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Physicochemical analysis of cholesterol-reduced egg yolk powder and its application in mayonnaise

[1]Fauziah, C. I., [1*]Zaibunnisa, A. H., [2]Osman, H. and [2]Wan Aida, W. M.

[1]*Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia*
[2]*Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia*

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Abstract

The aim of this study were to find out the physicochemical characteristics of cholesterol-reduced egg yolk powder and its application in the production of mayonnaises. Cholesterol-reduced egg yolk powder (CREYP) were prepared from removal of cholesterol by formation of cholesterol:β-cyclodextrin inclusion complex. The physicochemical characteristics of CREYP and NEYP were foaming capacity (FC):1.96%,4%; foaming stability (FS):96.48%,94.55%; emulsion capacity (EC):59.82%,58.43% and emulsion stability (ES):43.94%,41.48% respectively. Whereas the viscosity of CREY, NEY and commercial mayonnaises were 8000, 4768 and 6747 cP respectively. The lightness (L^*), redness (a^*), yellowness (b^*), saturation (C^*) and hue angle (h°) values for CREYP and NEYP results showed significantly different ($p>0.05$) for all chroma values with CREYP showed higher L^* and h° values but lower in a^*, b^* and C^* values showing that the yellow colour of NEYP lessened. Commercial mayonnaise appeared to be lighter and less yellowish than CREY mayonnaises with L^* and b^* values of commercial to CREY mayonnaises were L^*:78.34;63.78% and b^*:8.29;14.98% respectively. It can be concluded that CREYP can be used as replace to the NEYP and whole liquid egg yolk with enhance nutritional values. The results obtained from this study will be very useful for producing CREYP.

Introduction

Cholesterol has always been a constituent of concern for the mankind. Cholesterol is the most abundant steroid in animals and is supposed to be the causative agent for coronary heart diseases like arthrosclerosis and gall stones (Kanchana and Sekar, 2011; Fauziah *et al*., 2013). There are good reasons for long-standing recommendations that dietary cholesterol should be limited to less than 200 mg/day; a single large egg yolk contains approximately 275 mg of cholesterol (more than a day's worth of cholesterol), to lower the risk of coronary heart disease is to limit cholesterol intake to less than 200 mg/day (Spence *et al*., 2012). Food intake are now not only intended to provide necessary nutrients, but also to prevent nutrition-related diseases and also to improve physical and mental well-being of the consumers (Santos *et al*., 2011). Average consumption of eggs in the developed country has steadily decreased. It is partly due to a concern that dietary cholesterol may contribute to the incidence of coronary heart disease, hence this negative concerns related to yolk cholesterol have simulated researches to decrease cholesterol content in egg yolk (Lim *et al*., 2006). It can be assumed that cholesterol-lowering and cholesterol-free products will gain increasing relevance in the coming years (Santos *et al*., 2011).

Eggs have become one of the vital staple foods in the world. Egg yolk contains approximately 50% solids. The major constituents of the solid matter are lipids (65–70% on dry basis) and proteins (30% on dry basis), consisting of proteins in solution referred to as livetins, lipoprotein particles including high-density lipoproteins, HDLs, low-density lipoproteins, LDLs, and phosvitin (Li-Chan *et al*., 1995). Triacylglycerols (TAG, 66%) is the largest fraction of yolk lipids, followed by phospholipids (PL, 30%) and cholesterol (CL, 5%) (Sofia *et al*., 2006).

The major applications for egg yolk in the food industry are the manufacture and stabilization of emulsions. Native egg yolk from domestic hens consists of mixtures of lipids and proteins noncovalently bound in the form of large lipoprotein complexes. These complex confers to egg yolk having a high emulsifying capacity (Moros *et al*., 2002). Mayonnaise is an emulsions and one of the most widely used sauces in the world, where it is a mixture of egg, vinegar, oil and some other ingredients. It is a typical oil-in-water emulsion with

*Corresponding author.
Email: *nisha@salam.uitm.edu.my*

high oil content, and among its ingredients, egg yolk is most critical for the stability of the product (Laca *et al*., 2010). Food emulsions, such as mayonnaise or salad dressings, are typically stabilized by egg yolk and its has been widely used as emulsifier because it imparts desirable flavours, mouthfeel and colours (Moros *et al.*, 2002). One of the major trends in the food industry is to reduce the fat content of dressings, which has led to the popularity of "reduced fat", "low fat", or "fat free" versions of traditional products (McClemets 2005). However, fat as a food component contributes to flavour, appearance and shelf-life of the food products. Thus, it is difficult to mimic the quality of classic products when manufacturing low-fat foods (Sabaghian *et al.*, 2014).

