The effects of novel pasteurization technologies on egg product functionalities

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Egg products are protein ingredients with multiple functional properties such as gelling, foaming, binding adhesion and emulsification. Because of such properties, these ingredients are desirable in many foods, e.g. bakery products, meringues, meat products and cookies.
Pasteurization of liquid egg products is commercially performed in conventional high-temperature-short-time equipments, that can negatively affect product functionalities. The recent development of alternative processing technologies, such as high hydrostatic pressure, ultraviolet radiation, pulsed electric fields, high pressure homogenization, microwave-assisted pasteurization, and ohmic heating, offers the potential to inactivate microorganisms, reduce loss of essential nutrients, and maintain functional properties.
The aim of this review is the discussion of results reported in the literature about the effects of these novel pasteurization technologies on egg product functionalities. Moreover, preliminary results of an ongoing research aiming at the study of the ohmic heating effects on gelling and whipping properties of liquid whole egg will be illustrated.

Keywords: egg products; functional properties; pasteurization technologies.

Introduction

Egg products are protein ingredients with multiple functional properties such as gelling, foaming, binding adhesion and emulsification. Because of such properties, these ingredients are desirable in many foods, e.g. bakery products, meringues, meat products and cookies.

Unfortunately, the role of eggs and egg products acting as vehicles for foodborne diseases is still a major public concern in the United States and Europe (EFSA and ECDC, 2012; Jackson *et al.*, 2013). Thus, food manufacturers often substitute shell egg with liquid egg products due to their easier handling and lower microbiological risk (Nemeth *et al.*, 2011). Besides good agricultural and handling practices, pasteurization treatments are needed to achieve the required sanitary level for liquid egg products and to extend their shelf life. The prevalent treatment applied in the egg industry is thermal pasteurization due to its high efficacy in inactivating pathogens (Espina *et al.*, 2014). American recommendations aim at 5 decimal reductions of *Salmonella* in egg yolk and whole egg. This is achieved with time/temperature combinations between 3.5 min, 60°C and 3.5 min, 61.1°C. These treatment conditions are lower than those applied in Europe, where a 5-6 min holding time at 65°C or higher is typical. However, these milder heat treatments applied to egg white are not always sufficient to reach 5-6 decimal reduction of *Salmonella enteritidis* and *Listeria monocytogenes* (Lechevalier *et al.*, 2011).

Unfortunately, egg components are very sensitive to high temperatures, so attention must be paid to avoid the protein coagulation entailing deleterious effects against egg quality. Some of these effects, like the loss of foaming, emulsifying and gelling capacities, may limit liquid egg product utility as food ingredients (Espina *et al.*, 2014). Therefore, there is increasing demand for alternative pasteurization methods that ensure safety while decreasing product degradation. Novel thermal and non-thermal technologies have been designed to meet these requirements.

Technologies including ohmic and microwave heating are promising alternatives to conventional methods of heat processing. Such technologies are regarded as volumetric forms of heating, in which thermal energy is generated directly inside the food. Advantages over conventional methods include not having to rely on limiting heat transfer coefficients and the requirement of high wall temperatures. Other advantages include improved retention of quality and nutritional parameters, shorter processing times and higher yields (Cullen *et al.*, 2012).

The term "non-thermal processing" is often used to designate technologies that are effective at ambient or sublethal temperatures, such as pulsed electric fields (PEF), high hydrostatic pressure, UV light, or high pressure homogenization. Although the term "non-thermal" is used to classify these technologies, many of these approaches may result in temperature rises and efficacy is commonly found to have a synergistic effect with temperature. Consequently, temperature rises may be expected or even desired to be industrially practical (Cullen *et al.*, 2012).

The aim of this review is the discussion of results reported in the literature about the effects of these novel pasteurization technologies on egg product functionalities. Moreover, preliminary results of an ongoing research aiming at the study of the ohmic heating effects on gelling and whipping properties of liquid whole egg are illustrated.

