

## Effect of Freezing-Thawing and Stabilizers on the Phase Behavior of Egg Micro-Particles and Quality Attributes of Liquid Egg

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### ABSTRACT

Freezing can adversely affect the quality of liquid egg through complex inter-particle interactions of egg micro-particles. The effect of pectin, PG-Alginate (PGA), Arabic Gum, maltodextrin and defrost temperature on the rheological properties, microstructure and color of frozen egg was studied. The fresh sample exhibited a light yellow color with high values of  $L$  and positive values of  $a$  and  $b$ . Freezing resulted in a reduced  $L$  value with slight changes in  $a$  and  $b$ . The additives could enhance the lightness with the closest values of  $L$  to that of the fresh sample detected for PGA and pectin. Fresh egg with a low viscosity exhibited near-Newtonian flow behavior. However, the freezing-thawing significantly increased the viscosity. Accordingly, the flow behavior index of the frozen samples was decreased significantly along with a sharp increase in the consistency index, revealing a pseudo-plastic behavior. All frozen samples exhibited higher viscosities than the fresh sample. Fresh samples contained evenly distributed micro-particles ranging from 0.05 to 5.50  $\mu\text{m}$  centering at 1.27  $\mu\text{m}$ . Freezing resulted in aggregated particles with significantly larger sizes. Maltodextrin significantly reduced the particle size. Further reduction was achieved by the addition of pectin, PGA, and Arabic gum. Smallest particle size distributions were achieved at pectin and PGA concentrations of 0.25 and 0.1%, respectively. The results of this study can be employed for the development of new products based on frozen egg with no added sugar or salt, while maintaining the physical and functional properties of the final product.

**Keywords:** Arabic gum, Flow behavior, Microstructure, Pectin, PG-Alginate.

### INTRODUCTION

Hen's egg has been known from the ancient times as an important food product for humans. Egg has gained major attentions in the food sector research and development due to its wide spread consumption and its application as an ingredient for a varied range of processed foods (Surai and Sparks, 2001).

Salmonella poisoning outbreaks related to raw eggs (shell egg), as a serious concern in terms of food safety, technological restrictions in consumption of this type of egg, as well as the development of food

industry and food processing and preservation methods, has led to increased production and supply of processed eggs (whole eggs, yolks and whites) (Mukhopadhyay and Ramaswamy, 2012).

Freezing is an excellent food preservation method preventing microbial, chemical and physical alterations by reducing water activity and temperature (Galletto *et al.*, 2010). However, appropriate design of the processing conditions is very critical to avoid tissue damage and changes in the functional properties of frozen food products. Changes in the rheological and functional properties are caused by changes

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in the molecular arrangement of egg compounds, dehydration, and changes in the concentration of electrolytes. Many researches have been focused on the prevention of these undesirable changes (Lovelock, 1957; Smith, 1961). Factors, such as the freezing rate, supercooling degree, thawing rate, thawing temperature, and ice crystal characteristics are critical to minimize the freezing damage (Ballin and Lametsch, 2008; Muela *et al.*, 2012). Application of different additives such as sugars, salts, and enzymes, and also the physical and mechanical treatments have been among different methods tried by researchers (Chang *et al.*, 1977; Kaloyereas *et al.*, 1962; Powrie *et al.*, 1963).

Food stabilizers, thickeners, and gelling agents, which are obtained from a wide range of natural raw materials, control moisture and provide structure, flow, stability and eating qualities to food products (Imeson, 2011). Among these materials are hydrocolloids, which prevent the formation of ice and sugar crystals (Dickinson, 2003). The use of hydrocolloids as stabilizers, thickeners and water preservatives agents in food is dependent on the type and intensity of polysaccharides interactions with other ingredients such as proteins (Hemar *et al.*, 2002; Ibanoglu and Erçelebi, 2007). Boye *et al.* (1997) showed that hydrocolloids can be effective in prevention of proteins aggregation and coagulation during heat treatments. As temperature changes can cause undesirable changes in food texture, the hydrocolloid stabilizers can absorb water and prevent the ice formation (Flores and Goff, 1999). Chanamai and McClements (2002) reported that high molecular weight hydrocolloids, with the ability of water absorption, can form a thick layer around the particles and prevent their accumulation under the unfavorable conditions such as thermal shock and high salt concentrations.

According to our bibliography, until now, no Scientific study has been reported about the effect of adding hydrocolloids on the properties of frozen eggs. So, due to

limitations in consumption of the egg products containing salt and sugar in different food industry, and also according to the aforementioned ability of hydrocolloids in controlling ice crystals and texture, the main objective of this study was to evaluate the effect of four additives, including maltodextrin, pectin, Propylene Glycol Alginate (PGA) and Arabic Gum, in various concentrations on color, rheological properties, and microstructure of frozen-thawed whole egg. In addition, we aimed to study the effect of thawing temperature on the mentioned properties and evaluate the microstructure to explore the behavior of egg micro-particles.

## MATERIALS AND METHODS

### Raw Materials

Fresh eggs (Hy-Line breed, single mass around 45 g, after laid within 7 days) were obtained from Telavang Co. (Tehran, Iran) and stored under refrigeration at 8°C until use. Propylene Glycol Alginate (PGA), Arabic gum, and high methoxyl pectin were provided by ZamZam Iran Co (Tehran, Iran). Maltodextrin (DE= 20) was purchased from Peyvandi Co. (Tehran, Iran).

### Sample Preparation

The eggs were manually broken and, after removing the chalaza, transferred to a stomacher bag and then homogenized for 2 minutes at 230 rpm in a laboratory mixer. In order to achieve uniform samples, in each series of sample preparation, at least twenty eggs were used. Before each treatment, to obtain the corresponding final samples, the additives were added directly to the raw whole egg according to Table 1 while the samples were being mixed (IKA Eurostar Power control Visc, Germany). After preparing of different samples, they were packed in polyethylene containers (3 samples for each formulation) with 300 mL

**Table 1.** The concentration (% wt) of additives in whole egg.

Additive	Percentage (% wt)
Maltodextrin	0, 2
Pectin	0, 0.1, 0.25, 0.5
PGA	0, 0.1, 0.25, 0.5
Gum Arabic	0, 1, 2, 3

volume and used for freezing. The freezing process was performed in a laboratory freezer (Pars, Tehran, Iran) at  $-18 \pm 2^\circ\text{C}$  and before thawing the samples they were kept for a week at  $-18^\circ\text{C}$ . The frozen eggs were thawed at 4 or  $20^\circ\text{C}$  and were subsequently analyzed. To ensure that the core of the samples reached the desired temperature, a thermocouple was placed in the center of each container.