Despite having high cholesterol content in liquid egg yolk, fresh eggs are however, difficult to transport because of their bulkiness, fragility, and highly perishable nature. Egg in powder form, provides a near complete solution to these (Ndife *et al*., 2010). Therefore the objectives of this study was to determine the effect of removal of cholesterol on the physicochemical properties of cholesterol-reduced egg yolk powder (CREYP) and its application in mayonnaises.

Materials and Methods

CREY powder preparations

The pH (pH 10.5) and °Brix (2.9) of liquid egg yolk was adjusted using pharmaceutical grade, Sodium Hydroxide, NaOH 1M and purified water respectively. Sample of liquid egg yolk was adjusted continuously using homogenizer (IKA Ultra Turrax T25 Homogenizer, UK) at 210±10 rpm without foaming. The treated liquid egg yolk then heated to 50°C by using laboratory shaker waterbath (17-55L Shaking Water Baths) at 100 rpm for an hour. Treated-liquid egg yolk (5 gm) was mixed with 15 mM β-CD using a vortex mixer (Laboratory equipment, VELP, Italy) at 1600 rpm for 10 minutes and continued in laboratory shaker waterbath at 100rpm in 50°C for 20 minutes. The β-CD-egg yolk solution prepared was cooled to 8-10°C within 1 hr and centrifuged at 5000rpm at 5°C for 15 mins (Hettich Universal 320, Benchtop centrifuge, Germany). Insoluble β-CD-cholesterol inclusion complex in the form of sediment was separated from the supernatant. This supernatant was adjusted to pH 6.0 with food grade citric acid. Supernatant (at 15 mM β-CD which is the cholesterol-reduced egg yolk; and without β-CD addition which is the normal egg yolk) was dried in oven dryer at 44°C for 4 hr to produce the cholesterol-reduced egg yolk powder (CREYP) and normal egg yolk powder (NEYP) and allowed to cool. The egg flakes were scooped, milled and sieved with a 60 mm mesh (Kumaravel *et al.*, 2012). The egg powders were kept at 4°C in tightly sealed container until further analysis.

Preparation of mayonnaise

CREYP/NEYP were prepared as described earlier, and all other ingredients used to prepare the mayonnaise, such as soybean oil, eggs, vinegar, sugar, lemon and salt were purchased from the local supermarket. Mayonnaise recipe was modified from Shen *et al.* (2011) with the following ingredients in percentage (w/w): CREYP/NEYP 12%, vinegar (5% (w/v) acetic acid) 3%, soybean oil 50%, salt 1.0%, sugar 14%, water 15% and lemon juice 5%. The mayonnaise was prepared separately by mixing the egg powder and vinegar together. Then all other ingredients were added except for oil and were stirred homogeneously by a mechanical overhead stirrer. Oil was added very slowly, while stirring at 1600 rpm for 1 min, and will be followed by 2000 rpm for another 4 min. The emulsion (prepared mayonnaises) was stored in plastic bag and was left 1 day in chillers at 7°C prior testing (Shen *et al*., 2011).

Foaming capacity and foaming stability of CREYP and NEYP

Foaming capacity and foaming stability were determined as described by Baljeet *et al.* (2014) with slight modifications. CREYP and NEYP (1.0 g) was added to 50 ml distilled water at 30 ± 2°C in a separated graduated cylinder. The suspension was mixed and shaken for 5 min to foam. The volume of foam after whipping for 30 s was expressed as foaming capacity. The volume of foam was recorded 30 min after whipping to determine foam stability as percent of the initial foam volume.

Foaming capacity (FC) was determined as:

$$\frac{\text{Volume of foam (AW) - Volume of foam (BW)}}{\text{Volume of foam (BW)}} \times 100$$

AW: After whipping, BW: Before whipping.

