Investigations on the emulsifying properties of egg white protein

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ABSTRACT

Egg white proteins (EWP) have excellent foaming and gelling functional properties. However, their emulsifying properties are considered poor when compared to soy proteins or milk proteins. Some studies have attributed the poor emulsifying properties to the hydrophobic amino acid groups buried deeply in the interior of the protein conformational structure which is crucial for emulsification. Several methods, such as heat treatment, acid/acid-heat treatment, Maillard reaction, phosphorylation and enzymatic hydrolysis, have been used by some researchers to improve the emulsifying properties of EWP. Preliminary experiments carried out in this study showed that oil-in-water (O/W) emulsions prepared with egg white liquid (EWL) generated lots of visible large aggregates, which no other study has reported. Therefore, it was important to investigate the factors responsible for the formation of these aggregates. Investigations into improving EWP's emulsifying properties could offer opportunities in developing unique and well-defined egg white-based emulsions.

The objective of this research project was to produce egg white emulsions with little or no aggregates. This thesis comprises three main parts. The first part focused on the effects of pH and heat treatment on protein aggregation and partial denaturation of proteins in EWL. The second part investigated the effects of heat treatment, oil concentration and protein concentration on the reduction of large visible aggregates in emulsions prepared with EWL. The third part studied the effect of enzymatic hydrolysis on the degree of hydrolysis and emulsifying properties of EWP hydrolysates. The emulsifying properties of original EWP and EWP hydrolysates were characterised in terms of size and zeta (ζ)-potential of emulsion droplets and emulsion stability (e.g. turbidity, microscopic examination and phase separation).

Firstly, an experimental study was carried out to evaluate the effect of pH on protein aggregation and precipitation in EWL containing different protein concentrations (0.5, 1, 2, 3, 4, 5 and 10% w/w). It was found that at all the protein concentrations used and at pH less than around 5, ζ-potential values were all positive but decreased as pH increased from 2 to 5. At pH 5, ζ-potential was close to zero (this is the pI of most egg white proteins), while, at pH levels above 5, ζ-potential became negative and increased as pH increased from pH 5 to 11. The spectral absorbance (turbidity) of emulsion samples was also

measured at 600 nm which revealed that for all protein concentrations, turbidity was observed to be higher at acidic pH of 3, 4 and 5, indicating the aggregation of EWP. At alkaline conditions of pH 7, 8, 9 and 10 the EWL solutions remained to be transparent. The effect of heat treatment and holding time on the denaturation of EWP in EWL was also studied at different temperatures (57-62^{o}C) and heating times (0-19 minutes). Higher turbidity due to protein aggregation was observed as temperature increased from 57 to 62^{o}C and the heating time increased from 5 to 19 minutes. It is therefore concluded that EWL can be safely pasteurized with little or no denaturation or aggregation at around 57-58^{o}C for less than 5 minutes. At 60^{o}C, it was observed that EWL began to thicken and after 5 minutes coagulation and gelation occurred rapidly.

Studies were also carried out to determine the cause of visible large aggregates formed in emulsions prepared with EWL using various factors, such as heat treatment, oil concentration and protein concentration. It was found that heat treatment (60^{o}C for 30 minutes) of 1% (w/w) EWP solution prior to homogenisation had no effect on reduction of aggregates in emulsions containing 5, 10, 15 and 20% (w/w). However, the formation of aggregates was reduced significantly as oil concentration was reduced to 5%. Therefore, the effect of lower oil concentrations (1, 3, 5, 6, 7 and 10% w/w) on the formation of aggregates in emulsions prepared with 1% or 3% EWP concentrations was also investigated. Little or no visible aggregates were formed when emulsions were prepared with 1% EWP and $\leq$ 5% oil or 3% EWP and 1% oil. Therefore, the results indicated that both protein and oil concentrations played a significant role in the formation of visible aggregates in emulsions prepared with EWP as an emulsifier.

The effect of EWP concentrations (0.1, 0.3, 0.5, 0.8, 1 and 2% w/w) on the formation and properties of 5% oil emulsions at ~pH 8 was then investigated. It was discovered that little or no aggregates were produced in emulsions when prepared at 0.1-1% EWP while large aggregates were formed at 2% EWP concentration. The size of emulsion droplets was observed to increase significantly from 242.1 to 703.7 nm as protein concentration increased from 0.1 to 2%. ζ-potential was however not significantly affected by protein concentration and ranged from -35.3 to -39.2 mV. The emulsions prepared were also heat treated at 60-90^{o}C for 30 minutes. No sign of instability with a significant change in the size of emulsions due to heat treatment was observed from all emulsion samples prepared at different EWP concentrations (0.1 - 2%). However, phase separation of the emulsions

was observed upon freezing at -20°C and thawing at 4 and 20°C, respectively, at all protein concentrations used. Also, the stability of emulsions was affected by the addition of salts, such as $CaCl_2$ (5-100 mM) and NaCl (50-600 mM), with an increase in droplet size and phase separation. However, the emulsions were relatively more stable to salt-induced flocculation, especially against NaCl, at higher protein concentration (1-2%) than lower protein concentrations (0.1-0.8%). Lastly, the effect of pH 2-10 was also determined from the emulsions prepared at 1% EWP and 5% oil. Extensive droplet aggregation was observed at pH 4 and 5 as expected which is around the pI of most egg white proteins. On the other hand, it was not observed at extremely acidic pH 2.0 and alkaline pH 9-10 and in the control emulsion prepared at pH 8.3.