### Rheological Properties

The rheological properties of the whole egg samples were measured by a viscometer (Brookfield DV II+Pro, Brookfield Engineering Lab. Inc., MA, USA) equipped with a cylindrical spindle (LV-1) (cylinder diameter 18.84 mm, length 115 mm, beaker diameter 86.30 mm and 40 mL of sample volume). A shear ramp test was performed and the power law model was used to calculate the rheological parameters.

$$\tau = k\dot{\gamma}^n \quad (1)$$

Where,  $\tau$  is shear stress (Pa),  $\dot{\gamma}$  is shear rate ( $\text{s}^{-1}$ ),  $k$  is consistency coefficient ( $\text{Pa s}^n$ ) and  $n$  is flow behavior index. In each formulation for each prepared samples at least 3 measurements were done.

### Color and Microstructure

The color of the samples was measured using a calibrated image processing system equipped with a DNT color camera (DNT, Germany) based on reflectivity and the Image Pro Plus software (Ver. 6.2, Media Cybernetics Inc., Washington, US). The Lab color parameters (L, a, and b) were

calculated and were used to study the samples.

The microstructure of the samples was analyzed using a Leica S20 microscope (Leica, Germany) equipped with a digital camera. The magnification of the system, including the microscope and the camera, was 1,000 folds. For particle size analysis, the samples were first diluted 100 times in distilled water and were mixed using a vortex. Then, one drop of the sample was placed on a microscope slide and was analyzed in the phase contrast mode. The images were first calibrated and then processed using the Image Pro Plus software to calculate the size of micro-particles. For each prepared sample, at least 3 measurements were done.

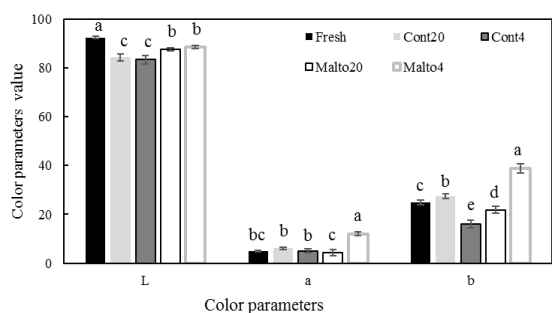
### Statistical Analysis

Statistical analysis was carried out using the SAS software (Ver. 9.1.3, NC, USA). Analysis Of Variance (ANOVA) test was done to find differences between the treatments and means (LSD) comparison test was performed, if necessary (95% confidence). Differences were presented by alphabetic letters.

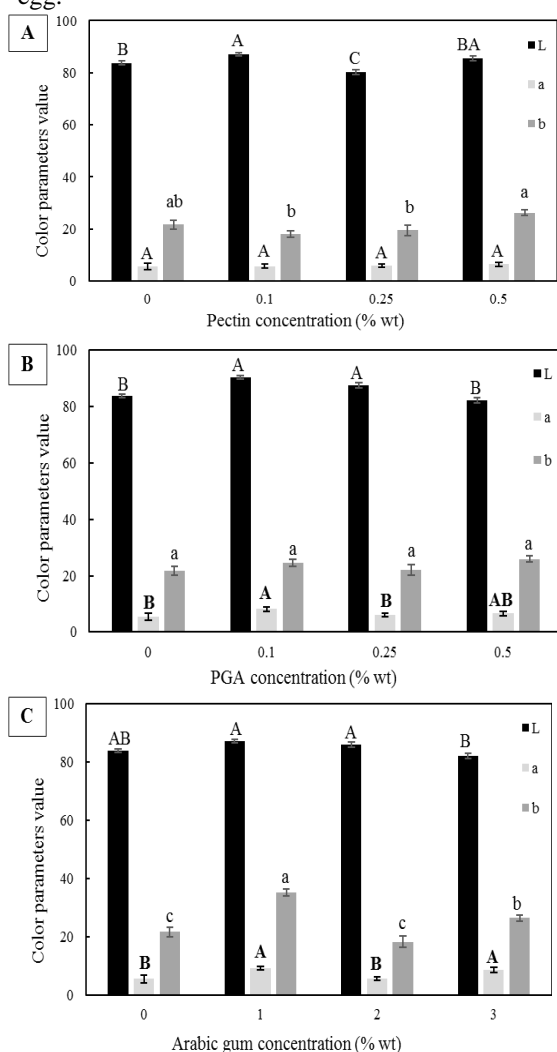
## RESULTS AND DISCUSSION

### Color

In Figures 1 and 2, the color parameters of different whole egg samples containing different stabilizers and processed at different thawing temperatures are shown. The color components  $L$ ,  $a$ , and  $b$  for the fresh sample were 92.43, 4.85, and 24.83, respectively. This observation indicated that the fresh whole egg sample analyzed in this study exhibited a light yellowish color developed from the combination of egg yolk and egg white. Statistical analysis revealed that the freezing-thawing cycle reduced  $L$  value significantly ( $P < 0.05$ ) indicating that the process resulted in a darker product.



**Figure 1.** The effect of maltodextrin addition and thawing temperature on the color parameters attributes of whole frozen-thawed egg.



**Figure 2.** The effect of pectin (A), PGA (B) and Arabic gum (C) concentrations on the color parameters of whole frozen-thawed egg samples.

Usually, darker colloids are produced when the size of the suspended particles become larger and reflect the light to increase the cloudiness (Min *et al.*, 2005). No significant differences were detected between *L* values for different thawing temperatures ( $P < 0.05$ ). The redness was not affected by the freezing process, but the yellowness (*b* value) was increased significantly ( $P < 0.05$ ) for the samples thawed at 20°C. However, a decrease of *b* value was observed for the samples thawed at 4°C. Maltodextrin increased the value of *L* in comparison with the control frozen samples. This could be attributed to the lower degree of aggregation and will be discussed in the next sections. The presence of maltodextrin resulted in decreased values of both *a* and *b* factors for the thawing temperatures of 20°C, while *a* and *b* were increased when the samples were thawed at 4°C. It should be noticed that the final color perception of liquid egg mostly depends on the chemical and physical properties, interaction of the ingredients and their conformation (Min *et al.*, 2005). For example, Su and Lin (1993), in a study on the effect of heat treatment on the color of egg, reported that denaturation of specific proteins could alter the transparency or turbidity of the samples depending on the type and intensity of the product. Therefore, color changes in egg during freezing could be explained by the changes in the water state from free and available water to frozen water that resulted in the concentration of the micro-particles and ingredients of the liquid whole egg.