Foam Stability (FS) was determined as:

$$\frac{\text{Volume of foam after 30 min whipping}}{\text{Volume of foam after initial whipping for 30 sec}} \times 100$$

Emulsion capacity and emulsion stability of CREYP and NEYP

The emulsion property was determined by method determined following the method used by Baljeet *et al*. (2010). The emulsion (1 g egg yolk powder, 10 ml distilled water, 10 ml soybean oil) was prepared in a calibrated centrifuged tube. The emulsion was centrifuged at 2000 rpm for 5 min. The ratio of the height of the mixture was calculated as the emulsion capacity expressed in percentage.

The stability of the emulsions was assessed using accelerated ageing as described by Anton *et al.* (2000). Aliquots of the emulsions (10 ml) were stored at ambient temperature (22°C) for 24 h, and then centrifuged in graduated centrifuging tubes at 2000 g for 30 min at 10°C. After the centrifugation, the volume of emulsion creamed at the top and the volume of sediment water phase were both noticed and the percentage of creaming was calculated as follows:

$$\% \text{ Emulsion stability (ES)} = 100- [(V_c / V_t) \times 100]$$

Where V_c, is the volume of the creamed phase and V_t the volume of the creamed phase + volume of the aqueous phase.

Determination of colour

Colour values (L^*, a^*, b^*, C^*, h°) of CREYP, NEYP, and mayonnaises were measured in triplicate by a reflectance colorimeter (Minolta Chroma Meter CR-300, Minolta Italia S.p.A., Milano, Italy) using illuminant source C. The equipment was calibrated by using white standard ceramic tile (Reference No. 1353123. Y=92.7, x=0.3133, and y=0.3193) (Bianchi *et al*., 2006). The L^* in the colour system is representing the lightness while a^* and b^* are colour coordinates whereby +a is the red, -a is the green, +b is the yellow and –b is the blue direction. Chroma (C^*) is a measure of intensity or saturation and calculated as $[(a^*)^2 + (b^*)^2]^{1/2}$ and represents saturation whereas h° represents hue angle which is derived from the two coordinates a^* and b^* and determined as $\tan^{-1} b^*/a^*$ (Bianchi *et al*., 2006). Hue angle (h°) is expressed on a 360° grid where 0°=bluish-red, 90°=yellow, 180°=green and 270°=blue (Wrolstada *et al*., 2005) . Freshly prepared sample was stored for one day prior colour determination.

Viscosity of mayonnaises

The determination of mayonnaise's viscosity was conducted by using a viscometer (Brook field digital viscometer model DV-1). The sample was added into the test viscometer cup and run at 750 rpm. The viscosities values of the sample were recorded once it stable and the measurement were repeated in triplicate (Shen *et al*., 2011).

Statistical analysis

Mean values from the 3 separate experiments or replicate analysis were reported. The statistical significance of observed differences among three treatment means evaluated by analysis of variance (ANOVA) and the comparison of means were carried out by Duncan's multiple range test ($p<0.05$) by using SPSS 10 for Windows. Comparison of means between two group of samples were carried out by independent t-test using SPSS 10 windows.

Results and Discussion

Foaming capacity and foaming stability

Figure 1 show the influence of the nature of CREYP and NEYP on the foam capacity and foam stability. There was significantly different ($p>0.05$) in foam capacity (%) of CREYP prepared from reduction of cholesterol using β-CD:cholesterol inclusion complexes and NEYP with foam capacity values of 1.96 and 4.00% respectively. Foam capacity of NEYP was higher than CREYP due to high protein contents of NEYP. Protein in egg yolk helps in contribution of foaming of foods. The foam volumes increased with protein concentration and decreased with holding time. LDL was found to be responsible for foaming capacity (Zayas, 1997).

Egg yolk having low protein content and high lipid than egg white. The lipid content egg yolk powder must have played a significant role in foam ability. There were several studies done to evaluate the foaming capacity of egg yolk powder comparison with egg white powder. Sheikh *et al.* (2009) reported the foaming capacity of egg yolk powder with 0.064-0.142cm^3/gm which were definitely lower than egg white powder itself. This report supported with study done by Ndife *et al*. (2010).

Foaming stability of CREYP was significantly higher ($p>0.05$) than in NEYP with 96.48% and 94.55% respectively. Even the foam capacity of CREYP was lower than NEYP, however CREYP did maintain in standing of foam rather than in NEYP.

