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Egg white drying: Influence of industrial processing steps on protein structure and functionalities

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Abstract

Spray-dried egg white is commonly used as a food ingredient for its foaming and gelling properties. However, these properties are obtained thanks to dry-heating of egg white powder, which is necessary to offset the harmful effects of spray-drying process on egg white functionality. The purpose of the present work is to identify the processing steps responsible for the damages to egg white functional properties, and to understand the mechanisms that occur in order to limit these effects and to reduce dry-heating time. Two trials were performed and the measurements of egg white protein conformation and gel firmness were significantly different from one trial to another, thus emphasizing great variations in raw material characteristics. In spite of this trial effect, processing steps significantly modified egg white foaming properties. The most critical step was the spray-drying one that strongly damaged foaming properties. During this step, heat transfers and air–product interface area rather than shear rates were responsible for these changes. Then, it was the pumping and filtering steps that had also a considerable effect, due to the generation of shear rates and stainless steel–product interfaces, responsible for foaming property damages. On the other hand, concentration and desugarization steps had an interesting improving effect on egg white foaming properties.

Keywords: Egg white; Foam; Gel; Protein structure; Spray drying; Heating; Shearing; Interface

1. Introduction

Egg white is a desirable ingredient for many foods such as bakery products, meringues and meat products, because of its excellent foaming and gelling properties. These properties are mainly due to egg white proteins that represent more than 80% of dry matter (Li-Chan, Powrie, & Nakai, 1995). Dehydrated egg products offer many advantages: longer shelf life, lower storage and transport costs, specific functional properties. However, during the production of egg white powder, the proteins are subjected to several processing steps with thermal, physical, interfacial and chemical treatments that may damage egg white functional properties (Mine, 1995). Proteins denature at least partially while they are subjected to heating (Kato, Tsutsui, Matsudomi, Kobayashi, & Nakai, 1981), shearing (Forsythe & Bergquist, 1951), air–liquid interface (Lechevalier et al., 2003; Lechevalier, Croguennec, et al., 2005). These treatments may explain at least part of the damages in egg white functional properties observed in industry and that is what this study aims at demonstrating.

Heating of egg white powder at 75–80 °C for 10–15 days is widely used in industry to offset functional property losses resulting from the spray-drying process: both gelling and foaming properties improve after such a dry-heating treatment (Handa, Hayashi, Shidara, & Kuroda, 2001; Kato, Ibrahim, Watanabe, Honma, & Kobayashi, 1989; Mine, 1996). However, it would be interesting to identify

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the critical steps of the drying process, to understand the mechanisms responsible for the functional property losses in order to limit their harmful effects, and thus to reduce storage time at high temperature. Hammershoj, Peters, and Andersen (2004) carried out such a study, in which they found a loss of gelling properties from raw egg white to powder that was offset by the final dry-heating. However, that study did not include analysis of protein structure and the process studied included extraction of lysozyme, which is not general in the egg white powder production.

The objective of the current study was thus to highlight critical processing steps of a classical drying process of egg white to explain the functional property modifications through the protein structure changes, and also to identify the kind of treatments (thermal, physical, interfacial, chemical) essentially responsible for the processing step effects.

2. Experimental

2.1. Samples

Egg white samples were collected at a French egg product company (Igreca, Seiches/Loir, France) at 11 steps along the spray-drying process of egg white, as shown in Fig. 1. The sampling at step 10 was performed by collecting egg white directly at the nozzle outlet, before drying, in order to obtain egg white that was only sprayed. This was done to distinguish between spraying and drying effects. All sampling was repeated twice with an interval of one month. Analyses were initiated on the day of sampling and were performed in triplicate.

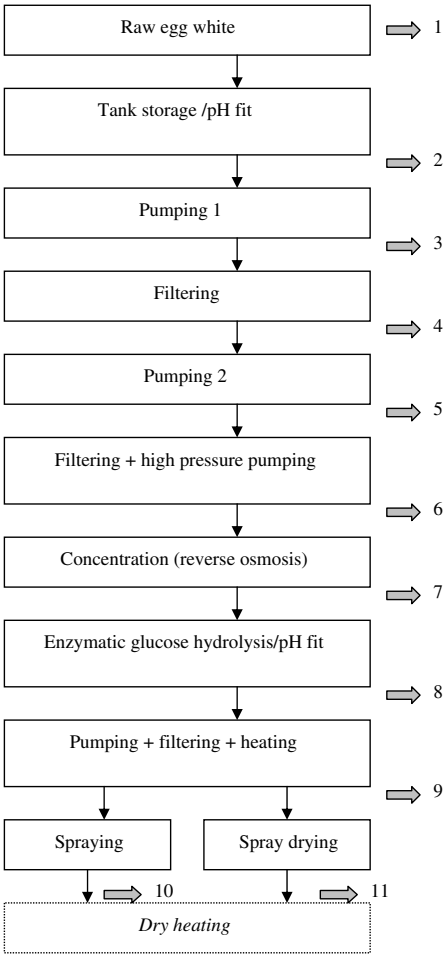

Fig. 1. Diagram of the egg white spray-drying process. The numbers 1 to 11 indicate the processing steps where samples were collected.

2.2. Chemical analyses

The dry matter (DM) content of the samples was analyzed by heating (106 °C, 18 h) 5 g of egg white mixed with 25 g of sand. The pH of liquid egg white and reconstituted egg white powder was measured using a Consort P600 pH meter. The DM and pH data were used to prepare the sample solutions for further analyses at standard concentration and pH conditions (120 $g.l^{-1}$ and pH 9.3, respectively). DM adjustments were done using deionised water; pH adjustments were done with NaOH 1 M.

