



## The Role of Novel Pasteurization Approaches on Altering Functional Properties of Egg Proteins<sup>#</sup>

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ARTICLE INFO	ABSTRACT
<p><sup>#</sup>This study was presented as an oral presentation at the 4th International Anatolian Agriculture, Food, Environment and Biology Congress (Afyonkarahisar, TARGID 2019)</p> <p>Review Article</p> <p>Received : 30/06/2019 Accepted : 06/11/2019</p> <p>Keywords: Egg Egg products Protein functionality Novel pasteurization techniques Foaming</p>	<p>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, <i>Salmonella enterica</i> serovar Enteritidis and <i>Salmonella</i> 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.</p>

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### 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, ovotransferrin, 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 loss their solubility and coagulate. Foaming properties is negatively affected 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 Ramaswanay, 2013; Uygun-Saribay 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 the 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  $\alpha$ -helix to disordered structure and intermolecular  $\beta$ -sheet content was decreased as a result of irradiation (Uygun-Saribay 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  $\alpha$ -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)	<ul style="list-style-type: none"> <li>• Similar foaming capacity and stability (2.5 kGy)</li> <li>• Deteriorated color parameter (protein denaturation)</li> <li>• Generation of sulfur-containing volatiles</li> </ul>	Min et al., 2012
0, 1, 2, 3 kGy Irradiation	Egg white	<ul style="list-style-type: none"> <li>• Increased foam capacity, however, decreased foam stability</li> <li>• Decreased viscosity (separation of o glikocid from ovomucin)</li> </ul>	Uygun Saribay and Köseoğlu, 2012
0, 1, 2, 3 kGy Irradiation	Egg yolk	<ul style="list-style-type: none"> <li>• Loss of zeaxanthin and lutein (due to free radicals)</li> </ul>	Uygun Saribay et al., 2014
0, 1, 2, 3 kGy Irradiation	Egg white	<ul style="list-style-type: none"> <li>• Decreased viscosity</li> <li>• Decreased intermolecular <math>\beta</math> sheet content <math>\alpha</math>-helix to disordered structure</li> </ul>	Uygun Saribay et al., 2017

No protein coagulation and lowered aggregation of proteins
No noticeable change in protein functionality
Lysozyme inactivation (higher electrostatic fields) (loss of $\alpha$ helix structure)
35 kV/ 1200 $\mu$ s induced the unfolding of tertiary structure, thus cleaving of disulfide bonds observed.
Application of PEF more than 400 $\mu$ s (25 kV) caused generation of insoluble lysozyme, ovalbumin and ovotransferrin aggregates.
No color changes
Foaming ability as much as raw egg (up to 32 kV)
Better gel stability (unfolding of secondary structure)
Increased emulsion capacity (partial unfolding of proteins)

Figure 1 Changes in functional properties of egg affected from PEF treatment (Zhao et al., 2009; Marco-Mol' es et al. 2011; Monfort et al., 2012; Wu et al., 2014; Liu et al., 2017)

PEF is a suitable pasteurization application for liquid food, and its working principles can be explained as applying external electrical fields for a short time (Yogesh, 2016). When the PEF and thermal treatment is compared, it can be stated that while heat treatment cause and opaque appearance due to thermal coagulation of proteins, PEF did not change the color of egg white. Also up to 32 kV/cm PEF application exhibit foaming capacity as good as fresh egg white; however, after 37 kV/cm protein granules denature and degrade so, foaming capacity is affected negatively (Marco-Moles et al. 2011). Other changes in the functional properties of egg proteins affected by PEF treatment are given in Figure 1. Wu et al., (2015) reported that insoluble aggregates formed by thermal treatment contained ovotransferrin, lysozyme, and ovalbumin. Solubility of proteins is more affected by heat treatment than PEF. Electric field did not change the turbidity however, as the application time is increased turbidity is also increased due to interaction between protein molecules (Wu et al., 2015). Structure and function of lysozyme were not decreased by the action of PEF (35 kV/cm for 300  $\mu$ s in sodium phosphate buffer) (Zhao and Yang, 2008).