With neglect in protein of CREYP, lipid content affects stabilising of the foam. Several authors reported that foaming by proteins is greatly influenced by the presence of lipids in the system because they adsorb and spread at the air-water interface and displace the protein molecules, or they may induce local thinning of the films between the bubbles. Additionally, they may break up the protein-protein interactions in the interfacial film and destroy its elasticity (Paraskevopoulou *et al*., 1997). Foaming formed with proteins are rigid and visco-elastic whereas foaming by lipids tend to form a dense with reduced

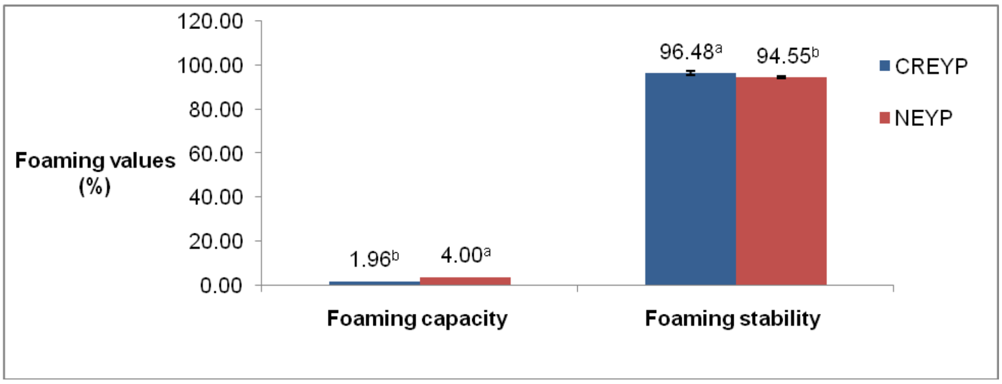

Figure 1. Foaming capacity and stability (%) of two types of egg yolk powder (CREYP: cholesterol-reduced egg yolk powder and NEYP: normal egg yolk powder).
* Small letters a, b indicate significant different (p>0.05) between two types of egg yolk powder

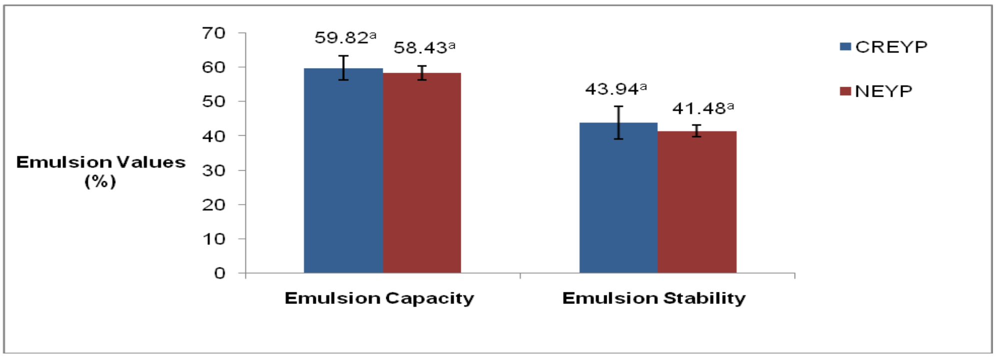

Figure 2. Emulsion capacity and stability (%) of two types of egg yolk powder (CREYP: cholesterol-reduced egg yolk powder and NEYP (control): normal egg yolk powder)
* Small letters a, b indicate significant different (p>0.05) between different types of egg yolk powder.

interfacial tension. Foam becomes less stable when both polar lipids and proteins are at the interface. The visco-elasticity arising from intermolecular interaction between proteins was reduced by lipid molecules and the mobility of the lipid is affected by the protein (Wang, 2009). Reduced total lipid content in CREYP helps in stabilising foam bubbles whereas NEYP with higher total lipid content destabilise the foam bubbles which eventually reduced its foam stability.

