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Evaluation of nontranslucent functionality of refitted proteins in mayonnaise-like emulsions using selected enzyme- refitted proteins and their respective non-parse controls

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The study investigated whey protein isolate (WPI), casein, and enzyme-modified WPC in a O/W emulsion. Enzyme-modified samples were selected based on the sensory evaluation and their functionality was tested in mayonnaise-like emulsion and compared to the mayonnaise emulsion prepared using egg components. Casein and hydrolyzed casein were not used for making mayonnaise-like emulsion because of their undesirable taste and poor emulsion stability. At a given concentration of protein, egg components provide smaller oil drops and creamier mayonnaise compared to WPC and WPI. However, WPC provides a thicker mayonnaise-like emulsion compared to the egg proteins and WPI. Both WPC and WPI provided products with much thicker texture. However, the texture was not creamy compared to the emulsion containing the egg components. Enzyme-modified WPC samples are significantly less functional than the enzyme-modified WPI samples. Emulsifying functionality in mayonnaise was decreased as the level of WPI or enzyme-modified WPI was decreased. At all protein levels studied, protease (Multifect Neutral)-treated WPI is more functional than transglutaminase-treated WPI. Enzyme modification, in general, leads to a decrease in the emulsifying functionality of WPC and WPI in mayonnaise-like emulsions.

Keywords: whey proteins, WPC, WPI, casein, non-hydrolyzed controls

INTRODUCTION

Mayonnaise is an oil-in-water (O/W) emulsion where egg proteins including lipoproteins act as emulsifiers. Composition and emulsifying properties of whole egg and egg yolk have been widely studied (Powrie and Nakai, 1990; Baldwin, 1990; Nakai and Li, 1989). In products such as mayonnaise, the functional contribution of egg is difficult to replace. As far as the emulsifying functionality is concerned, egg yolks are the most functional components of the whole egg owing to the presence of lipoproteins. Egg whites (albumen) are typically less functional. Mayonnaise emulsions are stabilized primarily through steric forces. Because of their large size, protein and lipoprotein molecules form a thick layer (~140 in thickness) around oil droplets (Ford, Borwankar, Martin and Holcomb, 1997) and prevent the close approach of the surfaces of the adjacent oil droplets in an emulsion.

Isoelectric point of egg whites and egg yolks are 5.4 and 5.3, respectively (Riddick, 1968). Although they possess an overall positive charge at the pH of mayonnaise (pH = 3 to 4), stabilization through an electrostatic mechanism is not likely because of the presence of salt (high ionic strength suppresses repulsion charge).

Mean drop size of oil droplets in mayonnaise ranges from 2-10 μm. The phase volume of the oil (internal phase) in mayonnaise is very high (75-82%) that is past the point of hexagonal close packing limit (74.05%) of spheres. Thus, the oil drops are forced close together and in an extreme case, the spherical shape of the oil drops is deformed. It is possible to incorporate the internal oil phase beyond 74.05% in mayonnaise because (1) the egg yolk possesses exceptional emulsifying functionality, (2) the oil drops are deformable, and (3) there exists a distribution of oil droplet sizes thus leading

to a more efficient packing.

Mayonnaise has thick texture because of the high internal phase volume and smaller droplets. Hence, the need does not arise to stabilize it against creaming. However, it is formulated to provide maximum stability against coalescence because the oil drops are in close proximity to one another. Thus, a strong, thick, pliable membrane is needed around the oil droplets in order to stabilize the emulsion against coalescence. Mayonnaise exhibits viscoelastic rheological behavior and also possesses a yield stress. Egg white proteins are also partly responsible for yield stress as they have an ability to gel.

Although egg possesses excellent functional properties, it suffers from some disadvantages such as high cholesterol content and susceptibility to microbial contamination. Furthermore, some people are allergic to egg proteins and some vegetarians cannot consume products that contain egg or egg components. These considerations have led to a search for egg replacers and egg extenders (Roberts, 1978; Chess, 1980).

In the U.S., based on the standard of identity, only egg components are allowed as emulsifiers in mayonnaise. However, other proteins/emulsifiers may be used for non-standardized mayonnaise (mayonnaise-like product). Generally, proteins such as casein and skim milk products that have flexible, random coiled structure precipitate upon acidification and lose their emulsifying properties. This makes the emulsification with oil and the production of high fat emulsions with directly acidified solutions difficult. However, proteins such as whey, soy, and pea proteins that have compact, inflexible structures do not precipitate upon acidification and provide emulsion with higher viscosity.

There have been some investigations of preparation of oil-in-water emulsions with high fat content (mayonnaise consistency) using emulsifiers other than egg yolk. Emulsions prepared with low molecular weight emulsifiers such as ethoxylated monoglycerides, diacetyl tartaric acid ester of mono- and diglycerides, and hydrolyzed lecithins have low viscosity (Trueck and Campbell, 1999). Emulsions having higher viscosity can be produced using polyoxyethylene-(20)-sorbitan monostearate (polysorbate 60) at concentrations higher than 1%, but the taste is unacceptable. Viscosity increase in oil-in-water (O/W) emulsions also can be obtained using thickening or gelling agents such as polysaccharides, gums or cold swelling starches. However, the use of such gelling agents often leads to undesirable texture.