In Figure 2-A, the effect of different concentrations of pectin on the color changes of frozen-thawed egg samples is displayed. The lowest and the highest applied concentrations (0.1 and 0.5%, respectively) increased the *L* value of the samples while the sample with 0.25% pectin reduced this color component. Evaluation of a component of color showed no significant effect of pectin on this parameter. The same could be observed for *b* values excluding the concentration of 0.5% pectin that increased

the  $b$  value, significantly. MacDougall (1982) indicated that changes in the lightness of food can be resulted from changes in the configuration of proteins including denaturation. Microparticles and their interaction can also affect the lightness strongly and they have proteins on their surface. In general, changes in the structure and configuration of food components can affect the reflection and absorption of light.

In Figure 2-B the effect of different concentrations of PGA on the color parameters of whole egg samples is displayed. In comparison with the control frozen-thawed sample, the samples containing PGA showed higher values for lightness with the peak value of lightness observed for 0.1 and 0.25% PGA. No direct relationship was observed between PGA concentration and  $a$  value but the maximum  $a$  value was detected for the concentration of 0.1%. The yellowness component,  $b$ , was not affected by PGA concentration.

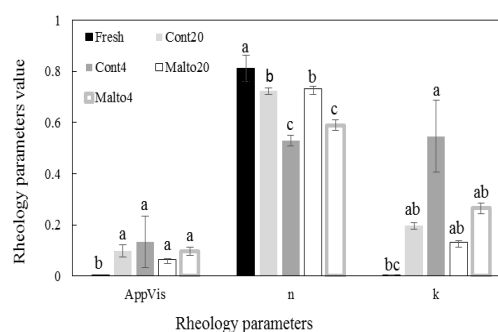
The effect of Arabic gum on the color parameters are displayed in Figure 2-C. The results indicated that the highest lightness ( $L$  value) was observed for the sample containing 1% Arabic gum ( $L=87.17$ ) followed by the sample with 2% gum. Further increase in the gum concentration resulted in the reduced  $L$  value. It means that upon the addition of Arabic gum the  $L$  value was increased, but a reverse relationship between the gum concentration and  $L$  value was observed. The yellowness was also at its maximum for 1% gum (35.24) but higher concentrations gave lower  $b$  value. However, the  $b$  value of all concentrations was higher than the control sample. Similar to the results observed for PGA, the changes in  $a$  value did not exhibit a distinct pattern.

MacDougall (2002) reported that changes in the visible color might not only be affected by the direct influence of processing conditions on the pigments but may arise from their indirect effect on other ingredients in the food sample. Although during the processing of egg pigments such as xanthophyll may undergo oxidative or heat induced degradation, the results of this

study suggested that the main factors determining the color changes in the frozen-thawed egg samples could be configuration and interaction of egg particles and proteins. In most of the samples studied here,  $a$  and  $b$  were affected to a smaller extent in comparison with  $L$ . The color component of  $L$ , as described previously, represents the lightness or darkness and accounts for light absorption or reflection. This behavior of light is mainly controlled by larger colloidal particles. About the effects of thawing temperature (4 and 20°C) on color parameters, no significant differences were observed for pectin, PGA, and Arabic gum containing samples.

### Rheological Properties

The rheological properties of frozen-thawed samples are displayed in Figures 3 and 4 for different conditions. As indicated in Figure 3, the apparent viscosity, flow behavior index, and consistency index of the fresh whole egg samples were 1.75 mPa s, 0.81, and 0.0025 Pa s, respectively. The results showed that the fresh samples exhibited a near Newtonian behavior with low viscosities, complying with the results of the literature. Review of the literature indicated that whole egg has been known to exhibit a Newtonian rheological behavior at temperatures below 60°C (Hamid-Samimi



**Figure 3.** The effect of maltodextrin addition and thawing temperature on the rheology parameters attributes of whole frozen-thawed egg.



and Swartzel, 1985; Hmid-Samimi *et al.*, 1984; Scalzo *et al.*, 1970). Atilgan and Unluturk (2008) reported that whole egg was a near-Newtonian fluid at room temperature. Freezing and thawing of whole egg resulted in a significant increase in the apparent viscosity for both thawing temperatures of 4 and 20°C with values reaching 132.61 and 97.87 mPa s, respectively. Meanwhile, flow behavior index exhibited a sharp decline such that at the thawing temperature of 4°C its value decreased to 0.53. Accordingly, consistency index showed a significant increase after thawing. These observations suggested that the freezing-thawing process changed the nature of the fresh sample and produced a viscous material with a distinct non-Newtonian behavior. This behavior was dominant for the thawing temperature of 4°C, probably due to the prolonged low temperature exposure. Studies have shown that denaturation, coagulation, and gelation of proteins and granules in whole egg caused by different processes could alter the viscosity considerably (Dawson and Martinez-Dawson, 1998; Herald and Smith, 1989; Hmid-Samimi *et al.*, 1984). The results of color measurement confirmed this observation and, as will be discussed in the next sections, microstructural measurements also support this result. The components originating from yolk have been confirmed to play a major role in this regard (Jaax and Travnicek, 1968; Moussa *et al.*, 2002).

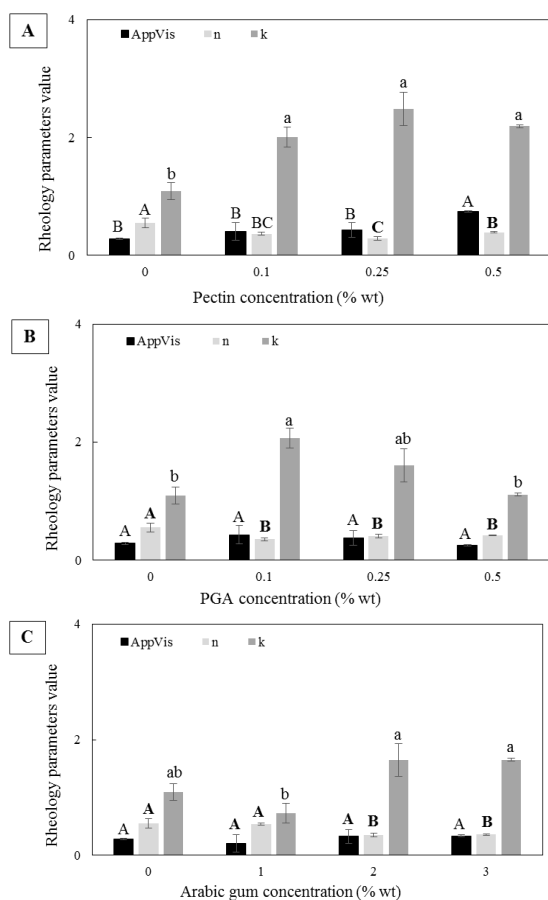
As shown in Figure 3, Maltodextrin was able to prevent the gelation of whole liquid egg to some extent and lower consistencies were achieved. However, this assumption needs further microstructural investigations that will be discussed later.

