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Original research

Thermal aggregation of egg white proteins as affected by saccharides

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A B S T R A C T

Thermal characteristics of egg white proteins (EWP) may differ in the presence of saccharides. Therefore, the influence of saccharides including carboxymethyl cellulose (CMC), pectin, sucrose and maltodextrin and heating time on physicochemical characteristics of EWP as a whole were studied. Investigation of Heat Coagulation Time (HCT), solubility, turbidity and protein secondary structure of heat-treated EWP solutions, indicated the reasonable characteristics of heat-treated protein as affected by saccharides (especially maltodextrin). Heat stability of EWP was increased in the presence of either CMC, pectin, sucrose or maltodextrin, as shown by a decrement in turbidity and an increase in solubility and HCT. The Fourier transform infrared spectroscopy analysis was also utilized to observe the EWP's secondary structure in the presence of saccharides before and after heat treatment. Analyses manifested the enhanced heat stability of proteins in the presence of saccharides by indicating fewer changes in egg white's physicochemical properties.

Keywords: Egg white, Saccharides, Thermal characteristics, Protein aggregation

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1. Introduction

Egg white have been applied as a good source of protein and its wide variety of functional properties, hence, further promotion of functional properties is beneficial for industrial uses. To be insured of egg white products microbial safety and reduce contamination by pathogenic microorganisms, the egg whites are subjected to heat processes such as pasteurization (Aragon-Alegro et al., 2005), which may affect industrial processes of egg-based products which defines their shelf life relevant to microbial growth (Kiosseoglou & Paraskevopoulou, 2005; Wang et al., 2009). As proteins are commonly sensitive to heat treatment, their industrial usage has been limited due to functional properties loss caused by protein denaturation.

Due to heat, the hydrogen bonds especially between the polypeptide chains of the protein molecule break, the polypeptide chains unfold and hydrophobic reactive groups subject to the external surface of the protein (Mine, 1995). In the next step, the unfolded protein molecules rearrange by hydrophobic and electrostatic interactions. Inter-molecular disulfide crosslinking strengthens the arranged protein network moreover. Thirdly, a gel is produced which gets harder because of the formation of hydrogen bonds (Mine, 1995).The gel network is affected by pH, ionic strength and the type of additives (Croguennec et al., 2002).

In many food products, saccharides are present beside proteins. Alongside their contribution to the structure and texture, saccharides may have some consequences on the heat stability of food formulations (Doublier et al., 2000; Maroziene & De Kruif, 2000).Thermal characteristics of heat-treated proteins differ by the presence of saccharides. Coagulation of protein could be postponed by saccharide which behaves like a shell surrounding protein without any interaction or interacts with a protein which inhibits protein–protein aggregation and increases the solubility of protein or disorders the protein structure and decrease the heat stability of the protein (Doublier et al., 2000).

It appears more practical to investigate EWP gelation in the presence of common food industrial additives. CMC, pectin, sucrose and maltodextrin are typical saccharides that have been widely utilized in food (Togrul & Arslan, 2004), meanwhile do not have harmful effects on human health (Su et al., 2010). These saccharides are applied as highly effective additives to improve the processing properties of products in foodstuffs (Zhu et al., 2013).

To the authors' knowledge, there are no published studies systematically studying the influence of saccharides on thermal denaturation properties of EWP as a whole concentrating on the effect of the additives on hampering protein aggregation. Therefore, the purpose of this study was to gain knowledge on the effect of type (CMC, pectin, sucrose and maltodextrin), saccharide concentrations (0, 0.5 and 1%) and heat treatment time (0, 1 and 2

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minutes) on turbidity, solubility and HCT of EWP. FT-IR spectroscopy analysis was also applied to evaluate the proteins' secondary structure as affected by saccharides before and after heat treatment. This new knowledge would be useful for upgrading pasteurization and sterilization techniques to lessen the negative impact of heat on technological properties of egg white.

2. Material and Methods

2.1. Chemicals

Hen eggs were obtained from Telavang .Co (Tehran, Iran). Pectin (high methoxyl), sucrose and maltodextrin (Dextrose Equivalent: 18) were procured from Merck (Darmstadt, Germany). CMC was supplied by DaeJung Chemicals and Metals Company, Ltd. (Gyeonggi-do, South Korea). Other chemicals and solvents were of analytical reagent grade and distilled water was also applied all over the experiments.