Nakajima et al (2006) and Mikami et al (1981) have prepared mayonnaise-like products using soy proteins or modified soy proteins. A mayonnaise product having a continuous aqueous phase and a dispersed oil phase and that uses a combination of soy protein and whey protein was claimed by Bodor and Petten (2007). Kolen and Golosinec (1975) described the preparation of emulsified oil dressings with serum protein that is treated to denature predetermined level of the protein. Trueck and Campbell (1999) have claimed a mayonnaise-like product containing an emulsifier other than egg yolk including milk and vegetable proteins and small molecule emulsifiers. Holst et al (1996) also described partially denatured whey proteins and their use as emulsifiers for making a mayonnaise-like product. Denaturation at a degree between 70 and 80% are claimed. The emulsion is claimed to have the consistency of a highly viscous mayonnaise and to have smooth texture and good stability and taste.

Three normally used strategies to modify functional properties of proteins are: chemical modification, heat treatment, and enzymatic modification (Vojdani and Whitaker, 1994). Chemical modifications are not popular because of higher costs and difficulty in gaining consumer acceptance and getting the ingredient approved from regulatory agencies. Heat treatment, on the other hand, has limited success in improving the functionality of protein (Gao et al., 2005). Enzyme hydrolysis of proteins can enhance their functional properties. Choosing the right type and amount of enzyme and conditions of hydrolysis are critical for enhancing their functional properties. Enzymatic modification of proteins has been reviewed extensively (Panyam and Kilara, 1996; Margot et al., 1994; Arai and Fujimaki, 1991; Reimerdes, 1990; Adler-Nissen, 1985).

For enzyme-catalyzed hydrolysis, a suitable enzyme is added to the protein solution, and then held for a time and at a temperature (and at a pH) sufficient to achieve the desired degree of hydrolysis. Typically, "degree of hydrolysis" is defined as the amount, in percentage, of peptide bonds that have been cleaved during the hydrolysis step (Mellqvist and Mellqvist, 1989) . Enzymes suitable for hydrolyzing proteins such as whey are known in the literature, and are typically proteases (Mellqvist and Mellqvist, 1989; Margot et al., 1998; Martinez et al., 1996; Faigh et al., 1989). Proteolytic enzymes cleave proteins into peptides and amino acids and change their physicochemical properties. This process can alter their functional properties over a wide range of pH. Hydrolysis of whey proteins is a subject of several investigations (Mellqvist and Mellqvist, 1989; Edens, 2007; Schlothauer et al., 2006; Schlothauer et al., 2005; Hudson et al., 2001).

The objective of our study was to determine if the emulsifying functionality of milk proteins, viz. whey protein concentrate (WPC), whey protein isolate (WPI), and casein can be enhanced by their modification using enzymes. First, the enzymes were screened with a variety of protein substrates and O/W emulsions were prepared using the control (unmodified) and enzyme-modified proteins. Emulsifying functionality of modified proteins was further evaluated in mayonnaise-like emulsions using the selected enzyme-modified proteins and their respective non-hydrolyzed controls.

MATERIALS

Whey protein concentrate (WPC) containing 80% protein was obtained from Leprino Foods (Denver, CO), whey protein isolate

Table 1. Supplier, action, activity, and source of various enzymes used.

Enzyme	Supplier	Action	Activity	Source
Enzeco alkaline protease (EAP)	Enzyme Dev. Corp.	Protease	690K DU/g	*B. licheniformis*
Multifect neutral (MFN)	Genencor	Protease	1600 AU/g	*B. amylo liquefaciens*
Alcalase (ALC)	Novozymes	Protease	2.4 AUA/g	-
Deamizyme (Deam)	Amano	Deamidase	-	*Aspergillus* sp.
Transglutaminase activa GB (TG)	Ajinomoto	Protein cross linking	100 A/g	-
Flavorzyme	Novozymes	Aminopeptidase	1000 LAPU/g	*Aspergillus* sp.

WPI; BiPro JE198- 4-420) containing 92% protein was procured from Davisco (Eden Prairie, MN), and natural casein isolate (261-B; indicated as Cas; nearly 100% protein) was obtained from Glanbia (Monroe, WI). Various enzymes were obtained from different suppliers as indicated in Table 1.

METHODS

Enzymatic hydrolysis of proteins

Five different enzymes, as shown in Table 1, were screened with WPC, WPI, and casein (Cas) in the presence and absence of Flavorzyme, an enzyme preparation containing a mixture of peptidases. Samples for making O/W emulsions were selected based on informal sensory testing which included taste and odor. To 100 ml of 10% protein powder/substrate in a sterile screw-cap bottle, 0.1% enzyme was added and incubated at 60°C overnight (20 h) in a shaking incubator and deactivated the enzyme at 70° C for 30 min. These solutions were freeze-dried and stored at room temperature, in a closed container, for further evaluation. Treatment variables were as follows:

(1) Protein + EAP (2) Protein + EAP + Flavorzyme
(3) Protein + MFN (4) Protein + MFN + Flavorzyme
(5) Protein + ALC (6) Protein + ALC + Flavorzyme
(7) Protein + Deam (8) Protein + Deam + Flavorzyme
(9) Protein + TG (10) Protein + TG + Flavorzyme

Preparation of O/W emulsions

Initially, oil- in-water (O/W) emulsions were prepared using 50 g of 1% protein solutions and 50 g of soybean oil using a PowerGen 700D rotor-stator homogenizer (Fisher Scientific, Pittsburgh, PA) operating at 20,000 rpm for 1 min. Samples for the emulsion work were chosen based on the sensory results. 200 ml beaker containing 50 g of 1% protein solution was positioned such that the homogenizing head of the PowerGen was in the beaker. PowerGen was started and 50 g of soybean oil was added slowly over 20 sec and the PowerGen was stopped after 1 min.

