



Effect of dry heat treatment of egg white powder on its functional, nutritional and allergenic properties



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ABSTRACT

Egg white is a key ingredient in many food products as it combines high nutritional quality with excellent functional properties. However, it is also one of the leading causes of food allergy in childhood. Dehydrated egg white is a common form of industrial egg white. To increase the functional properties, egg white powders are heated which may change protein antigenicity and susceptibility to digestion. The present work highlighted the effect of a wide range of dry heating rates (from 1 to 10 days between 60 and 90 °C) simultaneously on the interfacial properties, antigenicity and susceptibility to *in vitro* digestion of egg white proteins. Thanks to a powerful statistical methodology, i.e. multiple factor analysis (MFA), that enables to consider all the data in a common space, intermediate dry heating treatments (2–5 days at 70 °C or 1–2 days at 80 or 90 °C) were found to be good compromise to improve egg white functionality without enhancing too much protein resistance to digestion or protein antigenicity.

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1. Introduction

Egg white is a key ingredient in many food products as it combines high nutritional quality (Seuss-Baum et al., 2011) with excellent functional properties (Lechevalier et al., 2011). However, egg white is also one of the leading causes of IgE mediated food allergy in childhood (Moneret-Vautrin, 2008).

Egg white is commercialised as liquid, frozen or spray dried, the last one being a common and convenient form. After spray-drying, egg white powders are stored in hot rooms under controlled temperature (over 60 °C) and humidity conditions during a few days to ensure their microbiological safety. This industrial practice, called dry heating, is also very effective to improve the gelling and

foaming properties of egg white when performed at higher temperatures (over 70 °C) (Kato et al., 1989, 1990a,b). Many studies have thus been carried out to investigate the effect of dry heating on foaming and gelling properties of either egg white, or purified egg white proteins (Baron et al., 2003; Hammershoj et al., 2006b; Desfougères et al., 2008; Matsudomi et al., 1991; Mine, 1996, 1997). Fewer studies were dedicated to egg white emulsifying properties (Kato et al., 1989). Most of these studies were performed at a given dry heating temperature, often 80 °C, for 5–10 days. Talansier et al. (2009) explored the consequences of different time-temperature tables (from 1 to 7 days and 60–80 °C) on the interfacial properties of egg white and its protein structure. These authors highlighted that mild treatments (between 70 °C for 3 days, and 80 °C for 1 day) offered a good compromise to optimize both foaming capacity and foam stability.

Because of the well-known relationship between protein functionality and protein structure, many studies were also performed to understand the effect of dry heating treatment on the structure of egg white proteins (Hammershoj et al., 2006a; Matsudomi et al., 2001; Nicorescu et al., 2011; Van der Plancken et al., 2007; Watanabe et al., 1999; Xu et al., 1998). Protein aggregation and an increase in protein surface hydrophobicity were the main changes

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noticed by the authors. Recently, Desfougères et al. (2011a) highlighted chemical modifications of lysozyme induced by dry heating (formation of one to five cyclic imide rings) leading to the increase in the protein net charge and hydrophobicity but without any changes in protein secondary or tertiary structures. These modifications were correlated with the strong increase of the lysozyme interfacial properties after dry heating. These improved properties were due to a faster and more efficient adsorption of the protein at the air-water interface, and to the thick and strong viscoelastic interfacial film the dry heated lysozyme created (Desfougères et al., 2011b). Undoubtedly, dry heating modifies protein properties, and one can thus wonder if such changes may alter other protein properties such as digestibility and/or antigenicity.

The high content of essential amino acids in egg white proteins and the high bioavailability of these proteins are of great benefit to human nutrition. However, the effects of industrial processing such as dry heating on the nutritional quality of egg white proteins are poorly documented. Some studies considered the effect of dry heating on the *in vitro* digestibility of proteins as it is a prerequisite to nutritional quality. Schmidt et al. (2007) did not find any significant effect of dry heating duration at 70 °C on the *in vitro* digestibility of egg white proteins. Watkins and Veum (2010), however, showed a negative effect of dry heating (30 min at 121 °C) on the growth performance of neonatal pigs. Some studies also investigated the heat treatment effect on the digestibility of purified proteins in solution. Jimenez-Saiz et al. (2011) thus highlighted an increase of the *in vitro* digestibility of ovalbumin treated up to 15 min at 95 °C, whereas the digestibility of ovomucoid was not affected by this treatment.

In the same way, the changes in protein structure induced by dry heating may hide, destroy or disclose protein allergenic epitopes. Moreover, the potential changes in protein digestibility can also change the ability to sensitize and elicit the immune response (Jimenez-Saiz et al., 2011). The effect of heat treatment on the allergenic properties of egg proteins has been focused on cooking since some children with egg allergy tolerate cooked eggs (Hirose et al., 2004; Jimenez-Saiz et al., 2011; Julia et al., 2007; Lemon-Mulé et al., 2008; Liu et al., 2013; Mine and Zhang, 2002; Shin et al., 2013; Tong et al., 2012; Urisu et al., 1997). In most cases, physico-chemical changes caused by cooking enhanced either a decrease or no significant effect on protein allergenicity, depending on the susceptibility of the proteins to unfold and lose their conformational epitopes. But up to now, no study was performed to test the effect of dry heating on egg white antigenicity or allergenicity. Only Escudero et al. (2013) tested dehydrated egg white in oral food challenges as it is microbiologically safer than raw egg white. As they tested commercial dehydrated egg white, one can think it was dry heated egg white, even if the process conditions were not mentioned by the authors. They showed that dehydrated egg white was not less allergenic than raw egg white.

Up to now, the few studies carried out on dry heating of egg white powder focused on the effect on either functional properties or protein digestibility or again protein immunoreactivity or allergenicity. The present work proposes a multicriteria approach by testing a wide range of industrial dry heating conditions simultaneously on protein interfacial properties, antigenicity and digestibility. The aim is to determine the treatment conditions that optimize protein interfacial properties neither decreasing their digestibility nor increasing their antigenicity. To do so, a powerful statistical tool, i.e. multiple factor analysis (MFA) was applied to the data set. As explained by Lechevalier et al. (2015), MFA considers several sets of variables simultaneously as active elements, in order to take all of them into account in the definition of the distance between the conditions tested. Moreover, as a multivariate method, MFA reduces the complexity of the dataset and is less affected by

data distribution or variance heterogeneity than univariate methods. The originality of this work rests on the use of this method that allows considering several treatment conditions on many measurements of different nature (protein interfacial properties, digestibility, immunoreactivity, ...).

2. Material and methods

2.1. Experimental design

Raw egg white powder (93.7% of dry matter) was supplied by Igreca (Seiches sur le Loir, France). The powder was aliquoted into 17 samples of 100 g. Each sample was subjected to one dry heating treatment according to the following experimental design: complete factorial design 4^2 with 4 temperatures (60, 70, 80 and 90 °C) and 4 durations (1, 2, 5 and 10 days); the control sample was maintained at 20 °C.

2.2. Protein solubility

Egg white solutions were prepared by dissolution of 1 mg of egg white powders in 1 ml of distilled water, and equilibration for 1 h at room temperature under continuous stirring with a magnetic stir bar. Protein content was measured in these solutions, and in the corresponding supernatants obtained after centrifugation for 20 min at 10,000g, and 10 °C. Protein content was determined according to Markwell et al. (1978) after calibration with bovine serum albumin as a standard. Protein solubility was calculated as follows:

$$\text{solubility}(\%) = \frac{C_s}{C_i} \times 100 \quad (1)$$

where C_s and C_i are the protein concentrations (in g.l^{-1}) in supernatant and initially, respectively.

2.3. Interfacial properties

2.3.1. Langmuir isotherms at air-water interface

Measurements of the surface pressure (Π) - surface area (A) isotherms have been performed by compression - expansion cycles using the Wilhelmy plate method as described by Rannou et al. (2015). Egg white powders were solubilised in 50 mM phosphate buffer pH 7, NaCl 300 mM, and stirred for 2 h at 20 °C, before centrifugation for 20 min at 10,000g and 10 °C. The final concentration of supernatant was adjusted to 1 mg ml^{-1} after measurement of protein content according to Markwell et al. (1978). One hundred microliters of 1 mg ml^{-1} protein solutions were dropped with a micrometric syringe all along the surface of the phosphate buffer. In order to allow protein spreading, adsorption and rearrangements, samples were dropped 30 min before compression. The compression speed was maintained constant at $40 \text{ cm}^2 \text{ min}^{-1}$, i.e. low enough to prevent secondary effects due to the barrier displacement. Three replicated isotherms were performed for each sample. The surface corresponding to an interfacial pressure of 10 mN m^{-1} (S_{10}) was selected as the indicator of the sample behaviour at the air-water interface.

2.3.2. Emulsifying properties

2.3.2.1. Preparation of oil-water emulsions. Emulsions were prepared as described by Rannou et al. (2015). The aqueous phase was the supernatant adjusted at 15 mg ml^{-1} obtained after centrifugation for 20 min at 10,000g and 10 °C of egg white powders dissolved at 20 mg ml^{-1} in 50 mM phosphate buffer pH7, NaCl 300 mM. For each sample, emulsions were done in triplicate.

2.3.2.2. Droplet size distribution. The droplet size distribution (DSD) was determined as described by Rannou et al. (2015) and modified as follows: in order to measure the individual droplet size, 250 μL of emulsion were diluted in 4750 μL of 1% SDS (w/vol) to avoid flocculation.

Emulsions were manually mixed and equilibrated for 1 h at room temperature before measurement. The refractive index of oil was 1.475, and the imaginary part of the refractive index (due to absorption) was fixed at 0.01. Values of the volumetric mean diameter, $d_{4,3}$, were calculated as follows:

$$d_{4,3} = \frac{\sum n_i d_i^4}{\sum n_i d_i^3} \quad (2)$$

where n_i is the number of droplets with a diameter d_i .

