OVONUTRIAL project: technologies impact on nutritional value and allergenicity of egg proteins

Nau F. [1]*, Gaudichon C.[2], Sanders P.[3], Guérin-Dubiard C.[1], Dupont D.[1]

[1]1Agrocampus Ouest, INRA, UMR1253 Science et technologie du lait et de l'œuf, F-35042 Rennes
[2]AgroParisTech, INRA, UMR914 Physiologie de la Nutrition et du Comportement Alimentaire, F-75231 Paris
[3]AFSSA, UR731, Laboratoire d'études et de recherche sur les médicaments vétérinaires et désinfectants, F-35302 Fougères

* Corresponding author: Francoise.Nau@agrocampus-ouest.fr

Abbreviated title: OVONUTRIAL project

Summary

OVONUTRIAL is a French 3-year research project (2008-2010), gathering interdisciplinary teams (8 scientific and medical partners) specialized in biochemistry and egg science, human nutrition, immunochemistry and food allergy respectively, and the major French eggproducts companies, joined in an association. The project aims to evaluate the effects of some major industrial processing (pasteurisation, drying, dry-heating) on the nutritional value and antigenicity of egg proteins, related to the induced protein modifications. One of its feature is that it is devoted to complex matrix (whole egg) submitted to realistic (industrial) technological treatments. Moreover, the project aims to provide new data by using the most recent methods for the evaluation of nutritional and allergenic properties. Especially, protein quality will be evaluated during the postprandial phase in humans, using an original approach that has been developed in that aim to intrinsically label egg proteins with 15N and 13C. Reactivity profiles of sera from egg allergic patients against both major and minor egg proteins will be analysed according to clinical data; a prospective and long term study will so be initiated on a patient cohort.

Keywords: egg protein, nutritional value, allergenicity, pasteurisation, spray-drying, dry-heating