Drop size distributions and viscosities were measured for all the emulsions. 15 ml aliquots of each emulsion were stored in a centrifuge tube for a shelf life evaluation. Samples to investigate/characterize mayonnaise functionality were chosen based on the particle size and emulsion stability data of these emulsions.

Preparation of mayonnaise-like emulsions

Model mayonnaise-like emulsions were prepared according to the compositions showed in Table 2 using the modified whey proteins (WPC and WPI). Control product, for comparison, contained salted whole eggs and salted egg yolk. Levels of the salt, sugar, oil, and vinegar in test samples were comparable to that of the control sample containing egg ingredients. Freeze-dried enzyme-hydrolyzed protein source was first dissolved in water in a Model N-50 Hobart Stand-mixer bowl (The Hobart Mfg. Company, Troy, OH). For preparing the control, a mixture of egg yolk and whole egg was used. Sugar and salt were dissolved in this mixture while continuing to stir. Soybean oil was then added in small proportions with the stirring speed set at 2. One minute after the incorporation of the oil, vinegar (120 Grain) was added to the Hobart bowl and stirring was continued for one more minute. The coarse emulsion, thus obtained, was homogenized in a lab-scale high shear (rotor-stator) homogenizer (internally built). The product was filled in 16 oz glass jars and stored at room temperature.

Drop size distribution

In an emulsion, there exists a range of drop size distribution depending on the nature (structure) and amount of the emulsifier used and the method used for emulsification. Hence, mean diameter is used to characterize the drop size. Tighter distribution typically yields more stable emulsion. Drop size also influences the flavor release and appearance of an emulsion-based product - smaller drops increase opaqueness while larger drops impart translucent appearance to the emulsion. In addition, drop size influences viscosity of the emulsion - smaller the drop size the thicker (higher viscosity) the emulsion.

Drop size distribution was determined by the Horiba LA 500 (Horiba Instruments, Irvine CA) laser diffraction particle size distribution analyzer. 1 g of a O/W emulsion or mayonnaise emulsion sample, as the case may be, in a 20 ml vial was dispersed homogeneously (using a vortex mixer) with 9 g of 1% sodium dodecyl sulfate (SDS) solution. SDS helps to break the emulsion aggregates into individual drops. The result of the analysis is a volume weighted distribution characterized over the size limits of the optical configuration used.

Viscosity

Viscosity of O/W emulsions was measured at room temperature (21°C) using a Brookfield Model DVI+ viscometer (Brookfield Engineering Laboratories, Inc., Stoughton, MA) utilizing the Spindle # S27 at 500 x g for 1 min. Viscosity of mayonnaise-like emulsions was measured using a Haake VT-24 viscometer (Haake, Karlsruhe, Germany) and a 1” × 1” vane at 1 min and room temperature (21°C). Yield stress of mayonnaise emulsion was the maximum value observed in each case.

Emulsion stability

Emulsion stability of the O/W emulsions was determined by storing the emulsion in a 15 ml plastic centrifuge tube at the room temperature for a period of 5 weeks and observing the separation

Table 2. Composition of Mayonnaise-Like Emulsion Prepared Using Enzyme-Modified WPC/ WPI.

Ingredients	WPC (%)	WPC/MFN (%)	WPC/TG (%)	WPI (%)	WPI/MFN (%)	WPI/TG (%)	50% WPI (%)	50% WPI/MFN (%)	50% WPI/TG (%)	30% (
Soybean oil	78.62	78.62	78.62	78.70	78.70	78.70	78.70	78.70	78.70	78
Water	16.12	16.12	16.12	16.17	16.17	16.17	16.65	16.51	16.51	16
Sugar	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.
Salt	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.
Salted egg yolk	0	0	0	0	0	0	0	0	0	
Salted whole egg	0	0	0	0	0	0	0	0	0	
WPC	0.81	0	0	0	0	0	0	0	0	
WPC/MFN	0	0.81	0	0	0	0	0	0	0	
WPC/TG	0	0	0.81	0	0	0	0	0	0	
WPI	0	0	0	0.68	0	0	0.34	0	0	0.
WPI/MFN	0	0	0	0	0.68	0	0	0.34	0	
WPI/TG	0	0	0	0	0	0.68	0	0	0.34	
Vinegar (120 Gr)	2.5	2.5	2..5	2.5	2.5	2.5	2.5	2.5	2.5	2
Total	100	100	100	100	100	100	100	100	100	1

of oil and aqueous phase at the end of 5 weeks period. In cases where there is no oil separation, the tubes were centrifuged at 1000 rpm in a bench-top centrifuge and observed for any oil separation. Higher the oil separation, less stable is the emulsion.

RESULTS AND DISCUSSION

Generally, all the enzyme-modified milk protein samples where Flavorzyme was not used deve-loped bitterness. The use of Flavorzyme reduces the bitterness of the peptides produced through protease reaction. However, casein modified with EAP, ALC, and TG enzymes exhibited bitterness even in the presence of Flavorzyme. Hence, those samples were not used for further studies.