2.3. Foaming properties

Foam was obtained at room temperature by whipping 200 ml of egg white in a Hobart N-50 mixer (Hobart Foster, Kampenhout, Belgium) at speed 3 during 2 min. Foam density was calculated from the mass of a given volume of foam, and foam stability as the percentage of liquid held in foam after 1 h, as suggested by Lechevalier, Périnel, et al. (2005).

2.4. Gelling properties

Egg white gels were prepared by heating 300 ml of egg white in plastic tubes (Krehalon, Deventer, Netherlands) in a water bath at 80 °C for 1 h, and subsequently cooled at room temperature for at least 4 h. After removing the tubes, cylindrical samples (2.9 cm diameter, 2 cm high) were cut using two parallel metal wires.

A uniaxial penetration test was performed until gel fracture on a TA-XT2 texture analyser (Rheo, Champlan, France) with a 1.7 cm diameter plate probe and a penetration speed of 1 $mm.s^{-1}$. Recordings of force (N) and displacement (m) were used to calculate work (J) on gel according to Hammershoj, Larsen, Ipsen, and Qvist (2001). The values obtained for work on gel were considered as gel firmness.

A uniaxial compression test was performed to measure the gel water holding capacity (WHC). A 5 mm displacement was maintained 30 s using a TA-XT2 texture analyser with a 5 cm diameter plate probe; compression speed was of 1 mm s^{-1}. The cylindrical gel was weighed before (mass M_1) and after (mass M_2) compression. The WHC was determined by Eq. (1), with M_0 the water content of the cylindrical gel (g) before compression.

$$\mathrm{WHC}\ (\%) = \frac{M_0 - (M_1 - M_2)}{M_0} \times 100 \qquad (1)$$

2.5. Protein structure analyses

Protein conformation in egg white were followed by measurements of the temperature of denaturation (TD) using differential scanning calorimetry (DSC), intrinsic fluorescence, protein surface hydrophobicity and absorbance variations (ΔA) in near and far UV by circular dichroism (CD).

DSC was performed using a Q1000 differential scanning calorimeter (TA Instruments, Paris, France). An amount of 90 µl of egg white was poured in hermetic stainless steel pans, and water was used as the reference. The temperature was scanned from 4 °C to 120 °C at a heating rate of 2 °C min^{-1}. The peak endotherm temperature for ovalbumin (ova), s-ovalbumin (S-ova) and ovotransferrin (ovt) were determined using TA data analysis software.

Intrinsic fluorescence measurements were performed using a spectrofluorimeter LS50B (Perkin–Elmer, Norwalk, USA). Egg white samples were diluted 600 times in 67 mM phosphate buffer pH 7.0 in order to be in the linearity domain of fluorescence versus protein concentration. The solutions were excited at 295 nm and emission spectra were registered between 305 and 415 nm with 1% attenuation. Excitation and emission slits were 15 nm. The fluorescence intensity and wavelength for maximal emission (λ) were registered.

Protein surface hydrophobicity was measured using the fluorescence probe anilino-1-naphtalene-8-sulfonate (ANS) and the same spectrofluorimeter as previously described. An amount of 15 µl of a 8 mM ANS solution were added to 1 ml of the egg white samples diluted as mentioned above. ANS fluorescence intensity was measured at 470 nm after excitation at 390 nm. Excitation and emission slits were 2.5 nm.

CD spectra were obtained on egg white samples diluted 100 times in 67 mM phosphate buffer pH 7.0 using a CD 6 spectropolarimeter (Y. Jobin, Paris, France). Far UV CD spectra were recorded from 180 to 250 nm with a 0.02 cm light path. Near UV CD spectra were recorded from 250 to 330 nm with a 1 cm light path. Each spectrum was the average of three scans integrated with the data processor CD6DOS (Y. Jobin, Paris, France). The values of ΔA measured at 295 nm in near UV were chosen to express tertiary structure changes while the values of ΔA measured at 222 nm in far UV were considered as representative of protein ellipticity changes.

2.6. Processing steps characterization

The type of energy (none, mechanical or thermal) transferred to the product, the nature of interfaces encountered by the product (none, air or stainless steel) and the presence or absence of shear rates were determined for each steps (Table 1).

2.7. Statistical analysis

Data were subjected to statistical analysis by the general linear model (GLM) procedure of Statgraphics Plus® (Manugistics, Rockville, MD, USA). The model of analysis was:

$$Y_{ij} = a_i + b_j + ab_{ij}, \quad (2)$$

where "*a*" is the processing step *i* (1,...,11) effect, "*b*" is the trial *j* (1,2) effect and "*ab*" is the interactive effect between the processing step *i* of the trial *j*. Processing step effect was defined as a fixed effect, whereas, trial effect was defined as a random effect. No replication effects were found, and therefore, excluded in the model. The LS-means were calculated and differences regarded as significant at a minimum 95% level ($p < 0.05$).

Data were also subjected to principal component analysis (PCA) using Copri procedure of SPAD® (Decisia, Pantin, France). PCA is a multivariate statistical method, which transforms a set of possibly correlated variables into new variables, which are mutually orthogonal (uncorrelated) linear combinations of the original variables. These new variables are called principal components (PC). Each principal component is defined by the coefficients in the linear combination of the original variables. Differences were classified by hierarchical cluster analysis (HCA) using Parti-decla procedure of SPAD® (Decisia, Pantin, France).

