck, Wehr-Öfflingen, Germany). The concentration needed to ensure a preset ERH was determined using the polynomial function for calculating the vapor pressure of a NaOH−H_2O system proposed by Olsson et al. (*13*), based on the Antoine equation and literature data. This function is valid in the temperature range of 70−150 °C and the molality range of 0−58 mol of NaOH/kg of H_2O.

Sorption Isotherms. Samples (~90 mg in glass weighing containers) of freeze-dried egg white (FDEW) samples stored above P_2O_5 at room temperature were transferred to hermetically sealed glass containers containing a specific concentration of an aqueous NaOH solution rendering a predefined ERH (10−70%) at room temperature (20 °C) or 80 °C. Constant temperature at 80 °C was ensured in a heating oven with forced convection (FD 53, Binder, Tuttlingen, Germany). Samples were kept under these conditions until equilibrium was reached (verified by mass determination). The amount of water absorbed by the samples is defined as the difference between the mass of the rehydrated sample and that of the dry sample (stored above P_2O_5). Moisture content (W) was expressed as grams of water per gram of dry matter.

Dry-Heating of FDEW. FDEW stored above P_2O_5 (~500 mg) was transferred to hermetically sealed glass containers containing an aqueous NaOH solution of a specific concentration at room temperature (20 °C) leading to a predefined ERH, and thus moisture content, derived from the sorption isotherms. The ERH values selected rendered moisture contents in the range of 0.5−10 g of H_2O/100 g of FDEW.

After equilibration (1 week), samples were placed above an aqueous NaOH solution (under specific conditions of ERH) resulting in the same moisture content at 80 °C (based on the sorption isotherm data). The samples were stored at constant temperature and moisture content for various periods of times (maximum of 21 days) in a heating oven with forced convection (FD 53, Binder, Tuttlingen, Germany). Periodically, samples of dry-heated FDEW (DH-FDEW) were removed from the oven, sealed, and cooled to room temperature, where the amount of water absorbed by the sample was calculated from the mass of the rehydrated sample and the initial mass of the sample (after storage above P_2O_5). In **Table 1**, the average moisture contents during dry-heating of DH-FDEW at 80 °C for all sets of moisture contents used in this study are listed. Except for the DH-FDEW dry-heated at an average moisture content of 6.8%, the moisture content was constant throughout the dry-heating period. The average values of all sets of moisture contents considered were significantly different ($\alpha = 0.05$). The moisture content range of this study (0.8−9.9%) comprises the moisture contents during dry-heating of egg white or ovalbumin reported by other researchers (*7*, *10*, *11*, *14*, *15*). Although those dry-heating experiments were performed in sealed vials, a decrease of moisture content was described at elevated temperatures (100−120 °C) (*11*). In our study, dry-heating was performed on open systems, but constant moisture content was assured by equilibration above concentrated NaOH solutions.

Next, the DH-FDEW samples were dehydrated for 1 week above P_2O_5 at room temperature prior to further analysis, to avoid the effect of differences in moisture content of samples dry-heated at different levels of ERH on the determination of the physicochemical and foaming properties.

Determination of Physicochemical Properties. *Determination of Denaturation Temperature and Enthalpy.* Denaturation temperature and enthalpy were determined using a Perkin-Elmer DSC 7 instrument (Perkin-Elmer, Boston, MA). Samples of DH-FDEW solution (55 μL, 100 g/L) prepared in 0.1 M phosphate buffer (pH 7.6) were transferred to stainless steel pans (Perkin-Elmer) and hermetically sealed. Samples were analyzed at a heating rate of 10 °C/min in a temperature range of 10−120 °C. An empty pan was used as a reference (*16*).

The denaturation temperature (T_d, °C) is defined as the temperature at peak maximum. The denaturation enthalpy (ΔH, J/g FDEW) was calculated from the peak area of the thermogram using Pyris software (Perkin-Elmer, 1996). Samples were measured in duplicate.

Determination of Protein Solubility and Turbidity. Dry-heated samples (10 g of DH-FDEW per liter in 0.1 M phosphate buffer, pH 7.6) were centrifuged for 15 min at 19900*g* and 4 °C. The protein content of the supernatant, after a 10-fold dilution in 0.1 M phosphate buffer (pH 7.6), was determined using Sigma Procedure No. TRPO-562 (*16*). Solubility is expressed as the percentage of protein remaining in the supernatant as compared to that in the untreated sample (FDEW).

The transmittance of a 1 g/L DH-FDEW solution in 0.1 M phosphate buffer (pH 7.6) was measured at 600 nm. A turbidity of 0% corresponds to a completely clear solution (transmittance in comparison to the blank is 100%).

Determination of Protein Aggregation. The aggregation behavior of egg white proteins during dry-heating was studied using a microfluidic, chip-based automated electrophoresis system. The Experion System (Bio-Rad Laboratories, Hercules, CA) was used for SDS-PAGE-like analysis of the DH-FDEW solutions. For protein separation, Experion Pro260 chips and reagents were used (Bio-Rad Laboratories, Hercules, CA). Samples (4 μL) of the DH-FDEW solutions (2 g/L in 10 mM phosphate buffer, pH 7.6, prepared in milliQ water) or of Experion Pro260 Ladder were mixed with 2 μL of Experion Pro260 Sample Buffer containing glycerol and lithium dodecyl sulfate, with or without 6.25% 2-mercaptoethanol. After 5 min of boiling, the samples were diluted with 84 μL of milliQ water. Then, 6 μL of this dilution was transferred to the Experion Pro260 chip, previously primed with Experion Pro260 Gel containing methylurea, SDS, and dimethyl sulfoxide in the Experion Priming Station. The molar masses and approximate concentrations of the proteins were determined using the positions and heights of the Experion Pro260 Ladder fluorescent peaks.