O/W emulsions (50% oil and 50% aqueous phases) were prepared using the control (unmodi-fied) and enzyme-modified WPC, WPI, and casein

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(Cas). The oil drop size distribution, mean oil drop size, and viscosity data for these O/W emulsions are shown in Figures 1, 2, and 3 respectively. The appearance of stable emulsions at the shelf life of 5 weeks is depicted in Figure 4. O/W emul-sions prepared using casein, enzyme-modified caseins, WPC modified with Deam, MFN, and ALC had very large oil drops, lower viscosity, were unstable, and separated into the oil and water phases. All the samples of enzyme-modified WPI, and control WPC and WPI exhibited smaller oil drops and produced stable O/W emulsions (see Figure 4). WPC modified with TG although had smaller oil drops, some oil separation (free oil) was observed. The O/W emulsions containing WPI modified with MFN and TG produced a thicker emulsion. In general, WPC- and WPI-treated with MFN and TG produced better O/W emulsions than those treated with other enzymes.

Hence, these sy in mayonnaise-l properties were sions were also teins levels for and TG. Conce were the same Calculated mois of the mayonn tabulated in Tab yolk/whole egg proteins (contrib pH, viscosity, specific area o mayonnaise-like and enzyme-m sented in Table upon decreasing the lower bufferin

Table 3. Calculated moisture, fat, protein and salt of mayonnaise-like emulsion prepared using control and enzyme-modified WPC and WPI.

	Control (with egg components)	WPC	WPC/MFN	WPC/TG	WPI	WPI/MFN	WPI/TG	50% WPI	50% WPI/MFN	50% WPI/TG	
Moisture (%)	18.29	18.32	18.32	18.32	18.37	18.37	18.37	18.71	18.71	18.71	1
Fat (%)	78.71	78.70	78.70	78.70	78.71	78.71	78.71	78.71	78.71	78.71	7
Protein (%)	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.32	0.32	0.32	
Salt (%)	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	

for the various mayonnaise-like emulsions are presented in Figure 5 and mean and median drop diameters are illustrated in Figure 6. Figure 7 shows specific area for the different mayonnaise samples.

The control sample containing egg components had the smallest drops and highest specific area. Mean drop diameter of the sample prepared using WPC was closer to the control sample containing the egg components (typical mayonnaise). However, drop sizes of the emulsions prepared using TG-modified WPC was much larger than the control sample and those formed using MFN-treated WPC destabilized and separated into an oil and water phases. Hence, no tests were performed on the mayonnaise sample prepared using MFN-treated WPC. In the case of WPI, oil drop diameters were slightly larger and specific area was slightly smaller than the control samples containing egg components or WPC. The WPI samples modified with MFN and TG exhibited larger drops and smaller specific areas compared to the WPI control sample. Mayonnaise-like emulsions were also prepared at two lower levels of WPI and enzyme- treated WPI.

At all levels of protein, MFN-treated WPI seems to provide smaller drop size and larger specific area compared to the respective controls. This indicates that th treated WPI is s treated WPI. Fur and specific are the protein co however, enzym an adverse effe of milk proteins.

Viscosity and mayonnaise-like control and MFN are illustrated in pared using eg behavior in term

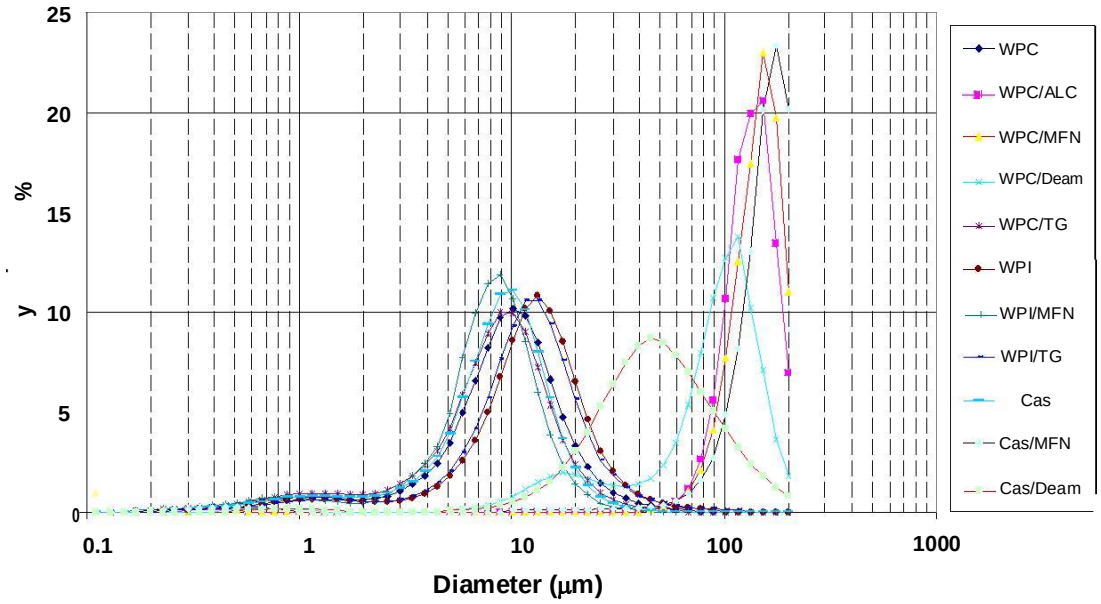

Figure 1. Drop size distribution for the various O/W emulsions.