Table 1
Kind of energy transferred to egg white, nature of interfaces encountered by egg white and presence/absence of shear rates for each step of egg white powder process

Step	Energy	Interface	Shear rates
1 (Raw egg white)	None	None	Absence
2 (Tank storage, pH fit)	None	Air + stainless steel	Absence
3 (Pumping)	Mechanical	Stainless steel	Presence
4 (Filtering)	None	Stainless steel	Presence
5 (Pumping)	Mechanical	Stainless steel	Presence
6 (Filtering, pumping)	Mechanical	Stainless steel	Presence
7 (Concentration)	Mechanical + thermal	Stainless steel	Presence
8 (Desugarization)	None	Air + stainless steel	Absence
9 (Pumping, filtering, heating)	Mechanical + thermal	Stainless steel	Presence
10 (Spraying)	Mechanical	Air + stainless steel	Presence
11 (Spray drying)	Mechanical + thermal	Air + stainless steel	Presence

To identify the kind of treatment (energy, interface or shear rates) responsible for processing step effects, data were subjected to the GLM procedure of Statgraphics Plus® (Manugistics, Rockville, MD, USA). The model of analysis was:

$$Y_{ijkl} = e_i + i_j + s_k + b_l, \quad (3)$$

where "e" is the type of energy i (0 = none, 1 = mechanical, 2 = thermal + mechanical) effect, "i" is the type of interface j (0 = none, 1 = stainless steel, 2 = air + stainless steel) effect, "s" is the presence/absence of shear rates k (0 = absence, 1 = presence) effect, and "b" is the trial l (1,2) effect. The type of energy, the type of interface and the presence/absence of shear rates effects were defined as fixed effects, whereas, trial effect was defined as a random effect. No replication effects were analyzed because of the lack of degree of freedom. The LS-means were calculated and differences regarded as significant at a minimum 95% level ($p < 0.05$).

3. Results and discussion

3.1. Influence of trials

pH was significantly different from one trial to another ($p < 0.01$) (Table 2a). This was due to the decrease of pH during the second step that was stronger for trial 2 (Fig. 2). It can be noticed that after desugarization (step 8), pH values were back to the same level.

Trial also had an effect on gel firmness and all protein conformation measurements (Table 2a). This result was particularly well illustrated by the PCA (Fig. 3a and b). PCA indeed showed two groups of samples, corresponding to both trials, separated by PC 1, which explained 53.8% of the variability (Fig. 3a). This PC was strongly correlated with gel firmness (82%) and all measurements of protein structure: ΔA at 222 nm (96%), ΔA at 295 nm (−95%), protein surface hydrophobicity (93%), TD of s-ovalbumin (94%), TD of ovalbumin (85%), TD of ovotransferrin (70%), intrinsic fluorescence intensity (−67%) and λ at 295 nm (−69%) (Fig. 3b). Thus, from one batch to another, egg white proteins had different conformations that were probably responsible for the different gelling properties. The stiffest gels were obtained when the denaturation temperature, the surface hydrophobicity and the ellipticity of the protein was high, whereas, measurements at 295 nm (fluorescence intensity, maximal emission wavelength, absorbance variation in CD) were low. Parts of these results are in agreement with the literature since Handa et al. (2001) showed that gel firmness increased with protein surface hydrophobicity. On the opposite, Kato, Ibrahim, Watanabe, Honma, and Kobayashi (1990) showed that gel firmness increased with the decrease of denaturation temperature and ellipticity of egg white proteins, but they were working on single protein solutions and not with egg white, as in the present study.

Unfortunately, no data were available on the egg used since they came from the international market, and thus, these results can just be noticed. However, they are very similar to those we observed in a previous study (Lechevalier, Périnel, et al., 2005). In that case, the differences between the trials were attributed to the different origins of the eggs and also to different storage lengths, in agreement with the literature (Hammershoj et al., 2001; Hammershoj & Qvist, 2001; Hammershoj, Larsen, Andersen, & Qvist, 2002; Kreuzer, Jaenecke, & Flock, 1995). On the opposite, Hammershoj et al. (2004) found, to their own surprise, no significant differences between the six batches they studied.

3.2. Influence of processing steps

Processing steps had a significant effect on pH, dry matter content, foaming properties and intrinsic fluorescence measurements (intensity and λ_{295nm}) (Table 2a).

Most of the pH and dry matter content variations were intentional: the twofold and fivefold increase of DM after reverse osmosis concentration (step 7) and spray-drying (step 11), respectively, and the 1.7 pH unit decrease between first and second steps and between steps 7 and 8 (Table 2 b). However, an increase of 0.6 pH unit after spray-drying was noticed. This was not intentional but was expected since Hill (1964) explained it by egg white decarbonatation. At intermediate pH level (6.0–7.5), the carbon dioxide is, indeed, incompletely evolved during pH adjustment and is further expelled during the drying process, thus leading to a pH increase.

Processing steps had a significant effect on egg white foaming properties ($p < 0.05$ for foam density and $p < 0.1$ for foam stability) (Table 2a). During the process, foaming properties were firstly progressively damaged between steps 1 and 6 (foam density increased by 37%, whereas, foam stability decreased by 17%) (Table 2b). Then between steps 6 and 10, foaming properties progressively improved, recovering the loss they had undergone during the first part of the process. At last, step 11 definitely damaged foaming properties: foam density increased by 64% and foam stability decreased by 20%. This step, (spray drying) was thus decisive for egg white powder foaming properties.