2.3.2.3. Emulsion stability. Emulsion stability towards creaming was determined as follows: 10 ml of emulsion were poured into 15 ml graduated tubes and stored at room temperature (around 18 °C) for 24 h. After centrifugation at 2,000g for 30 min at 10 °C, the volume of the separated aqueous phase at the bottom of the tube was recorded. The creaming index (Cr_i) was calculated as follows:

$$Cr_i = \frac{V_a}{V_t} \times \frac{100}{\phi} \quad (3)$$

where V_a is the volume of separated aqueous phase, V_t is the total volume of emulsion in the tube after centrifugation, and ϕ is the volumic fraction of the aqueous phase used to prepare the emulsion (i.e. 0.7). The higher the creaming index, the lower the emulsion stability, since it assumes a high release of aqueous phase due to a high compaction of droplets with centrifugation.

2.4. Protein *in vitro* digestion and characterization of digested samples

2.4.1. *In vitro* digestion

Each dry heated sample and the control sample were subjected to an *in vitro* digestion model which mimics successive gastric and intestinal stages of digestion in the adult human as proposed by Dupont et al. (2010) and detailed by Lechevalier et al. (2015). One ml of protein solution (10 g l⁻¹) was aliquoted before digestion (gast ti), 15s after gastric enzyme addition (gast t+), at the end of the gastric stage (gast tf) and at the end of the intestinal stage (int tf).

2.4.2. Degree of hydrolysis (DH)

The DH was calculated from the measurement of amino groups released during *in vitro* digestion. It is a quantitative and global indicator for the extent of protein hydrolysis. Free amino groups (NH₂) released during egg white hydrolysis were determined using OPA (orthophthalaldehyde) according to Church et al. (1983). The OPA assay was carried out by adding 75 μL of sample to 1.5 ml OPA/ β mercaptoethanol reagent. The absorbance of the mixture was measured after 2 min exactly, at 340 nm with a 6505 UV–Vis spectrophotometer (Jenway, Staffordshire, UK). A standard curve was prepared using methionine (Sigma). The total NH₂ was determined in non-dry heated egg white powder after complete hydrolysis in 6 N HCl at 110 °C for 24 h (L_{tot}). The degree of hydrolysis (DH) was calculated using the following formula:

$$\% DH = (L_t - L_0) / L_{\text{tot}} \times 100 \quad (4)$$

where L_t is the amount of free NH₂ after t min hydrolysis, L_0 is the

amount of initial free NH₂ (before digestion), and L_{tot} is the maximum amount of free NH₂, i.e. after total acid hydrolysis. Free NH₂ was expressed in mg per g of protein. Measurement were done in triplicate.

2.4.3. Electrophoresis

The digested samples were analysed by SDS-PAGE on 12.5% polyacrylamide gels, according to Laemmli (1970), modified as explained by Lechevalier et al. (2015).

Samples were distributed onto 34 gels. For each dry heated egg white (one temperature, one duration) and the control, one gel was prepared with the samples of the gastric stage, and another gel was prepared with the samples of the intestinal stage.

The gels were Coomassie Blue stained, and digitised using the Image Scanner III device (GE Healthcare Europe GmbH). ImageQuant software (GE Healthcare) was used to quantify the band by Gaussian fitting, and to match the gels. All matches were checked manually and corrected if necessary. The band volume was used as the response variable in the statistical analysis. Up to 22 bands per sample could be identified (Supplementary Data 1).

The final data set was composed of 68 different samples: 17 powders $\times$ 4 digestion times (gast ti, gast t+, gast tf, int tf) (Supplementary Data 1). The band volumes measured at gast ti were used to characterize sample protein composition after dry heating treatment and before digestion; they were analysed separately by principal component analysis as described in section 2.6.2.

2.4.4. Pre-processing of the data

As explained by Lechevalier et al. (2015), raw electrophoresis data are not suitable for processing by statistics: pre-processing of these data was thus necessary. Briefly, few values were missing in the final dataset, because not all the electrophoretic bands were systematically present and hence not systematically detected by ImageQuant software in all samples. In such cases, a zero value was assigned manually after checking the absence of the corresponding band on the gel.

Since the 68 samples were not randomized on the 34 gels, band volumes were normalized using the 30 kDa band of the LMW kit to avoid any differences in staining from one gel to another. The volume of this band on the gel prepared with the digested gastric samples of the control powder was taken as the reference value ($Gel_{\text{ref}}BV$). The volume of the 30 kDa band for each of the other 33 gels was called Gel_nBV ($1 < n < 33$). The correction coefficient (CC) for the gel n was then calculated as follows:

$$Gel_nCC = \frac{Gel_nBV}{Gel_{\text{ref}}BV} \quad (5)$$

All the band volumes of the Gel_n were then multiplied by the Gel_nCC coefficient.

The band volumes at the four digestion stages characterized hydrolysates protein composition and were analysed by multiple factor analysis as described by Lechevalier et al. (2015) to highlight dry heating treatment effect on the progress of the *in vitro* digestion. The coordinates of the sample on the first two principal components of this multiple factor analysis (called “digestion MFA”) were used as variables for the “global MFA” performed in this study (§2.6.4).

2.5. Protein antigenicity

Inhibition ELISA using ovomucoid, ovotransferrin or lysozyme-specific monoclonal antibodies was performed in order to determine the residual immunoreactivity of each protein after egg white dry heating, according to Dupont et al. (2010) Ovomucoid,

ovotransferrin and lysozyme-specific monoclonal antibodies were raised by immunizing mice with whole egg following the procedure previously described by Johansson et al. (2009). Then, ELISA plates (NUNC, Maxisorp, Roskilde, Denmark) were coated with $5 \mu\text{g ml}^{-1}$ ovomucoid (Sigma Aldrich, Saint Quentin Fallavier, France), ovotransferrin (EPS, Occhiobello, Italy) or lysozyme (Liot, Annezin les B  thune, France) in 0.1 M bicarbonate buffer pH 9.6 ($100 \mu\text{l}$ per well), and incubated for 1 h at 37°C . Wells were rinsed between each incubation stage with three changes of $300 \mu\text{l}$ phosphate-buffered saline, 0.05% Tween 20 (PBS-T), using an automatic microplate washer (EL50™ Filter Microplate Washer, BioTek®, USA). Blocking of the remaining binding sites was performed with $250 \mu\text{l}$ porcine gelatin (Sigma-Aldrich, Germany) at 10 g l^{-1} in PBS-T for 1 h at 37°C . Serial dilutions of ovomucoid, ovotransferrin and lysozyme in PBS-T were used as standards (concentrations ranging from 0 to $200 \mu\text{g ml}^{-1}$, 0 to $50 \mu\text{g ml}^{-1}$ and 0 to $1 \mu\text{g ml}^{-1}$, respectively).

Egg white was reconstituted from the different powders in order to keep constant protein concentration. Reconstituted egg white solutions diluted in PBS-T (from 1:1000 to 1:10,000) were incubated in test tubes with specific monoclonal antibodies (v:v) for ovomucoid, ovotransferrin or lysozyme diluted at 1:2,500, 1:2000 and 1:1,000, respectively and incubated for 1 h at 37°C . One hundred microliters of each mixture were then added to each ELISA plate well and further incubated for 1 h at 37°C . Wells were then incubated with anti-mouse IgG-alkaline phosphatase (Sigma-Aldrich, Germany) diluted 1:5000 in PBS-T. Finally, the reaction was initiated with $100 \mu\text{l}$ of 1 mg ml^{-1} p-nitrophenyl phosphate (KPL, Gaithersburg, Maryland, USA) diluted in 1 M diethanolamine-HCl pH 9.8, 1 mM MgCl_2 , 0.1 mM zinc acetate. After 1 h at 37°C , the absorbance was read at 405 nm against a blank using a microplate spectrophotometer (ELx800TM BioTek, USA). Results were expressed as ovomucoid, ovotransferrin or lysozyme concentration in samples using a four-parameter logistic (sigmoidal) function. The measurements were performed in duplicate.

2.6. Statistical analysis

2.6.1. ANOVA

ANOVA was used to test the effect of dry heating time and temperature on each measurement. The normal distribution of the data was first tested using a Shapiro Wilk normality test, and the homoscedasticity of the samples was tested using the Levene's test for homogeneity of variance of R language (R Development Core Team, 2007). Since all variables fitted these ANOVA hypotheses, the following linear model procedure of R was applied:

$$Y_{ij} = T_i + t_j + T.t_{ij} \quad (6)$$

where, T is the temperature ($20, 60, 70, 80, 90^\circ\text{C}$); t the treatment time ($0, 1, 2, 5, 10 \text{ d}$); and $T.t$ the interaction. Differences were regarded significant at minimum level of 95% ($p\text{-value} < 0.05$). The LS-Means were calculated and compared using multiple comparisons by means of LSD with the “agricolae” package of R software (R Development Core Team, 2007).

2.6.2. Principal component analysis (PCA)

To analyse the effect of dry heating on all egg white proteins at once, PCA was applied to the matrix with electrophoresis band volumes at gast ti stage as active variables, and the 17 samples as individuals (Supplementary data 2). Dry heating temperature and time were added as supplementary variables.

PCA transformed the observed variables into a new set of independent variables called principal components (PCs) which were uncorrelated linear combinations of the original variables. The first

component was the one which explained most of the variance in the data. A large part of the information in the data could thus be plotted in 2-D or 3-D graphs defined by the PCs.

PCA was performed using the FactoMineR package of the R software (R Development Core Team, 2007; Husson et al., 2008). The variables were automatically standardized (mean centred and scaled) by the software to give them all the same importance.