Analysis of rheological parameters for different defrost temperatures revealed that the changes were dominant for the thawing temperature of 4°C, probably due to a prolonged process time required for the complete thawing. Thawing at higher temperatures can increase the rate of heat transfer and, therefore, a quicker process can be achieved. However, thawing at higher

temperatures can increase the risk of microbial spoilage and in some cases it may be necessary to use refrigerator temperatures for defrosting the samples to avoid microbiological risks.

According to Figure 4-A, no significant differences were observed between the samples containing 0.1 and 0.25% pectin and the control sample, but an increase in the apparent viscosity was detected for the sample with 0.5% pectin. In addition, the flow behavior index exhibited a declining trend by increasing the concentration of pectin. The consistency index of egg samples was also increased by the addition of pectin. These observations indicated that the frozen-thawed samples containing pectin were thick pseudo-plastic materials. Since pectin is considered as a gelling and thickening agent, it could be expected that it can create a network within the samples. Particle size evaluations might help to clarify the role of pectin versus the role of particle aggregation in the rheological behavior of the samples.

In Figure 4-B, the effect of different concentrations of PGA is shown. The concentration in the range of 0.1 to 0.5% did not show a significant effect on the apparent viscosity in comparison with the control sample, while the flow behavior index decreased significantly by the addition of PGA. Consistency index was increased by the addition of 0.1% PGA and reached its peak. Further increase in the PGA concentration caused a reduction in the consistency index in a linear manner. Increasing the concentration of gums usually increases the apparent viscosity and consistency of a product (Kayacier and Dogan, 2006). However, a diverse relationship between the concentration and consistency was detected in the case of PGA in frozen-thawed whole egg. Initially, it could be noticed that, in addition to the gum concentration, freezing induced aggregation and gelation of egg particles, which is important for the determination of the consistency of the product. Secondly, such a behavior is usually observed when the added



**Figure 4.** The effect of pectin (A), PGA (B) and Arabic gum (C) concentrations on rheology parameters of frozen-thawed whole egg samples.

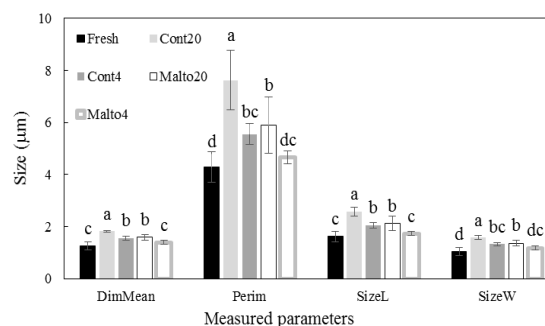
gum undergoes interactions (such as protein-poly-saccharide interaction) with the ingredients of the sample (Huang *et al.*, 2012).

Due to the desirable functions of Arabic gum in food products and emulsions, it has been widely used in different formulations. This gum provides lower viscosities at similar concentrations compared to other polysaccharides and, therefore, can be used at higher concentrations in the final product (Buffo *et al.*, 2001; Saha and Bhattacharya, 2010). The effect of different concentrations of Arabic gum on the rheological properties of frozen-thawed egg is shown in Figure 4-C. The results of this study indicated that the

apparent viscosity was not affected by the addition of this hydrocolloid. For flow behavior index, only concentrations of 2 and 3% were effective and reduced this parameter. Consistency was decreased for 1% gum and increased for 2 and 3% gum, although no significant differences were detected between the consistency index of the control sample and the samples containing the gum. Similar to color section, the effect of thawing temperature on rheological parameters of samples containing pectin, PGA, and Arabic gum was not significant.

### Microstructure and Particle Size Analysis

The size of particles and colloids can directly affect different properties of liquid whole egg including viscosity, flow-ability, texture, functional properties (such as emulsifying and foaming properties), and color (Afoakwa *et al.*, 2008). Since the above mentioned properties depend on the structure and conformation of egg constituents, especially proteins and lipoproteins, the micellar behavior of egg was studied by light microscopy and image processing. The particle size analysis indicated that the particles in the fresh sample ranged from 0.05 to 5.50  $\mu\text{m}$  centering at 1.27  $\mu\text{m}$  (mean diameter). The measured particle size parameters, as shown in Figure 5, were DimMean, Perim, SizeL, SizeW and the corresponding values for the



**Figure 5.** The effect of thawing temperature and maltodextrin addition on the particle size attributes of whole frozen-thawed egg.



fresh sample were 1.27, 4.30, 1.63, and 1.05  $\mu\text{m}$ , respectively. After the freezing and thawing processes, all the measured values were increased significantly at both thawing temperatures of 4 and 20°C. The largest particle sizes were detected in the samples thawed at 20°C.

As mentioned in the rheological properties section, egg components are sensitive to temperature changes and, in particular, subzero temperatures can induce major structural changes and aggregation due to the conversion of liquid water to frozen water. Since proteins play an important role in the properties of both egg yolk and egg white, it appears that these components might possess a key function during the structural changes throughout the freezing process.

The Low Density Lipoprotein (LDL) incorporated in egg granules is sensitive to low temperatures and, therefore, during freezing (especially at temperatures below -6°C), the water layer existing at the vicinity of the molecules is eliminated in the form of ice. The water layer is critical for the stability of the particles and its removal through the freezing process results in the conformational changes in the molecules at the surface of particles and eventually leads to the aggregation and gelation of egg (Cotterill, 1986; Wakamatu *et al.*, 1983). Telis and Kieckbusch (1997) reported that the breakage of LDL micelles could be considered as the initial step of structural changes of egg during freezing and by the development of freezing within the product; apo-proteins could be dehydrated resulting in the aggregation of LDL. Kurisaki *et al.* (1980) reported that the release of the active components from the surface of LDL during the freezing-thawing cycle and then incorporation of new exposed active sites in the inter-particle linkage and aggregation could be considered as the main cause of structural changes in egg. However, other researchers such as Wakamatu *et al.* (1982) disagreed with the sole role of the above mentioned mechanism for the gelation of egg and supposed that the information

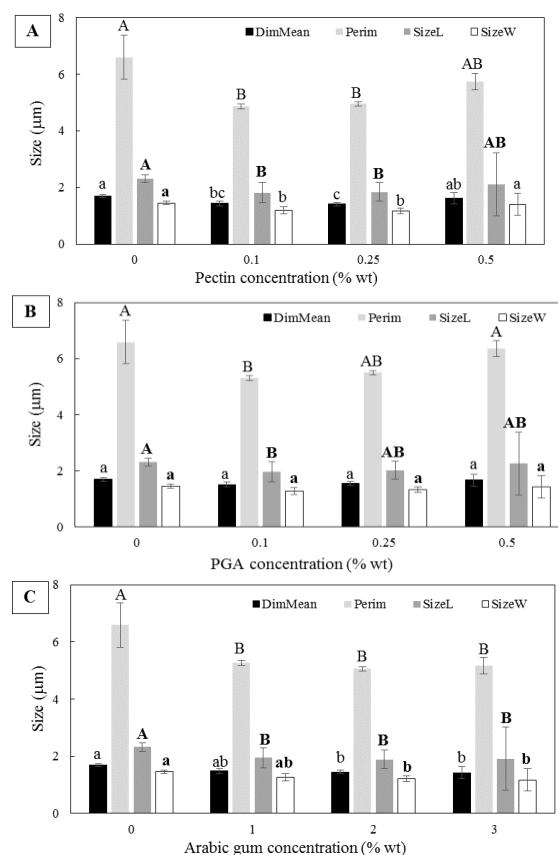
regarding the particle-particle interactions is not enough to describe the structural changes in liquid egg. In addition to that, whole egg consists of both egg yolk and egg white and therefore it is likely that proteins originating from white can also play a role in the structural changes.