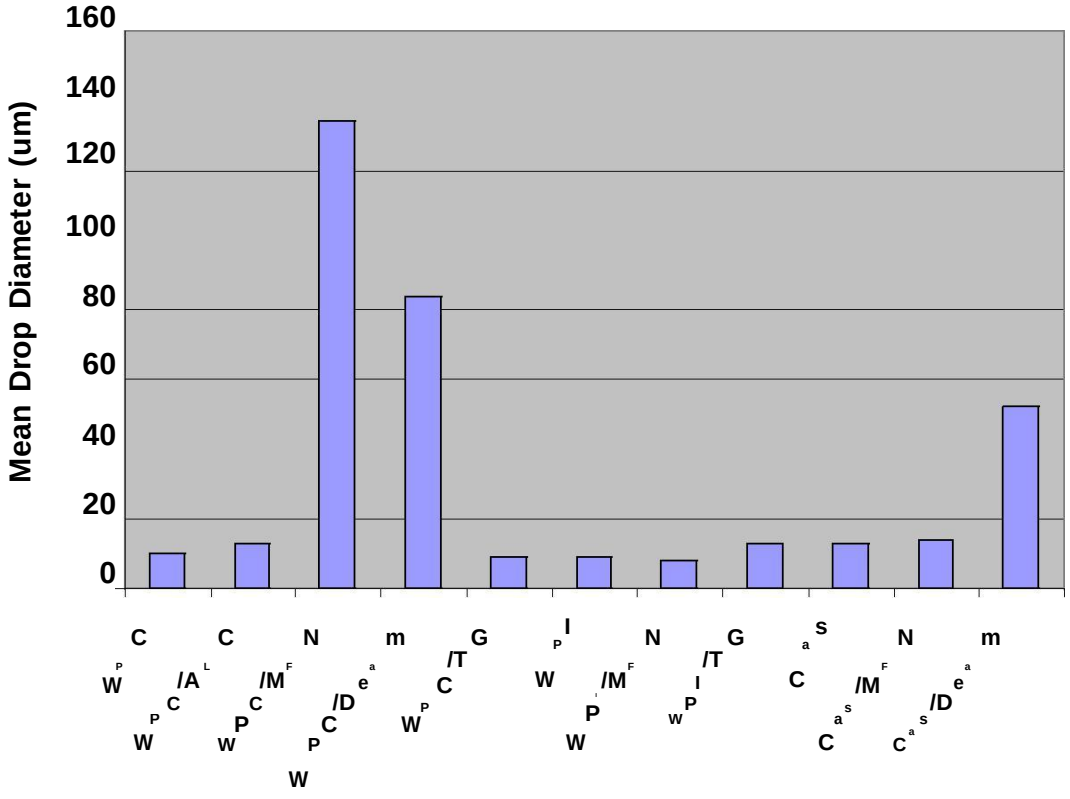

Figure 2. Mean drop diameter of various O/W emulsions.

Samples made using control WPC and WPI exhibited much higher viscosity and yield stress, but had chunky texture similar to mayonnaise prepared with egg whites. The WPC yielded slightly higher viscosity but similar yield stress compared to WPI. The WPI samples modified with MFN and TG exhibited lower viscosity and yield stress compared to the WPI control. Both viscosity and yield stress decreased with a decrease in the level of protein.

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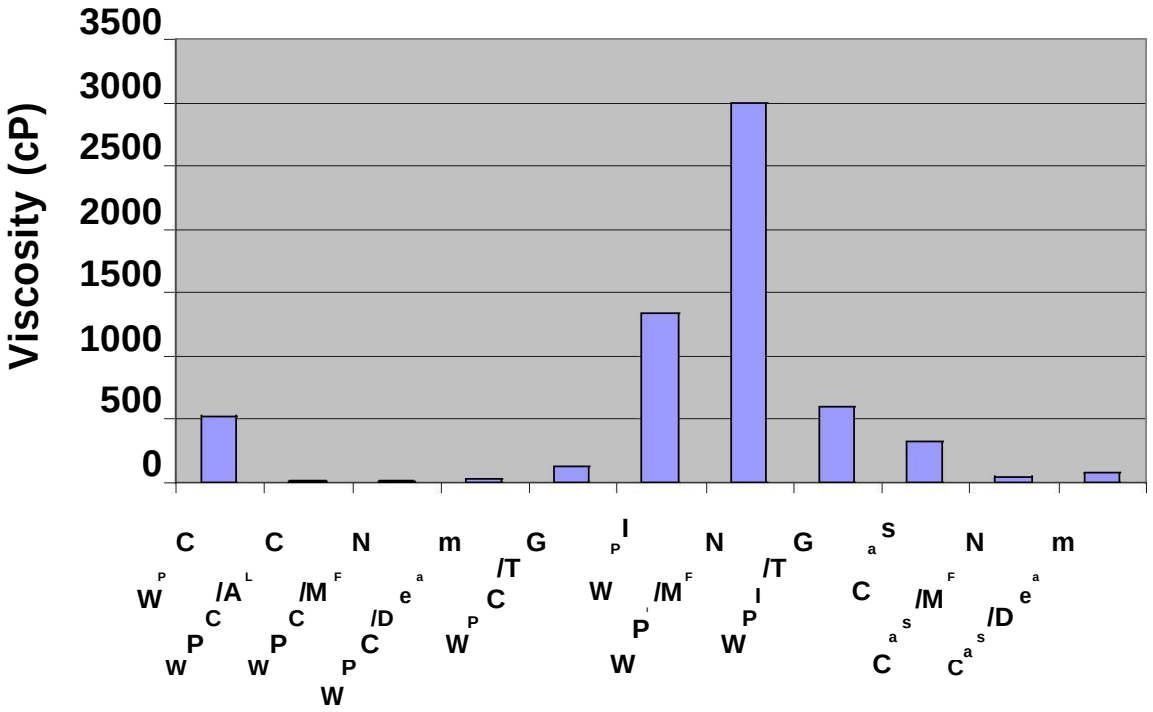

Figure 3. Viscosity of Various O/W emulsions.