2.2. Sample preparation

Egg white solutions were prepared as previously described (Nasabi et al., 2017). Briefly, egg white solutions (20 mg protein/ml defined by Bradford technique (Kruger, 1994),were blended with 0.2 M sodium phosphate buffer (pH 7.0) including different CMC, pectin, sucrose and maltodextrin concentrations at a proportion of 1:1 (v/v), in which the final concentrations were 0.5 and 1% for each material.

2.3. Thermal aggregation of EWP

The solutions (10 mg protein/ml, pH 7.0) with various additive concentrations were put in cuvettes located in an oil bath. Egg white solutions were heated at 70ºC (± 1ºC) and a thermometer was applied to detect the temperature of the solution. Tubes were gathered after 0, 1 and 2 minutes and were cooled instantly with iced water (0–2ºC).

2.4. Turbidity evaluation

The solutions were put in cuvettes (light path length of 1 cm). The turbidity was measured with the absorbance at 590 nm (Spectrum Instruments, SP-UV 500DB, Shanghai, China). Absorbance of the unheated solutions was used to bring the turbidity to zero (Kitabatake & Kinekawa, 1995).

2.5. Determination of HTC

The HCT of the egg white was evaluated at 75ºC (Davies & White, 1966). The time that passed between placing samples in the oil bath and the first visible inception of coagulation is recorded as the HCT.

2.6. Evaluation of protein solubility

The heated samples were centrifuged at 20000 g (Universal 320, Hettich Zentrifugen, Germany) for 20 min at 4°C (Nasabi et al., 2017). The protein solubility was demonstrated as the percentage of protein in the supernatant to the total protein content determined by the Bradford method (Kruger, 1994).

2.7. FT-IR spectroscopy analysis

Samples were freeze-dried and mixed with potassium bromide and made into tablets. The FT-IR spectroscopy was performed applying a Perkin Elmer FT-IR spectrometer frontier (Perkin Elmer Co., MA, USA). The sample was put in an infrared cell with ZnSe windows with controlled temperature (Duarte et al., 2002).

2.8. Statistical analysis

All the samples were prepared in three independent trials and analyzed in triplicate. Data were introduced as mean value with standard deviations. Analysis of variance (ANOVA) was carried out and the mean comparisons were run by Duncan's multiple range tests (Steel and Torrie 1980). Statistical analysis of all data was conducted using the statistical program SAS software (version 9.2, SAS institute, Cary, NC). The significance of $p < 0.05$ was applied in the evaluations.

3. Results and Discussion

3.1. Turbidity

Turbidity of solutions with different additives' concentrations (0.5 and 1%) which were heated for 1 and 2 min is presented in Fig. 1. The temperature of 70ºC was chosen to be close to gelation onset. Due to the formation a coagulum accompanied by the rigid gel structure as consequences of heating, turbidity is often used as an indicator of egg white gelation. The variety of turbidity is owing to various sizes and amounts of protein aggregation (Demetriades & McClements, 1998).

As it's shown in Fig. 1 turbidity of egg white increased by the increase in heat treatment time but turbidity was reduced, applying higher quantities of additives. Maltodextrin was the most effective additive in preventing turbidity augmentation of heat-treated egg white solution. Liu and Zhong (2012) investigated that maltodextrin could keep the whey protein isolate solution transparent even heated at 88°C for 2 min. Sucrose could weakly prevent turbidity increase in egg white solutions compared with other saccharides used in this study. Although, decelerating the gelation rate of proteins in the presence of sucrose has been previously reported which was ascribed to an increase in viscosity of the continuous phase, causing a decrease in the repetition of protein-protein collisions (Kulmyrzaev et al., 2000; Rich & Foegeding, 2000; Baier & McClements, 2001).

CMC and pectin showed similar activity in suppressing solution turbidity in both 0.5 and 1% concentration after 1 minute and in 0.5% concentration after 2 minutes heat treatment, but CMC showed more efficiency in higher concentration (1%) after 2 minutes heat treatment (Fig. 1b). Zhang et al. (2012) investigated the heat-induced aggregation of whey protein isolate in the presence of pectin at near neutral pH. At a high concentration of pectin (pectin: protein weight ratio > 0.2), the turbidity of the mixture decreased during heating due to increased interaction between the negatively charged pectin and positively charged zones of the proteins, which limits the protein–protein interactions.

Table 1. Solubility of heat-treated egg white proteins as affected by additives.