In general, there are two main theories describing the aggregation of micelles in egg during freezing. The first theory explains the aggregation by the hydrophobic interactions after the exposure of hydrophobic regions during the dehydration of proteins (Rao and Labuza, 2012). The second theory relies on the disulfide bonds and assumes that during the freeze-thaw cycle, the sulfhydryl groups hidden within the structure of the proteins are exposed by the disassociation of the protein structure and form intermolecular bonds (Ferry, 1948). Kiosseoglou and Paraskevopoulou (2005) implied that the primary mechanism of egg gelation were hydrophobic interactions while covalent disulfide bonds could support the gelation by their secondary effect. In addition, it has been assumed (Au *et al.*, 2015) that not only the components present in the granules but also plasma proteins could play a role in the gelation phenomenon. Au *et al.* (2015), according to the earlier studies as well as their own results, supported the first theory and proposed that after the formation of ice crystals followed by the elimination of water layers from the vicinity of the egg particles, the hydrophobic sites that were not exposed in the normal situation became available for protein-protein interactions. There are also other factors affecting the gelation and its extent including the rate of freezing and thawing, storage temperature, stabilizers, homogenization, etc. (Cotterill, 1986; Lopez *et al.*, 1954; Pearce and Lavers, 1949; Powrie *et al.*, 1963; Zeidler, 2002). In this regard, some researchers reported that the gelation of egg yolk is reduced when faster freezing and thawing processes are used. This could be because of less damages to the structure of proteins and reduced ionic changes in the product (Lai, 2006; Powrie *et al.*, 1963; Xiong, 1997).



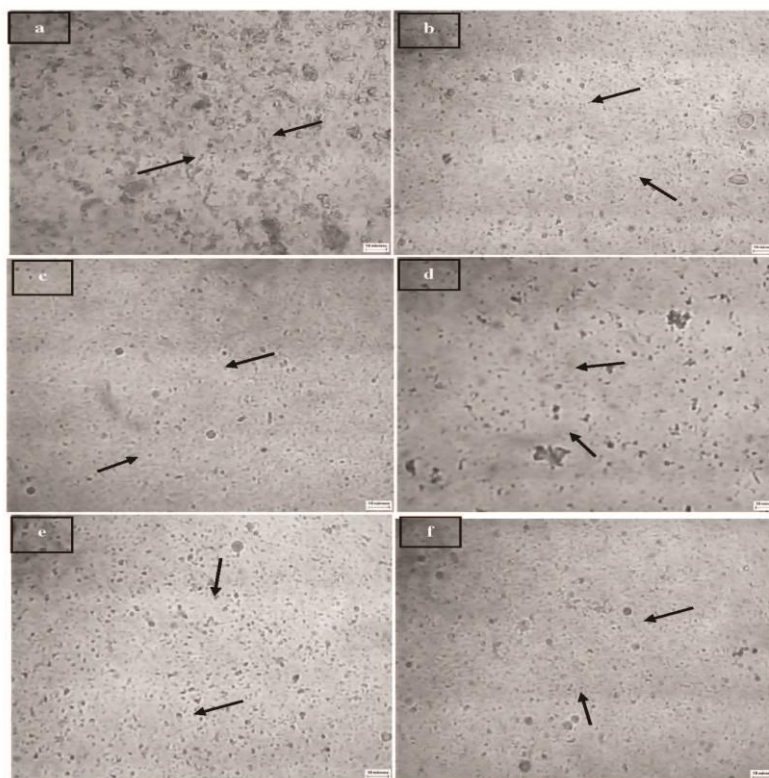
In Figure 5, the particle size analysis of the samples containing maltodextrin is displayed. The results indicated that maltodextrin was able to significantly decrease the particle size of the samples at both defrost temperatures. The results of microstructure visualization also confirmed this observation (Figure 7). According to the hydrophilic nature of maltodextrin, this substance was able to absorb some parts of free water and keep it from being frozen and, therefore, could reduce the aggregation of particles in whole egg.

In frozen food products, pectin and its derivatives have been used to limit ice crystal size, reduce drip loss, and improve the texture and stability (Thakur *et al.*, 1997). No reports have been found in the literature studying the behavior of pectin in egg products. In Figure 6-A, the effect of different concentrations of pectin on the particle size distribution of whole frozen-thawed egg is depicted. The mean particle diameter was decreased by the addition of pectin at the level of 0.1 and 0.25% with the lowest diameter observed for the latter concentration (1.41  $\mu\text{m}$ ). Other particle size parameters including perimeter, length, and width were also decreased but they were not significantly different among these concentrations. Further increase in the concentration of pectin increased the ice crystal size. Hydrocolloids are macromolecular structures and can limit particle aggregation through different mechanisms. Among these are the steric stabilization, viscous effect, and three-dimensional network formation. Pectin has been known to exhibit all of these mechanisms in different products (Laurent and Boulenger, 2003; Pérez *et al.*, 2000; Surh *et al.*, 2006; Thibault and Ralet, 2001; Yilmazer *et al.*, 1991). Very high concentrations of hydrocolloids can cause instabilities through the depletion flocculation mechanism (McClements, 2000). It seems that, according to the results of our studies, pectin was able to provide a steric stabilization at concentrations of 0.1 and 0.25% by reducing the size of the



**Figure 6.** The effect of pectin (A), PGA (B) and Arabic gum (C) concentrations on the particle size of whole frozen-thawed egg samples.

particles and inhibiting them from aggregation, but the concentration of 0.5% resulted in larger particles possibly due to the depletion fluctuation mechanism. Further investigations might be necessary to validate these observations. It should be noted that the functions of pectin strongly depend on its molecular weight, the degree and pattern of esterification and polysaccharide chains. Furthermore, other factors such as temperature, pH, and ions can affect the properties and behavior of pectin (Löfgren *et al.*, 2005). The pectin used in this study was a high methoxyl pectin, which can act as a steric stabilizer since it contains both hydrophobic and hydrophilic sites. It can also create a three dimensional network at high concentrations (Fishman *et al.*, 2007).