The PCA and HCA plots in Fig. 3 illustrated these results. PC 2 that explained 17% of the variability, was strongly correlated with foam stability (−90%) and foam density (95%) (Fig. 3b). Hence, it can be termed foaming properties component. This PC enabled to separate the different clusters of samples determined by HCA (Fig. 3a). First of all, it can be noticed that raw egg white (step 1) had different foaming properties from one trial to another. This confirmed the variability of raw material already mentioned above. Step 2 resulted in an improvement of foaming properties for the first trial, whereas, it resulted in damage of foaming properties for the second trial. A same step can thus have opposite effects according to the state of the product on which it is performed. However, after step 2, foaming properties were equivalent for both trials. This

Table 2
Effect of trials and processing steps on egg white functional properties and protein structure tested by general linear model

(a)	Trial	Processing step	Trial × processing step	R^2 (%)
DM	0.2252	$<10^{-4}$	$<10^{-4}$	99.99
pH	0.0045	$<10^{-4}$	–	96.50
Foam properties				
Density	0.4157	0.0124	$<10^{-4}$	90.85
Stability	0.1146	0.0962	0.0022	74.04
Gel properties				
Firmness	0.0003	0.6071	$<10^{-4}$	96.32
WHC	0.9418	0.4172	$<10^{-4}$	76.41
Protein conformation				
$T_{\text{-ova}}$	0.0004	0.5477	–	78.38
$T_{\text{-ovt}}$	0.0027	0.7312	–	69.04
$T_{\text{-Sova}}$	$<10^{-4}$	0.8762	–	90.04
Intrinsic fluorescence intensity	0.0005	0.0599	$<10^{-4}$	94.92
$\lambda_{295\text{nm}}$ (nm)	0.0007	0.0938	0.8797	39.37
Surface hydrophobicity	$<10^{-4}$	0.1357	$<10^{-4}$	98.44
$\Delta A_{295\text{nm}}$	$<10^{-4}$	0.3967	0.0153	91.06
$\Delta A_{222\text{ nm}}$	$<10^{-4}$	0.8919	0.0005	94.40

(b)	Trials		Processing steps										
	1	2	1	2	3	4	5	6	7	8	9	10	11
DM (g kg^{-1})	229.7	220.6	117.6^{a}	151.3^{ab}	117.6^{a}	115.6^{a}	118.2^{a}	146.0^{a}	214.1^{c}	186.4^{bc}	189.3^{c}	189.5^{c}	931.0^{d}
SEM	0.49	0.49	1.16	1.16	1.16	1.16	1.16	1.16	1.16	1.16	1.16	1.16	1.16
pH	7.5^{a}	7.1^{b}	9.27^{a}	7.54^{b}	7.45^{b}	7.55^{b}	7.53^{b}	7.64^{b}	7.76^{b}	6.04^{d}	6.09^{d}	6.29^{d}	6.89^{c}
SEM	0.03	0.03	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
Foam properties													
Density (g l^{-1})	148.2	154.2	123.4^{a}	139.8^{abc}	143.5^{abc}	163.3^{bc}	170.9^{cd}	169.5^{c}	150.7^{abc}	126.5^{ab}	142.0^{abc}	126.4^{a}	207.2^{d}
SEM	5.00	5.00	11.73	11.73	11.73	11.73	11.73	11.73	11.73	11.73	11.73	11.73	11.73
Stability (%)	36.7	39.1	41.3^{abc}	38.0^{abcde}	39.1^{abcde}	34.7^{cde}	34.2^{de}	35.4^{bcde}	37.5^{abcde}	41.9^{ab}	42.5^{a}	40.2^{abcd}	32.2^{e}
SEM	0.95	0.95	2.24	2.24	2.24	2.24	2.24	2.24	2.24	2.24	2.24	2.24	2.24
Gel properties													
Firmness (J)	0.041^{a}	0.017^{b}	0.036	0.029	0.035	0.032	0.034	0.025	0.035	0.028	0.027	0.029	0.011
SEM	0.003	0.003	0.007	0.007	0.007	0.007	0.007	0.007	0.007	0.007	0.007	0.007	0.008
WHC (%)	99.289	99.284	99.054	99.478	99.275	99.278	99.294	99.201	99.396	99.330	99.273	99.246	99.327
SEM	0.043	0.041	0.097	0.108	0.097	0.097	0.097	0.097	0.097	0.097	0.097	0.097	0.108
Protein conformation													
$T_{\text{-ova}}$ (°C)	81.1^{a}	79.3^{b}	80.8	80.5	80.7	80.4	79.9	80.3	80.0	79.5	79.6	79.4	80.9
SEM	0.2	0.2	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
$T_{\text{-ovt}}$ (°C)	64.6^{a}	63.3^{b}	64.5	64.8	63.7	63.7	63.2	63.8	63.4	64.2	64.3	64.5	64.8
SEM	0.2	0.2	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
$T_{\text{-Sova}}$ (°C)	87.4^{a}	84.7^{b}	85.6	86.5	86.4	86.3	86.1	85.6	86.1	85.8	85.8	85.7	86.6
SEM	0.2	0.2	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Intrinsic fluorescence intensity (AU)	209.8^{a}	232.5^{b}	239.4^{a}	229.6^{abc}	220.4^{abcd}	236.0^{ab}	209.4^{cd}	224.4^{bcd}	212.1^{cd}	221.5^{abc}	213.9^{bcd}	196.9^{d}	229.5^{abc}
SEM	3.2	3.2	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
$\lambda_{295\text{nm}}$ (fluo) (nm)	342.05^{a}	342.19^{b}	342.05^{ab}	342.25^{d}	342.1^{abc}	342.11^{abcd}	342.12^{abcd}	342.21^{cd}	342.14^{bcd}	342.19^{bcd}	342.09^{abc}	342.11^{abcd}	341.98^{d}
SEM	0.02	0.02	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Surface hydrophobicity (AU) SEM	647.5^{a} 11.8	323.9^{b} 11.8	545.2 27.7	492.0 27.7	528.0 27.7	510.7 27.7	437.9 27.7	484.2 27.7	458.2 27.7	445.6 27.7	447.5 27.7	455.5 27.7	537.8 27.7
$\Delta A_{295\text{nm}}$ ($\times 10^{-4}$)	−2.97^{a}	−0.02^{b}	−1.37	−1.52	−1.53	−1.39	−1.26	−1.09	−1.47	−1.63	−2.11	−2.31	−0.76
SEM	0.17	0.17	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
$\Delta A_{222\text{ nm}}$ ($\times 10^{-4}$)	−1.66^{a}	−5.07^{b}	−3.15	−3.45	−3.19	−3.38	−3.64	−3.22	−3.0	−3.15	−4.07	−3.33	−3.4
SEM	0.19	0.19	0.44	0.44	0.44	0.44	0.44	0.44	0.44	0.44	0.44	0.44	0.44