Determination of Surface Hydrophobicity. The surface hydrophobicity of the soluble protein fraction (supernatant obtained after 15 min of centrifugation of a 10 g/L DH-FDEW solution in 0.1 M phosphate buffer, pH 7.6, at 19900*g* and 4 °C) was determined using the fluorescent probe ANS (anilinonaphtalenesulfonic acid) (*17*), as described earlier (*16*).

Determination of Sulfhydryl Content. The concentration of sulfhydryl (SH) groups of the DH-FDEW was determined using Ellman's reagent [5′,5-dithiobis(2-nitrobenzoic acid), DTNB] (*18*). Both the total (10 g/L DH-FDEW solution in 0.1 M phosphate buffer, pH 7.6) and soluble (supernatant of the same solution after 15 min of centrifugation at 19900*g* and 4 °C) protein fractions were considered. Changes in total and exposed sulfhydryl group contents were measured in duplicate using the procedure described earlier (*19*). The amount of buried sulfhydryl groups (inaccessible to DTNB) was calculated by subtracting the amount of exposed SH groups from the total amount of SH groups. The sulfhydryl contents are expressed as the percentage of total sulfhydryl groups present in FDEW.

Determination of Foaming Properties. Foaming properties were determined as described earlier (*20*). In short, a 50-mL volume of DH-FDEW solution (10 g/L in 0.1 M phosphate buffer, pH 7.6) was whipped for 5 min at 1300 rpm, using a laboratory stirrer with microprocessor-controlled constant speed (OST basic, IKA, Staufen, Germany). The foam produced and any remaining liquid were gently transferred into a graduated glass cylinder (3.5-cm diameter, 31-cm height, graded up to 0.250 L) using a small plastic spatula and a plastic

broad-neck funnel, within 5 min. Any air pockets were removed by holding up the base of the cylinder with one hand and the top of the cylinder with the other hand. With the cylinder held upright, two quick downward shakes were given (*21*).

The foam-forming ability (FA) is defined as the foam volume (in liters) 5 min after the end of whipping. The liquid in foam (LF) is expressed as the volume of liquid retained in a fixed volume of foam 5 min after the end of whipping. Drainage of liquid was recorded for 1 h. The foam stability (FS) is defined as the percentage of liquid still present in the foam after 1 h compared to that present 5 min after whipping. Samples were determined in triplicate.

RESULTS AND DISCUSSION

Sorption Isotherms of FDEW. The relationship between the moisture content of the FDEW and the ERH (10−70%) of the surrounding atmosphere was studied at two different temperatures (20 and 80 °C). The moisture content of the FDEW increased with increasing ERH, as could be expected (data not shown). At 80 °C, the Brunauer−Emmett−Teller (BET) model could accurately describe the relationship between ERH and FDEW moisture content

$$W = \frac{W_m C_b (\mathrm{ERH}/100)}{(1 - \mathrm{ERH}/100)(1 + C_b \mathrm{ERH}/100)}$$

where W represents the mass (in grams) of water absorbed per gram of solid, W_m is the mass (in grams) of water in the form of a monolayer, and C_b is a parameter related to the heat of sorption of the monolayer.

At the lower temperature, however, the relationship between ERH and FDEW moisture content was modeled according to the GAB equation, developed by Guggenheim, Anderson, and de Boer

$$W = \frac{W_m C_G K (\mathrm{ERH}/100)}{[1 - K(\mathrm{ERH}/100)][1 - K(\mathrm{ERH}/100) + C_G(\mathrm{ERH}/100)]}$$

where W represents the mass (in grams) of water absorbed per gram of solid; W_m is the mass (in grams) of water in the form of a monolayer; and C_G and K are parameters related to the heat of sorption of the monolayer and intermediate layer, respectively. The GAB equation is an extension of the BET model, additionally taking into account the weak interaction with the solid of water molecules in the intermediate state in comparison with the interaction of the monolayer with the solid. Two different models were used for the sorption isotherms, as the purpose was to relate the moisture content and ERH requirements quantitatively. The GAB model did not give a good prediction of the sorption isotherm at 80 °C, probably because the interaction between the solid and the water molecules in the intermediate state is negligible at this temperature. The sorption isotherm parameters, reported in Table 2, were determined by nonlinear regression analysis (*22*). As could be expected, at higher temperature, a lower moisture content could be observed for the same ERH.

Using these sorption isotherms, the concentration of aqueous NaOH solution at room temperature (20 °C) needed to obtain the same moisture content as generated by an atmosphere with a predefined ERH at 80 °C could be calculated. This approach avoids changes in moisture content when the samples are transferred from 20 to 80 °C.