Time of heat treatment	0 (min)			1 (min)			2 (min)		
Additive/Concentration	0%	0.5%	1%	0%	0.5%	1%	0%	0.5%	1%
CMC	84.73	84.20	83.99	25.03	39.80	43.18	13.33	20.10	22.34
Pectin	84.73	84.28	84.33	25.03	38.62	42.51	13.33	20.37	23.00
Maltodextrin	84.73	84.68	84.62	25.03	55.86	59.05	13.33	28.07	34.33
Sucrose	84.73	84.72	84.51	25.03	30.19	36.00	13.33	16.08	17.98

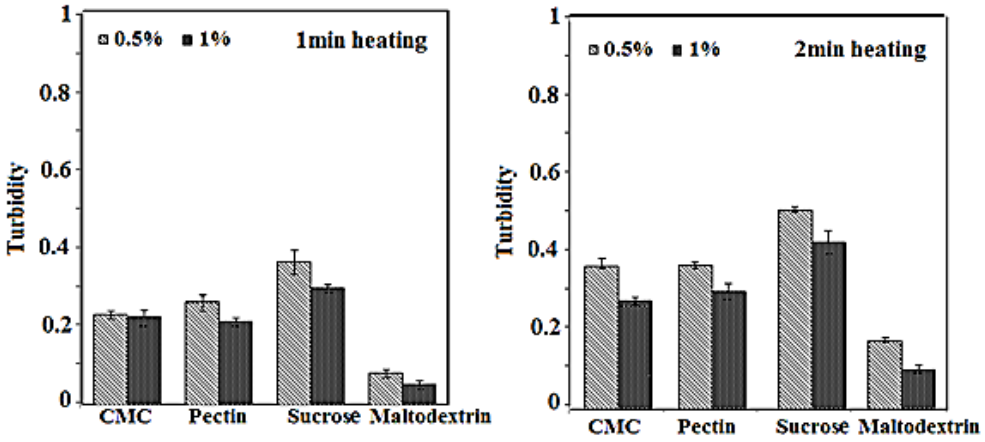

Fig. 1. Turbidity of egg white solutions (with 0.5 and 1% additives) heated for 1 and 2 min.

3.2. *Solubility*

High protein solubility is required to provide the demanded functional properties in food industry. A decrease in protein solubility affects negatively its functional characteristics. Accordingly, it can be applied as one the principal factors in observing proteins in food (Mechado et al., 2007). Solubility of solutions (0, 0.5 and 1% of additives) heated for 0, 1 and 2 min is presented in Table 1.

Although, remarkable changes in solubility were not observed in the presence of additives before any heat treatment, presence and increase in saccharides concentrations promoted significant increases in the solubility of EWP after 1 and 2 minutes heat treatment (Table 1). As proteins are the major constituents of egg white's dry matter (Deleu et al., 2015), the drastic augmentation in solubility by increasing time of heat treatment is a result of denaturation and aggregation of proteins such as ovalbumin which includes 54% of EWP (Desert et al., 2001). Maltodextrin was the most effective additive in solubility decrease inhibition of heat treated EWP. Protein solubility of solutions containing 0.5% and 1% maltodextrin were duplicated comparing with the sample without any additive after 1 and 2 minutes heat treatment. Nevertheless, sucrose had the least promoting effect on EWP's solubility among the saccharides.

It was supposed that such stabilization could be because of the organizing a network of aggregated proteins with the saccharides trapped inside (Turgeon et al., 2007). The alteration of proteins that causes protein denaturation is limited in the presence of saccharides and the slow dynamics surrounding protein protects it (Lopez-Diez & Bone, 2004). Wagoner and Foegeding (2017) also declared that high methoxyl pectin could form a polysaccharide shell around protein aggregate core. As previously mentioned, the improved stability of whey proteins against thermal aggregation by sucrose was also proposed to be related to the increased viscosity of continuous phase, which decreases the frequency of protein-protein encounters and increases protein unfolding temperature (Kulmyrzaev et al., 2000).