(a) p-Values given by the analysis of variance and (b) LS-means ($n = 6$) and standard error of the mean values.
Letters mean in a row with different superscripts are significantly different at $p < 0.05$.

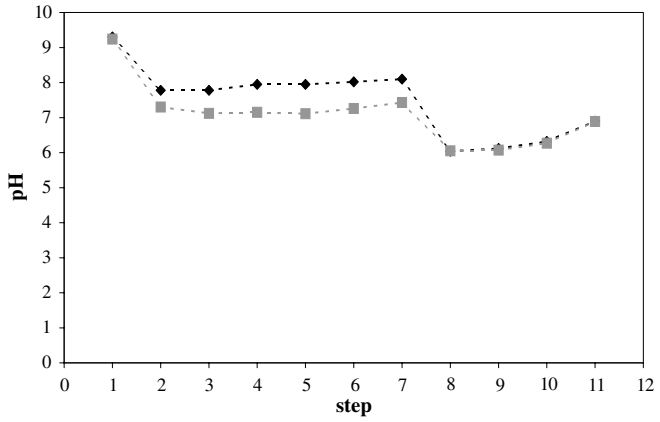

Fig. 2. pH of egg white as function of processing steps for both trials (trial 1 in black and trial 2 in grey). Standard deviations are indicated but are too small to be seen ($n = 3$).

pH fit enabled thus to abolish the variability due to different raw materials. It should be noticed here that all samples were standardized in DM and pH before analyses; the foaming properties variations previously mentioned were then probably due to modifications of protein conformation, irreversible even after readjustment to original pH. A significant red shift in maximal emission wavelength after excitation at 295 nm was indeed measured (Table 2b), assuming protein unfolding. Such results were already mentioned by Liang and Kristinsson (2005) but with more drastic pH variations: decreasing egg white pH to 3.5 and then increasing it to 8.5 improved significantly egg white foaming properties. These authors found a good correlation between this improvement and an increase in protein surface hydrophobicity measured with PRODAN (6-propionyl-2-(*N*,*N*-dimethyl-amino)naphthalene). In the present study, on the opposite, protein surface hydrophobicity was not correlated with foaming properties (correlation coefficients were 5% and 33% between surface hydrophobicity and foam density and foam stability, respectively) since it was correlated with PC1, whereas, foaming properties were correlated with PC2 (Fig. 3b). However, we used ANS to measure protein surface hydrophobicity and Haskard and Li-Chan (1998) and Alizadeh-Pasdar and Li-Chan (2000) showed that the results for protein surface hydrophobicity differ according to the probe used, and especially pH and ionic strength may have different effects on protein surface hydrophobicity according to the probe used. This may explain the difference between our results and those of Liang and Kristinsson (2005).

Filtering and pumping steps (3, 4, 5, and 6) progressively damaged foaming properties (Table 2b), resulting in foaming properties slightly lower than the average for both trials (Fig. 2a). During these steps, λ_{295nm} tended to increase, whereas, the signal of aromatic residues in CD and protein surface hydrophobicity tended to decrease, assuming protein aggregation that would be responsible for the loss of foaming properties. Aggregated proteins have indeed a higher hydrodynamic radius, a lower surface hydrophobicity and a lower flexibility that are harmful for protein diffusion and rearrangement at the air–water interface, and thus for foaming properties (Damodaran, 1997; Gekko & Yamagami, 1991; German, O'Neill, & Kinsella, 1985; Kato et al., 1990; Kato & Nakai, 1980).

Foaming properties, then, progressively improved during the steps of concentration, desugarization and spraying (steps 7, 8, and 10), as suggested by the rather high coordinates of these steps on PC2 (Fig. 3a): foam density decreased by 25%, whereas, foam stability increased by 13.5% between steps 7 and 10 (Table 2b). Concentration was performed in this study by reverse osmosis and foaming properties were measured after a fit of the dry matter content. Previous studies mentioned damaging (Conrad, Mast, Ball, Froning, & Mac Neil, 1993) or no (Froning, Wehling, Ball, & Hill, 1987) effect of reverse osmosis on egg white foaming properties. However, these studies were carried out on the powder obtained from the concentrate and not, as in the present study, on the concentrated egg white itself. It is thus not excluded that possible modifications of foaming properties, due to concentration, could be hidden by the drastic effect of the spray-drying step on these properties, thus explaining the different results between the present study and those mentioned above.