The effect of moisture content on the denaturation temperature of FDEW was studied on samples stored at different levels of ERH at 20 °C. Approximately 6 mg of the FDEW was transferred to stainless steel pans (Perkin-Elmer) and hermetically sealed. Samples were analyzed at a heating rate of 10 °C/min in a temperature range of 50−200 °C. Denaturation temperature was determined as described in the Materials and Methods section. Denaturation temperature increased linearly with decreasing moisture content from 107 °C at 14% moisture content to 155 °C at 1% (data not shown). These results agree with the common knowledge that protein thermal stability is enhanced at decreasing water availability. It therefore can be expected that, at a fixed dry-heating temperature, the degree of denaturation will be less pronounced under conditions of low ERH (and thus moisture content). Because the denaturation temperatures in the dry state are much higher than those observed for differential scanning calorimetry of FDEW solutions [typically a denaturation temperature of 84 °C is obtained for ovalbumin (*16*)], dry-heating at 80 °C will cause only mild denaturation. However, it has to be kept in mind that the time scale of DSC experiments differs significantly (heating at 10 °C/ min) from that in the dry-heating experiments (1−21 days). Therefore, denaturation during dry-heating can be more pronounced than expected according to the DSC thermograms.

Table 2. Parameters of the BET and GAB Equations Describing the Relationship between Moisture Content and ERH of FDEW

	20 °C	80 °C
	BET Model	
W_m (g of H_2O/ g of dry matter)	NA[a]	0.0301 ± 0.0015[b]
C_b		7.279 ± 2.55[b]
adj r^2		0.967
	GAB Model	
W_m (g of H_2O/ g of dry matter)	0.0727 ± 0.0060[b]	NA
C_g	7.72 ± 1.42[b]	
K	0.8491 ± 0.040[b]	
adj r^2	0.992	

[a] NA = not applicable. [b] Approximate standard error from nonlinear regression analysis.

Effect of Moisture Content during Dry-Heating on Physicochemical Properties of FDEW. In the food industry, dehydration of egg white is typically achieved by spray-drying instead of freeze-drying (*1*). The heating and atomization needed for spray-drying causes some degree of denaturation in the egg white proteins. Therefore, the physicochemical properties of the spray-dried egg white are somewhat different than those of the FDEW used in our study. However, the purpose of this study was to define the effect of dry-heating on egg white proteins in a native-like state. Hammershoj et al. (*23*) investigated the effect of the different processing steps in the production of dried egg white powder. They showed that spray-drying does in fact lead to changes in the functional properties of the dried egg white, as a result of protein denaturation.

Protein Solubility and Turbidity. Below 4.9% moisture content, dry-heating did not induce a significant ($\alpha = 0.05$) loss of protein solubility within the heating period studied (21 days). At higher moisture contents, however, dry-heating had a detrimental effect on protein solubility. A progressive decrease with increasing dry-heating time and moisture content was observed (**Figure 1**). At the highest moisture content studied (9.9%), the FDEW was virtually insoluble after merely 1 day of dry-heating. This result is of practical interest, as FDEW commonly has to be resuspended prior to formulation. Therefore, moisture content during dry-heating is a determining process parameter for the functionality of the resulting egg white

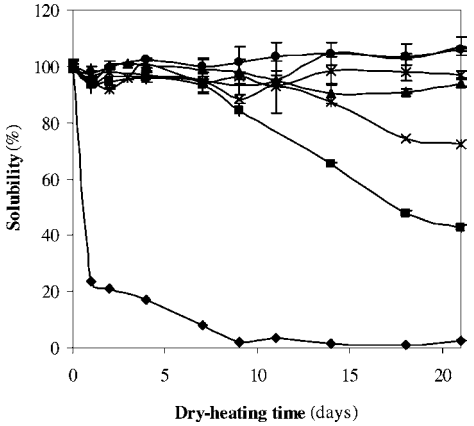

Figure 1. Protein solubility (%) of FDEW dry-heated at 80 °C and average moisture contents (sets 1–7) of (+) 0.8, (●) 2.4, (*) 3.7, (▲) 4.9, (×) 6.8, (■) 8.2, and (◆) 9.9 g of H_2O/100 g of FDEW(mean values; error bars represent standard deviations of duplicate measurements).

powder. Preferentially, dry-heating at 80 °C should be performed on dried egg white with a moisture content below 6.8%. Furthermore, the pronounced changes in protein solubility occurring over a rather narrow moisture content range (between 6.8% and 8.2% moisture content) call for strict control of temperature and ERH during dry-heating.

This time-dependent loss of solubility is in agreement with the dry-heating experiments discussed in the literature. Kijowski et al. (*6*) did not observe any loss of solubility for glucose-free dried egg white for dry-heating conditions of 60−80 °C during 0−10 days and a moisture content of 8.2%. However, when glucose was not removed from the egg white prior to dry-heating, a significant decrease of soluble protein content was observed, which was more pronounced at longer dry-heating time and higher dry-heating temperature. Therefore, it is indispensable to remove sugars prior to drying or dry-heating in order to obtain a high technological value. In food industry, desugarization of egg white is achieved by applying bacterial or yeast fermentation or by using an immobilized glucose oxidase (in combination with catalase) (*24*, *25*). In our study, sugars (along with small ions) were removed by dialysis. When heated at 80 °C for up to 1 week, ovalbumin (moisture content of 5.7%) does not show solubility loss of significant importance (*15*).

