The Role of Novel Pasteurization Approaches on Altering Functional Properties of Egg Proteins#

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A B S T R A C T

Eggs are important components of the human diet due to their low cost, high protein content and protein related technological features. High digestibility of egg proteins makes it possible to consume alone in the assay of nutritive values. Binding, emulsifying, foaming, gelling, and thickening properties of egg proteins provide an opportunity to use egg in various food products as an ingredient. Therefore, the consumption of egg is increasing with each passing day, however, Salmonella enterica serovar Enteritidis and Salmonella Typhimurium infections have been reported to be egg-born. These serious infections are originated from direct consumption of eggs or unpasteurized food products in which the egg yolk/albumen is added to the formulations such as mayonnaise, salad dressings or merengues. In order to prevent these infections, aforementioned microorganisms must be eliminated from the environment by pasteurization. Commercial pasteurization process is applied with hot water or vapor. Commercial processes include high temperature/short time or low temperature/long-time pasteurization. Although heat treatment is considered the most reliable method in terms of microbiological safety, high temperature and/or long time applications may have adverse effects on functional and nutritional properties of egg proteins. To ensure the microbiological safety of products without sacrificing technological or nutritional properties, researches have been centered upon innovative techniques such as irradiation, pulsed electric field, high hydrostatic pressure, and radiofrequency applications. This review is aimed to bring out the amendments occurred in the egg protein structures in consequence of aforementioned pasteurization methods.

Introduction

A whole egg consists of 8-11% shell, 56-61% egg white and 27-32% egg yolk. Eggs comprise of 64% egg white and 36% egg yolk in the absence of shell. Egg yolk provides most of the essential amino acids, important vitamins, folate and some micronutrients including choline while egg white contains ovalbumin, ovomucine, ovaltransferrin, lysozyme, and globulin that are responsible for technological properties of egg-based products. Considering these reasons use of egg became a requirement for young or elder people diet and food product formulations (Kusum et al., 2018). Functional properties of egg proteins in foods can be sorted as binding, gelling, emulsifying, clarifying and foaming in meat loafs, custards, salad dressing, broths and angel cakes respectively (Stadelman et al., 2017). Despite the positive effects of egg proteins on health and technology, contamination of egg and eggshell through processing and nutritional composition is a major problem (Whiley and Ross, 2015). S. enterica serotypes Typhimurium and Enteritidis are the most common microorganisms responsible for infections. Since some products added egg did not heat-treated, there might be an egg-related foodborne salmonellosis risk depending on microbial quality of egg (Gantois et al., 2009; Moffat and Musto, 2013). According to the Centers for Diseases Control and Prevention reported total 53 egg born Salmonella infections with high fever, vomiting, diarrhea and headache symptoms from 2016 to 2018. Even though none of the cases eventuated with death, the need for pasteurization of the egg used as raw material has emerged (CDC, 2016; CDC, 2018). Liquid egg white, egg yolk, and shell egg require 56.7°C /3.5 min., 61.1°C /3.5 min, and 57°C/75 min. for inactivation or inhibition of Salmonella spp. (Hou et al., 1996; FDA, 2000). However application of such a high temperature or long-time induces deterioration of tertiary structure of globulins irreversibly.
As a result of this effect, proteins lose their solubility and coagulate. Foaming properties is negatively affected at temperatures between 54-60°C as well as texture and taste. These kinds of changes are not preferred in food matrices generally (Cunningham, 1995; Campbells et al., 2003; Akkouche et al., 2012; Uysal et al., 2017).

Various novel pasteurization techniques have been demanded recently to eliminate the disadvantages triggered by heat treatment and also to ensure the microbiological safety of the products. Irradiation, pulsed electric field, ultrasound, high hydrostatic pressure and radiofrequency are some non-conventional applications that allow avoiding high temperatures in novel pasteurization techniques (Marco-Moles et al., 2011; Singh and Ramaswany, 2013; Uysal et al., 2017; Sheng et al., 2018). In this review, it was aimed to summarize the changes in egg proteins functionality by new pasteurization techniques.