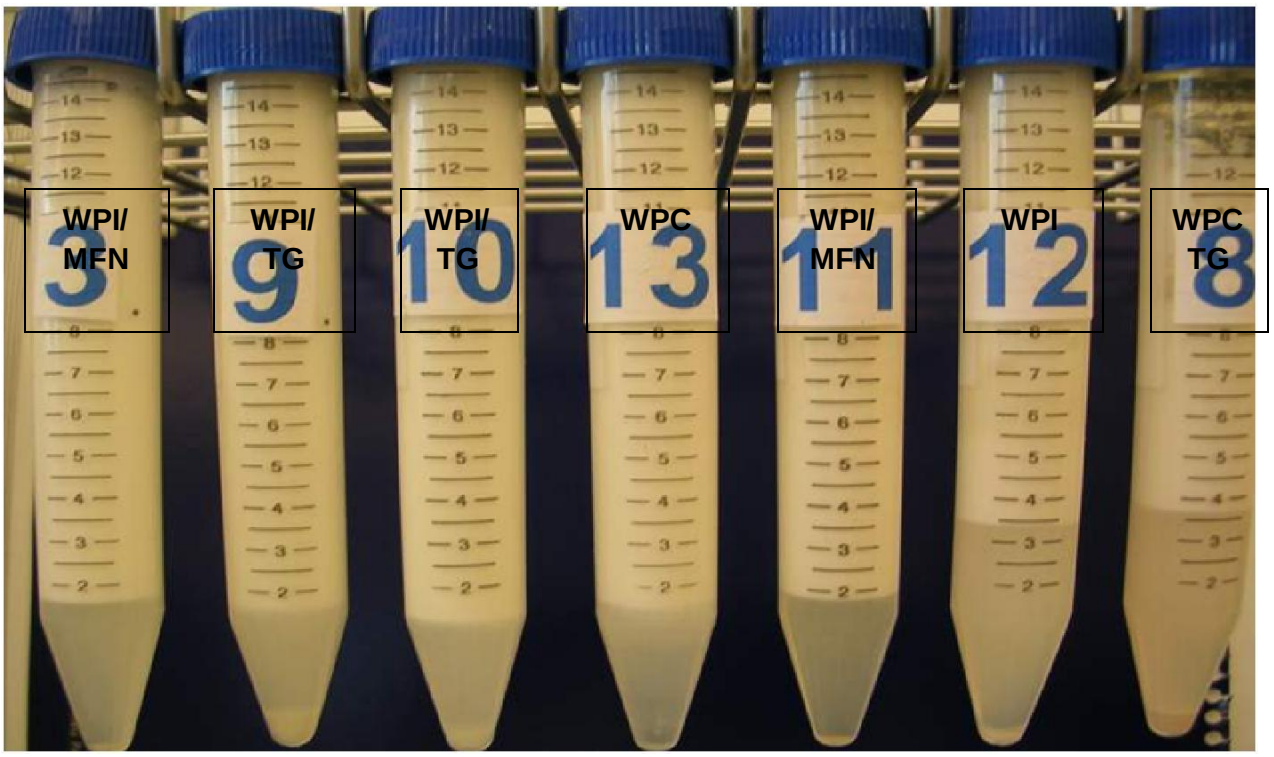

Figure 4. Various O/W emulsions which are stable.

At all three protein levels studied, MFN-modified WPI provided higher viscosity and yield stress compared to the TG-modified WPI. In future work, the effect of combination of the enzymes on the emulsifying functionality will be investigated.