The pH of the egg white prior to dry-heating strongly affects the residual protein solubility. When dry-heating at a pH level above 10 (corresponding to a pH level of 7.6 before spray-drying), the resulting protein solubility decreases progressively at longer dry-heating time, down to less than 15% at 75 °C and 8.5% moisture content, whereas at lower pH levels, no significant decrease is observed up to 1 week (*8*). In our study, the pH of the FDEW prior to dry-heating was 7.65. Xu et al. (*10*) observed no loss of protein solubility of egg white dry-heated at 120 °C within a time period of 6 h at an initial moisture content of 5.4%. Furthermore, they observed that short-time dry-heating (1−6 h) impeded the formation of insoluble aggregates when the reconstituted DHEW was heated for 3.5 min at 60 °C. Correspondingly, addition of this DHEW to fresh egg white inhibited the heat coagulation of the latter (*10*, *11*).

In concurrence with the decreased soluble protein content, an increased turbidity of the reconstituted egg white was observed (Table 3). At moisture contents above 6.8%, turbid suspensions consisting of large protein particles were obtained. Both phenomena indicate the formation of large, insoluble aggregates during dry-heating at moisture contents from 4.9% upward. For most practical applications, it is desirable that reconstituted egg white consist of a clear solution. Therefore, at 80 °C, dry-heating should preferentially be performed on dried egg white with a moisture content below 6.8%, as above this value, highly turbid suspensions are obtained upon reconstitution.

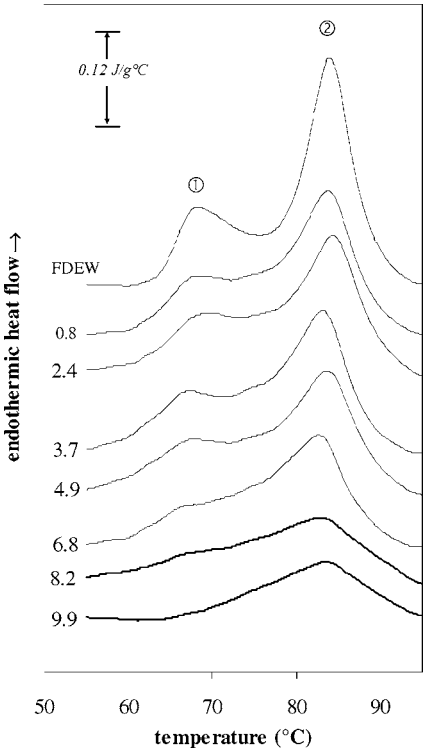

Figure 2. DSC thermograms (heating rate 10 °C/min) of FDEW solutions (100 mg/mL in phosphate buffer, pH 7.6) dry-heated at 80 °C for 4 days at different moisture contents (g of H_2O/100 g of FDEW). Peak 1 corresponds to the denaturation of ovotransferrin and lysozyme, and peak 2 corresponds to the denaturation of ovalbumin.

Table 3. Turbidity at 600 nm of Resuspended DH-FDEW Solutions (5 mg of Protein/mL of Phoshpate Buffer, pH 7.6) after 1 Week of Dry-Heating at 80 °C and Constant Moisture Content

moisture content (g of H_2O/100 g of FDEW)	turbidity (%) at 600 nm
FDEW	11.8 ± 0.9
0.8	12.2 ± 1.3
2.4	11.4 ± 0.7
3.7	15.4 ± 1.5
4.9	15.9 ± 0.8
6.8	20.0 ± 0.8
8.2	26.9 ± 1.5
9.9	71.6 ± 7.3

Denaturation Enthalpy and Temperature. When 100 g/L samples were analyzed (**Figure 2**), FDEW showed two endothermic peaks, corresponding to the unfolding of lysozyme and ovotransferrin (indistinguishable) (69.5 °C) and ovalbumin (84 °C) (*26*). Dry-heating for 4 days at 80 °C resulted in a broadening of the two peaks, a minor shift of the peak temperatures to lower temperature, and a marked decrease in denaturation enthalpy. Similar results were also observed by Kato et al. (*4*) and Matsudomi et al. (*15*). These authors suggested that the broadening of the peaks indicates the presence of partially unfolded conformations of the protein. These intermediates can promote intermolecular interactions during foaming or heat-induced gelation of the resuspended DHEW.

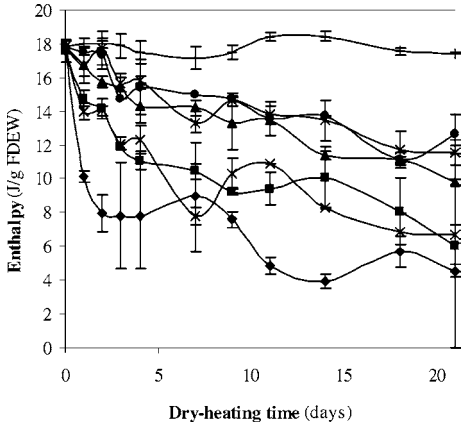

Figure 3. Denaturation enthalpy (J/g of solids) of FDEW dry-heated at 80 °C and average moisture contents (sets 1–7) of (+) 0.8, (●) 2.4, (*) 3.7, (▲) 4.9, (×) 6.8, (■) 8.2, and (◆) 9.9 g of H_2O/100 g of FDEW (mean values; error bars represent standard deviations of duplicate measurements).