In liquid whole eggs, 25 kV/cm, 105 $\mu$ s ve 35 kV/cm, 45 $\mu$ s PEF application only decreased 3 logs of *Salmonella enteritidis* ve *Salmonella senftenberg* counts (Monfort et al., 2010); however, using PEF with heat and/or additives (lowering the surface tension) enhanced the foaming and emulsion ability of eggs, while loss of proteins were decreasing (Monfort et al., 2012; Espina et al., 2014).

HHP technology which is applying pressures to the product between 100 and 1000 MPa has drawn attention from food industry due to the opportunity of low or moderate temperatures application during the process in order to eliminate the microorganisms without affecting the nutritional quality of foods (Koutchma, 2014; Naderi et al., 2017). Secondary structure of proteins is affected by high pressures, while tertiary and quaternary structure can be affected above 100 MPa. Since protein functionalities

are provided by tertiary structure, high-pressure treatment brings about some changes in the quality of proteins (Tewari and Juneja, 2008). High-pressure treatment provokes proteins denaturation and coagulation through pressure effect (Andrassy et al., 2006). However, effects of pressure on the proteins functional properties are changeable, for instance Van Der Plancken, et al. (2007) observed that foaming stability and capacity is improved through the exposure of sulfhydryl groups by the effect of high pressures as 500-700 Mpa while another study showed that 100-500 MPa decreased the solubility, emulsifying activity, free sulfhydryl content and surface hydrophobicity of egg yolk proteins (Yan et al., 2010). In another study, it was observed that high pressure coagulated the egg whites, also increasing pressure and application time resulted gelation of proteins like egg patties. Also pressure increased the viscosity of egg yolks (Singh and Ramaswamy, 2013). Finally, high pressure can improve the foaming properties; however, it can also lower the emulsion capacity compared to heat treatment (Monfort et al., 2012). The foam density is positively affected by the high-pressure process; however, extensive unfoldings in proteins due to the pressure can also cause a decrease in foam stability (Singh and Ramaswamy, 2015). The changes in proteins reported by other researchers as a result of HHP are presented in Table 2.