2.6.3. Hierarchical clustering analysis (HCA)

To complete PCA, HCA was performed in order to visualize and highlight the similarities between individuals. Briefly, HCA consists in calculating the dissimilarity, usually called the distance, between individuals (Meunier et al., 2007). In the present study, Ward's method was used for hierarchical clustering with the Euclidean distance as a similarity measure. The distances were calculated from the 5 first principal components only to suppress the noise in the data that is in the last principal components.

2.6.4. Multiple factor analysis (MFA)

MFA is a multifactorial analysis that enables to visualize in a common space samples characterized by several groups of measurements (Escofier and Pag  s, 1998). Since MFA balances the influence of the groups on the first principal dimension by dividing the weights of the variables/columns of a group by the first eigenvalue of the separated PCA of this group, each group of measurements has the same weight in the analysis. MFA provides thus the classical results of principal component methods. PCA characteristics and interpretation rules are retained. A graph of the groups allows global comparison of the groups and evaluation if the relative positions of the individuals are globally similar from one group to another. It also allows the comparison of partial groups and assesses whether they provide the same information.

In the present study, MFA was performed on the table containing all the previously described measurements dispatched in four groups (Supplementary data 3): the volumes of each SDS PAGE band before hydrolysis in “protein profiles” group; the protein solubility, the surface pressure (S_{10}), the droplet size distribution ($d_{4,3}$) and the emulsion stability (Cri) in “protein interfacial properties” group; the degree of hydrolysis at the end of gastric (DH_{gast}) and intestinal (DH_{int}) stages and the coordinates of the samples on the first 2 PCs of the “digestion MFA” in the “protein digestion” group; the ovomucoid, ovotransferrin and lysozyme concentrations determined by inhibition ELISA in the “protein antigenicity” group. The rows represented the control and the 16 dry heated samples. Dry heating temperature and time were added as supplementary variables.

This MFA provided thus a representation of the diversity of dry heating treatment by taking into account the protein profile before hydrolysis, the protein interfacial properties, their susceptibility to digestion and their antigenicity. The superimposed representation of dry heated samples described by each one of the four groups of variables on the subspace of the global analysis generated by PCs highlighted the similarities and discrepancies between samples according to the properties considered.

3. Results and discussion

3.1. Dry heating induced egg white protein aggregation and hydrolysis

Protein aggregation due to dry heating is usually associated to lower protein solubility. In the present study, while protein solubility in the control sample was 99%, it varied from 88 to 99% for the dry heated samples (Supplementary data 3), but without significant effect ($p < 0.05$) of either dry heating temperature or time

Table 1Significant levels of variables and their interaction for the different analytical parameters and model R² and p-value.

	Temperature	Time	Temperature x time	R ²	p-value
solubility	NS	NS	NS	0.05	0.88
PCA PC1	NS	*	*	0.54	0.015
PCA PC2	NS	NS	NS	0.13	0.59
S₁₀	**	•	*	0.79	0.0001
d_{4/3}	***	•	*	0.86	7 10⁻⁶
Cr_i	NS	***	***	0.78	0.0001
Lysozyme antigenicity	**	NS	NS	0.45	0.45
Ovomucoid antigenicity	NS	NS	NS	0.15	0.53
Ovotransferrin antigenicity	NS	***	***	0.8	8 10⁻⁵
DH gast	NS	**	**	0.66	0.0021
DH int	NS	NS	NS	0.305	0.18
Digestion MFA PC1	NS	**	**	0.72	0.0007
Digestion MFA PC2	NS	NS	NS	0.08	0.75

• Significant effect at P < 0.10; * significant effect at P < 0.05; ** significant effect at P < 0.01; *** significant effect at P < 0.001; NS, no significant effect.

Measurements in bold characters are significantly (p < 0.05) affected by heat treatment temperature and/or time.

(Table 1). In most previous works, the decrease of solubility was lower than 5% (Baron et al., 2003; Desfougères et al., 2008; Kato et al., 1989; Matsudomi et al., 2001; Mine, 1997; Xu et al., 1998) but Van der Plancken et al. (2007) observed a decrease of more than 10% after dry heating at 80 °C of egg white powder containing more than 5% humidity. In the present study, non-heated powder contained 6% humidity that could probably explain the loss of solubility observed after dry heating.

Egg white protein profile was analysed by SDS-PAGE for each dry heated sample. Principal component analysis (PCA) was performed on the dataset described in Supplementary materials 2 (i.e. the volume of the protein bands measured on SDS-PAGE gels before digestion (gast ti samples)). The graph of the variables, i.e. of the electrophoretic band volumes, on the first two dimensions is given in Fig. 1A. PC1 and PC2 explain 30% and 26% of the variability of the data set, respectively. PC1 is positively correlated to the volume of the band at 164, 152, 93 and 10 kDa, and negatively correlated to the volume of the band at 130 and 112 kDa (Fig. 1A). Dry heating temperature is also correlated to PC1 at 49%. PC2 is positively correlated to the volumes of the band at 130, 112, 71, 55, 42, 21 and 14 kDa.

The graph of the individuals is given in Fig. 1B. PC1 contrasted (right side) samples dry heated over 2 days at 90 °C and 2 days at 70 °C (cluster 3), with all other samples (clusters 1 and 2) (left side). PC2 contrasted (upper part) medium dry heated samples (cluster 2) with (lower part) slightly (left side) and strongly (right side) dry heated samples (clusters 1 and 3, respectively). PC2 thus represents a quadratic effect of dry heating treatment.

The effect of dry heating temperature and time was tested on the coordinates of the individuals on PC1 and PC2. Dry heating time and the interaction (temperature x time) have a significant effect (p < 0.05) on the coordinates of the individuals on PC1 (Table 1). A strong dry heating treatment (i.e. high temperature and long-time) thus increases the volume of the bands at 164, 152, 93 and 10 kDa, while it decreases the volume of the bands at 130, 121, 112 and 42 kDa, indicating protein aggregation during dry heating treatment. Especially, the band at 93 kDa was attributed to dimer of ovalbumin. Several studies previously highlighted such aggregation resulting from dry heating of either egg white (Hammershoj et al., 2006a; Handa et al., 2001; Kato et al., 1989; Mine, 1996; Nicorescu et al., 2011; Van der Plancken et al., 2007; Watanabe et al., 1999; Xu et al., 1998); or purified proteins (Desfougères et al., 2011a; Matsudomi et al., 2001). The aggregation is usually assigned to disulfide bonds, hydrophobic interactions, and/or non disulfide covalent bonds (Desfougères et al., 2011a; Hammershoj et al., 2006a; Kato et al., 1989; Matsudomi et al., 2001; Van der Plancken et al., 2007; Xu et al., 1998). In the present study, the

electrophoresis performed in denaturing conditions confirms the involvement of covalent bonds. Especially, the free SH group of the ovalbumin molecule would explain that this protein participates in such covalent aggregates through SS bonds, consistently with the decrease of the volume of the ovalbumin band (42 kDa) when dry heating time increased.

A striking result was the increase of the 10 kDa band volume when dry heating temperature increased (Fig. 1A), suggesting protein hydrolysis during the heat treatment. But the present study did not enable to determine which protein was hydrolysed, since several bands decreased when the intensity of dry heating increased: 130, 121, 112 kDa that corresponded to aggregates and 42 kDa that corresponded to ovalbumin. Matsudomi et al. (2001) mentioned a possible hydrolysis of ovalbumin after dry heating for 3 days at 80 °C. On the opposite, Mine (1996) did not notice any protein hydrolysis when egg white dry heating was performed at 75 °C. However, in the present study, the higher temperature used (i.e. 90 °C) could explain the apparition of the 10 kDa band.

Although protein aggregation due to dry heating is well described in the literature, the interest to use PCA to analyse SDS PAGE gels was to highlight the proteins the most involved in the one hand, and clustering the dry heating conditions with the same effect on the other hand. Dry heating temperature clearly increases protein aggregation when it reaches 90 °C. For lower temperatures, it is rather dry heating time that matters (difference between samples from cluster 1 and 2 along PC2), and the band concerned are not the same than the one affected by temperature (along PC1). We can thus assume that depending on the dry heating rate, aggregates formed are not of the same nature which would then lead to different functional properties, immunoreactivity and sensitivity to hydrolysis.

3.2. Egg white dry heating improved interfacial properties

Interfacial properties were measured both on 2D through surface pressure-area isotherms and 3D through emulsifying properties.

Surface pressure-area isotherms of films made with egg white proteins were shifted towards higher pressures and areas when the intensity of dry heating treatment increased (Fig. 2). Dry heating thus favoured the spreading of egg white proteins at the air-water interface, making dry heated proteins more surface-active than control proteins. Talansier et al. (2009) already mentioned an increase of egg white protein surface activity after dry heating for 7 days at 75 °C, or for 3–5 days at 80 °C. Similarly, Desfougères et al. (2011b) mentioned a much more surface-active lysozyme after dry heating for 7 days at 80 °C. However, these studies did not test