In another part of the study, the effects of enzyme type (bromelain, ficin and papain), enzyme concentration (0.3, 0.5, 1, 2 and 4% w/w; enzyme/substrate (E/S) ratio) and hydrolysis time (0, 30, 60 and 120 minutes) on the degree of hydrolysis (DH) of EWP were investigated by diluting EWL containing 10% EWP to different EWP concentrations followed by adding enzymes into the EWL solutions. DH was observed to increase significantly ($p < 0.05$) with increasing enzyme concentration and hydrolysis time. A significant difference ($p < 0.05$) among the different types of enzymes was only observed from the samples with 4% E/S ratio at 120 minutes of hydrolysis time. Papain yielded the highest DH of 7.69% while bromelain and ficin yielded similar DH levels of 5.03% and 4.99%, respectively. The results of SDS-PAGE revealed that the protein bands corresponding to ovalbumin and ovotransferrin disappeared due to their enzymatic hydrolysis into smaller peptides but it was not significantly different between the samples treated with different E/S ratios and hydrolysis reaction times.

The effects of enzyme concentration, DH and hydrolysis time on the emulsifying properties of hydrolysed EWP prepared with bromelain and ficin were investigated. Surprisingly, enzymatic hydrolysis significantly improved the appearance of emulsions prepared with EWL containing hydrolysed EWP by producing an emulsion free of aggregates compared to the control emulsions prepared from original EWP which had lots of large aggregates in it. For example, emulsions containing 10% oil and various EWP concentrations (1, 5 and 10%) prepared with hydrolysed EWP (4% E/S, DH 5.16%) yielded smaller droplet size (0.66-0.98 μm) than those of original EWP emulsions (1.22-39.35 μm). However, phase separation occurred immediately after preparation at all protein concentrations (1, 5 and

10%) used while phase separation occurred in only emulsions stabilised with 5 and 10% original EWP. When the emulsions were heat treated at 60-90ºC for 0-30 minutes, gelation occurred in the emulsions prepared with 5 and 10% EWP concentrations while the emulsions prepared with 1% EWP had no gelation but had aggregation and phase separation after heat treatment. Emulsions prepared with 1% E/S ficin (DH 4.03% and 4.96%, respectively, after 2 and 4 hours of hydrolysis time) yielded smaller droplets size (0.75-0.87 μm) than droplet size (6.40-7.37 μm) of emulsions prepared with 1% E/S bromelain (DH 4.10% and 4.87% after 2 and 4 hours of hydrolysis time). Droplet size decreased as hydrolysis time increased from 2 to 4 hours for both ficin and bromelain hydrolysates with phase separation occurring the following day after the preparation of emulsions. Thus, DH and enzyme type had some influence on the emulsifying properties of EWP hydrolysates.

In conclusion, this study demonstrated that egg white emulsions can be prepared with little or no aggregates using low oil (≤5%) and low protein (1%) concentrations and by enzymatic hydrolysis of EWP. Emulsions containing 5% oil prepared with a relatively higher protein concentration (1-2%) were more stable to destabilization to ionic strength (salt concentration), especially against NaCl. These could lead to production of egg white protein based-emulsions with distinct appearance and characteristics.

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LIST OF SYMBOLS

$D_{4,3}$	Sauter mean diameter
$D_{3,2}$	Volume mean diameter
μm	micrometre
^{o}C	Degree Celsius
mM	Millimolar

LIST OF ABBREVIATIONS

AEP	Acid treated egg protein
AHEP	Acid-heat treated egg white protein
ANOVA	Analysis of variance
BEWPH-	Bromelain egg white protein hydrolysates
$CaCl_2$	Calcium chloride
CLSM	Confocal Laser Scanning Method
Da	Dalton
DH	Degree of hydrolysis
DLS	Dynamic Light Scattering
E/S	Enzyme/Substrate
EWP	Egg white protein
EWPH	Egg white protein hydrolysate
EW	Egg white
EWL	Egg white liquid
FEWPH	Ficin egg white protein hydrolysate
HCl	Hydrochloric acid
HEWP	Heat-treated egg white protein
HMW	High molecular weight
HPLC	High Performance Liquid Chromatography
kDa	Kilodalton
LMW	Low molecular weight
MPa	Mega pascal
NaCl	Sodium chloride
NaOH	Sodium hydroxide
nm	nanometre
OEWP	Original egg white protein
OPA	Ortho-phthalaldehyde
O/W	Oil-in-water emulsion
PDI	Polydispersity Index
pI	Isoelectric point
SDS	Sodium dodecyl sulphate
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis

SEM	Scanning Electron Microscopy
SLS	Static Light Scattering
TEM	Transmission Electron Microscopy
W/O	Water-in-oil emulsion
β-lg	Beta-lactoglobulin
ζ-potential	Zeta-potential