Emulsion capacity and emulsion stability

The emulsion capacity (EC) and emulsion stability (ES) of CREYP and NEYP are as shown in Figure 2. In our study, the EC and ES was not significantly ($p \leq 0.05$) influenced by cholesterol-reduced egg yolk powder. EC for CREYP and NEYP were 59.82% and 58.43% respectively. Emulsion capacity (EC) also can be defined as a molecule's ability to act as an agent that facilitates solubilisation or the dispersion of two immiscible liquids (Bouaziz *et al.*, 2014). The different in EC between CREYP and NEYP were due to the change of LDL components. Many researchers stated that LDL is the main contributor to the emulsifying properties (Mine *et al.*, 2000). Egg yolk protein serve as surface-active agent which forms an interfacial film, by orienting the polar portion of the molecule to the aqueous phase and non polar to the oil phase, thus helping to form a stabilized emulsion (Oloyede *et al.*, 2004).

Anton *et al.* (2003) proven that this apoprotein (referred to LDL) showed a high proportion of amphipathic α-helix chains, explaining the high capacity of this apoprotein to adsorb at the oil-water interface. Cholesterol removal from egg yolk in this study does not affects the property of apoprotein of LDL as surface-active agent in reducing interfacial tension between oil and water and finally resulted in CREYP having the same EC with NEYP.

ES for CREYP and NEYP were 43.94% and 41.48% respectively. Emulsion stability refers to the ability of an emulsion to resist changes in its properties over time: the more stable the emulsion, the more slowly its properties change (McClements, 2005). Removal of cholesterol from egg yolk using β-CD results in reduction of cholesterol and lipid content, however our study showed that there were no affects to the emulsifying activity of the CREYP as compared to NEYP.

This results showed CREYP having hydrophobic sites exposed to the continuous phase which is similar as with NEYP, something that leads to degree of interlinking between segments projecting from neighbouring drops and eventually; forming ES with

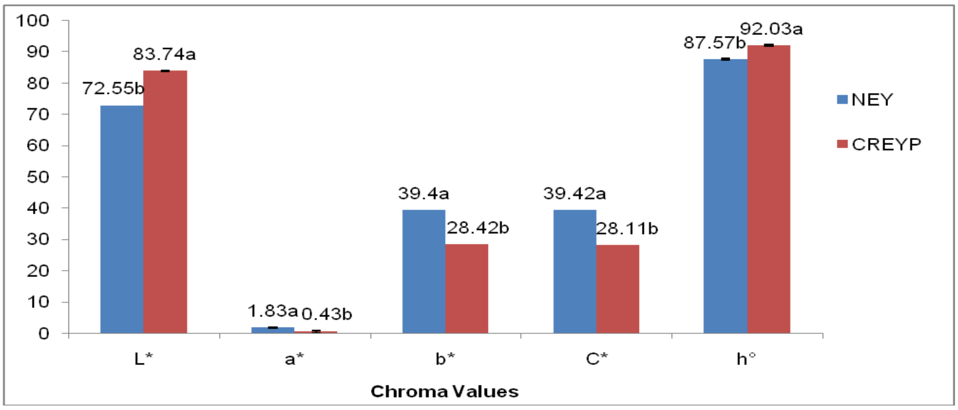

Figure 3. Chroma values of different egg yolk powder (CREY-cholesterol-reduced egg yolk, NEY: normal egg yolk).

* Small letters a, b indicate significant different (p>0.05) between two type of egg yolk powder.

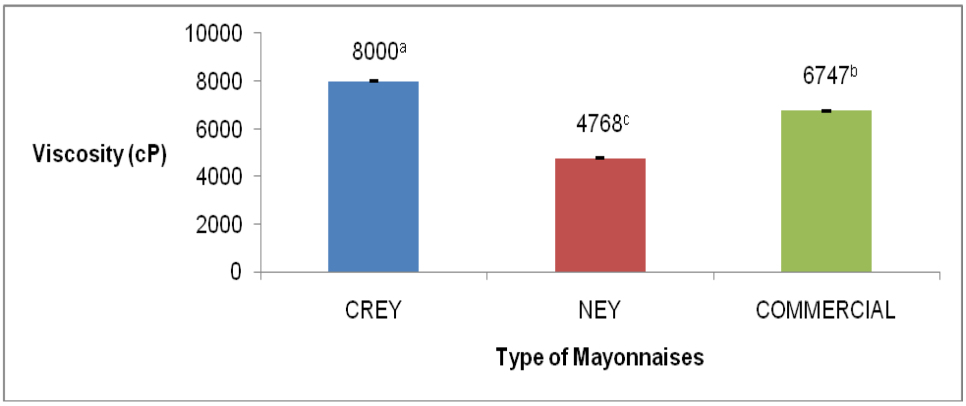

Figure 4. Viscosity of mayonnaises from different types of egg yolk powder (CREY-cholesterol-reduced egg yolk, NEY: normal egg yolk)