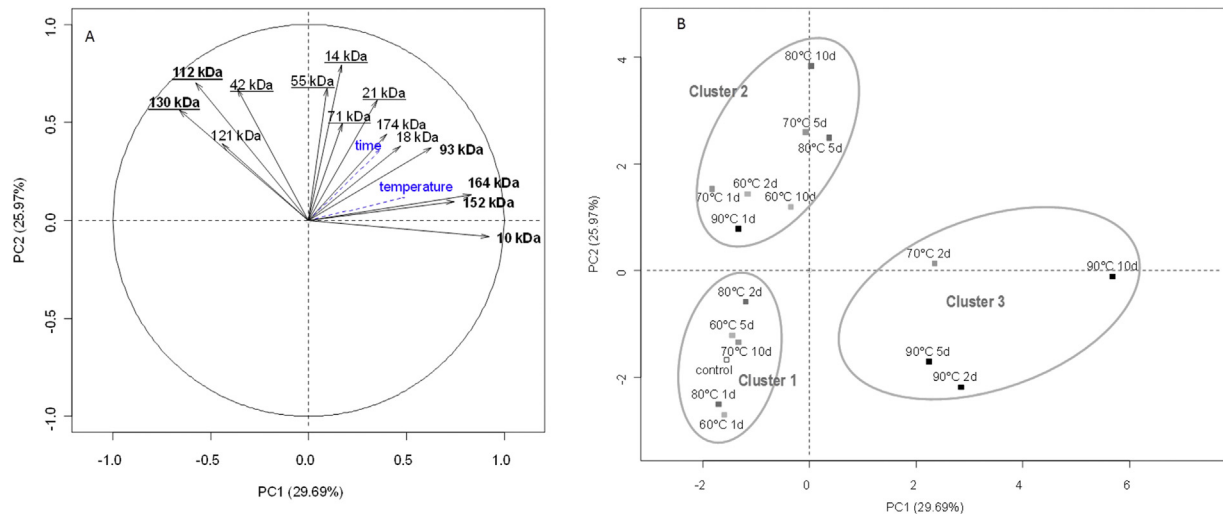


Fig. 1. A) Projection of variables (electrophoresis band volumes identified by their molecular weight) onto the plane defined by the first two principal components (PCs) of Principal Component Analysis (PCA). The coordinates of each variable are the correlation coefficients with the two first PCs: the closer the arrow to the circle, the better the representation of the variable. Variables significantly correlated with PCs 1 and 2 are in bold characters and underlined, respectively. Of the variables significantly correlated with PCs 1 and 2, the correlations between two variables were all the highest, given that the angle between their directions was small. B) PCA map of individuals projected on the 2D plane defined by PC1 and PC2. Individuals in different ellipses are considered as significantly different by HCA.

higher temperature than 80 °C. On the contrary, [Hammershoj et al. \(2006b\)](#) did not notice any effect of dry heating parameters (fluidized bed from 90 to 130 °C during 10 min to 3 h) on egg white surface pressure. In this study, the time of heating was thus much shorter than the one applied in the present study, which could explain this result. Therefore it is indeed dry heating time-temperature rate that matters to improve protein interfacial activity as indicated by ANOVA results ([Table 1](#)).

The interfacial properties of egg white proteins were also investigated for emulsifying activity, as measured by droplet size and creaming index. Droplet size significantly ($p < 0.05$) decreased when the intensity of dry heating increased ([Fig. 3](#)). Up to the author's knowledge, no data are available in the literature on the effect of dry heating on egg white emulsifying properties, however, [Talansier et al. \(2009\)](#) and [Nicorescu et al. \(2011\)](#) mentioned a decrease of foam bubble size when the intensity of dry heating

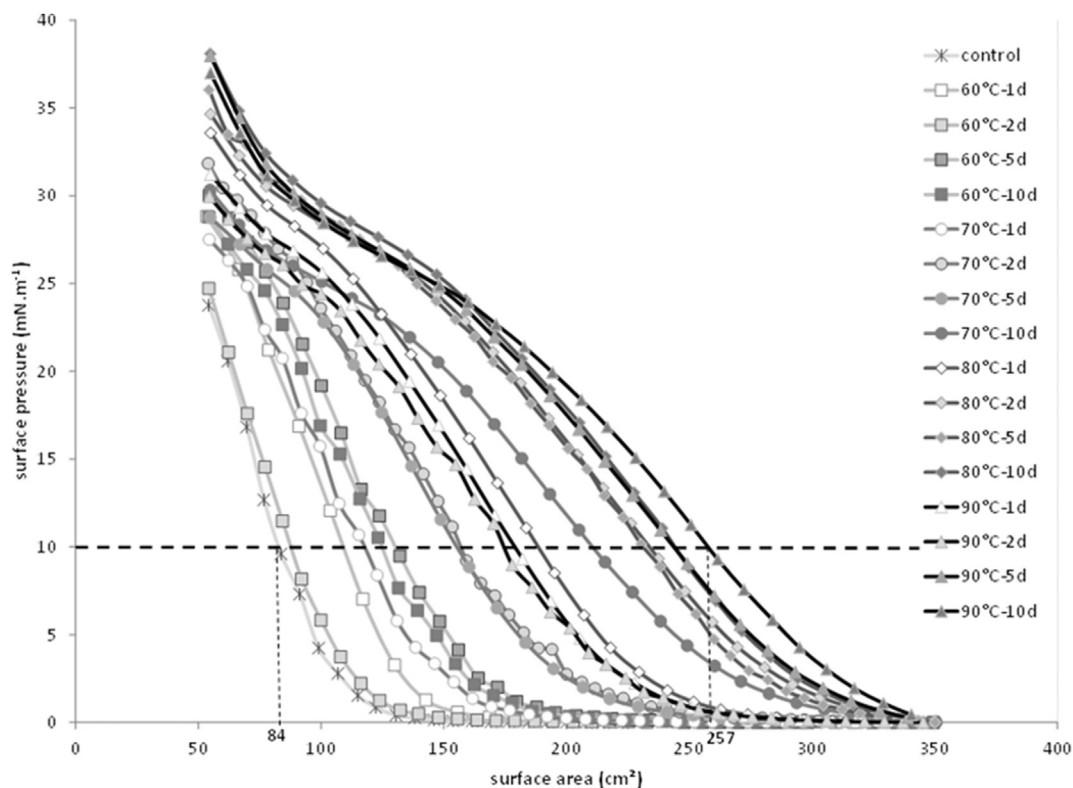


Fig. 2. Surface pressure – surface area isotherms obtained by the compression of the film after adsorption during 30 min of 100 µg egg white proteins. S_{10} values were recorded along the dashed line for the 17 samples.

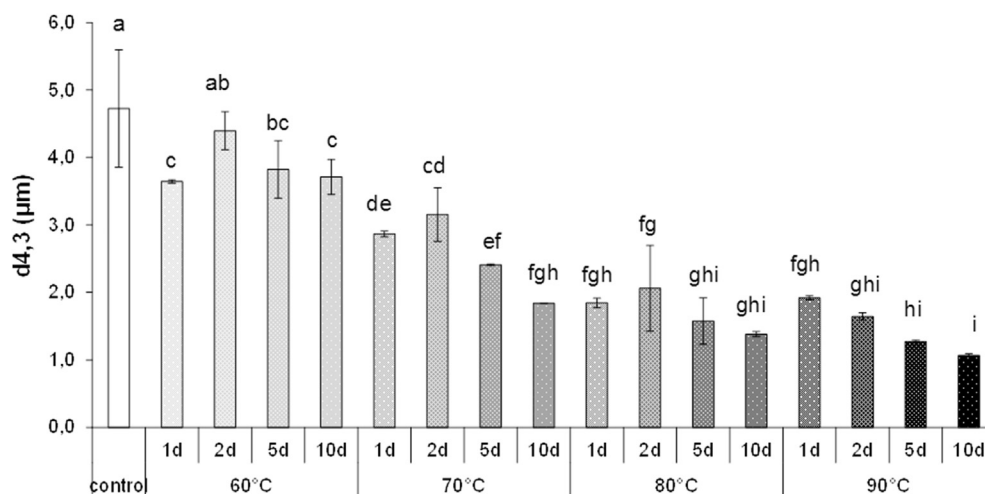


Fig. 3. Droplet size distribution of emulsions (O:W/30:70) prepared with 1.5% egg white proteins as a function of dry heating temperature and time. Samples with different letters are significantly different (LSD test, $p < 0.05$).

treatment increased. Considering the creaming stability of emulsions, the performances of egg white dry heated at 60 °C were significantly ($p < 0.05$) lower than the one of the control sample (Fig. 4); this may result from the decrease of the protein solubility which was the highest for these samples. But when dry heating temperature was 70 °C or higher, creaming stability increased with dry heating intensity (i.e. Cr_i decreased); creaming stability was significantly ($p < 0.05$) higher for egg white dry heated for 5 and 10 days at 90 °C than for the control sample. The increase of emulsion stability observed after intense dry heating treatment was consistent with the decrease of the droplet size resulting from such heat treatment, since it was established that smaller the droplets, more stable the emulsions (McClements, 2007). As for the decrease of emulsion droplet size, it could be explained by the increase of protein surface-activity (McClements, 2004). Finally, it can be noticed that the present study was in agreement with the one of Kato et al. (1989) who also obtained an increase in emulsifying activity and emulsion stability with dry heating treatment time at 80 °C.

As dry heating treatment also generates aggregation, one can thus wonder what the role of aggregates in interfacial properties was. In the present study, aggregates were removed before

interfacial measurements and emulsifying; the improvement of interfacial properties was thus only due to changes in soluble proteins. Similar results were observed by Desfougères et al. (2008) who obtained stable foams after dry heating (7 days at 80 °C) of lysozyme, irrespective of the presence or not of aggregates. These authors demonstrated that large bulk aggregates did not intervene in foam formation or stability, and hypothesized that the increase of lysozyme propensity to aggregation favoured its aggregation at the interface, thus stabilizing air bubbles. On the opposite, heat treatment of lentil protein isolate resulted in poor emulsion stability due to molecular aggregation that decreased protein diffusion coefficient (Joshi et al., 2012).

Our results showed that dry heating may also induce partial hydrolysis of egg white proteins. Many studies performed on a wide variety of proteins have shown that partial hydrolysis improves the emulsifying properties by reducing protein molecular weight, increasing protein solubility, and increasing protein surface hydrophobicity (Lam and Nickerson, 2013). Partial hydrolysis due to egg white dry heating could then have contributed to the improvement of the emulsifying properties observed in the present study.