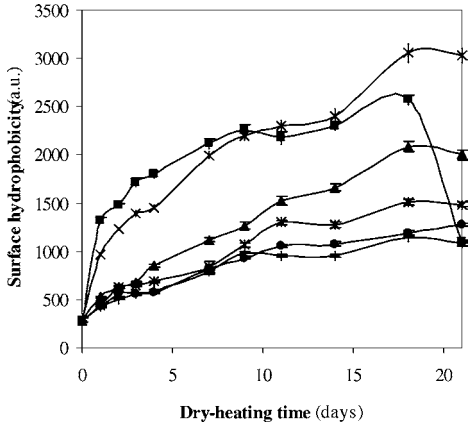

Figure 4. Surface hydrophobicity (au) of the soluble fraction of FDEW dry-heated at 80 °C and average moisture contents (sets 1–7) of (+) 0.8, (●) 2.4, (*) 3.7, (▲) 4.9, (×) 6.8, and (■) 8.2 of H_2O/100 g of FDEW (mean values; error bars represent standard deviations of duplicate measurements).

The decrease in denaturation enthalpy indicates partial unfolding of the egg white proteins during dry-heating (Figure 3). With increasing dry-heating time, the loss of protein structure was more pronounced. Both ovalbumin and lysozyme were affected in their protein structure, as evidenced by a simultaneous decrease of the individual peaks (Figure 2).

As could be expected on the basis of the decrease in thermal stability with increased moisture content, the extent of protein denaturation was larger at the higher levels of moisture content (**Figures 2** and **3**). In a time scale of days, FDEW did show considerable loss of protein structure, even at 80 °C, although the denaturation temperature of ovalbumin in FDEW was as high as 124 °C at the highest moisture content (9.3%) investigated in this study. Under these conditions, only 25% of the initial denaturation enthalpy (in solution) remained after prolonged dry-heating.

Mine (*7*) demonstrated that dry-heating of egg white proteins at alkaline pH results in the formation of a broad population of intermediate protein conformations, as the endothermic peaks were broader for egg white dry-heated at pH 9.40 than for that dry-heated at pH 6.88. Furthermore, with increasing pH, the decrease in denaturation enthalpy is more pronounced.

Surface Hydrophobicity. The surface hydrophobicity of soluble proteins increased strongly when the FDEW was subjected to prolonged dry-heating (**Figure 4**). Thus, loss of protein structure, as evidenced by the decrease in denaturation enthalpy, was accompanied by exposure of the previously hidden hydrophobic groups. At higher moisture contents, the increase in surface hydrophobicity of the soluble protein fraction was more pronounced, except for FDEW dry-heated at 8.2%. At this moisture content, the maximum level of surface hydrophobicity attained was somewhat lower, probably because of the significant loss of protein solubility. The surface hydrophobicity of FDEW dry-heated at 9.9% moisture content could not be determined because of the low protein content.

This increased surface hydrophobicity can lead to better hydrophobic interactions during the stabilization of heat-induced aggregates and the formation of protein-stabilized foams and emulsions for the rehydrated DH-FDEW. In comparison to the increase of surface hydrophobicity observed after heat (*16*) or high-pressure treatment (*27*) in solution, dry-heating only induced a mild exposure of the buried hydrophobic groups. Because ANS is anionic and because small ions were removed during dialysis prior to freeze-drying, the measured surface hydrophobicity might be somewhat different than when the ions would still be present (*17*).

These results are supported by the work of Matsudomi et al. (*15*), who measured the surface hydrophobicity of ovalbumin after dry-heating at 80 °C for up to 10 days at a moisture content of 5.7%, but using the hydrophobic probe *cis*-parinaric acid instead of ANS. Likewise, an increased exposure of hydrophobic groups in dry-heated spray-dried egg white (80 °C, 7.5% moisture content) has been observed, leading to improved emulsifying properties (*2*). At higher pH, the increase in hydrophobic groups is more pronounced, until the majority of the proteins become insoluble (*8*).

Sulfhydryl Content. Inaccessible thiol groups in ovalbumin become exposed by unfolding of the protein during heating (*28*). This is observed for egg white proteins not only in solution (*19*), but also when heated in the dry state. **Figure 5** illustrates the changes in the total and exposed SH contents of FDEW during dry-heating. The mild unfolding of egg white proteins indicated by increased surface hydrophobicity is supported by the exposure of buried SH groups (Figure 5A). With increasing dry-heating time and increasing moisture content, the degree of exposure increased. However, the increase in exposed SH groups was not as pronounced as observed at the same temperature (80 °C) in solution (up to 70% of the total SH content) (*19*). These exposed SH groups are more reactive and can participate both in SH−SS exchange and in oxidation reactions. The latter was evidenced by a gradual decrease of total SH groups (**Figure 5B**) that was more pronounced at high moisture content. At low solubility (i.e., at moisture contents above 4.9%), the major fraction of both the total and exposed SH groups remained in the insoluble protein fraction (data not shown).

The exposure and successive reaction of SH groups was also observed in ovalbumin heated in the dry state (80 °C, moisture content of 5.7%) (*15*). In spray-dried egg white, the exposure of previously buried SH groups and their subsequent oxidation was more pronounced when dry-heating (75 °C, 8.5% moisture content) was performed at high pH (*8*).