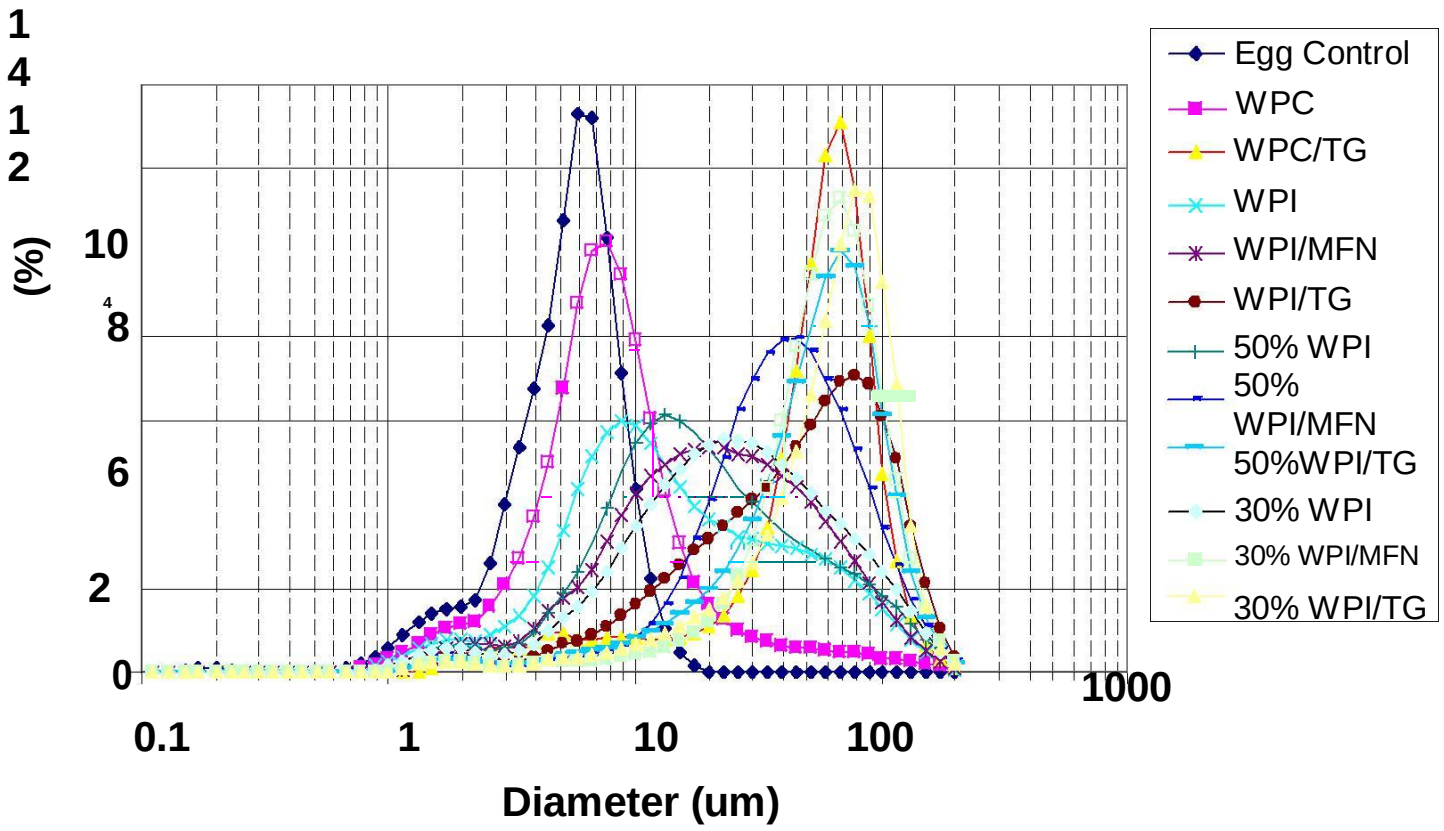

Figure 5. Drop size distribution for the various mayonnaise-like emulsions.