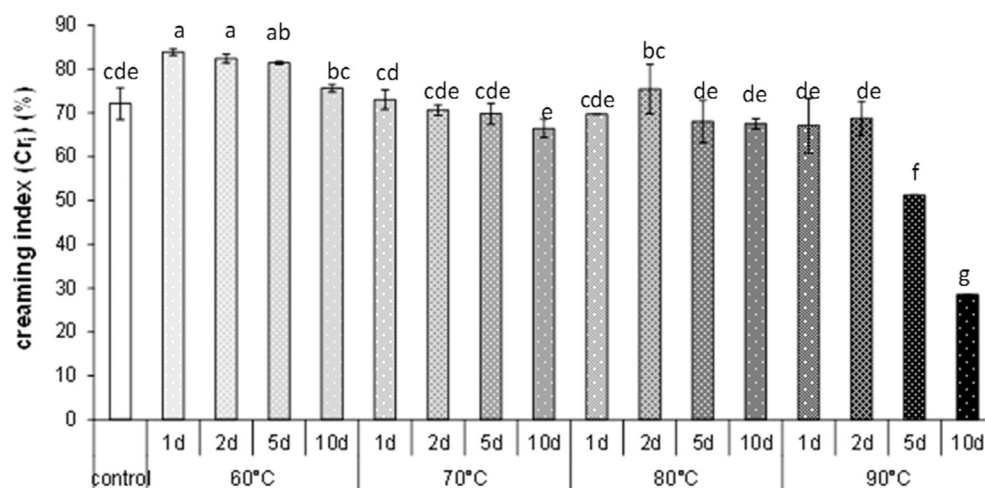


Fig. 4. Index of creaming of emulsions (O:W/30:70) prepared with 1.5% egg white proteins as a function of dry heating temperature and time. Samples with different letters are significantly different (LSD test, $p < 0.05$).

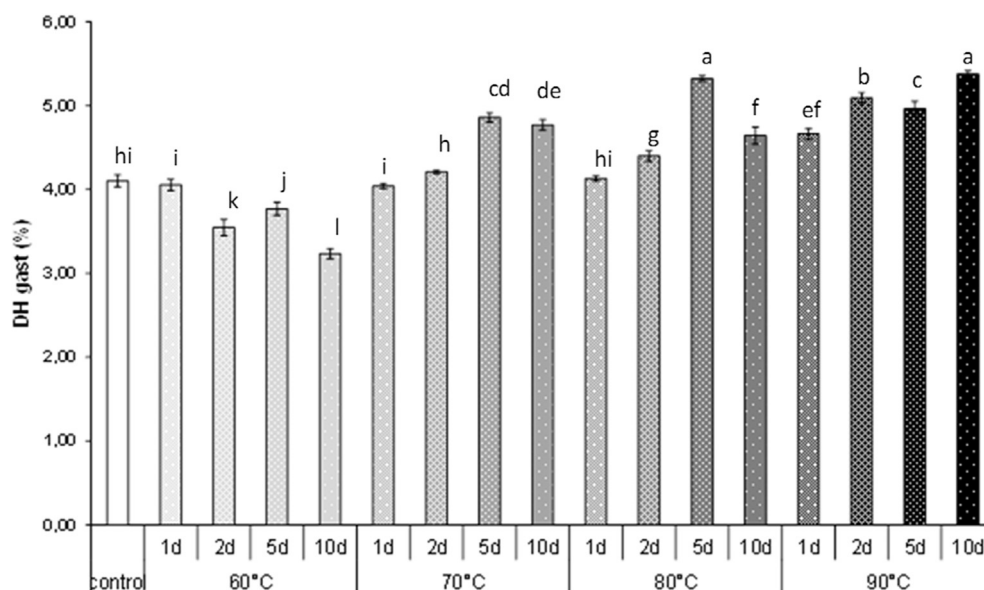


Fig. 5. Degree of hydrolysis at the end of the gastric stage of egg white proteins *in vitro* digestion, as a function of dry heating temperature and time. Samples with different letters are significantly different (LSD test, $p < 0.05$).

Many studies also mentioned the role of protein flexibility in the improvement of interfacial properties (Desfougères et al., 2011b; Joshi et al., 2012; Tang and Shen, 2013), since protein flexibility increase can ease structural unfolding at the interface, lateral interactions between adsorbed proteins, and formation of viscoelastic interfacial layers (Tang and Shen, 2013). Thus, Desfougères et al. (2008, 2011a, 2011b) showed that dry heating increased lysozyme flexibility which formed thus thicker and more viscoelastic multilayer films than native lysozyme. It could thus be hypothesized that an increase of protein flexibility resulting from egg white dry heating could also participate in the increase of interfacial properties here measured.

3.3. Slight to moderate dry heating treatments favour protein *in vitro* digestion, whereas strong treatments disfavour

Protein *in vitro* digestion was characterized by the measurement of the degree of hydrolysis and by protein and peptide profiles in SDS PAGE.

The degree of hydrolysis was measured at the end of the gastric (DH_{gast}) and the intestinal (DH_{int}) stages of the protein *in vitro* digestion. Dry heating time and time $\times$ temperature interaction have a significant effect on DH_{gast}, but no significant effect was observed on DH_{int} (Table 1). At the end of the gastric stage, the degree of hydrolysis of powders dry heated for 2 days or longer at 60 °C was significantly lower than the control one, whereas the one of samples dry heated for 5 or 10 days at 70, for 2 days or longer at 80 °C and for any time at 90 °C was significantly higher (Fig. 5). This result was in agreement with Kato et al. (1990a) who highlighted an increase in ovalbumin and ovotransferrin susceptibility to proteolysis when dry heating time at 80 °C increased. These authors explained such an increase in susceptibility to proteolysis by the enhancement of protein flexibility due to dry heating. Schmidt et al. (2007) also observed an increase in egg white proteolysis with an increase of dry heating temperature between 100 and 105 °C for 10 min. However, these authors did not obtain any change of protein “digestibility” after dry heating up to 14 days at 70 °C.

Protein and peptide profiles of egg white digests were characterized by SDS-PAGE, and quantified by the band volumes at four digestion stages (gast ti, gast t+, gast tf and int tf). Since the analysis of these data by multiple factor analysis (MFA) was the subject of Lechevalier et al. (2015), only the main conclusions are here summarized (supporting by Tables 2 and 3 and figures in supplementary data 4).

The progression of *in vitro* digestion was modified according to the degree of protein unfolding and/or aggregation. *In vitro* digestion tended to decrease the heterogeneity of sample electrophoretic patterns overall but it occurred either at the very beginning of the gastric stage or throughout the gastric stage or again during the intestinal stage, depending on the heat treatment to which the sample had been subjected. At the end of digestion, three groups of

Table 2

Digestion MFA: significant correlation coefficients ($p < 0.05$) between band volumes at a given sampling time and PC1. NS = non-significant. Band molecular weights in italics = bands that appeared during digestion.

	Gast ti	Gast t+	Gast tf	Int tf
174 kDa	NS	NS	NS	NS
164 kDa	+0.78	+0.78	NS	NS
152 kDa	+0.92	+0.50	NS	NS
130 kDa	−0.55	NS	NS	NS
121 kDa	NS	+0.67	+0.67	+0.64
112 kDa	NS	NS	NS	NS
93 kDa	+0.70	+0.82	+0.75	+0.88
71 kDa (ovotransferrin)	NS	NS	NS	NS
55 kDa	NS	+0.52	+0.68	+0.77
42 kDa (ovalbumin)	NS	NS	NS	+0.65
41 kDa	NS	+0.55	−0.54	NS
40 kDa	−0.64	+0.64	NS	NS
35 kDa	NS	NS	+0.69	NS
32 kDa	−0.66	NS	NS	NS
30 kDa	+0.66	+0.62	+0.71	NS
21 kDa	NS	NS	NS	NS
18 kDa	NS	NS	+0.65	NS
15 kDa	NS	NS	NS	NS
14 kDa (lysozyme)	NS	NS	NS	+0.58
10 kDa	+0.91	NS	NS	+0.63
9 kDa	NS	+0.87	NS	NS

samples were obtained: all samples dry heated at 60 °C and the sample dry heated for 1 day at 70 °C were more hydrolysed than the control; samples dry heated for more than 2 days at 80 °C or 90 °C were less hydrolysed than the control; samples dry heated for more than 2 days at 70 °C or 1 day at 80 or 90 °C were as hydrolysed as the control. Since dry heating of egg white powder enhances protein aggregation, it caused different courses of *in vitro* digestion, depending on the dry heating treatment applied: slightly to moderately dry heated samples were more prone to *in vitro* digestion than the non-treated sample, whereas strongly dry heated samples were the most resistant. It can therefore be assumed that, after low to medium dry heating, protein unfolding or increased flexibility make *in vitro* digestion easier, whereas after high dry heating treatment (over 80 °C for 2 days), protein aggregation slows down *in vitro* digestion.

3.4. Egg white dry heating effect on protein antigenicity depended on the protein considered

The residual antigenicity of proteins was tested using ELISA inhibition method for lysozyme, ovomucoid and ovotransferrin. Though ovalbumin is the major egg white protein, its antigenicity has not been tested here since we did not manage to obtain monoclonal antibodies with a sufficient degree of purity.

In the present study, no significant effect of dry heating treatment was observed for ovomucoid (Table 1). Even more, IgG-immunoreactivity of ovomucoid appeared not significantly modified by dry-heating, consistently with many studies reported the resistance of ovomucoid immunoreactivity to heat treatment in solution (Fu et al., 2010; Jimenez-Saiz et al., 2011; Shin et al., 2013; Urisu et al., 1997). Despite, Mine & Zhang (2002) observed a decrease after 15 min at 95 °C in solution. Such discrepancies between studies could be attributed to the different sources of antibodies and techniques used (Julia et al., 2007).