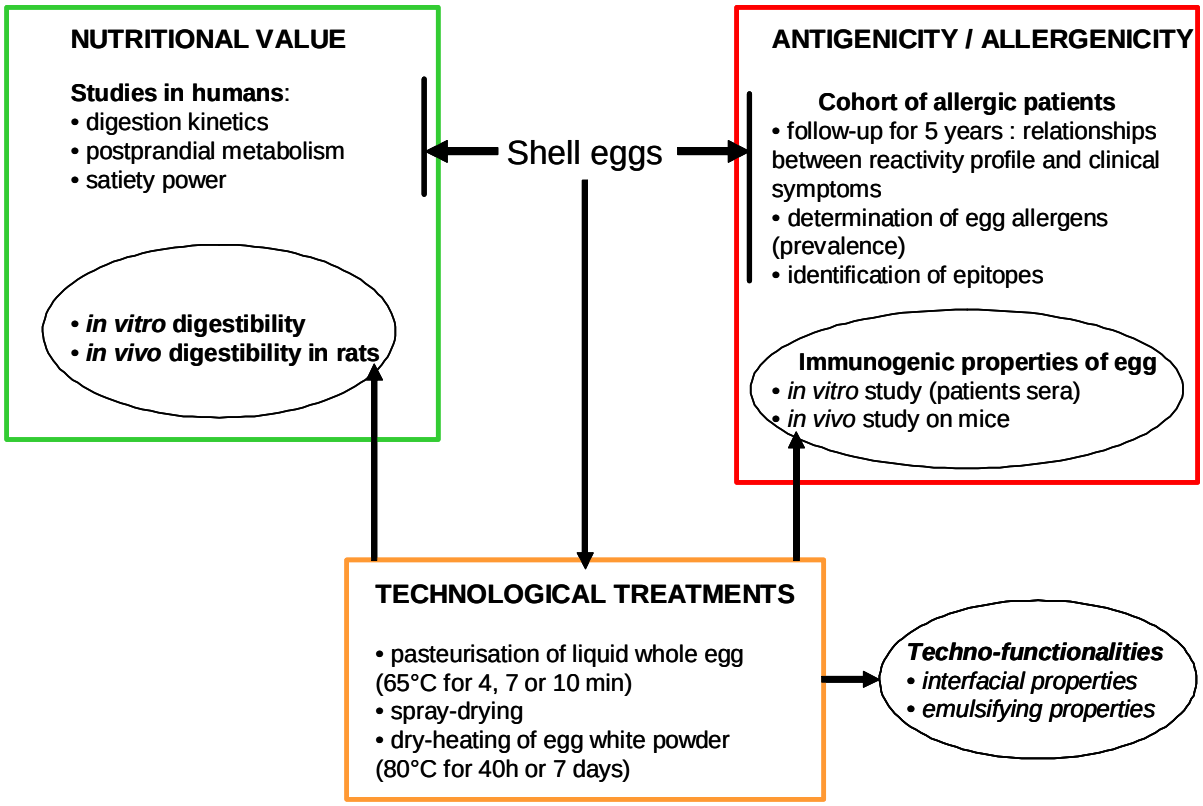
Introduction

Egg is one of the most consumed by human amongst animal products, because of its technofunctional properties, as well as its nutritional quality recognized as high. Especially, due to their excellent scoring patterns, egg protein had the status of “reference protein” in the 70’s and 80’s. But it is now unquestionable that protein quality could not be approached on their unique ability to sustain dispensable amino acids for protein synthesis: several criteria such as physiological and metabolic response to protein intake are necessary to take in account; for instance, digestion kinetics play a role in nutritional quality of proteins (Dangin et al., 2001; Bos et al., 2003). In vivo studies are then required, but very few are available about egg protein. Moreover, whereas egg is more and more consumed as industrial egg products, the impact of egg transformation on its nutritional quality has been very selectively studied (Allemeersch, 1983; Cook and Briggs, 1986; Satyanarayama Rao, 1987). For milk products, industrial technological treatments can affect protein quality through a modulation of digestive kinetics (Lacroix et al., 2006).

Egg allergy is the most frequent food allergy in childhood (50%), especially under 3 years-old (70%) (Moneret-Vautrin, 2008). Egg allergy symptoms are most often cutaneous or respiratory, but anaphylactic reactions also exist. Egg allergy is then definitely an important and serious public health question, especially as egg is very difficult to avoid in diet. Many researches have been devoted to egg allergy, enabling identification of the major egg allergens. But questions still remain about potential markers for prediction of outcome of egg allergy. Moreover, if it is known that some patients allergic to raw egg accept cooked egg (Eigenmann, 2000; Romeira et al., 2003), the impact of industrial technologies used by egg industry has not been studied; especially, no data are available about a strong and widespread treatment that is egg white dry-heating.

OVONUTRIAL project proposes to update and complete scientific data on nutritional and allergenic properties, needed to objectively position egg proteins amongst the other food proteins (Figure 1). Especially, because of the development of egg transformation, an assesment of the impact of industrial processes on these properties appears necessary. Such data should be useful to egg product industry to define the most appropriate technological strategies to optimize product quality, considering the nutritional and/or allergenic questions. Simultaneously, some technofunctional evaluations will be achieved, to ensure that the treatments efficient for nutritional and/or allergenic properties, are not deleterious to the technological quality of egg.

Figure1. Global organisation of the OVONUTRIAL project.