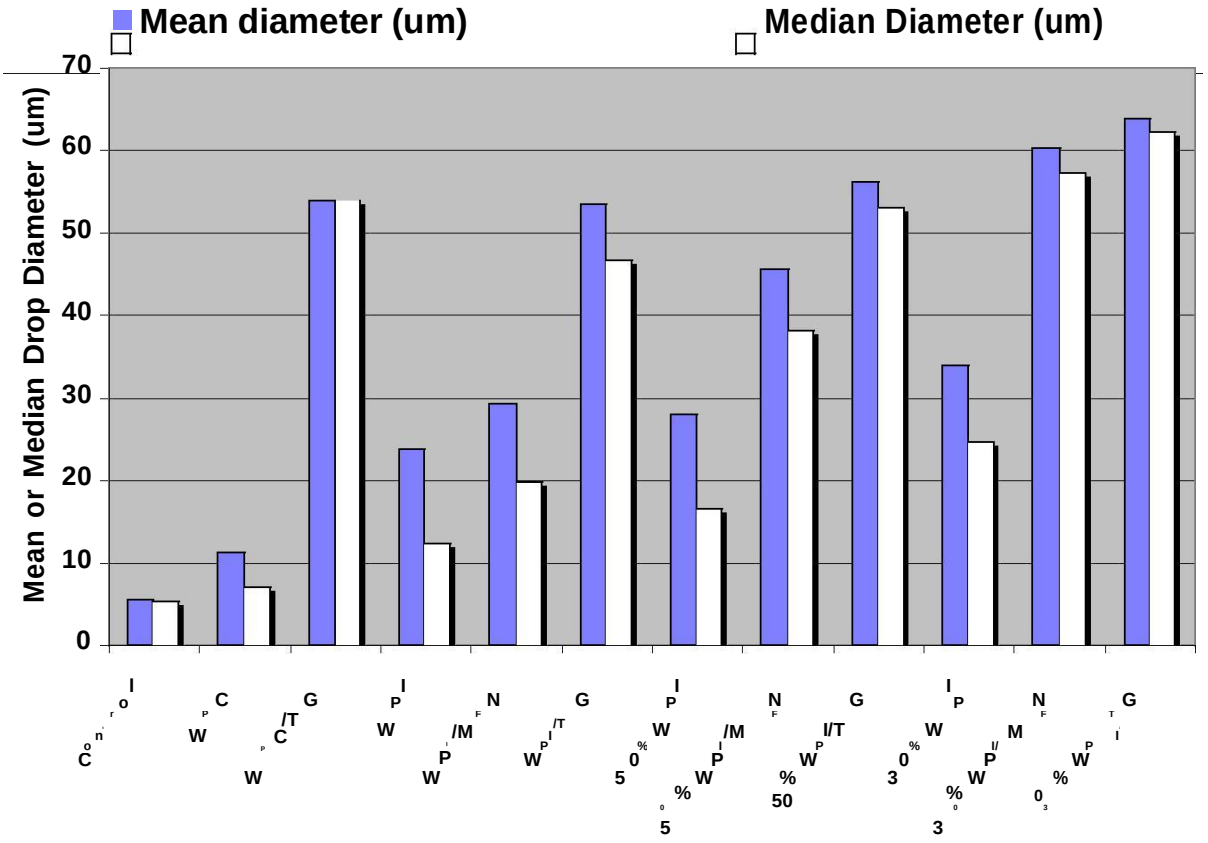

Figure 6. Mean and median drop diameters for the various mayonnaise-like emulsions.

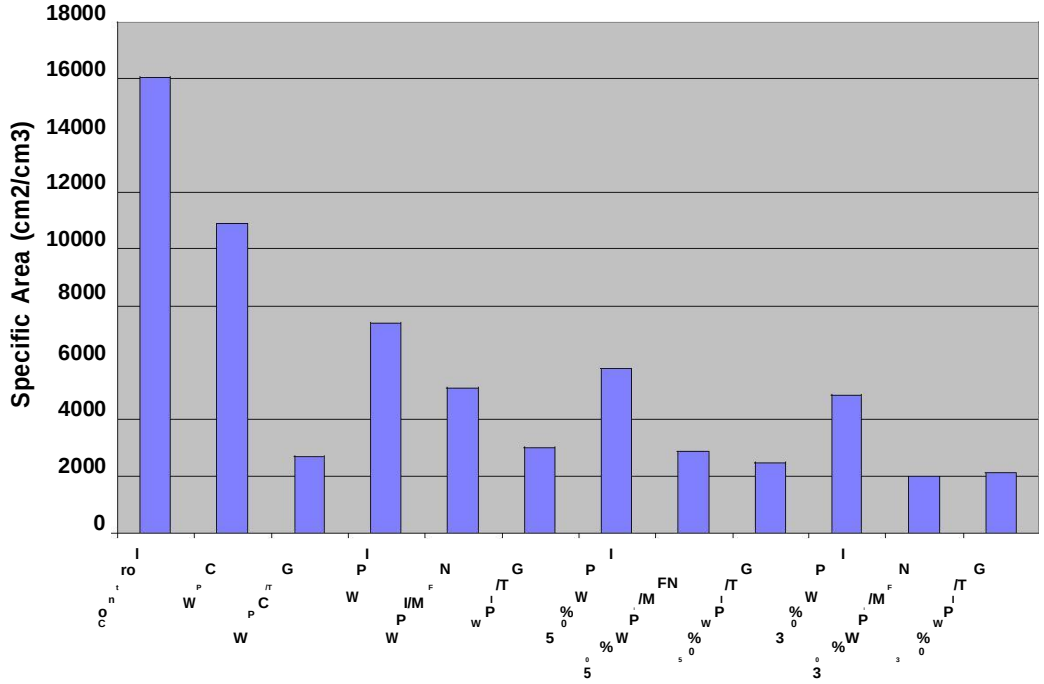

Figure 7. Specific areas for the various mayonnaise-like emulsions.

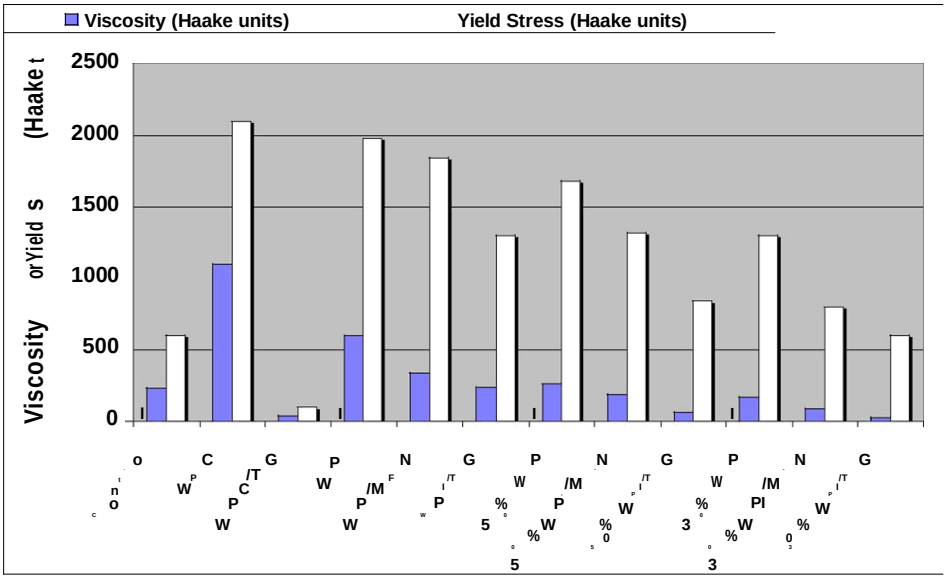

Figure 8. Haake viscosity and yield stress for the various mayonnaise-like emulsions.

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