However, dry heating temperature has a significant effect ($p < 0.05$) on lysozyme antigenicity (Table 1); the apparent concentration of lysozyme decreased when dry heating temperature increased (Fig. 6A). Dry heating is known to modify from one to five amino acid residues into succinimidyl residues, to increase lysozyme surface hydrophobicity, to enhance dimerization, and to decrease its lytic activity (Desfougères et al., 2011a). An effect on its

antigenicity was thus not surprising even if the immunochemical answer is strongly dependent on the type of monoclonal antibody used. Mine and Zhang (2002) also obtained a less antigenic form of lysozyme after heating in solution for 15 min at 95 °C when testing against human IgG. However, when testing against human IgE or rabbit IgG, no significant difference between native and heated lysozyme was measured. These results are in agreement with Urisu et al. (1997) who neither noticed any significant difference in the level of IgE antibodies to lysozyme despite different conditions of heating since they performed their heat treatment in solution for 60 min at 90 °C.

Considering ovotransferrin, dry heating time and time $\times$ temperature interaction were the main factors, with highly significant effects (Table 1). Surprisingly, the apparent concentration increased in that case when the intensity of dry heating treatment increased (Fig. 6B). Ovotransferrin is known to be the most sensitive to heat treatment among egg white proteins (Kato et al., 1990b). Tong et al. (2012) showed that the potential antigenicity towards IgG and IgE of ovotransferrin correlated well with its conformational changes induced by heat treatment in solution. Antigenicity increased with the unfolding of the structure (heating up to 1 h at 55–60 °C in solution) and then decreased with the cleavage and rearrangement of disulphide bonds (heating over 10 min at 70 °C). Shin et al. (2013) also noticed a strong decrease in ovotransferrin antigenic activity after egg white boiling over 30 min. The increase in ovotransferrin antigenicity observed in the present study, i.e. after dry heating for more than 2 days at 80 °C could thus be attributed to the unfolding of the protein.

In the present study, the evolution of egg white antigenicity was assessed with monoclonal antibodies specific for ovomucoid, lysozyme or ovotransferrin. In contrast to polyclonal antibodies that may exhibit cross-reaction with other egg white proteins, monoclonal antibodies are highly specific probes that interact with specific epitopes i.e. small motifs made by the association of few amino acids of the protein. Therefore, the modifications observed on the antigenicity of each egg protein studied can be unambiguously attributed to ovomucoid, lysozyme or ovotransferrin modifications. However, depending on the domain where the epitope is located, modification of the antibody-antigen interaction may vary. Indeed, if the structure of the domain is unchanged by dry heating, no modification of the antigenicity of the protein will be observed. Nevertheless, it is interesting to note that the results obtained in the present study are in agreement with the literature and reinforce the consequences of dry heating on egg white protein structure already observed.

3.5. Integrative statistical analysis to optimize dry heating treatment of egg white powders

MFA was performed on the dataset that contained all the previously described measurements, with the row representing the 17 dry heated samples. Dry heating temperature and time were added as supplementary variables. Four groups of measurements were constituted: “protein profiles”, “protein interfacial properties”, “protein digestion” and “protein antigenicity” as described in section 2.6.4. The first PC explains 35% of the variability of the dataset. It is positively correlated to dry heating temperature, S_{10} , electrophoresis band volumes of the 164, 152, 93 and 10 kDa components, DH gast, DH int, digestion MFA.PC1, and ovotransferrin antigenicity; it is also negatively correlated to Cr_i and $d_{4.3}$ (Fig. 7A). As shown in Fig. 7B, PC1 contrasted (left side) the control and slightly to moderately dry heated samples, with (right side) strongly dry heated samples. Dry heating thus induced protein aggregation since the volumes of the bands at 164, 152 and 93 kDa increased. It also favoured protein hydrolysis since the volume of the band at

Table 3
Digestion MFA: significant correlation coefficients ($p < 0.05$) between band volumes at a given sampling time and PC2. NS = non-significant. Band molecular weights in italics = bands that appeared during digestion.

	Gast ti	Gast t+	Gast tf	Int tf
174 kDa	NS	NS	NS	NS
164 kDa	NS	NS	NS	NS
152 kDa	NS	NS	NS	NS
130 kDa	+0.5	+0.70	+0.60	NS
121 kDa	+0.55	NS	NS	NS
112 kDa	+0.77	NS	NS	NS
93 kDa	NS	NS	+0.56	NS
71 kDa (ovotransferrin)	NS	NS	NS	NS
55 kDa	+0.73	+0.74	NS	NS
42 kDa (ovalbumin)	+0.71	+0.76	+0.78	NS
41 kDa	NS	NS	NS	NS
40 kDa	NS	NS	NS	NS
35 kDa	+0.53	NS	NS	NS
32 kDa	NS	NS	NS	NS
30 kDa	NS	NS	NS	NS
21 kDa	NS	+0.65	NS	NS
15 kDa	NS	NS	NS	NS
14 kDa (lysozyme)	+0.59	+0.70	+0.70	NS
10 kDa	NS	NS	NS	NS
9 kDa	NS	NS	NS	NS

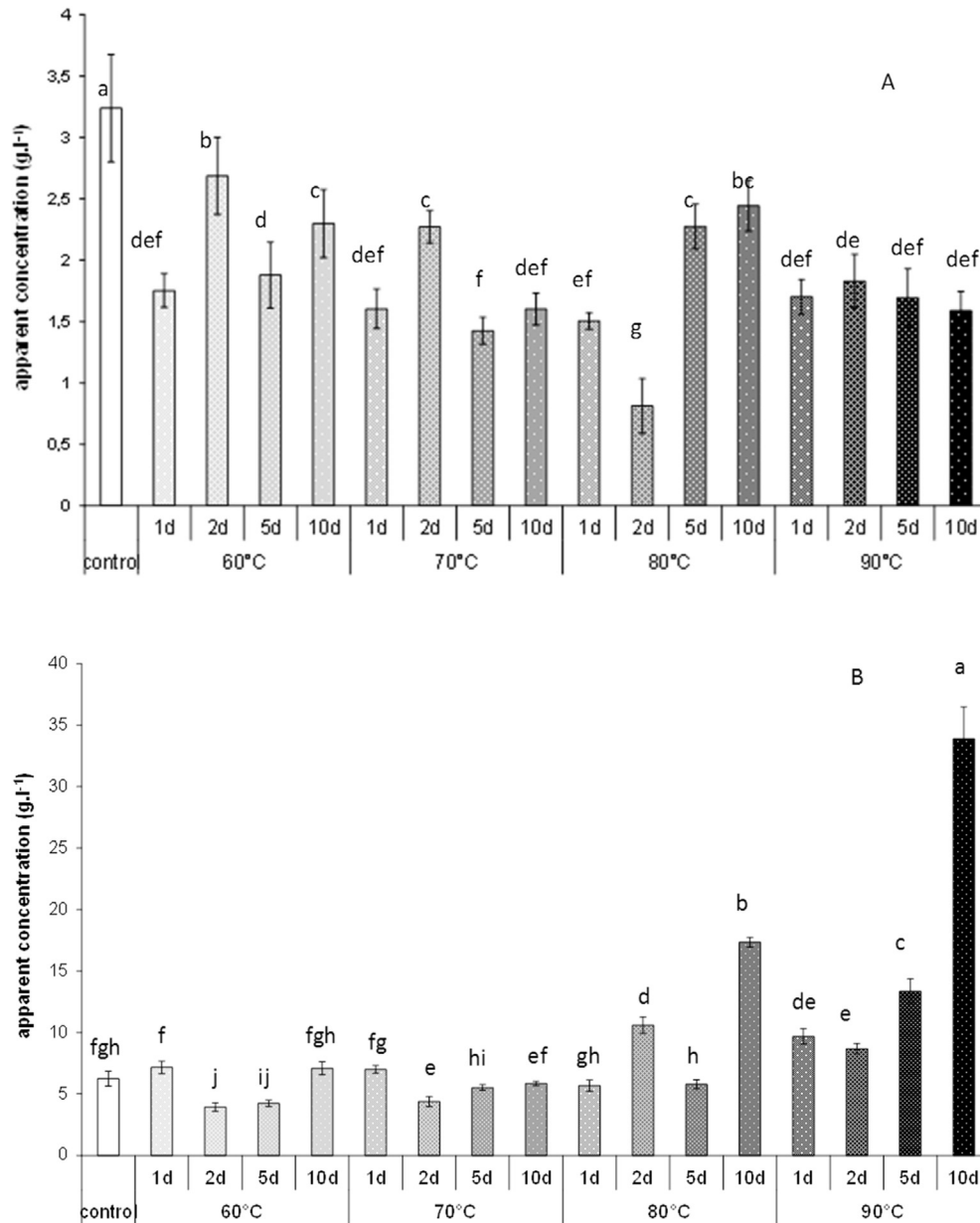


Fig. 6. Lysozyme (A) and ovotransferrin (B) apparent concentration determined by ELISA inhibition as a function of the intensity of the dry heating treatment. Samples with different letters are significantly different (LSD test, $p < 0.05$).

10 kDa increased when dry heating intensity increased. Moreover, dry heating enhanced protein interfacial properties since dry heated proteins were more surface-active (S_{10} increased) and generated more stable emulsions (Cr_i decreased) with smaller droplets ($d_{4,3}$ decreased). The effect of dry heating treatment on protein digestion depended on the treatment intensity: slightly to moderately dry heated samples had lower coordinates on the PC1 of “digestion MFA” than strongly dry heated samples (over 5 days at 80 °C or 2 days at 90 °C) (Fig. 7B). According to the results of digestion MFA described in section 3.3 and Lechevalier et al. (2015), the 121, 93, 55, 42 and 14 kDa components were thus less hydrolysed in the strongly dry heated samples assuming a decrease in protein sensitivity to digestion in these samples compared to the control or lower treated samples. Protein antigenicity was also modified by dry heating, but in different ways depending on the

protein considered; when dry heating intensity increased ovotransferrin antigenicity increased, whereas it decreased for lysozyme and remained constant for ovomucoid.