Materials and methods

Raw whole egg was provided by Cascina Italia S.p.A. (Bergamo, Italy). Ohmic treatments were carried out at the Experimental Station for the Food Preserving Industry (SSICA, Parma, Italy), using a pilot plant by Emmepiemme S.r.l. (Piacenza, Italy) equipped with three ohmic heater columns. Two different temperature-time combinations were tested: 68°C-1.4 min; 71°C-0.6 min. As a reference, also a sample of whole egg pasteurized by a traditional thermal treatment (65°C, 3.5 min) was analyzed (Cascina Italia S.p.A.).

Foaming and gelling properties of whole egg were evaluated following Alamprese *et al.* (2012). Flow curves were determined by using a Physica MCR 300 rheometer (Anton Paar GmbH, Graz, Austria), supported by the software Rheoplus/32 (v. 3.00, Physica Messtechnik GmbH, Ostfildern, Germany) and equipped with a 50 mm cone-plate geometry (CP50-1). Shear rate ranged between 20 and 500 s^{-1}. Results are expressed as apparent viscosity at 290 s^{-1} and represent the average of two replicates.

Experimental results were processed by one-way analysis of variance (ANOVA) with least significance difference (LSD) test to evaluate significant differences among the averages (Statgraphics Plus 5.1, Statistical Graphics Corp., Herndon, VA, USA).

Results and discussion

Pulsed Electric Field

High intensity pulsed electric field (PEF) processing involves the application of short pulse (1-10 μs) of high voltage (typically 20-80 kV/cm) to food materials located between two metal (usually stainless steel) electrodes. Electric fields can cause changes to cell membranes causing their rupture (direct mechanical damage, the electric breakdown theory), or destabilizing the lipid and proteins layers of cell membranes, resulting in pores (electroporation theory). The damaged cell membrane loses its selective semi-permeability, which allows water to enter the cell, and results in excessive cell volume swelling, and ultimately leads to cell rupture and inactivation of the organism (Chen *et al.*, 2010). High quality and microbial stable liquid whole egg, immediately after PEF treatment and during refrigerated storage, has been reported (Dunn and Pearlman, 1987; Qin *et al.*, 1995). No apparent changes were observed in most of the egg physical and chemical properties except for a drop in viscosity and an increase in colour. Also, a sensory panel did not find detectable differences between the sensory attributes of scrambled eggs made with fresh and PEF-treated eggs (Qin *et al.*, 1995). Ma *et al.* (2000) compared the independent effects of PEF, high hydrostatic pressure, and thermal processing on the sensory, physical, chemical, and microbiological attributes of liquid whole egg. The results led to the conclusion that PEF (40 μs at 48 kV/cm) is suitable for industrial implementation, since the quality attributes of liquid whole egg treated by this method were higher compared to other methods studied. However, Monfort *et al.* (2010) demonstrated that PEF technology by itself at temperatures lower than 35°C is not a suitable technology for pasteurizing liquid whole egg. Energetic levels greater than 250 kJ/kg would be required, but these are expected to have a prevalent thermal effect on inactivation and, more importantly, probably affect the functional properties of the product. Thus, the most recent studies on PEF applied to liquid whole egg suggested to adopt a combined approach based on PEF, mild heat treatment and antimicrobial compounds (Monfort *et al.*, 2012; Espina *et al.*, 2014). Positive synergistic effects on bacteria inactivation and sensory properties were demonstrated, even with an improvement in foaming and emulsifying capacity.

High Hydrostatic Pressure

High hydrostatic pressure (HHP) refers to the application of hydrostatic pressure ranging from 100 to over 800 MPa to foods in order to inactivate spoilage and pathogenic microorganisms. Liquid or solid foods, with or without packaging, are placed in the pressure vessel. The closed pressure vessel is then filled with a pressure transmitting fluid (usually water or a dilute aqueous solution) that is compressed by a pump or pressure intensifier. The hydrostatic pressure distributes uniformly throughout the pressure vessel and equally in all directions of the food surfaces. Treatment times, once constant high pressure levels are achieved, can range from a millisecond pulse to over 20 min, and initial treatment temperatures can range from 0 to 90°C. HHP generally produces better results for the pasteurization of foods when combined with initial temperatures around 45-50°C. HHP is believed to cause pressure sensitive non-covalent bonds (hydrogen, ionic, and hydrophobic bonds) to break, leading to microbial injury and death (Chen *et al.*, 2010).