* Small letters a, b, c indicate significant different (p>0.05) between different types of mayonnaises.

no significantly difference ($p\leq0.05$) than NEYP. The results implies that cholesterol reduction treatment to the egg yolk did not disrupt the emulsifying properties of egg yolk however can improve the emulsifying properties of the native egg yolk itself. Overall, this effect leads to the conclusion that CREYP can be used as replace to the NEYP and whole liquid egg yolk for the emulsifying stability as well as the food ingredients emulsifier.

Chroma values of egg yolk powder

The colour analysis of CREYP and NEYP (Figure 3) were confirmed by the data obtained using Minolta Chroma Meter CR-300 (Japan) to detect the lightness (L^*), redness (a^*) and yellowness (b^*), saturation, (C^*) and hue angle (h°) values of egg yolk powder. Reduction of cholesterol from egg yolk resulted in production of egg yolk powder (CREYP) with reduced in yellow colour. CREYP showed higher L^* values which in turn posses egg yolk powder with much lighter colour than NEYP. Decreasing of redness (a^*) and yellowness (b^*) colour intensity correlate with reduction of yellowness of yolk and finally makes the CREYP became less yellowness than NEYP.

The colour of egg yolk is attributed to fat-soluble carotenoids (xanthophylls; including lutein, zeaxanthin, β-cryptoxanthin and minor amounts β-carotene) (Li-Chan and Kim, 2008). It appeared that the lightness (L^*), redness (a^*) and yellowness (b^*), were influenced with reduction of cholesterol. Lipid-soluble pigments of egg yolk probably were being trapped by β-CD cavities during the cholesterol removal process.

NEYP with yellow coloured sample going from thick yellow to light yellow (towards green) of CREYP with increasing h° values. Whereas C^* values of egg yolk powder showed that as egg yolk pigment concentration reduced, the C^* values lessened. The yellow-orange colour of yolk is due to carotenoid pigments, primarily xanthophyll with minor amount of carotene (Li-Chan and Kim, 2008). Awad *et al.* (1997) reported that β-carotene concentration of egg yolk powder treatment with β-CD were lower than those of untreated egg yolk powder. Except for deficiency in colour, these results suggest that CD can be used to produce a low cholesterol egg product with functional properties similar to those of the

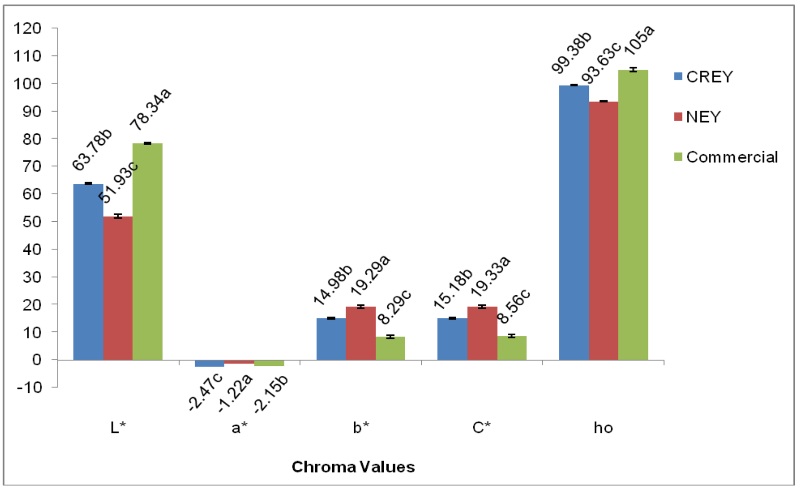

Figure 5. Colour analysis for different type of mayonnaise. (NEY (normal egg yolk powder, CREY (cholesterol-reduced egg yolk powder) and commercial mayonnaise).

* Small letters a, b, c indicate significant different (p>0.05) between different type of mayonnaises

original liquid egg yolk.