In HHP and high intensity ultrasound (HIU) methods, foaming properties of albumen which are based on ovomucin network degradation, protein unfolding, and aggregation are affected. Under HHP and HIU treatments, ovomucin degradation produces small particles with higher solubility and flexibility (Gharbi and Labbari, 2018). Disruption of ovomucin network, unfolding and recovery of disrupted ovomucin by the formation of SS bonds and aggregation of proteins increases the surface hydrophobicity or viscosity thus, foam properties enhance (Van der Plancken et al., 2007; Brand and Kulozik, 2016; Brand et al., 2016; Naderi 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	<ul style="list-style-type: none"> <li>• Maximum foaming capacity (350 MPa) (protein aggregation up to a specific point)</li> <li>• Generation of sulfhydryl groups</li> <li>• Increased hydrophobicity- foaming capacity</li> </ul>	Yang et al., 2009
100 MPa, 1,3,5 min. HHP	Liquid whole egg	<ul style="list-style-type: none"> <li>• Lower viscosity compared to heat-treated samples</li> <li>• Increased foaming capacity (unfolding of sulphhydryl groups)</li> </ul>	Patrignani et al., 2013
600-900 MPa (0-15 min) HHP	Liquid egg white	<ul style="list-style-type: none"> <li>• Pressure-induced gels were soft and highly elastic</li> <li>• Full set egg gels with improved physicochemical characteristics and without any cooked flavors.</li> </ul>	Singh and Ramaswamy 2013
400,600-800 MPa HHP	Egg white	<ul style="list-style-type: none"> <li>• Increase its pepsin digestibility at 800 MPa to a greater extent than did the thermal treatment at 95 °C.</li> <li>• Increased protein digestibility</li> <li>• Less potential egg born food allergy due to increased pepsin digestibility.</li> </ul>	Hoppe et al., 2013
350-550MPa 5-15 min HHP	Liquid whole egg Liquid egg white	<ul style="list-style-type: none"> <li>• The highest level of pressure treatment (550 MPa for 15 min) was sufficient to cause complete gelatinization</li> <li>• Egg yolk to turn brighter yellow, and egg white to become translucent and white in color.</li> <li>• The optimum values of color, viscosity, viscoelasticity and foaming were found at around 550MPa pressure treatment for 5 min</li> </ul>	Singh and Ramasawamy, 2015
400 MPa 600 s HHP	Liquid whole egg Liquid egg yolk	<ul style="list-style-type: none"> <li>• Denaturation of 40% of egg yolk's proteins</li> <li>• Aggregation and separation of protein groups</li> <li>• Viscous egg yolk and whole egg</li> </ul>	Tóth a 2016
200-350 MPa (5 min) HHP	Liquid whole egg Liquid egg white Liquid egg yolk	<ul style="list-style-type: none"> <li>• No detectable protein denaturation</li> <li>• Most pressure-sensitive egg type was liquid egg white</li> <li>• Similar foaming ability to raw samples</li> <li>• No color changes between treatments</li> </ul>	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	<ul style="list-style-type: none"> <li>• The higher viscosity of egg yolks compared to control groups at the end of the storage.</li> <li>• Higher foaming capacity compared to control samples</li> <li>• Power levels of 300W and 450W of ultrasound treatments had improved internal quality of fresh eggs during storage, but negative effect on shell strength.</li> </ul>	Caner and Yüceer, 2015
20 kHz, 34–36 and 45–48 W/m <sup>2</sup> for 20–40 min US	Ovalbumin	<ul style="list-style-type: none"> <li>• Increased surface hydrophobicity and decreased surface net charge</li> <li>• Higher emulsion and foaming capacity</li> <li>• Increased gelation temperatures of ovalbumin</li> </ul>	Xiang et al., 2016
400 W 1,4,8, 12 ve 16 min.	Liquid egg white	<ul style="list-style-type: none"> <li>• A decline in <math>\alpha</math>-helices and an increase of <math>\beta</math>-sheets.</li> <li>• Secondary structure content is not affected by ultrasonication time.</li> </ul>	Zhu et al., 2018
0, 75, 150, 225 and 300 W 10 min. US	Egg yolk	<ul style="list-style-type: none"> <li>• Increased emulsifying, foaming and gel properties, however, decreased foam stability.</li> <li>• Increased free sulfhydryl content</li> <li>• Reduced the average particle size</li> </ul>	Xie et al., 2019
180 W 25 min. 0,15,30,45,6 0,75 and 50 day storage	Egg white	<ul style="list-style-type: none"> <li>• Increased foaming ability</li> <li>• Highest foaming ability was found in 60 day</li> <li>• Increased free sulfhydryl content and surface hydrophobicity, thus easier adsorption to the interface.</li> </ul>	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. Degradated 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 sulfhydryl 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 time of heat treatment could be reduced up to 60% by radiofrequency treatment in terms of *Escherichia coli* inhibition.

Geveke et al., (2018) found that using RF treatment (35 W, 6 min.) with hot water pasteurization (56.7°C 5, 10 and 15 min.) provided better inactivation of *Salmonella* than samples only treated with 56.7°C for 60 min without affecting the albumen quality. Angel cakes formulated with eggs pasteurized with RF+ 56.7°C 15 min. had better volume than samples treated with hot water for 60 min. Better foaming stability and lower whipping time are reported with combined use of RF and hot water treatment than hot water treated samples (Yang et al., 2019).

## Conclusion

Heat application to egg and egg products could meet the microbiological requirements based on the time and temperature parameters. High temperature applications or long application period lead to heat-induced changes in egg proteins that may cause loss of some functional and nutritional properties. In order to minimize these 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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