In the present study, we thus showed that longer the dry heating time and higher the temperature, better the interfacial properties. However, dry heating also changes egg white protein conformation, which impairs their sensitivity to proteolysis and their antigenicity. Thus, at the end of *in vitro* digestion mimicking the gastric digestion stage, the degree of hydrolysis increases when dry heating time and temperature increase. But at the end of the second digestion stage, mimicking the intestinal stage, the effect of dry heating time and temperature is no more significant ($p < 0.05$). Even more, for some proteins, the susceptibility to digestive enzymes is lower when dry heating intensity increases. Especially, the major egg white protein, i.e. ovalbumin is more resistant to

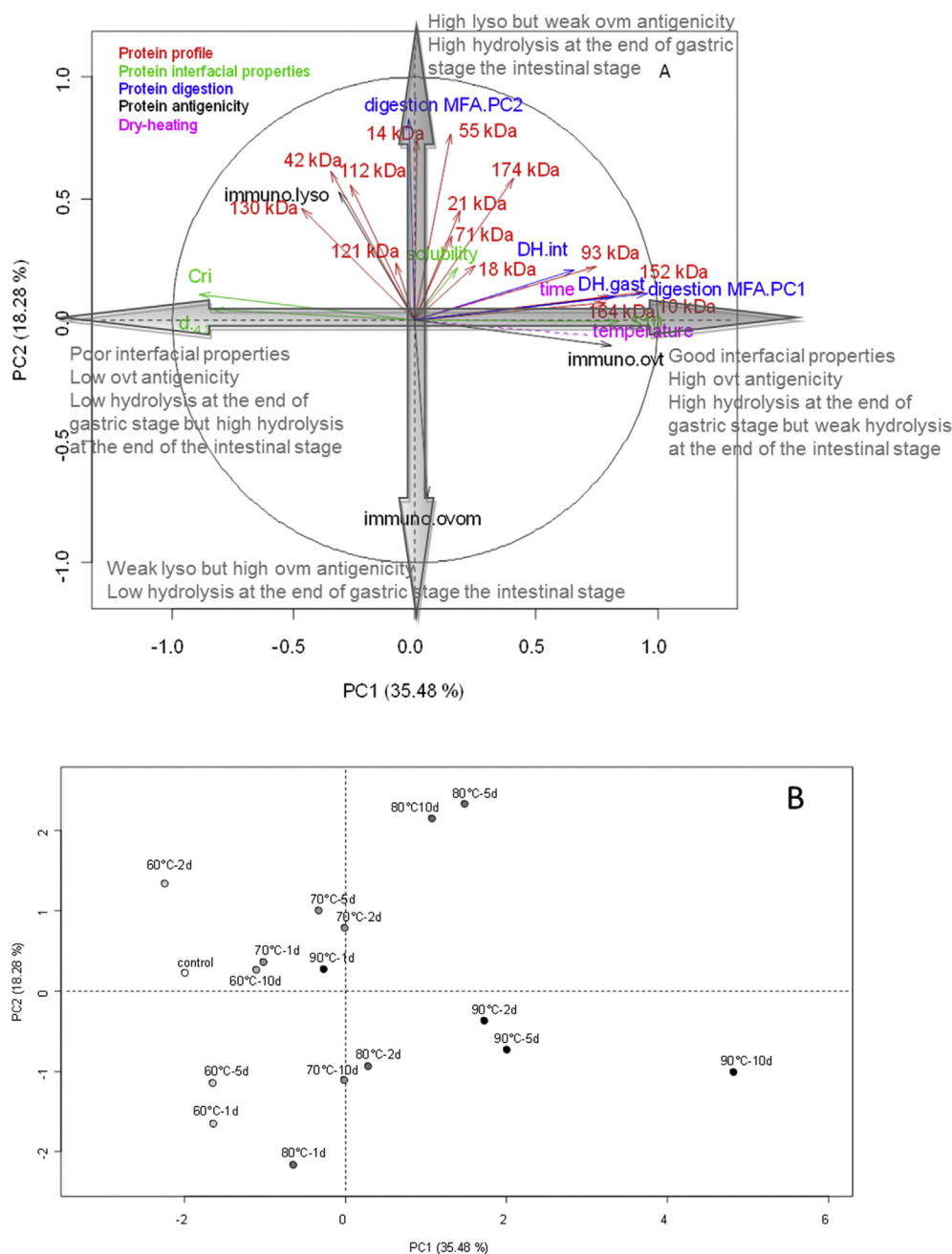


Fig. 7. A) Projection of variables onto the plane defined by the first two principal components (PCs) of the global Multiple Factor Analysis (MFA). B) MFA map of the individuals projected on the 2D plane defined by PC1 and PC2. Each dry heated sample is represented by the centroid of the four partial points that corresponded to each group of variables.

digestive enzymes when previously strongly dry heated. Such results suggest that *in vivo* digestion could be impaired by dry heating, at least for some proteins.

Moreover, the modifications of protein structure due to dry heating also modify protein antigenicity. The strongest effects are rather observed for the strongest treatment intensity, but depending on the protein it can either decrease (lysozyme) or increase (ovotransferrin) the protein antigenicity.

Finally, thanks to multiple factorial analysis that enabled to consider all the dimension of the study at once, a good compromise to improve the interfacial properties of egg white, without enhancing too much protein resistance to digestion and/or protein

antigenicity can be proposed: intermediate dry heating treatments of 2–5 days at 70 °C or 1–2 days at 80 or 90 °C for batches of 100 g of powder. However, industrial scale-up will require that these heating rates would be adapted since dry heating is performed on much larger quantities of powder than those used in the present study (about 25 kg per bags vs 100 g), thus temperature increase rate is much slower.

Funding source

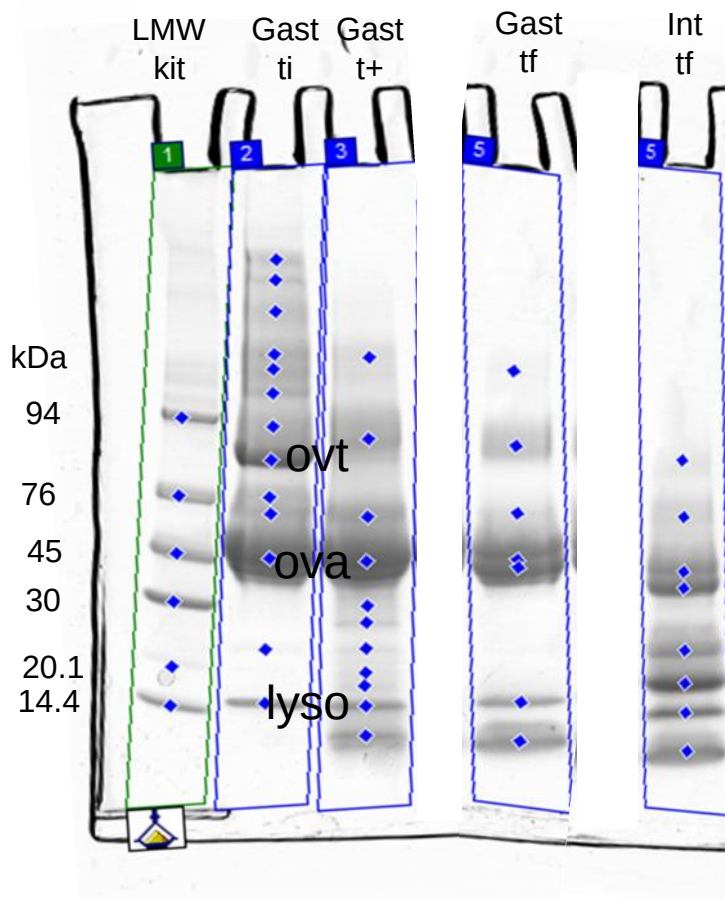
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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.jfoodeng.2016.09.022>.