The effects of novel pasteurization techniques on the egg proteins quality

Heat pasteurization is applied to eggs in order to guarantee the microbiological safety; however, heat processing should be controlled in case of the effect on the egg protein quality. For instance, normally, electrostatic forces lead to ovomucin-lysozyme interactions in egg white. This interaction exhibits reversible characteristics and has no adverse effect on the functional properties of egg white. However, application of pasteurization induces the formation of stable, rigid and insoluble complex. This complex lead to observe undesirable changes in foaming attributes such as more whipping time is required to ensure the equivalent foaming properties as much as fresh egg white (Garibaldi et al., 1968). Further changes in foaming ability may be associated with loss of conalbumins ability to foam correspondingly, cakes formulated with pasteurized eggs illustrated harder, sticky texture and lower volume (Hatta et al., 1996; Singh et al., 2019; Uysal et al., 2019).

Another important functional property of the egg which is affected by temperature is emulsification capacity provided by egg yolk. Due to the interactions of small fragments of livetins which unfolded more than optimum concentration with the effect of heat, generation of thick emulsion is observed in mayonnaises formulated with heat-treated (68°C) eggs more than 7 minutes (Guilmineau and Kulozik, 2007).

Also, the color of eggs is a significant characteristic that is affected from thermal treatments as a result of 3-dimensional gel structure formed and denaturation of proteins when the egg proteins meet with the heat (Min et al., 2012).

Considering all the changes provoked by heat researches directed to new alternatives less harmful methods such as irradiation, pulsed electric field (PEF), Ultrasound, High hydrostatic pressure (HHP) and radiofrequency (RF) applications.

Irradiation is a process that can inactivate Salmonella, Escherichia coli, Listeria from the eggshells in the absence of internal or external heat (Farkas and Mohacsi-Farkas, 2011). Irradiation can be applied to egg and egg products without changing the sensory or functional properties up to 3 kGy (Bakalinov et al., 2008). Better foaming capacity and more stable viscosity are provided by irradiated egg white proteins than heat-treated egg white proteins (Min et al., 2005), yet some studies also indicated that oxidative changes as a result of irradiation can depress the foaming properties (Arvanitoyannis, 2011). Irradiation causes conformational changes in the egg white proteins from α-helix to disordered structure and intermolecular β-sheet content was decreased as a result of irradiation (Uygun-Sarbay et al., 2017).

In a study carried out by Song et al. (2009), it was investigated that as the dose of irradiation increased, the foaming capacity of egg white is improved and same authors revealed that angel cakes formulated with 2 kGy irradiated egg white had the highest volume and height. Also, textural properties of angel cakes improved with the use of irradiated egg white. Increased foaming capacity could be associated with increased surface hydrophobicity and changes in α-helix structure. Despite the positive effects of irradiation, protein and lipid oxidation are under concern (Liu et al., 2009). Other researches regarding the effects of irradiation on the properties of egg proteins are given in Table 1.

Table 1 Researches focus the effects of irradiation applications on the properties of egg proteins

| Dose-application | Product | Results | References |
|------------------|---------|---------|------------|
| 0, 2, 5, 10 kGy-Irradiation | Shell egg (egg white analysed) | • Similar foaming capacity and stability (2.5 kGy)  
• Deteriorated color parameter (protein denaturation)  
• Generation of sulfur-containing volatiles | Min et al., 2012 |
| 0, 1, 2, 3 kGy Irradiation | Egg white | • Increased foam capacity, however, decreased foam stability  
• Decreased viscosity (separation of α-glikocid from ovomucin) | Uygun Sarbay and Köseoğlu, 2012 |
| 0, 1, 2, 3 kGy Irradiation | Egg yolk | • Loss of zeaxanthin and lutein (due to free radicals) | Uygun Sarbay et al., 2014 |
| 0, 1, 2, 3 kGy Irradiation | Egg white | • Decreased viscosity  
• Decreased intermolecular β sheet content α-helix to disordered structure | Uygun Sarbay et al., 2017 |
Figure 1 Changes in functional properties of egg affected from PEF treatment (Zhao et al., 2009; Marco-Moles et al., 2011; Monfort et al., 2012; Wu et al., 2014; Liu et al., 2017)
Table 2 Researches focus the effects of HHP application on the properties of egg proteins