Protein Aggregation. Under the conditions used in this study, dry-heating of FDEW induced the formation of insoluble aggregates as evidenced by the decrease in protein solubility with increasing dry-heating time and moisture content (**Figure 1**). In addition to these insoluble aggregates, soluble aggregates also were formed. In **Figure 6**, the simulated gel display based

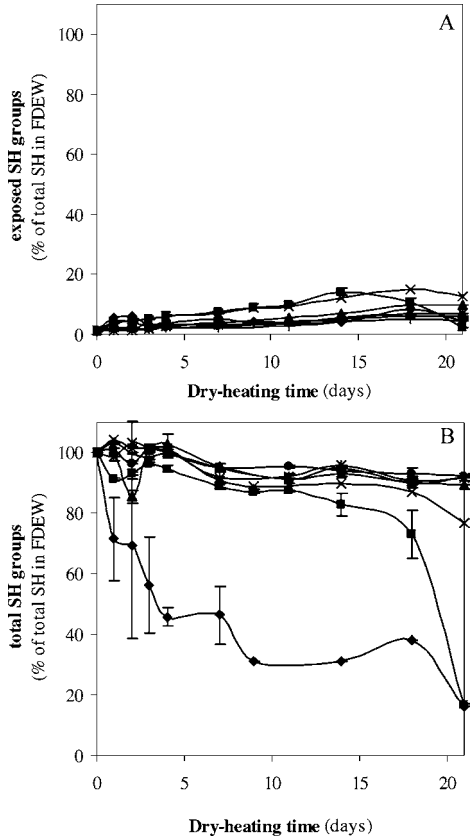

Figure 5. (A) Exposed and (B) total sulfhydryl contents (%) of FDEW dry-heated at 80 °C and average moisture contents (sets 1–7) of (+) 0.8, (●) 2.4, (*) 3.7, (▲) 4.9, (×) 6.8, (■) 8.2, and (◆) 9.9 g of H_2O/100 g of FDEW (mean values; error bars represent standard deviations of duplicate measurements).

on the electropherogram of microfluidic, chip-based SDS-PAGE is shown for the soluble protein fraction of FDEW dry-heated under different conditions of moisture content and dry-heating time. With increasing moisture content, a decrease in peak height of the three major peaks (lysozyme, ovalbumin, and ovotransferrin) was observed for FDEW dry-heated for 2 days at 80 °C. Concurrently, aggregates that could not be solubilized using SDS appeared (Figure 6a). The calculated molar masses of these aggregates amounted to 88 and 120 kDa (the latter is not highly visible in the gel display, but is clear from the electropherogram; data not shown), which indicates that multimers of ovalbumin and/or ovotransferrin are formed during dry-heating. The formation of these multimers was more pronounced at higher moisture contents, as evidenced by their increased intensity in the polyacrylamide gel. Apparently, polymers too large to migrate through the gel were also formed, as addition of the disulfide bond reducing agent β-mercaptoethanol led to an increase of the intensity of these bands (**Figure 6b**). Again, dry-heating at higher moisture contents resulted in the formation of higher amounts of soluble aggregates. As some of the aggregates could not be completely dissociated in the presence of β-mercaptoethanol, the type of binding cannot be attributed solely to the formation of disulfide bonds (resulting from the oxidation of exposed sulfhydryl groups, **Figure 5**, or disulfide-sulfhydryl exchange reactions) during dry-heating. The presence of tight binding forces in aggregates formed by dry-heating was also observed by other researchers (*2*, *10*, *15*). Time was another determining factor in the formation of protein aggregates during dry-heating (**Figure 6c**,**d**). With increasing time, the intensities of the bands corresponding to soluble aggregates increased.

Watanabe et al. (*11*) observed that the formation of these soluble aggregates prevented the further heat-induced aggregation of the resolubilized dry-heated egg white. Furthermore, the addition of dry-heated egg white (a few hours at 120 °C and 5.4% moisture content) to fresh egg white inhibited the occurrence of coagula upon heating (3.5 min at 60 °C) (*10*). These authors suggested that the formation of linear aggregates prevented the disulfide−sulfhydryl exchange reactions and hydrophobic interactions among the monomers and/or aggregates of ovotransferrin during pasteurization.

Effect of Moisture Content during Dry-Heating on Foaming Properties of FDEW. Because of the low resulting solubility at higher moisture contents, the foaming properties of DH-FDEW after dry-heating at 80 °C were studied only in the moisture content range of 2.9−7.3% (**Table 1**). In addition to the physicochemical properties, the foaming ability, foam density, and foam stability of the DH-FDEW obtained were studied. Although in the food industry foam testing is performed at 10% protein content, we chose to use a 1% sample to detect minor changes in the egg white proteins' physicochemical properties leading to differences in foaming properties in order to correlate them. At higher protein concentrations, these changes can be masked by the more pronounced protein−protein interactions. However, this means that the actual foaming properties of the DH-FDEW as applied in the food industry might be somewhat different.

The mild conformational changes in FDEW during dry-heating resulted in improved foaming ability (**Figure 7**). The foaming ability increased at longer drying times, until a plateau was reached. At higher moisture contents, the foaming ability improved faster. This is probably because of a more pronounced exposure of hydrophobic and SH groups (which can both contribute to the stabilization of the protein layer surrounding the air bubble), while conserving a high protein solubility (a maximal solubility loss of 10% was observed at 7.3% moisture content). In our previous study (*20*), it was shown that egg white solutions (10% v/v) processed under conditions that resulted in protein unfolding combined with high residual solubility and the presence of soluble aggregates (i.e., heating above 75 °C at pH 8.8 or pressure treatment at 400−700 MPa and 10−60 °C) exhibited improved foaming ability.