**Figure 7.** Microscopic micrographs of the microstructure of different egg sample: (a) Fresh; (b) Control; (c) Maltodextrin, (d) Pectin; (e) PGA, (f) Arabic gum.

The presence of hydrophobic propylene glycol groups in the chain of PGA renders it a valuable emulsifier with thickening properties. This stabilizer has also a good resistance to acids and is widely used in different food products as a thickening and emulsifying agent (Imeson, 2011). According to the surface activity and water binding capacity of this additive, its effect on the frozen egg was studied in this research and the results are shown in Figure 6-B. The addition of PGA reduced the size of microparticles with the lowest value observed for 0.1% concentration. Further increase in the concentration of PGA increased the size of particles. As described for pectin, in a similar way, PGA can incorporate into the steric stabilization since it is a surface active gum. It can also bond the free water, increase the viscosity and create the network. All of these mechanisms could play a role in the stabilization. However, the direct relationship between the

gum concentration and particle size implied that the emulsifying effect of PGA could be considered as the dominant stabilization mechanism (Imeson, 2011).

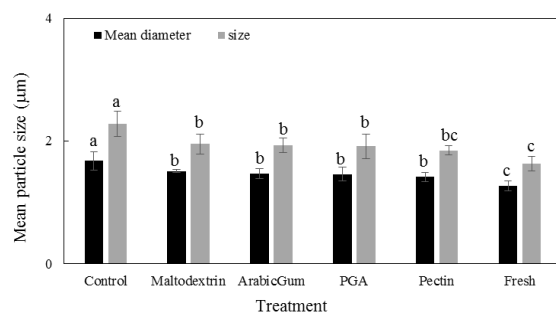
Figure 6-C shows the effect of Arabic gum concentration on the size of micelles in whole egg. At the gum concentrations of 2 and 3%, the particle size was significantly smaller than that detected for the control sample and the sample with 1% gum. In addition, the length and perimeter of particles was significantly decreased at the presence of Arabic gum, but no significant difference was observed between different concentrations. Arabic gum is also a surface active hydrocolloid. It is consisted of a protein part which is covalently linked to the carbohydrate section to form arabinogalactan-protein structures. Therefore, Arabic gum was assumed to be able to limit the particle to particle aggregation by its surface active

characteristics (McNamee *et al.*, 1998; Nakauma *et al.*, 2008).

Figure 8 compares the optimum concentration of each stabilizers used in this study with the fresh and frozen-thawed egg sample. The corresponding micrographs of the samples are displayed in Figure 7. As can be observed in both the measured size values and microstructural evaluations, the fresh sample contained the smallest particles. Freezing caused a considerable increase in the particle size and, as the micrographs indicate, micelles were aggregated in the control sample. The addition of maltodextrin, Arabic gum, PGA, and pectin reduced the size of particles in the frozen sample. The smallest particles could be achieved by the addition of pectin followed by PGA.

## CONCLUSIONS

The results of this study showed that freezing could decrease the lightness, increase the viscosity, and increase the particles size (through aggregation of particles in egg) of liquid whole egg. The application of maltodextrin, PGA, Arabic gum, and pectin could enhance the lightness, and the closest values of  $L$  to that of the fresh sample was detected for PGA and pectin. The additives could also inhibit particle aggregation during freezing. Maltodextrin was able to significantly reduce the particle size. Further reduction



**Figure 8.** Comparing the optimum concentration of additives in terms of mean diameter and particle size with fresh and control samples.

was achieved by the addition of pectin, PGA and Arabic gum. Smallest particle size distributions were achieved at pectin and PGA concentrations of 0.25 and 0.1%, respectively. The results of this study showed that the selected stabilizers could enhance the quality of frozen thawed whole egg and the findings can be employed for the development of new products based on frozen egg with no added sugar or salt, while maintaining the physical and functional properties of the final product.

## Nomenclature

AppVis	Apparent viscosity (Pa s)
Cont20	Control sample thawed at 20°C
Cont4	Control sample thawed at 4°C
DimMean	Mean diameter of particle
Fresh	Fresh sample
k	Consistency index (Pa s <sup>n</sup> )
Malto20	Sample containing 2% Maltodextrin thawed at 20°C
Malto4	Sample containing 2% Maltodextrin thawed at 4°C
n	Flow behavior index (-)
Perim	Perimeter of particle
SizeL	Length of particle
SizeW	Width of particle

## REFERENCES

1. Afoakwa, E. O., Paterson, A. and Fowler, M. 2008. Effects of Particle Size Distribution and Composition on Rheological Properties of Dark Chocolate. *Eur. Food Res. Technol.*, **226**: 1259-1268.
2. Atilgan, M. R. and Unluturk, S. 2008. Rheological Properties of Liquid Egg Products (Leps). *Int. J. Food Proper.*, **11**: 296-309.
3. Au, C., Acevedo, N. C., Horner, H. T. and Wang, T. 2015. Determination of the Gelation Mechanism of Freeze-Thawed Hen Egg Yolk. *J. Agri. Food Chem.*, **63**: 10170-10180.
4. Ballin, N. Z. and Lametsch, R. 2008. Analytical Methods for Authentication of Fresh vs. Thawed Meat—a Review. *Meat Sci.*, **80**: 151-158.