HHP treatment has many advantages: it has a minimal heat impact, gives products a similar shelf-life as pasteurisation but with better quality, needs a smaller amount of energy than thermal treatment and can be used to process packaged food. However, it is not typically a continuous process and requires large investment costs. Moreover, it can affect the structure of large molecules such as proteins. *Salmonellae* seem particularly sensitive to high pressure treatments in liquid whole egg. Coagulation occurs at over 300 MPa for egg white or 400 MPa for whole egg at 25°C. At higher temperatures, coagulation occurs at lower pressure (e.g. 1 h at 150 MPa and 45°C for whole egg). At a pressure range from 400 to 700 MPa an increase of egg white turbidity, surface hydrophobicity and susceptibility to enzymatic hydrolysis was observed; in the same time, protein solubility, total sulfhydryl group (SH) content and denaturation enthalpy decreased (Lechevalier *et al.*, 2011). No clear results are reported for foaming properties: Richwin *et al.* (1992) measured a higher foam expansion for egg white treatment up to 400 MPa, whereas Strohalm *et al.* (2000) did not notice any significant change in foam volume and little or no effect on foam density. In contrast, Iametti *et al.* (1999) obtained denser foams after 5 min at 600 MPa and 25°C.

Ultraviolet processing

Ultraviolet (UV) processing uses radiation from the UV region of the electro-magnetic spectrum to inactivate microorganisms in foods, water, and packaging materials. The typical wavelength for UV processing ranges from 100 to 400 nm. UV in the 200-280 nm region is believed to be most effective in inactivating bacteria and viruses. During UV irradiation, DNA molecules absorb UV light and a mutation in the DNA base-pairing occurs, hindering cell growth and reproduction (Chen *et al.*, 2010).
UV radiation can be a cost-effective alternative non-thermal process to achieve microbiologically safe liquid egg products with longer shelf life. The benefits of UV treatment include fewer changes in colour, flavour, taste or pH. UV treatment efficiency is higher when product absorbance is lower, thus a higher reduction of *E. coli* is obtained in egg white, compared with egg yolk and whole egg (Lechevalier *et al.*, 2011).

Manzocco *et al.* (2012) studied the effect of UV processing (10.6 and 63.7 kJ m^{-2}) on selected properties of egg white, demonstrating that the UV exposure induces the development of browning, the formation of large protein aggregates by disulfide exchange, and protein backbone cleavage. However, egg white proteins were differently sensitive to UV radiation. No changes in gelling temperature and gel firmness were observed. Independently on the UV dose, light treated egg white produced foams with higher stability. This effect was attributed to protein aggregates jamming in the fluid interstices between bubbles and/or to the higher viscosity of the aqueous phase. The latter was also associated to higher foam volume.

de Souza and Fernandez (2011; 2013) reported that short-wave UV processing (UV-C; 6.1 and 36.5 kJ m^{-2}) did not affect rheological data, and thermal and electrophoretic properties of egg white, whole egg or egg yolk, indicating that relevant protein denaturation or aggregation could be discarded. On the contrary, heat pasteurization caused an increase on the viscosity-shear rate dependency, and also a certain degree of coagulation could be observed in the flow behaviour diagrams. Browning due to Maillard reaction was perceptible in egg yolk and whole egg at low UV-C doses, but the corresponding browning indexes were always lower than in heat pasteurized egg fractions. Major changes were only due to lipid oxidation.

High pressure homogenization

High pressure homogenisation (HPH) is a promising technique, particularly suitable for continuous production of fluid foods, allowing the limitation of thermal damage. In general terms, during HPH, the fluid is forced through a narrow gap in the homogenizer valve, where it is submitted to a rapid acceleration. As a consequence, phenomena such as cavitation, shear and turbulence are simultaneously induced, leading to an instant temperature increase whose magnitude depends on the intensity of the applied pressure. HPH has been demonstrated to cause inactivation of bacteria and yeasts in several different foods. Recently, Patrignani *et al.* (2013) demonstrated the possibility to efficiently inactivate *Salmonella enterica* in liquid whole egg by HPH at 100 MPa for up to 5 passes. These authors also observed minor impact on product viscosity as well as an increase in foam capacity and stability.