Kato et al. (*3*) also observed an increased foaming power of spray-dried egg white (7.5% moisture content) due to dry-heating (80 °C). The increased foaming ability was shared by the individual globulin and ovalbumin fractions. The former, however, showed a higher foaming power. In a different study, on the other hand, a decrease in foam volume was observed for glucose-free dried egg white (8.3% moisture content) after dry-heating in the temperature range of 60−80 °C (*6*). A strong decrease in foam density (the amount of liquid trapped in a specific foam volume) was observed after the first day of dry-heating at all levels of moisture content studied, whereas at longer dry-heating times, the decrease was more gradual (**Figure 8**).

As the foams produced from both FDEW and DH-FDEW collapsed internally, the foam volume could not be measured as a function of drainage time. Consequently, foam stability had to be determined on the basis of the amount of liquid retained in the foam after a fixed delay time (1 h). Although the amount of liquid retained in a specified volume of foam did change as a result of dry-heating, the percentage of that liquid that drained from the foam after 1 h (71.8 ± 2.5%) was not significantly ($\alpha = 0.05$) influenced by either dry-heating time or moisture content.

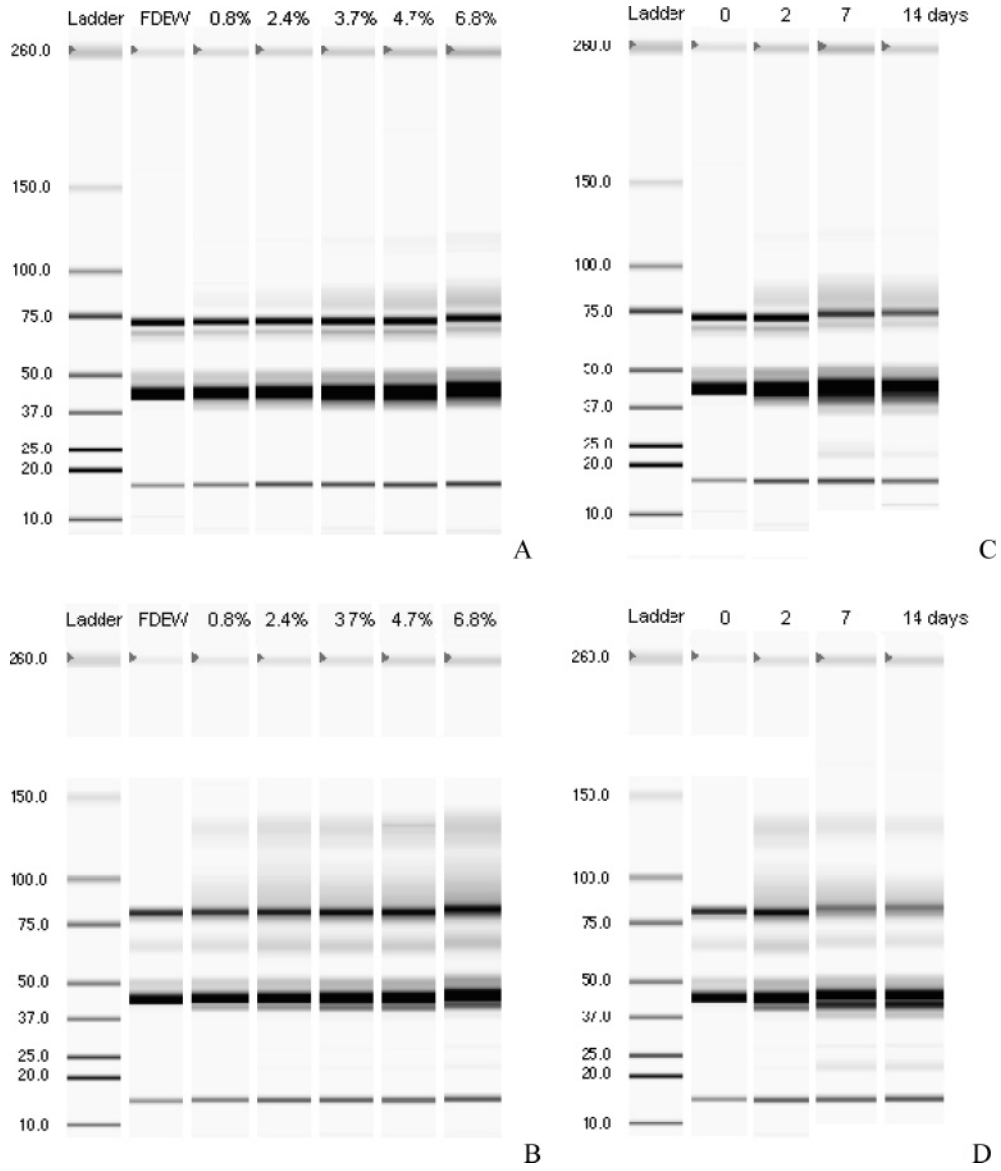

Figure 6. Gel display of (A,C) non-reducing and (B,D) reducing SDS-PAGE analyses of FDEW for a 1:10 dilution of the soluble fraction of a 20 g/L solution of FDEW dry-heated (A,B) for 2 days at different moisture contents or (C,D) at 3.7 g of H_2O/100 g of FDEW for different dry-heating times. The band at 260 kDa corresponds to an internal marker.