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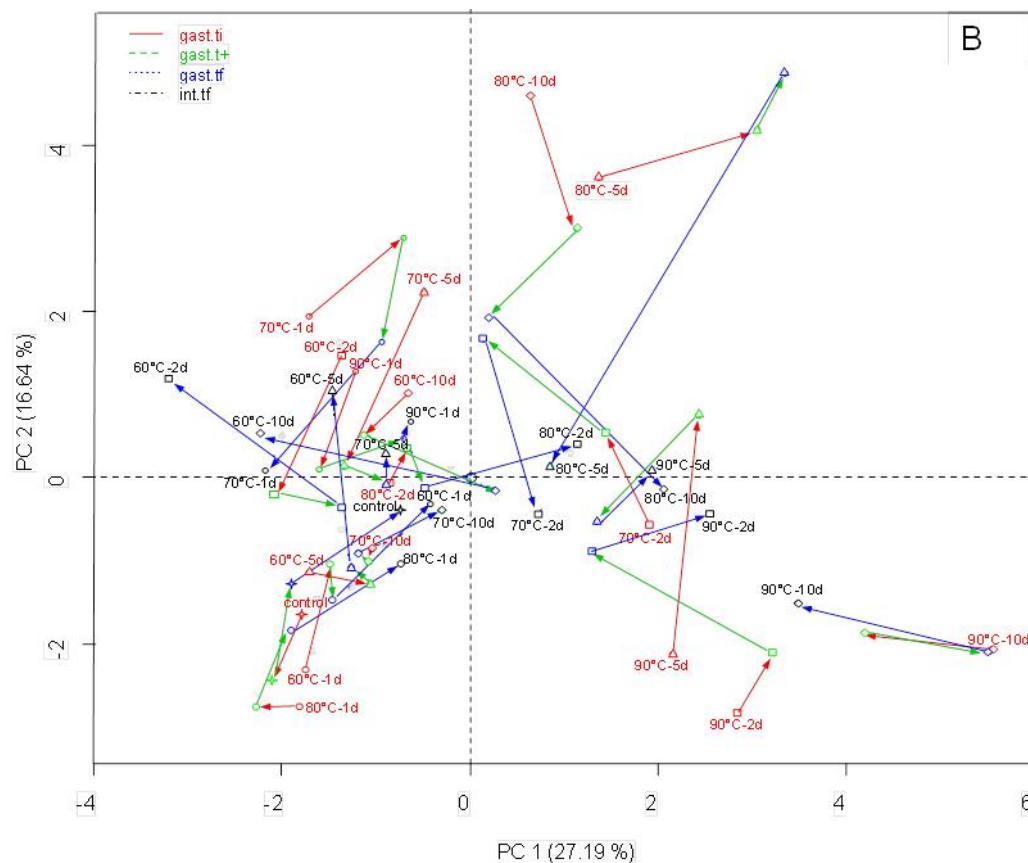
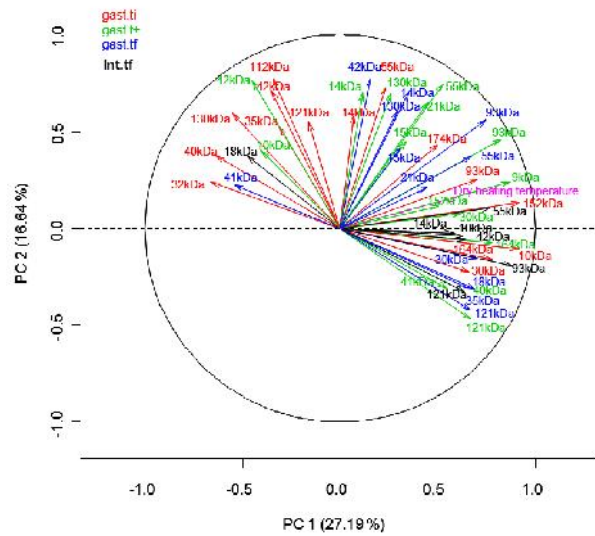
Supplementary data 1: *In vitro* digestion of egg white followed by SDS PAGE analysis for gastric and intestinal stages. Lane frames (gast ti, gast t+, gast tf and int tf) correspond to digestion times used for quantification by Imagequant software (before digestion: gast ti, 15s after gastric enzyme addition (gast t+), at the end of the gastric stage (gast tf) and at the end of the intestinal stage (int tf)). Bands marked with spots correspond to the quantified bands. Ovotransferrin (ovt), ovalbumin (ova) and lysozyme (lyso) bands are identified. The electrophoresis gel presented here as example show the digestion of egg white dry-heated at 80°C for 2 days.

Supplementary data 2: Table used to generate data set used for PCA, consisting of band volumes for the 14 electrophoresis bands quantified (from 174 kDa to 9 kDa) at gastric digestion time. Rows represent the 17 dry heating treatments (temperature (°C) – time (days)).

Dry heating treatment	Temperature	Time	Electrophoresis band volume (x10 ⁶)													
			174	164	152	130	121	112	93	71	55	42	21	18	14	10
	(°C)	(days)	kDa													
Control	20	0	8	19	8	28	12	12	23	111	65	213	0	0	28	0
60°C-1d	60	1	7	16	8	23	13	12	28	89	51	179	0	0	25	0
60°C-2d	60	2	39	25	0	68	0	24	61	129	52	331	4	0	35	0
60°C-5d	60	5	6	10	13	27	17	14	41	82	62	221	4	3	28	0
60°C-10d	60	10	14	26	16	33	20	21	45	98	72	250	4	8	43	0
70°C-1d	70	1	13	24	24	51	30	30	66	160	53	393	0	0	35	0
70°C-2d	70	2	29	18	33	0	0	0	61	91	72	232	6	8	39	13
70°C-5d	70	5	19	22	22	39	25	22	35	116	70	253	8	10	49	0
70°C-10d	70	10	22	17	38	31	34	24	45	83	52	181	0	0	28	0
80°C-1d	80	1	18	14	17	25	28	15	30	91	41	177	0	0	24	0
80°C-2d	80	2	22	17	39	39	34	24	58	88	59	216	3	0	24	0
80°C-5d	80	5	44	23	64	49	46	37	85	97	89	239	3	0	35	9
80°C-10d	80	10	34	27	49	47	42	43	73	121	88	309	4	4	42	5
90°C-1d	90	1	15	18	33	79	0	25	81	103	71	291	2	0	31	0
90°C-2d	90	2	38	37	65	0	0	0	33	99	53	172	0	0	32	17
90°C-5d	90	5	12	28	60	0	0	0	80	76	68	244	0	5	30	13
90°C-10d	90	10	26	61	71	0	0	0	141	150	52	151	6	6	30	15

Supplementary data 3: Table used to generate data set considered for “global MFA”, consisting of the band volumes for the 14 electrophoresis bands quantified (from 174 kDa to 9 kDa) for each of the 17 dry-heating treatments, at gast ti digestion times (“protein profiles” group), the protein solubility, the surface pressure, the droplet size distribution and the emulsion stability (“protein interfacial properties” group), the degree of hydrolysis at the end of gastric and intestinal stages, the coordinates on the first two PCs of the “digestion MFA” (“protein digestion” group) and the ovomucoid, ovotransferrin and lysozyme concentrations (“protein antigenicity” group).

Dry heating treatment	Temp. (°C)	Time (days)	Protein profiles Band volumes (x10 ⁶)														Protein interfacial properties				Protein digestion				Protein antigenicity		
			174 kDa	164 kDa	152 kDa	130 kDa	121 kDa	112 kDa	93 kDa	71 kDa	55 kDa	42 kDa	21 kDa	18 kDa	14 kDa	10 kDa	Solubility (%)	S ₁₀ (cm ²)	d _{4,3}	Cr _i (%)	DH gast (%)	DH int (%)	MFA PC1	MFA PC2	Ovm (%)	Ovt (%)	Lyso (%)
Control	20	0	8	19	8	18	12	12	23	111	65	213	0	0	28	0	99,0	84	4,7	72	4,10	5,49	-1,63	-1,43	3,24	1,56	6,24
60°C-1d	60	1	77	16	8	23	13	12	28	89	51	179	0	0	25	0	96,0	102	3,6	84	4,05	5,50	-1,68	-2,07	1,75	1,81	7,16
60°C-2d	60	2	39	25	0	68	0	24	61	129	52	331	4	0	35	0	90,4	87	4,4	82	3,55	5,89	-2,01	0,54	2,69	1,51	3,94
60°C-5d	60	5	6	10	13	27	17	14	41	82	62	221	4	3	28	0	88,3	129	3,8	81	3,77	6,36	-1,38	-0,60	1,88	1,89	4,24
60°C-10d	60	10	14	26	16	33	20	21	45	98	72	250	4	8	43	0	98,0	124	3,7	76	3,23	7,20	-0,94	0,49	2,30	1,90	7,08
70°C-1d	70	1	13	24	24	51	30	30	66	160	53	393	0	0	35	0	96,3	116	2,9	73	4,04	6,23	-1,39	1,65	1,60	1,80	7,00
70°C-2d	70	2	29	18	33	0	0	0	61	91	72	232	6	8	39	13	94,1	160	3,2	71	4,21	6,36	1,06	0,31	2,27	1,57	4,38
70°C-5d	70	5	19	22	22	39	25	22	35	116	70	253	8	10	49	0	97,5	155	2,4	70	4,86	5,67	-0,91	0,66	1,43	1,59	5,52
70°C-10d	70	10	22	17	38	31	34	24	45	83	52	181	0	0	28	0	93,0	210	1,8	66	4,77	6,39	-0,90	-0,78	1,60	1,85	5,86
80°C-1d	80	1	18	14	17	25	28	15	30	91	41	177	0	0	24	0	99,0	188	1,8	70	4,13	5,54	-1,28	-1,29	1,51	2,06	5,68
80°C-2d	80	2	22	17	39	39	34	24	58	88	59	216	3	0	24	0	96,7	234	2,1	75	4,40	6,38	-0,22	0,16	0,81	1,81	10,60
80°C-5d	80	5	44	23	64	49	46	37	85	97	89	239	3	0	35	9	99,0	231	1,6	68	5,32	8,24	2,16	3,22	2,28	1,62	5,79
80°C-10d	80	10	34	27	49	47	42	43	73	121	88	309	4	4	42	5	99,2	244	1,4	67	4,64	6,27	1,01	2,36	2,44	1,67	17,34
90°C-1d	90	1	15	18	33	79	0	25	81	103	71	291	2	0	31	0	93,9	179	1,9	67	4,66	5,68	-1,04	0,65	1,70	1,68	9,68
90°C-2d	90	2	38	37	65	0	0	0	33	99	53	172	0	0	32	17	95,5	175	1,6	69	5,09	8,16	2,48	-1,55	1,83	1,62	8,69
90°C-5d	90	5	12	28	60	0	0	0	80	76	68	244	0	5	30	13	96,3	243	1,3	51	4,96	6,84	1,97	-0,45	1,69	1,77	13,35
90°C-10d	90	10	26	61	71	0	0	0	141	150	52	151	6	6	30	15	94,5	257	1,1	29	5,38	7,45	4,69	-1,87	1,59	1,84	33,90



Supplementary data 4: A) Projection of variables (electrophoresis bands before digestion (red), at the beginning of the gastric stage (green), at the end of gastric stage (blue), and at the end of intestinal stage (black)) onto the plane defined by the first two principal components (PCs) of Multiple Factor Analysis (MFA). Only variables with a correlation coefficient >0.5 are presented. B) MFA map of the individuals projected on the 2D plane defined by PC1 and PC2. Each dry-heated sample is represented

by four partial points that correspond to characterization by one group of variables: gast ti (red), gast t+ (green), gast tf (blue) and int tf (black), respectively. Arrows illustrate the effects of digestion stages on the coordinates of the individuals, i.e. on their electrophoretic pattern.