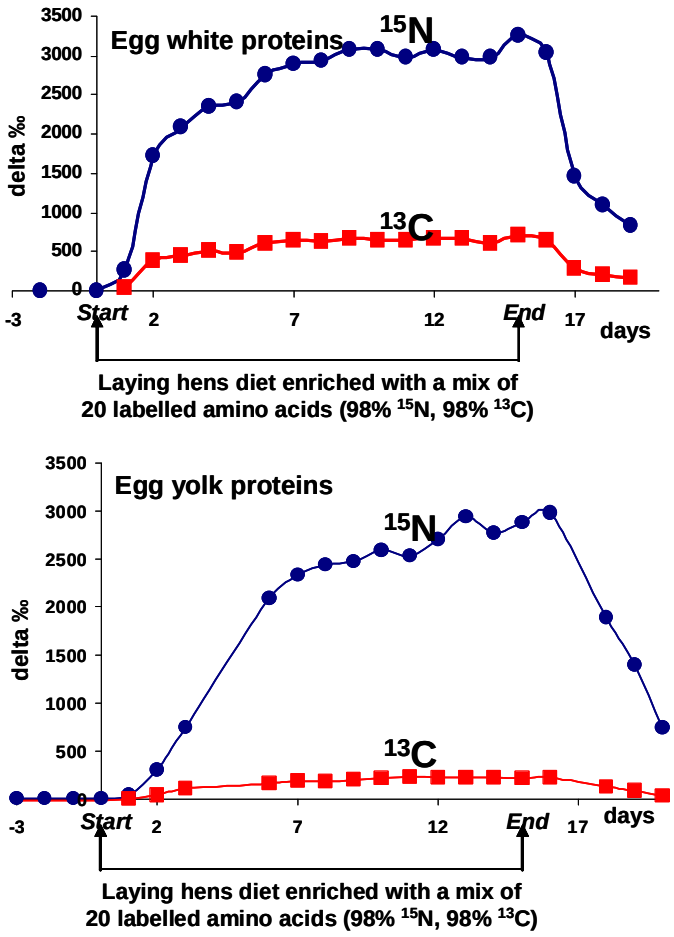
Nutritional value of egg proteins and effect of technologies on it

In vivo studies on human

The nutritional quality of egg proteins will be assessed on 8 healthy volunteers after the ingestion of an omelette (4 eggs equivalent) containing intrinsically ^{15}N and ^{13}C labelled proteins. During the 8 postprandial hours, respiratory exchanges will be registered, and blood and urine samples collected. Measurements from blood samples will be glycaemia, hormones, urea, amino acids, ^{15}N and ^{13}C enrichments in plasma protein, amino acids, urea and glucose; from urines, measurement of urea, ammonia, creatinine, ^{15}N enrichment of ammonia and urea will be performed. Since proteins have been shown to be the most satiating macronutrient (Bensaïd et al., 2002; Porrini et al., 1997), and because protein satiety power may depend on the protein source (Hall et al., 2003), it seems interesting to complete the very few and unclear data available about egg proteins. In parallel, the satiating power of egg proteins will then be tested on 30 volunteers in a cross-over design against milk proteins. Briefly, the delay between the test snack (egg or

cottage cheese) and the spontaneous demand of the following meal will be measured, as well as the spontaneous energy intake during the meal, the appetite rating and the circulating gut and pancreatic hormones. This study will be the first one to concomitantly study pancreatic and gastro-intestinal hormones, energy expenditure as well as the fate of dietary nitrogen and carbon skeleton from amino acids. The project should then enable to update the data concerning the protein value of egg assessed in humans on the basis of digestive kinetics and postprandial egg protein metabolism. It will then allow to compare egg protein quality with regard to other food proteins, measured in the same "real" conditions, that means in humans during the postprandial phase.

Figure 2. Kinetics of intrinsically ^{15}N and ^{13}C labelling of egg proteins by enrichment of laying hens diet with 0.4% of a 20 labelled amino acids mix (98% ^{15}N, 98% ^{13}C), from day 0 to day 15.