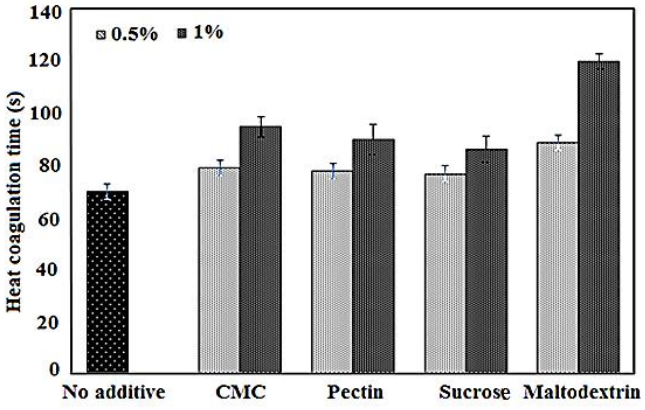

Fig. 2. Effect of saccharides on heat coagulation time of egg white solutions.

3.3. *HCT*

Turbidity Fig. 2 represents HCT of egg white solutions without any additives and in the presence of either CMC, pectin, sucrose or

maltodextrin with 0.5 and 1% concentrations. Presence of saccharides significantly promoted proteins HCT. Although, CMC, pectin and sucrose showed similar heat stabilizing activity with 0.5% concentration but they had different manners when the concentration increased (Fig. 2). Increase in heat stability was also detected in α-lactalbumin or β-lactoglobulin, when CMC was present (Capitani et al., 2007). Wong et al. (2015) revealed an alternation in denaturation temperature of egg white protein with sugars. In addition, sucrose increased the gelation temperature of bovine serum albumin because of thermal stabilization of the native protein (Baier & McClements, 2001). This increase is a consequence of unfavorable encounters of the saccharide with the denatured, unfolded form of the protein, versus to the folded form (Hemar et al., 2002). Other saccharides including lactose have been revealed to have the same outcomes on thermal stabilization of proteins, which as a result induce increase in time and temperature necessary for gelation (Rich & Foegeding, 2000).

Moreover, the heat stability depended on the saccharide concentration especially with maltodextrin such that increase in heat stability was observed as the saccharides concentration increased from 0.5 to 1%. Adding maltodextrin in both concentrations increased HCT of egg white solutions to a significantly more amount than that detected for a similar amount of other additives.

3.4. FT-IR analysis

FT-IR spectroscopy is a powerful method that has been successfully employed to study the secondary structural changes of protein during thermal denaturation (Barth, 2007). FT-IR analysis is also useful to study the protein–saccharide systems, as the chemical fingerprints of proteins and saccharide do not overlap significantly in diagnosed regions of the mid-infrared spectrum (Turner et al., 2002). For saccharides, a series of overlapping peaks positioned in the region of 953–1180 cm^{-1} originates from vibration modes including the stretching of C – C and C = O, and the bending mode of C – H bonds. These absorptions are weak in most proteins.

Fig. 3 shows the FT-IR spectra of different samples. The samples were gathered after 2 minutes heat treatment in 70˚C.We previously identified the FT-IR spectra of native EWP and heat induced egg white (Nasabi et al., 2017). According to our findings, the FT-IR spectrum of egg white before heat treatment sample showed the C = O stretching vibration of amide I that is related to α-helix structure at 1651 $cm^{-1}$and the band at 3303 cm^{-1} related to hydrogen bonded N – H stretching (Liana et al., 2013) which were in accordance with native EWP. For heat-treated egg white with no additive, the main band for α-helix absorbance was displaced to 1627 cm^{-1} and was reduced in intensity, which indicates α-helix to β-sheet conversion. There is also an absorbance increase and shift to higher wave number for the band originally absorbing at 1690 cm^{-1} (β-sheet). For heat-treated egg white with no additive the band at 3303 cm^{-1} related to hydrogen bonded N – H stretching vanished and another band is appeared at 3460 cm^{-1} which is related to non-hydrogen bonded N – H stretching. Besides, the appearance of a shoulder in proximity of 3490 cm^{-1} in the spectrum of aforesaid sample would be a manifestation of free N – H groups. The disulfide bond region was also found between 510 and 550 cm^{-1} (Stuart, 2004). Considering structural changes in protein, damage to EWP's secondary structure during heat treatment without additives appeared to be intense (Nasabi et al., 2017).

The FT-IR spectra of egg white samples as affected by saccharides with no heat treatment were alike with the native egg white ones (data not included), however conversions in intensity were detected as a result of structural conformation alteration. Hereby, additives before heat treatment protected proteins native secondary structure and inhibited unfolding. As it's shown in Fig. 3, differences in the spectra of the heat induced samples indicated that the use of different saccharides resulted in the production of various protein molecular conformations.