Desugarization also improved foaming properties (Table 2b). During this step, pH was lowered to 6.0 to enable enzymatic reaction. At this pH and in the absence of sugar, protein interfacial unfolding was favored (Damodaran, 1997). Proteins adsorbed more easily at the interface because of their higher flexibility (Gekko & Yamagami, 1991; Kato et al., 1989). In the present study, no significant effect of desugarization was observed on protein structure, however, the upward trends of intrinsic fluorescence intensity and variation of absorbance at 295 nm in CD during this step may highlight protein unfolding.

Sprayed egg white (step 10) showed good foaming properties, as suggested by the coordinates of step 10 on PC 2 (Fig. 3a). On the opposite, spray-dried egg white (step 11) showed the worse foaming properties. This step also significantly decreased intrinsic fluorescence intensity (Table 2b) and the same trend was observed for the variation of absorbance at 295 nm in CD and for protein surface hydrophobicity, showing the formation of aggregates, thus explaining foaming properties decrease. The harmful effect of spray drying on foaming properties seemed thus being due to heat transfer and not to shear rates during spraying, as suggested by Bergquist (1995). This author, indeed, attributed the loss of foaming properties to spray-drying process in itself but also to the physical treatments, especially spraying, it involved. On the other hand, Bergquist and Stewart (1952) did not notice any damage of spraying on foaming properties with droplets diameter lower than 15 μm, which was the case in the present study. This latter highlighted thus the great effect of heat transfers during spray-drying step; effects which were probably extended by the presence of wide air–product interfaces.

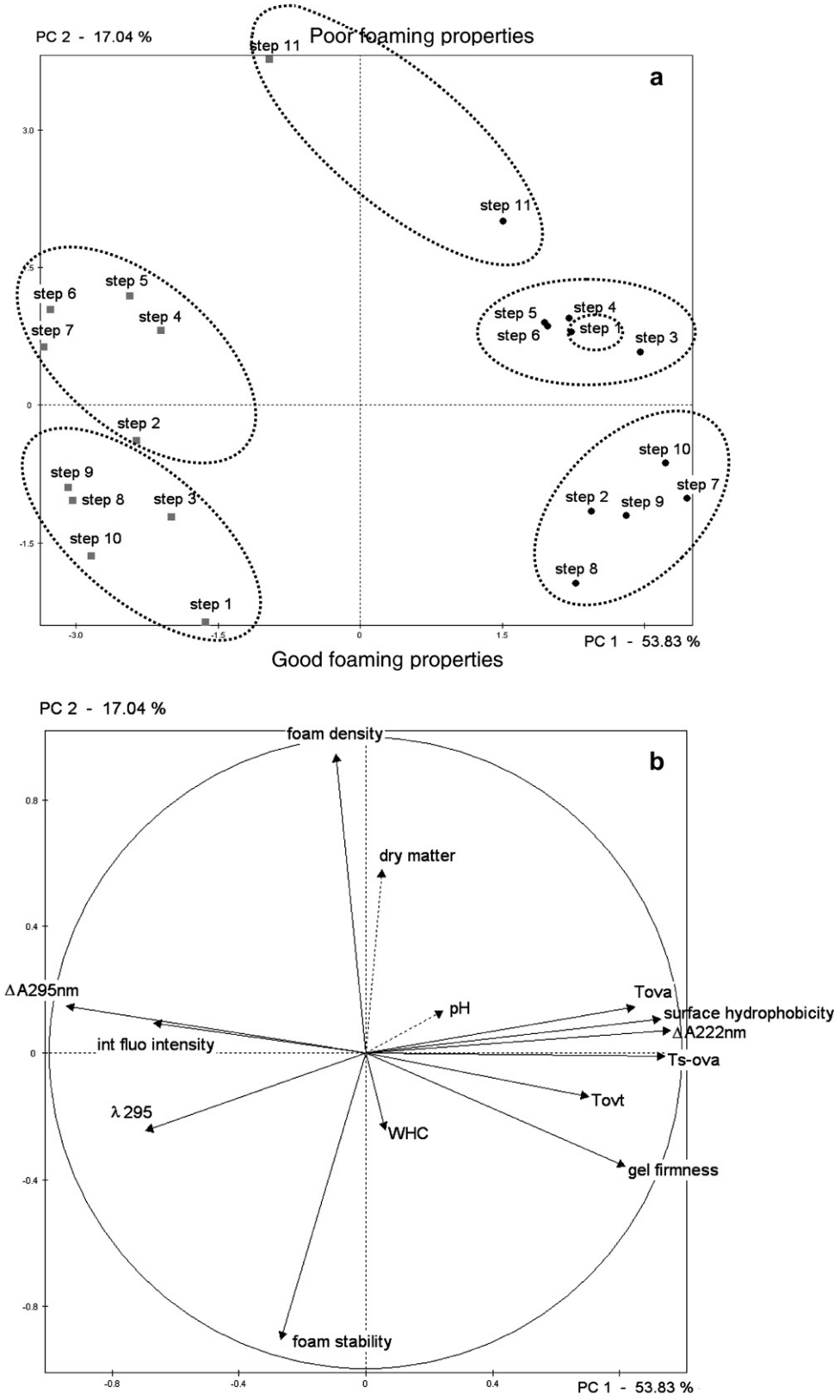

Fig. 3. PCA showing first two principal components (PC) of significant variables: A, similarity map determined by PCs 1 and 2 for samples (black circles: trial 1, grey square: trial 2). Samples in a same dotted circle belong to the same cluster determined by hierarchical cluster analysis; B, correlation circle of continuous variables (DM, pH, foaming and gelling properties, protein conformation).