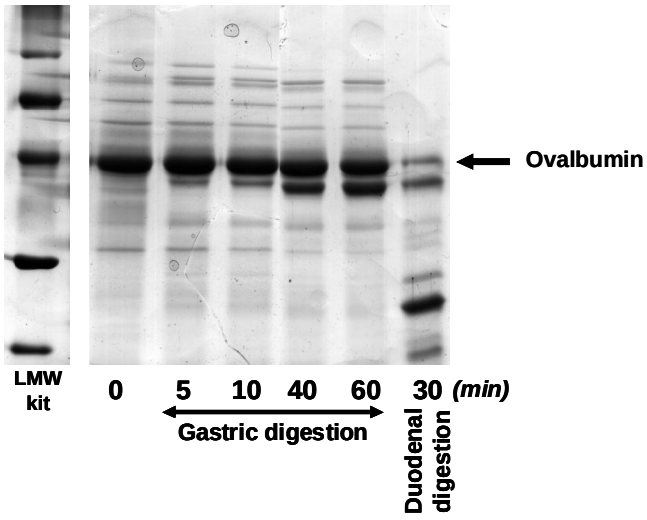
A previous labelling of egg proteins was performed by feeding hens with a diet in which only ^{15}N or ^{13}C leucine was incorporated (Evenepoel et al., 1997). Thus, labelling of proteins was unlikely to represent all amino acids. Our project should produce more representative results, since labelled egg proteins have been here produced by enrichment of laying hens diet with a mix of 20 labelled amino acids (98% ^{15}N, 98% ^{13}C). A preliminary study demonstrated that with a diet containing 0.4% of this mix, a plateau of labelling was obtained after 7 days for egg white proteins, and 9 days for yolk proteins (Figure 2). Moreover, the uniformity of proteins labelling was checked; the ^{15}N labelling of amino acids was homogeneous among the 12 amino acids investigated, despite a lower enrichment for aromatic amino acids. These results allowed defining the experimental conditions for labelled eggs production: 3 hens have been fed with a diet containing 0.3% of the mix, for 26 days; the objective was to produce 50 eggs for the *in vivo* nutritional study in humans.

Technologies impact

The technological treatments considered here are liquid whole egg pasteurization (none or 65°C for 4, 7 or 10 min), spray-drying, and dry-heating of egg white (none or 80°C for 40 h or 7 days) before mixing with yolk powder to reconstitute whole egg powder. Because of no previous data are available to direct the study, and because of the expensive costs of human tests, the effects of these technological treatments will be only screened *in vitro* and in rats. This study will then be a preliminary approach intended to identify new potential axis for research.

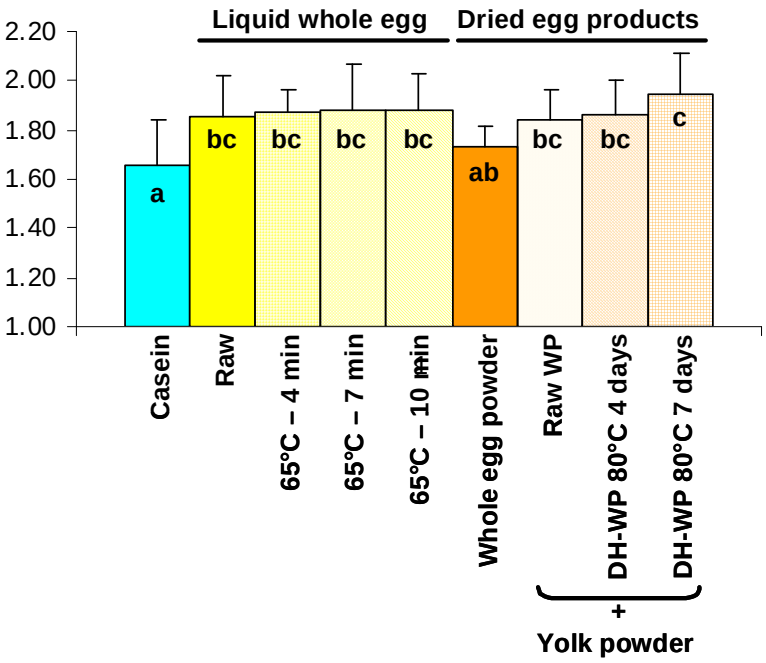
The *in vitro* digestion model used is a two-step model mimicking both gastric and duodenal digestions (Dupont et al., 2009). For the gastric digestion, egg products are dissolved in simulated gastric fluid adjusted to pH 2.5, to a final protein concentration of $10 mg.mL^{-1}$, and warmed to 37°C; porcine pepsin is then added to a final concentration of 182U/mg protein, and digestion is performed at 37°C for 60 min, before it is stopped by raising the pH to 7.5. Duodenal digestion is then carried out after pH adjustment at 6.5 and addition of bovine chymotrypsin (0.4U/mg protein) and porcine trypsin (34.5U/mg protein); digestion is performed at 37°C for 30min and stopped by adding inhibitors. The digestion progress is analysed by SDS-PAGE of samples collected all along the both digestion phases (Figure 3).