In the spectrum formulated in the presence of maltodextrin (Fig. 3a), the band related to C = O stretching of amide I shifted to 1648 cm^{-1} which is related to unordered structure (Jackson & Mantsch, 1995). As it is investigated, unfolded protein presents a broad amide I band placed near 1650 cm^{-1} that is a sign of unordered structure. Conversely, egg white formulated with sucrose showed the disulfide bond region near 530 cm^{-1} and a band at under 1620 cm^{-1} (Fig. 3b) which is often characterized as intermolecular β-sheets and protein aggregation (Barth, 2007). The wave number 3303 cm^{-1} assigned to hydrogen bonded N – H stretching vibration did not alter in the spectrum of maltodextrin containing sample compared with native egg white and versus heat-treated egg white with no additives and in the presence of sucrose (Fig. 3b). However, a decrease in band intensity by adding maltodextrin during heat treatment was observed. It has been declared that the number of similar groups in a molecule affects the relative strength of the relevant absorption band and the intensity of one band in comparison with another gives data on the extent of a defined functional group, besides type of the group (Miller et al., 2013).

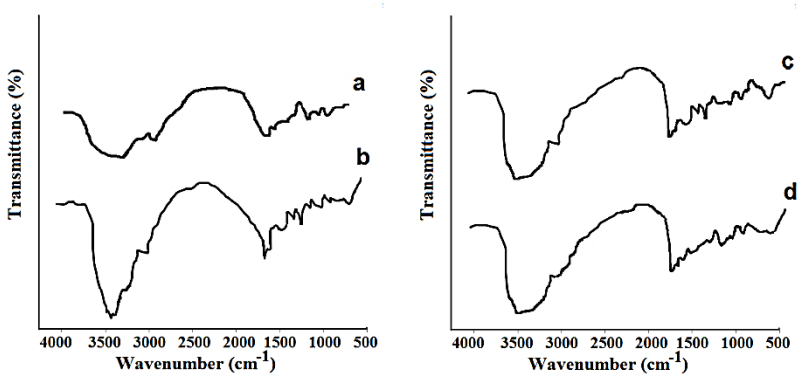

Fig. 3. FT-IR spectra of heat induced egg white in the presence of maltodextrin (a), sucrose (b), CMC (c) and pectin (d).

Obviously, it is derived that EWP molecules unfolded upon heating with maltodextrin. Therefore, with maltodextrin, Random coil increased while the α-helix and β-turns decreased. The FT-IR spectra of CMC and pectin are shown in Figs. 3c and d. The principal protein secondary structure portion in heat-treated egg white with CMC and pectin were α-helical and unordered protein structures. Although the disulfide band region were also observed with CMC and pectin, heat-treated egg white with no additive was rich in intramolecular β-sheets which offers the structural alterations were more severe without using additives. The addition of saccharides inhibited protein secondary structure rearrangement in a concentration dependent manner (data not included). Enomoto et al. (2007) declared that polysaccharides prevent the close interaction of protein molecules in either their native or denatured state. Accordingly, egg white containing maltodextrin result in a more native-like EWP's secondary structure. Therefore, the effect of maltodextrin in protecting of protein was better than that of other saccharides used in this study. This conclusion which to the best of our knowledge is presented for the first time is in line with the outcomes of the solubility, turbidity and HCT of the proteins.

4. Conclusion

Saccharides including CMC, pectin, sucrose and maltodextrin altered the thermal behavior of egg white solutions and the thermal stability of EWP as a whole were improved in the presence of saccharides and by the increase in their concentrations. This heat stabilizing effect was accompanied by an increase in proteins solubility and a decrease in egg white solutions turbidity. Addition of maltodextrin resulted in the most significant reduction in turbidity and augmentation in HCT and solubility of egg white, though sucrose could weakly promote egg white physico-chemical properties. FT-IR analysis confirmed that the change of the protein secondary structure was more obvious when egg white solution was heat-treated without any additives. While, in the presence of saccharides especially maltodextrin, the reduction of the α-helix structure and the increase of β-sheet content were reduced. The analysis of FT-IR spectra provided novel information about conformational changes in the protein. This study clearly declared the usefulness of employing FTIR as an analytical tool to assess proteins conformational changes. FT-IR spectroscopy analysis could be employed to efficiently observe the denaturation and aggregation phases of egg white. Besides, using EWP as a whole helps the study to be more applicable in industry.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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