3.3. Influence of the type of treatment

Processing steps considered individually had a significant effect only on foaming properties. Another way to analyze the results of this study is to consider the type of treatment applied at each step and to look at the treatment effect on egg white functional properties and protein structure (Table 3).

Shear rates were the only treatment that had a significant effect on foaming properties (Table 3a). They were thus damaged after steps that applied shear rates to the product: +33% for foam density, −16% for foam stability. This result is fortunately in agreement with processing step effect on foaming properties, since steps during which shear rates were applied were filtering and pumping steps (steps 3, 4, 5, 6 and 9). Few literatures are available about effects

Table 3
Effect of trials and kind of treatments on egg white functional properties and protein structure tested by general linear model

(a)	Energy	Interface	Shear rates	Trial	R^2 (%)
Foam properties					
Density	0.1859	0.3645	0.0092	0.3271	29.75
Stability	0.3013	0.4971	0.0137	0.0380	20.78
Gel properties					
Firmness	0.2843	0.0048	0.2754	$<10^{-4}$	70.57
WHC	0.2408	$<10^{-4}$	0.1221	0.9301	32.16
Protein conformation					
$T_{\text{-ova}}$	0.8510	0.5591	0.6041	0.0002	62.37
$T_{\text{-ovt}}$	0.8172	0.6648	0.5570	0.0005	62.24
$T_{\text{-Sova}}$	0.8529	0.6355	0.7904	$<10^{-4}$	86.24
Int fluo intensity	0.0007	0.0547	0.3327	$<10^{-4}$	62.45
$\lambda_{295\text{nm}}$ (nm)	0.4833	0.0397	0.0539	0.0006	28.36
Surface hydrophobicity	0.1840	0.0032	0.0235	$<10^{-4}$	92.95
$\Delta A_{295\text{nm}}$	0.8759	0.8502	0.7777	$<10^{-4}$	79.86
$\Delta A_{222\text{ nm}}$	0.7809	0.8726	0.9870	$<10^{-4}$	87.14

(b)	Energy			Interface			Shear rates		Trial	
	0	1	2	0	1	2	0	1	1	2
Foam properties										
Density (g l^{-1})	146.7	133.4	146.6	139.1	138.7	148.8	122.1[a]	162.4[b]	139.2	145.2
SEM	6.3	8.0	8.6	11.4	7.5	5.4	10.3	7.1	5.6	5.6
Stability (%)	37.5	40.5	40.8	39.9	40.2	38.6	43.0[a]	36.1[b]	38.4[a]	40.7[b]
SEM	1.1	1.4	1.6	2.1	1.4	1.0	1.9	1.3	1.0	1.0
Gel properties										
Firmness (J)	0.031	0.032	0.027	0.032[ab]	0.034[a]	0.024[b]	0.034	0.027	0.043[a]	0.018[b]
SEM	0.002	0.003	0.003	0.004	0.003	0.002	0.004	0.003	0.002	0.002
WHC (%)	99.219	99.200	99.273	98.999[a]	99.356[b]	99.338[b]	99.297	99.165	99.229	99.232
SEM	0.035	0.044	0.048	0.064	0.042	0.031	0.057	0.040	0.026	0.026
Protein conformation										
$T_{\text{-ova}}$ (°C)	80.51	80.12	80.25	80.84	80.01	80.03	80.05	80.53	81.18[a]	79.41[b]
SEM	0.38	0.48	0.52	0.69	0.45	0.32	0.62	0.43	0.34	0.34
$T_{\text{-ovt}}$ (°C)	64.14	64.08	64.32	64.28	63.94	64.32	64.40	63.96	64.84[a]	63.52[b]
SEM	0.31	0.40	0.42	0.56	0.37	0.26	0.51	0.35	0.28	0.28
$T_{\text{-Sova}}$ (°C)	86.08	85.79	85.93	85.60	86.06	86.15	85.84	86.03	87.30[a]	84.57[b]
SEM	0.30	0.39	0.41	0.54	0.36	0.25	0.49	0.33	0.27	0.26
Intrinsic fluorescence intensity (AU)	234.8[a]	212.4[b]	218.4[b]	230.0[a]	219.5[ab]	216.1[b]	218.3	225.4	210.5[a]	233.2[b]
SEM	3.1	3.9	4.1	5.5	3.6	2.6	5.0	3.4	2.7	2.7
$\lambda_{295\text{nm}}$ (fluo) (nm)	342.10	342.14	342.08	341.97[a]	342.22[b]	342.13[b]	342.20[a]	342.01[b]	342.04[a]	342.18[b]
SEM	0.04	0.05	0.05	0.07	0.05	0.03	0.07	0.05	0.04	0.04
Surface hydrophobicity (AU)	519.4	478.8	481.4	552.6[a]	451[b]	476.2[b]	459.7[a]	526.8[b]	655.0[a]	331.5[b]
SEM	12.2	15.4	16.6	22.0	14.5	10.3	19.8	13.7	10.8	10.8
$\Delta A_{295\text{nm}}$ ($\times 10^{-4}$)	−1.42	−1.57	−1.46	−1.36	−1.52	−1.57	−1.55	−1.42	−2.96[a]	−0.007[b]
SEM	0.20	0.25	0.27	0.36	0.24	0.17	0.33	0.23	0.18	0.18
$\Delta A_{222\text{nm}}$ ($\times 10^{-4}$)	−3.28	−3.26	−3.41	−3.19	−3.41	−3.34	−3.31	−3.32	−1.6[a]	−5.0[b]
SEM	0.18	0.22	0.24	0.32	0.21	0.15	0.29	0.20	0.16	0.16

(a) p-Values given by the analysis of variance and (b) LS-means ($n = 33$) and standard error of the mean values. Energy 0, 1 and 2 means absence, mechanical and thermal + mechanical, respectively. Interface 0, 1 and 2 means absence, stainless steel and air + stainless steel, respectively. Shear rates 0 and 1 means absence and presence, respectively.