5. Boye, J. I., Alli, I. and Ismail, A. A. 1997. Use of Differential Scanning Calorimetry and Infrared Spectroscopy in the Study of Thermal and Structural Stability of A-Lactalbumin. *J. Agri. Food Chem.*, **45**: 1116-1125.
6. Buffo, R., Reineccius, G. and Oehlert, G. 2001. Factors Affecting the Emulsifying and Rheological Properties of Gum Acacia in Beverage Emulsions. *Food Hydrocol.*, **15**: 53-66.
7. Chanamai, R. and McClements, D. 2002. Comparison of Gum Arabic, Modified Starch, and Whey Protein Isolate as Emulsifiers: Influence of Ph, CaCl<sub>2</sub> and Temperature. *J. Food Sci.*, **67**: 120-125.
8. Chang, C., Powrie, W. and Fennema, O. 1977. Studies on the Gelation of Egg Yolk and Plasma Upon Freezing and Thawing. *J. Food Sci.*, **42**: 1658-1665.
9. Cotterill, O. J. 1986. Freezing Egg Products. In: "Egg Science and Technology", (Ed.): W. J. Stadelmann, O. J. C. Consider revising!!! 4<sup>th</sup> Edition, The Haworth Press, New York, PP. 265-288.
10. Dawson, P. and Martinez-Dawson, R. 1998. Using Response Surface Analysis to Optimize the Quality of Ultrapasteurized Liquid Whole Egg. *Poult. Sci.*, **77**: 468-474.
11. Dickinson, E. 2003. Hydrocolloids at Interfaces and the Influence on the Properties of Dispersed Systems. *Food Hydrocol.*, **17**: 25-39.
12. Ferry, J. D. 1948. Protein Gels. *Adv. Protein Chem.*, **4**: 1.
13. Fishman, M. L., Cooke, P. H., Chau, H. K., Coffin, D. R. and Hotchkiss, A. T. 2007. Global Structures of High Methoxyl Pectin from Solution and in Gels. *Biomacromol.*, **8**: 573-578.
14. Flores, A. and Goff, H. 1999. Recrystallization in Ice Cream after Constant and Cycling Temperature Storage Conditions as Affected by Stabilizers. *J. Dairy Sci.*, **82**: 1408-1415.
15. Galetto, C. D., Verdini, R. A., Zorrilla, S. E. and Rubiolo, A. C. 2010. Freezing of Strawberries by Immersion in CaCl<sub>2</sub> Solutions. *Food Chem.*, **123**: 243-248.
16. Hmid-Samimi, M., Swartzel, K. and Ball, H. 1984. Flow Behavior of Liquid Whole Egg During Thermal Treatments. *J. Food Sci.*, **49**: 132-136.
17. Hamid-Samimi, M. and Swartzel, K. 1985. Maximum Change in Physical and Quality Parameters of Fluid Foods During Continuous Flow Heating. Application to Liquid Whole Egg. *J. Food Process. Preserv.*, **8**: 225-239.
18. Hemar, Y., Hall, C., Munro, P. and Singh, H. 2002. Small and Large Deformation Rheology and Microstructure of K-Carrageenan Gels Containing Commercial Milk Protein Products. *Int. Dairy J.*, **12**: 371-381.
19. Herald, T. and Smith, D. 1989. Functional Properties and Composition of Liquid Whole Egg Proteins as Influenced by Pasteurization and Frozen Storage. *Poult. Sci.*, **68**: 1461-1469.
20. Huang, G. -Q., Sun, Y. -T., Xiao, J. -X. and Yang, J. 2012. Complex Coacervation of Soybean Protein Isolate and Chitosan. *Food Chem.*, **135**: 534-539.
21. Ibanoglu, E. and Erçelebi, E. A. 2007. Thermal Denaturation and Functional Properties of Egg Proteins in the Presence of Hydrocolloid Gums. *Food Chem.*, **101**: 626-633.
22. Imeson, A. 2011. *Food Stabilisers, Thickeners and Gelling Agents*. John Wiley and Sons. Oxford, United Kingdom.
23. Jaax, S. and Travnicek, D. 1968. The Effect of Pasteurization, Selected Additives and Freezing Rate on the Gelation of Frozen-Defrosted Egg Yolk. *Poult. Sci.*, **47**: 1013-1022.
24. Kaloyereas, S. A., Novak, A. F. and Watts, A. B. 1962. Heat Sterilization of Liquid Eggs after Stabilization Treatment with Various Proteinases. *Poult. Sci.*, **41**: 284-288.
25. Kayacier, A. and Dogan, M. 2006. Rheological Properties of Some Gums-Salep Mixed Solutions. *J. Food Eng.*, **72**: 261-265.
26. Kiosseoglou, V. and Paraskevopoulou, A. 2005. Molecular Interactions in Gels Prepared with Egg Yolk and Its Fractions. *Food Hydrocol.*, **19**: 527-532.
27. Kurisaki, J. I., Kaminogawa, S. and Yamauchi, K. 1980. Studies on Freeze-Thaw Gelation of Very Low Density Lipoprotein from Hen's Egg Yolk. *J. Food Sci.*, **45**: 463-466.
28. Lai, L. 2006. Quality and Safety of Frozen Eggs and Egg Products. *Handbook Frozen Food Process. Packag.*, **155**: 503.
29. Laurent, M. and Boulenguer, P. 2003. Stabilization Mechanism of Acid Dairy Drinks (Add) Induced by Pectin. *Food Hydrocol.*, **17**: 445-454.