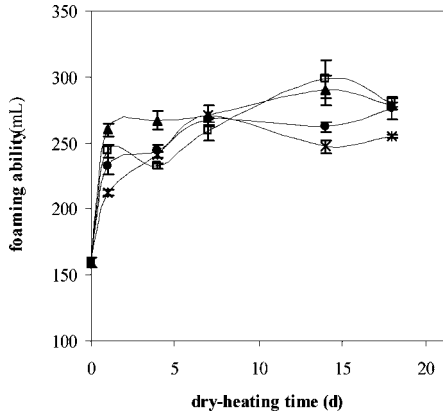

Figure 7. Foaming ability (mL) of FDEW dry-heated at 80 °C and average moisture contents (sets 8–11) of (*) 2.9, (●) 4.8, (▲) 6.1, and (■) 7.3 g of H_2O/100 g of FDEW (mean values; error bars represent standard deviations of triplicate measurements).

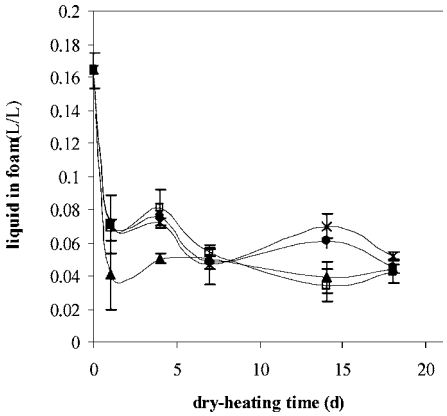

Figure 8. Liquid in foam (mL/mL) of FDEW dry-heated at 80 °C and average moisture contents (sets 8–11) of (*) 2.9, (●) 4.8, (▲) 6.1, and (■) 7.3 g of H_2O/100 g of FDEW (mean values; error bars represent standard deviations of triplicate measurements).

In **Table 4**, the pair-wise Pearson correlation coefficients of all foaming properties and physicochemical properties of the DH-FDEW solutions are listed. The foaming ability and the amount of liquid trapped in the foam are highly correlated. Kato

Table 4. Pearson Correlation Coefficients among Foaming and Physicochemical Properties of DH-FDEW[a]

	foaming ability	liquid in foam	foam stability
foaming ability	–	–0.971[b]	–0.070
liquid in foam	–0.971[b]	–	0.025
foam stability	–0.070	0.025	–
solubility	–0.217	0.247	–0.096
turbidity	0.700[b]	–0.620[b]	–0.343
enthalpy	–0.827[b]	0.772[b]	0.156
S_0	0.812[b]	–0.738[b]	–0.258
exposed SH	0.707[b]	–0.622[b]	–0.199
total SH	–0.804[b]	0.716	0.240

[a] Dry-heating at 80 °C for 1–18 days at 2.9–7.3% moisture content. [b] Significant at $p < 0.001$.

et al. (*3*) noticed a linear relationship between residual denaturation enthalpy and foaming power. This was not the case in our study, however. This might be due to the higher number of data points considered in our study (six) in comparison to that by Kato et al. (three). In our study, no (linear) relationship between protein solubility and foaming ability was observed. It has to be kept in mind that only dry-heating conditions resulting in high solubility were considered (2.9−7.3% moisture content). Foaming ability improves with increasing degree of unfolding (as evidenced by decreases in denaturation enthalpy and exposure of hydrophobic and sulfhydryl groups) until a plateau value is attained. Further unfolding does not appear to have an additional effect on the foam volume, although no detrimental effect was observed either.

In conclusion, the present study has shown that, in addition to temperature (*6*) and pH (*8*), moisture content is an important process parameter in the dry-heating of egg white proteins. At higher moisture contents, the degree of unfolding is more pronounced, as shown by a marked decrease in denaturation enthalpy and exposure of hidden SH groups and hydrophobic residues. However, at 80 °C, dry-heating should be performed on dried egg white with a moisture content below 6.8%, as the loss of protein solubility above this value is extensive. In addition to insoluble aggregates, soluble, strongly stabilized aggregates are also formed, especially at higher moisture contents. Partly, the oxidation of exposed sulfhydryl groups to form disulfide bonds is responsible for the formation of these aggregates. In comparison to heating in the liquid state, dry-heating induces mild changes in protein structure. As a result, the foaming properties (foaming ability and liquid in foam) are improved. The foam stability was not affected by heating of the egg white proteins in the dry state. Thus, dry-heating at relatively low moisture contents (below 6.8%) results in egg white powder with high solubility and improved foaming properties. A strict control of temperature and ERH during dry-heating is needed, as pronounced changes occur in the functionality of the DH-FDEW over a narrow range of moisture content. Although not within the scope of this work, the gelling properties of DH-FDEW will also depend on the moisture content during dry-heating. Kato et al. (*4*) demonstrated an inverse relationship between denaturation enthalpy and gel strength. Also, increased surface hydrophobicity leads to a stronger gel because of the increased possibility for hydrophobic interactions upon heating of the resuspended DH-FDEW. Therefore, at higher moisture contents, stronger gels can be expected for DH-FDEW. However, extensive insolubilization can lead to the formation of turbid coagula.

As this study was performed on unpasteurized, freeze-dried egg white, it has to be taken into account that both pasteurization and spray-drying (generally applied in the egg industry) will result in additional unfolding of the egg white proteins and thus alterations in the functional properties (*23*).

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Received for review August 17, 2006. Revised manuscript received October 31, 2006. Accepted November 14, 2006. I.V.d.P. is a postdoctoral researcher, funded by the Research Fund K.U. Leuven.

JF062370Y