| Dose-application | Product                      | Results                                                                                                                                                                                                 | References                         |
|------------------|------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------|
| 0-500 MPa 0,20 min. HHP | Liquid whole egg            | • Maximum foaming capacity (350 MPa) (protein aggregation up to a specific point)  
• Generation of sulfhydryl groups  
• Increased hydrophobicity-foaming capacity                                                                 | Yang et al., 2009                  |
| 100 MPa, 1,3,5 min. HHP | Liquid whole egg            | • Lower viscosity compared to heat-treated samples  
• Increased foaming capacity (unfolding of sulphydryl groups)                                                                                     | Patrignani et al., 2013            |
| 600-900 MPa (0-15 min) HHP | Liquid egg white            | • Pressure-induced gels were soft and highly elastic  
• Full set egg gels with improved physicochemical characteristics and without any cooked flavors.                                              | Singh and Ramaswamy, 2013          |
| 400,600-800 MPa HHP | Egg white                   | • Increase its pepsin digestibility at 800 MPa to a greater extent than did the thermal treatment at 95 °C.  
• Increased protein digestibility  
• Less potential egg born food allergy due to increased pepsin digestibility.                                                            | Hoppe et al., 2013                 |
| 350-550MPa 5-15 min HHP | Liquid whole egg  
Liquid egg white | • The highest level of pressure treatment (550 MPa for 15 min) was sufficient to cause complete gelatinization  
• Egg yolk to turn brighter yellow, and egg white to become translucent and white in color.  
• The optimum values of color, viscosity, viscoelasticity and foaming were found at around 550 MPa pressure treatment for 5 min | Singh and Ramasawamy, 2015         |
| 400 MPa 600 s HHP | Liquid whole egg  
Liquid egg yolk  
Egg white | • Denaturation of 40% of egg yolk’s proteins  
• Aggregation and separation of protein groups  
• Viscous egg yolk and whole egg                                                                                                                          | Tóth a 2016                       |
| 200-350 MPa (5 min) HHP | Liquid whole egg  
Liquid egg white  
Liquid egg yolk | • No detectable protein denaturation  
• Most pressure-sensitive egg type was liquid egg white  
• Similar foaming ability to raw samples  
• No color changes between treatments                                                                                                                      | Tóth et al 2017                   |

Table 3 Researches focus the effects of US application on the properties of egg proteins