30. Löfgren, C., Guillotin, S., Evenbratt, H., Schols, H. and Hermansson, A. -M. 2005. Effects of Calcium, Ph, and Blockiness on Kinetic Rheological Behavior and Microstructure of Hm Pectin Gels. *Biomacromol.*, **6**: 646-652.
31. Lopez, A., Fellers, C. and Powrie, W. 1954. Some Factors Affecting Gelation of Frozen Egg Yolk. *J. Milk Food Technol.*, **17**: 334.
32. Lovelock, J. 1957. The Denaturation of Lipid-Protein Complexes as a Cause of Damage by Freezing. *Proceedings of the Royal Society of London. Series B, Biol. Sci.*, **147**: 427-433.
33. Macdougall, D. 1982. Changes in the Colour and Opacity of Meat. *Food Chem.*, **9**: 75-88.
34. Macdougall, D. B. 2002. *Colour in Food: Improving Quality*. Woodhead Publishing.
35. McClements, D. 2000. Comments on Viscosity Enhancement and Depletion Flocculation by Polysaccharides. *Food Hydrocol.*, **14**: 173-177.
36. Mcnamee, B. F., O'riorda, E. D. and O'sullivan, M. 1998. Emulsification and Microencapsulation Properties of Gum Arabic. *J. Agri. Food Chem.*, **46**: 4551-4555.
37. Min, B., Nam, K., Lee, E., Ko, G., Trampel, D. and Ahn, D. 2005. Effect of Irradiating Shell Eggs on Quality Attributes and Functional Properties of Yolk and White. *Poult. Sci.*, **84**: 1791-1796.
38. Moussa, M., Martinet, V., Trimeche, A., Tainturier, D. and Anton, M. 2002. Low Density Lipoproteins Extracted from Hen Egg Yolk by an Easy Method: Cryoprotective Effect on Frozen-Thawed Bull Semen. *Theriogenol.*, **57**: 1695-1706.
39. Muela, E., Sañudo, C., Campo, M., Medel, I. and Beltrán, J. 2012. Effect of Freezing Method and Frozen Storage Duration on Lamb Sensory Quality. *Meat Sci.*, **90**: 209-215.
40. Mukhopadhyay, S. and Ramaswamy, R. 2012. Application of Emerging Technologies to Control Salmonella in Foods: A Review. *Food Res. Int.*, **45**: 666-677.
41. Nakauma, M., Funami, T., Noda, S., Ishihara, S., Al-Assaf, S., Nishinari, K. and Phillips, G. O. 2008. Comparison of Sugar Beet Pectin, Soybean Soluble Polysaccharide, and Gum Arabic as Food Emulsifiers. 1. Effect of Concentration, pH, and Salts on the Emulsifying Properties. *Food Hydrocol.*, **22**: 1254-1267.
42. Pearce, J. A. and Lavers, C. 1949. Liquid and Frozen Egg: V. Viscosity, Baking Quality, and Other Measurements on Frozen Egg Products. *Can. J. Res.*, **27**: 231-240.
43. Pérez, S., Mazeau, K. and Du Penhoat, C. H. 2000. The Three-Dimensional Structures of the Pectic Polysaccharides. *Plant Physiol. Biochem.*, **38**: 37-55.
44. Powrie, W., Little, H. and Lopez, A. 1963. Gelation of Egg Yolk. *J. Food Sci.*, **28**: 38-46.
45. Rao, Q. and Labuza, T. P. 2012. Effect of Moisture Content on Selected Physicochemical Properties of Two Commercial Hen Egg White Powders. *Food Chem.*, **132**: 373-384.
46. Saha, D. and Bhattacharya, S. 2010. Hydrocolloids as Thickening and Gelling Agents in Food: A Critical Review. *J. Food Sci. Technol.*, **47**: 587-597.
47. Scalzo, A., Dickerson, R., Peeler, J. and Read, R. 1970. Viscosity of Egg and Egg Products. *Food Technol.*, **47(24)**: 1301-1307
48. Smith, A. U. 1961. *Biological Effects of Freezing and Supercooling. with Foreword by as Parkes*. Monographs. Physiological Society, Edward Arnold, London, 462 PP.
49. Su, H. P. and Lin, C. W. 1993. A New Process for Preparing Transparent Alkalised Duck Egg and Its Quality. *J. Sci. Food Agri.*, **61**: 117-120.
50. Surai, P. and Sparks, N. 2001. Designer Eggs: From Improvement of Egg Composition to Functional Food. *Trend. Food Sci Technol.*, **12**: 7-16.
51. Surh, J., Decker, E. A. and McClements, D. J. 2006. Influence of pH and Pectin Type on Properties and Stability of Sodium-Caseinate Stabilized Oil-in-Water Emulsions. *Food Hydrocol.*, **20**: 607-618.
52. Telis, V. R. N. and Kieckbusch, T. 1997. Viscoelasticity of Frozen/Thawed Egg Yolk. *J. Food Sci.*, **62**: 548-550.
53. Thakur, B. R., Singh, R. K., Handa, A. K. and Rao, M. 1997. Chemistry and Uses of Pectin: A Review. *Critical Rev. Food Sci. Nutr.*, **37**: 47-73.
54. Thibault, J. F. and Ralet, M. C. 2001. Pectins, Their Origin, Structure and Functions. In: "Advanced dietary fibre technology", (Ed.): B. V. McCleary, L. Prosky, Blackwell Science, Oxford, UK, PP. 367-378.



55. Wakamatu, T., Sato, Y. and Saito, Y. 1982. Identification of the Components Responsible for the Gelation of Egg Yolk During Freezing. *Agric. Biol. Chem.*, **46**: 1495-1503.
56. Wakamatu, T., Sato, Y. and Saito, Y. 1983. On Sodium Chloride Action in the Gelation Process of Low Density Lipoprotein (Ldl) from Hen Egg Yolk. *J. Food Sci.*, **48**: 507-512.
57. Xiong, Y. L. 1997 Protein Denaturation and Functionality Losses. In: "*Quality in Frozen Food*", (Ed.): M. C. Erickson, Y. H. Chapman and Hall, New York, PP. 111-140.
58. Yilmazer, G., Carrillo, A. R. and Kokini, J. L. 1991. Effect of Propylene Glycol Alginate and Xanthan Gum on Stability of O/W Emulsions. *J. Food Sci.*, **56**: 513-517.
59. Zeidler, G. 2002 Further-Processing Eggs and Egg Products. In: "*Commercial Chicken Meat and Egg Production*", (Ed.): D. D. Bell, W. D. Weaver. 5<sup>th</sup> Edition, Kluwer Academic Publishers, Massachusetts, USA, PP. 1163-1197.

## اثر انجماد-انجماد زدایی و پایدارکننده های بر روی رفتار فازی ذرات میکرونی تخم مرغ و خواص کیفی تخم مرغ مایع

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### چکیده

انجماد به علت ایجاد میانکنش های پیچیده بین ذرات موجود در تخم مرغ می تواند باعث افت کیفیت این محصول شود. در این مطالعه، تاثیر پکتین، پروپیلن گلیکول آلژینات (PGA)، صمغ عربی، مالتودکسترین و همچنین درجه حرارت انجماد زدایی بر روی رنگ، رفتار رئولوژیکی و ریز ساختار تخم مرغ منجمد مورد بررسی قرار گرفت. نتایج رنگ نمونه تازه بیانگر روشنایی قابل توجه و رنگ متمایل به زرد آن با مقادیر L بالا و a و b مثبت بود. در اثر فرایند انجماد نمونه تا حدودی کدرتر شد و L کاهش یافت ولی a و b تقریباً ثابت بودند. استفاده از افزودنی ها حین انجماد منجر به بهبود روشنایی و افزایش معنادار L در مقایسه با نمونه های شاهد گردید و بهترین نتیجه برای پکتین و PGA حاصل شد. تخم مرغ کامل رفتار سودوپلاستیک نزدیک به نیوتنی با ویسکوزیته کم از خود نشان داد. انجماد و انجماد زدایی تخم مرغ منجر به افزایش چشمگیر ویسکوزیته گردید. همزمان اندیس جریان کاهش معنادار و ضریب قوام افزایش قابل توجهی نشان داد. تمام نمونه های منجمد ویسکوزیته بالاتری داشتند. نمونه های تازه حاوی ذراتی بین ۰/۰۵ تا ۵/۵۰ با میانگین ۱/۲۷ بودند. انجماد باعث اتصال ذرات به همدیگر و ایجاد ذراتی بسیار درشت تر گردید. حضور مالتو دکسترین توانست به صورت معناداری اندازه ذرات را در مقایسه با نمونه های شاهد کاهش دهد. افزودن صمغ عربی، PGA و پکتین توانست قطر ذرات را کاهش بیشتری بدهد. کمترین قطر ذرات در غلظت ۰/۲۵ درصد پکتین و ۰/۱ درصد PGA به دست آمد. نتایج این مطالعه می تواند در توسعه فرآورده های جدید تخم مرغ مایع منجمد بدون افزودن نمک و قند و در عین حال دارای خواص فیزیکی و عملکردی بهبود یافته، استفاده گردد.