Mayonnaises viscosity determinations

The effect of cholesterol reduction from egg yolk on the viscosity of mayonnaises produced from egg yolk powder production was studied (Figure 4). Comparison of the viscosity of these emulsions (mayonnaises) showed that viscosity of CREY mayonnaises was the highest (8000 cP) ($p>0.05$) among them (NEY mayonnaises (4768 cP) and commercial mayonnaise (6746 cP)). As common, commercial mayonnaise was prepared using liquid egg yolk.

The viscosity of a liquid is a measure of its resistance to flow: the higher the viscosity, the greater the resistance The highest viscosity of CREY mayonnaise indicated that the flow or movement of oil droplet was slow. Upon cholesterol removal, more LDL particles were expected to destabilised its spherical behaviour, with the release and disorganised of triglycerides, cholesterol, and cholesteryl esters from LDL. These free apoprotein and phospholipids protected the oils droplets from flocculated (McClements, 2005).When the interactions are long-range and repulsive, the emulsion viscosity will increased. Ghoush *et al.* (2008) reported that the emulsion viscosity of mayonnaise was decreased due to very rapid flocculation and/or coalescence of small droplets.

Temperature of drying at 44°C did not alter or denature all the protein properties of egg yolk, since the emulsifying properties of emulsions produced were conserved rather than using spray dried method with higher temperature which denature major protein properties of egg yolk. The results of this study was correlated with the study done by Froning *et al.* (1990), where extraction of egg yolk with CO2 at 306 atm/45°C did not affect the adsorption and stabilization properties of yolk proteins (Paraskevopoulou *et al.*, 1997).

The good emulsifying properties of egg yolk lipoproteins are attributed to their highly flexible structures, allowing great affinity and adsorption at oil-water interfaces, followed by their ability to form viscoelastic films (Moros, 2002). Bringe *et al.* (1996) notice that elimination of triglycerides and cholesterol from LDL does not affected the emulsifying properties of yolk.

CREY mayonnaises viscosity produced allows us to produce a cholesterol-reduced mayonnaise with rheological similar to commercial mayonnaise with minimizing the amount of CREYP addition to imitate the commercial mayonnaise rheology with respect to consumers preferences. Cholesterol removal from egg yolk using β-CD:cholesterol encapsulation did not affect the emulsifying properties even improve the rheology behaviour of normal egg yolk.

Mayonnaises colour determinations

The colour analysis of the CREY, NEY and commercial mayonnaises was shown in Figure 5. The lightness (L^*), redness (a^*), yellowness (b^*), saturation (C^*) and hue angle (h°) values for mayonnaise were analyzed and the results showed significantly different ($p>0.05$) for all these values between NEY, CREY and commercial mayonnaises. Commercial mayonnaise was lighter in colour (higher L^* values), followed by CREY and NEY mayonnaises. Commercial mayonnaise and CREY mayonnaises were less yellowish (lower b^* values) than NEY mayonnaise. This colour parameter can be detected easily by naked eye when the sensory analysis done on the colour attribute showed that there is significant different (p>0.05) between prepared mayonnaise and commercial mayonnaise.

CREY mayonnaise showed higher h° values as compared to NEY mayonnaise NEYP proved that the yellow colour of egg yolk being reduced. Whereas C^* values of mayonnaises showed that as egg yolk pigment concentration reduced, the C^* values lessened.

The colour of egg yolk is attributed to fat-soluble carotenoids (xanthophylls; including lutein, zeaxanthin, β-cryptoxanthin and minor amounts of β-carotene) (Li-Chan and Kim, 2008) which comes from the feeding which is corn feed (Gee, 2004). Noted that lipid are concentrated in the NEY, thus yolk pigment appeared mainly in NEY mayonnaise and this is the main reason of the differences in colour parameter between NEY mayonnaise and CREY mayonnaise.

The CREY mayonnaise was differing significantly in term of lightness (L^*), redness (a^*), and yellowness (C^*) from commercial mayonnaise and NEY due to the some component of fat-soluble carotenoids that might be extracted out together with the cholesterol during inclusion complex of cholesterol with β-CD.

Conclusion

Overall, this effect leads to the conclusion that CREYP can be used as replace to the NEYP and whole liquid egg yolk as the emulsifying food ingredients. Utilization of cholesterol-reduced egg yolk powder in food can be beneficial to food industry in production of healthy and nutritious products even minimizing the cost of loss in industry due to spoilage and improper handling of native egg yolk.

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