| Dose-application | Product                      | Results                                                                                                                                                                                                 | References                         |
|------------------|------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------|
| 200-300-450 W 2-5 min US | Shell eggs                  | • The higher viscosity of egg yolks compared to control groups at the end of the storage.  
• Higher foaming capacity compared to control samples  
• Power levels of 300W and 450W of ultrasound treatments had improved internal quality of fresh eggs during storage, but negative effect on shell strength. | Caner and Yüceer, 2015             |
| 20 kHz, 34-36 and 45-48 W/m2 for 20-40 min US | Ovalbumin                  | • Increased surface hydrophobicity and decreased surface net charge  
• Higher emulsion and foaming capacity  
• Increased gelation temperatures of ovalbumin                                                                                                        | Xiang et al., 2016                 |
| 400 W 1,4,8,12 ve 16 min. | Liquid egg white            | • A decline in α-helices and an increase of β-sheets.  
• Secondary structure content is not affected by ultrasonication time.                                                                                | Zhu et al., 2018                   |
| 0,75, 150, 225 and 300 W 10 min. US | Egg yolk                   | • Increased emulsifying, foaming and gel properties, however, decreased foam stability.  
• Increased free sulphydryl content  
• Reduced the average particle size                                                                                                                      | Xie et al., 2019                   |
| 180 W 25 min.  
0,15,30,45,60,75 and 50 day storage | Egg white                   | • Increased foaming ability  
• Highest foaming ability was found in 60 day  
• Increased free sulphydryl content and surface hydrophobicity, thus easier adsorption to the interface.                                             | Chen et al., 2019                  |
Ultrasonic (US) treatment is another non-thermal technology that could be used instead of heat pasteurization. Ultrasonic waves transmitted to the product via media. Its popularity is increasing each passing day (Yüceer and Caner, 2018). Researches regarding the ultrasound applications to egg products are given in Table 3. Ultrasonic treatment alters the functional properties of the proteins by damaging covalent bonds and disruption of large aggregates to small particles (Stefanovic et al., 2014). Foaming capacity is associated with partial protein unfolding, higher solubility, and smaller particle size. Also, ovomucin degradation with ultrasonic cavitation could decrease particle size and viscosity. Degraded ovomucin or partially unfolded proteins adsorb to interface and enhance the foaming capacity, however, foam stability is negatively affected by the reduction of viscosity (Stefanovic et al., 2017; Sheng et al., 2018; Gharbi and Labbafi, 2019). Arzeni et al., (2012) stated that ultrasonic treatment increased the emulsion stability while the dynamics of gelation and gel strength are not affected by ultrasonication. However, Ye et al., (2018) stated that gel stability is increased by 360W ultrasonication process. Quite higher foaming capacity is reported after 360 W ultrasound process. Approximately 5 fold higher foaming ability was found in samples treated with 360 W ultrasound waves than control groups. Solubility of proteins is increased through application of ultrasound, these findings are also proved by small aggregate and pores in scanning electron microscopy images (Sheng et al., 2018). Ultrasound process leads changes in tertiary structure due to increments in partial unfolding of ovalbumin and free sulphydryl groups, therefore, emulsifying and foaming abilities of ovalbumin are enhanced (Xiong et al., 2016).

Radiofrequency is another pasteurization method that may enhance the gelling properties of egg white without sacrificing the foaming capacity (Boreddy et al., 2014). Radiofrequency treatment is thought to be a method that can be applied in order to avoid the disadvantage of long-time heat treatment in shell eggs. In a study conducted by Geveke et al., (2017), shell eggs were pasteurized in a water bath with 600 MHz. As a result of time of heating the methods had undesirable changes, researches are focused on some novel approaches such as irradiation, pulsed electric field, ultrasound, high hydrostatic pressure and radiofrequency. All novel methods had favourable effects on foaming, emulsifying, gelling properties or solubility of egg proteins depending on the parameters (time, electric field, power or etc.). Irradiation process aggregates the proteins up to a specific level, by this effect a decrement in viscosity and increment in foaming capacity are observed, however, there are some suspicious thoughts due to increment in oxidation processes.

Since pulsed electric field method does not contain heat, no colour changes are taking place, as a matter of fact unfolding of proteins triggered by electric field enhance gelling and foaming properties. Despite the positive effects, microbiological safety of eggs processed with this method is under concern, thus it is recommended that PEF should be used with mild heat treatments.

HHP treatment positively affects the functionality of proteins depending on the pressure, however, increasing pressure may destruct the functional properties. Changes observed in ovomucin as a result of ultrasound treatment increase the foaming ability. Radiofrequency is a method that should be used with heat treatment. The radiofrequency treatment has minimal change on egg proteins as a result of reduced temperature and/or time.

It can be concluded that novel techniques can replace commercially available heat treatment, however, further researches are needed to focus the microbiological and functional changes in egg-containing products (cake, merengue, etc.) when the egg in the formulation treated one of these novel methods. These studies will show whether these methods can be used as a complete heat process replacer.

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