Figure 3. SDS-PAGE analysis of in vitro digests of egg proteins; example of raw liquid whole egg digestion.



The study in rat consisted in protein efficiency ratio (PER) measurements, calculated as the body weight gain of a rat divided by its protein intake, over 3 weeks. It was achieved on 9 groups of 6 growing male rats each; 8 groups were fed with one of the 8 processed whole egg, and compared to a control group fed with a casein-diet. Despite PER is a rough global indicator, slight differences could be observed between the different processed egg products: whole egg powder seems less efficient than liquid, and than mix of egg white powder and yolk powder; moreover, dry-heating of egg white increases PER. On the other hand, it is noticeable that egg is more efficient compared to casein (Figure 4).

Figure 4. Effect of technological treatments on protein efficiency ratio (PER) of whole egg; study on rats.



Allergenicity of egg proteins and effect of technologies on it

Three biological properties of egg proteins will be evaluated: antigenicity (ability of a protein to bind a specific antibody), immunogenicity (ability of a protein to elicit an immune response), and allergenicity (ability of a protein to generate an allergic response).

Impact of food processing on the antigenicity and immunogenicity of egg proteins

Monoclonal antibodies (Mabs) specific from native egg proteins have been raised by fusion experiments performed on mice directly immunised with liquid raw egg. This allowed the obtention of a panel of antibody-producing hybridomas specific towards the different major egg allergens; 16, 42, 58 and 15 hybridomas have been produced against ovalbumin, ovotransferrin, ovomucoide and lysozyme, respectively. Once specificity of Mabs determined, a simple inhibition ELISA will be applied to the raw and processed egg samples. According to the response that will be observed, 3 kinds of conclusion could be drawn: (1) the epitope has not been damaged since no signal modification in ELISA is observed between raw and processed material; (2) the epitope has been damaged by the process since a decrease of the signal in ELISA occurs; (3) an increase in the ELISA signal could be related to the opening of the protein structure, due to the process, and resulting in an easier access for the Mab.

In the same way, Mabs against processed eggs will be produced by immunisation of mice with the processed materials. Since processing should lead to neo-epitopes, it is expected that some of the Mabs produced will not recognize native egg proteins; directed against new epitopes generated by processing, these Mabs will be identified. It should highlight the peptidic sequences that are structurally modified by technological treatments.

Impact of food processing on the in vivo allergenicity of egg proteins

Since egg processing is expected to alter protein structure and its sensitivity to enzyme digestion, it could lower or increase allergenicity of egg, and especially thus of egg white. This will be studied in vivo using a sensitive Balb/c mouse model. Five groups of 8 female mice each will be fed with a diet in which proteins will be egg white, either raw

(control), or processed (pasteurised, spray-dried with or without dry-heating). During the sensitisation period (4 weeks), blood samples will be collected for IgE, IgG and histamine measurements; at the end of this period, the mice will be sacrificed for cytokines measurements and T lymphocyte proliferation assay; histological analysis of intestinal inflammation will be performed too.

Clinical follow-up of egg-allergic patients and characterization of the reactivity of their IgE towards egg proteins

Using a large cohort of 120 patients (children between 18 months and 7 years old), either sensitive or allergic to egg, this part of the study will aim at characterizing the patients sera: IgE profile reactivity will be drawn and epitopes will be determined; one of the objectives will be to identify potential allergens among minor egg white proteins. The process impact on IgE reactivity against egg proteins will be evaluated by competitive techniques, using egg products to determine which treatment affects the IgE recognition of egg proteins and to quantify these effects. Finally, the clinicians will also follow the patients up for 2-5 years to study the evolution of the pathology. The objective of this extra-study is to look for some putative indicators of the pathology evolution. Indeed, it would be very important for the patients to know if the allergy will be cured spontaneously, or if there is a risk of permanent allergy.

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