Panozzo *et al.* (2014) reported that HPH could be exploited to obtain safe egg white without impairing its multifunctional properties. In fact, multiple HPH passes (from 2 to 17 at 150 MPa) induced protein structural modifications by promoting partial aggregation, possibly due to protein unfolding and exposure of hydrophobic regions. These structural changes resulted in the formation of a weak and unstable protein network, that did not affect egg white foaming properties. In addition, the Authors demonstrated that by choosing proper processing conditions, some egg white functionalities, such as immunoreactivity and gel firmness, can be steered to obtain egg white with specific functional properties.

Microwave heating

Microwave heating is based, as most other alternative heating techniques, on electromagnetism. The coupling of the electromagnetic field (mainly the electric component) with the matter is responsible for the energy conversion into heat. Waves of frequencies between 300 MHz and up to 300 GHz are called microwaves. Industrial microwave systems operate at frequencies of 915 MHz and 2.45 GHz (Regier, 2014).

Microwave-assisted thermal processing is a novel technology that provides rapid volumetric heating. It overcomes the slow heating in conventional thermal processes, so that better product quality could be retained. A major challenge for developing microwave-assisted thermal processes is possible non-uniform heating patterns caused by the factors influencing the electromagnetic field, such as food properties, package geometry, and location of the product inside the microwave applicators. Because of uneven temperature distribution, microorganisms are not fully eradicated during microwave pasteurization. Destruction of microbes or enzymes by microwave or radio frequency waves at sublethal temperatures was explained by one or more of the following theories: selective heating, electroporation, cell membrane rupture and magnetic field coupling (Chandrasekaran *et al.*, 2013).

The application of microwave pasteurization has been largely applied to fluid foods. With the knowledge of dielectric properties, the appropriate conditions for applying microwave energy and desired process lethality could be obtained (Chandrasekaran *et al.*, 2013). Therefore, results of Zhang *et al.* (2013) about the effects of solid concentration and salt content on dielectric properties and electrical conductivity of liquid egg white and whole egg samples can be useful for practical applications of microwave-assisted pasteurisation of liquid egg products. In fact, to the best of our knowledge, no papers have been published yet on the effect of microwave-assisted pasteurisation on egg product functional properties. On the contrary, it has been demonstrated that pasteurization of in-shell eggs can be achieved with the help of microwaves without losing the shell integrity (Chandrasekaran *et al.*, 2013).

Ohmic heating

Ohmic heating occurs when an alternating electrical current is passed through a food, resulting in the internal generation of heat due to the electrical resistance of food. In this way ohmic heating is similar to microwave heating. However, in the case of ohmic heating, the penetration depth is virtually unlimited and heat penetration is more effective. The process depends on the electrical conductivity of the product (Swart *et al.*, 1993). This technique has many advantages: temperature is quickly achieved, there are low heat losses since thermal energy is generated directly within the food itself, and liquids are uniformly heated. This technology is particularly suitable for the processing of viscous liquid foods such as egg products (Lechevalier *et al.*, 2011). Ohmic heating is a very fast heating method for liquid whole egg: the product can be heated up to 60°C from 10°C in 105 s by applying a voltage gradient of 20 V/cm (Icier and Bozkurt, 2011). Moreover, the absence of moving parts (feed pump excluded) is an advantage for shear-sensitive food products (Swart *et al.*, 1993).

Bozkurt and Icier (2012) observed that temperature has a significant role during ohmic treatment of liquid whole egg, affecting the occurrence of the protein denaturation and the change in the rheological behaviour. They demonstrated that the liquid whole egg was more sensitive to temperature changes during conventional heating rather than the ohmic one.