[a,b] Mean in a row with different superscripts are significantly different at F-test level.

of shear rates on egg white functional properties. Since Forsythe and Bergquist (1951) and Thapon (1981), very little studies were devoted to this topic; generally, shear rate effects have been explained by ovomucin modifications. The results of the present study suggest that this protein is maybe not the only one involved, since shear rates also had a significant effect on protein surface hydrophobicity and maximal emission wavelength (λ_{295nm}) in intrinsic fluorescence (Table 3a). Some studies carried out on whey proteins (Oliva, Santovevena, Farina, & Llabres, 2003) and on human growth factor and human desoxyribonuclease (Maa & Hsu, 1997) showed that shear rate effects depend on the protein considered. For example, β-lactoglobuline underwent a reversible first order denaturation kinetic, whereas, human and bovine serum albumin underwent monomer–dimer transitions (Oliva et al., 2003). In the same way, human growth factor formed aggregates, whereas, no significant structure changes were noticed for human desoxyribonuclease (Maa & Hsu, 1997). In the present study, such conclusions can hardly be drawn because of the complexity of egg white, mixture of several proteins. However, the blue shift of λ_{295nm} and the increase in protein surface hydrophobicity with shear rates (Table 3b) suggested complex protein tertiary structure modifications since the blue shift suggested protein folding or aggregation, whereas, the increase in protein surface hydrophobicity rather assumed protein unfolding.

If processing steps did not have individual significant effect on egg white gelling properties, the kind of interface encountered by the product had a significant effect on these properties (Table 3a). Gel WHC significantly increased after steps where either stainless steel–product or both air– and stainless steel–product interfaces were present. On the other hand, gel firmness was significantly weaker (−31%) after steps where both air– and stainless steel–product interfaces were present compared with steps where none or only stainless steel–product interface existed. Gel WHC was thus modified by the presence of stainless steel–product interfaces, whereas, gel firmness was rather dependent on the presence of air–product interfaces. At the same time, intrinsic fluorescence intensity and protein surface hydrophobicity significantly decreased by 5–6% and 14–18%, respectively, whereas, λ_{295nm} increased by 0.25 to 0.15 nm once stainless steel–product or both air– and stainless steel–product interfaces were present (Table 3b). The modifications of gelling properties observed in the presence of stainless steel–product or both air– and stainless steel–product interfaces may thus be attributed to protein aggregation. Such results were already found in a previous study (Lechevalier, Périnel, et al., 2005). At the air–product interface, ovalbumin, ovotransferrin and lysozyme are able to aggregate thanks to electrostatic interactions and intermolecular disulfide bonds formation (Lechevalier, Croguennec, et al., 2005). As aggregation occurs before gel formation, an unstable fragile coagulum is formed, which explains gel firmness decrease (Ferry, 1948).

Surprisingly, the type of energy transferred to egg white did not have any significant effect on its functional properties. However, it had a significant effect only on intrinsic fluorescence intensity (Table 3a), that decreased by 7–10% once mechanical or both thermal and mechanical energy was transferred to the product, assuming protein aggregation (Table 3b). Thus, after energy transfer to the product, contact with interfacial area or shear rates, proteins seemed to aggregate. All these effects have been described in the literature (Johnson and Zabik, 1981; Lechevalier, Croguennec, et al., 2005; Liang and Kristinsson, 2005; Matsuda et al., 1981; Meyer and Potter, 1974).

4. Conclusions

This study highlighted, one more time, the great variation in raw material characteristics. This variability was particularly visible on egg white protein conformation and gelling properties. Surprisingly, there was no trial, and thus no raw material, effect on egg white foaming properties. This was due to the pH fit at the beginning of the process that standardized the product. On the other hand, industrial steps of egg white powder process were responsible for significant changes in foaming properties. They were damaged during the few first filtering and pumping steps, because of the shear rates and stainless steel–product interfaces they implement. Then, they were improved during concentration, desugarization and spraying. These steps had thus an interesting effect from an industrial point of view. However, we tested here only one kind of concentration and desugarization. A comparative study with the other existing methods would be of great interest. At least, foaming properties were definitely damaged during spray-drying step. This study confirmed thus, the harmful effect of spray drying and put it down to heat treatment associated with a wide interfacial area rather than shear rates existing in the nozzles.

In this study, interactions between the different types of treatment could not be considered because of a lack of degree of freedom. In this way, an outlook of this work would be to consider interactions between energy, shear rates and interface creation on functional properties changes. Ultimate step would be to consider the influence of each step on functional properties with a kinetic and energetic point of view with regard to residence time distribution of the product in the installation.

An attempt of structure–function correlation was performed during this study. It highlighted that protein structure measurements were well correlated with gel firmness but absolutely not with foaming property measurements. The mechanisms involved in egg white functional properties cannot thus be simply explained by protein structure measurements, in such a complex solution as egg white. Further experiments to explain the different kind of treatment effects on the structure of isolated egg white proteins are currently in progress to try to modelize the structure–function relationship.

According to this study, egg product manufacturers have now several levers to improve their process. They can indeed choose between a better control of the last step of drying, especially concerning thermodynamic parameters, and to make the most of the beneficial effect of concentration and desugarization.

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