Sanovo Technology Group (Montecchio Precalcino, VI, Italy) developed a wave ohmic process able to extend the shelf life of egg products, without decreasing functional properties. In particular, good yields in production of choux pastry and sponge cake have been obtained in terms of specific volume. However, a significant effect of treatment time and temperature on liquid whole egg viscosity was observed (personal communication).

In the preliminary trials carried out at SSICA, a part from rheological behaviour, foaming and gelling properties of whole egg products were also tested. The results obtained are reported in Tables 1 and 2. Whereas the traditional pasteurization treatment caused a significant ($p<0.05$) worsening of the foaming properties (Tab. 1), the ohmic heating beneficially affected the whipping performances of the liquid whole egg. These results are probably due to the reduced shear stress applied to the product in the ohmic pilot plant. Only the foam instability resulted to significantly ($p<0.05$) increase for both kind of treatments. As regards gelling properties (Tab. 2), only the gel load showed significant differences ($p<0.05$): both the product treated at 71°C-0.6 min in the ohmic plant and the traditionally pasteurized sample yielded stronger gels with respect to those obtained by raw products. These findings are in agreement with a previous work demonstrating that a partial ovalbumin denaturation improves gelling properties of egg products (Alamprese *et al.*, 2011). However, it has to be pointed out that with the ohmic heating a significant ($p<0.05$) increase of apparent viscosity of the whole egg was observed, directly influenced by the treatment temperature. This viscosity increase could be caused by a partial coagulation of the egg proteins, indicating that the applied treatments are too much severe for the heat sensitivity of the product. The temperature-time combinations were calculated in order to obtain the same pasteurization effect of a traditional treatment carried out at 70°C-1 min, that has been demonstrated as the condition able to guarantee the product safety.

Table 1 Influence of ohmic (O) or traditional (T) pasteurization treatment on foaming properties of whole egg (average ± s.d.).

Sample	Overrun (%)	Foam consistency (mA)	Foam instability (%)
Raw	413 ± 15^a	307 ± 1^b	71 ± 1^a
O-68°C-1.4 min	484 ± 3^c	294 ± 1^b	99 ± 3^c
O-71°C-0.6 min	465 ± 5^b	317 ± 1^c	78 ± 2^b
Raw	415 ± 23^b	326 ± 7^b	38 ± 1^a
T-65°C-3.5 min	324 ± 7^a	218 ± 3^a	96 ± 3^b

$^{a-c}$ different letters on the same column indicate significant differences ($p < 0.05$) between treated and raw samples.

Table 2 Influence of ohmic (O) or traditional (T) pasteurization treatment on gelling properties and apparent viscosity of whole egg (average ± s.d.).

Sample	Gel WHC (%)	Gel load (N)	Apparent viscosity (mPa s)
Raw	95.5±1.0[a]	13.2±0.3[a]	9.3±0.3[a]
O-68°C-1.4 min	95.9±0.5[a]	13.2±0.2[a]	13.9±0.1[b]
O-71°C-0.6 min	94.8±1.0[a]	13.7±0.3[b]	20.6±0.5[c]
Raw	93.1±4.1[a]	12.6±0.1[a]	10.7±0.6[a]
T-65°C-3.5 min	95.2±0.3[a]	13.2±0.3[b]	10.6±0.1[a]

[a-c] different letters on the same column indicate significant differences ($p < 0.05$) between treated and raw samples.
WHC, water holding capacity

In conclusion, the development of novel pasteurization technologies has widened the opportunities for food manufacturers of producing safety foods with improved functionalities. However, for large-scale applications, there are still some technical and economical issues to be overcome.

In the case of ohmic heating, our preliminary results demonstrated the potential of this technology in terms of whole egg functional properties, but they also evidenced a problem in protein denaturation that needs to be solved. The lower fluidity of the ohmic-treated product could in fact represent a major issue in production plant managing. Maybe, a combination of either novel and conventional processing technologies, which is termed “hurdle technology”, may represent a strategic approach to develop effective and efficient egg product pasteurization processes, able to maximize the synergistic effect on microbial inactivation and to minimize possible detrimental effects on functional properties.

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