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# Analytical Methods Antioxidative effect of purple corn extracts during storage of mayonnaise <sup>☆</sup>

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#### ABSTRACT

Anthocyanin is a powerful natural antioxidant. Purple corn husk is rich in anthocyanin. In this paper the antioxidative effect of anthocyanin-rich purple corn husk extract (PCHE) in mayonnaise during storage was studied. The antioxidative effect of the mayonnaise containing PCHE was evaluated by measuring peroxide values, *p*-anisidine values, total oxidation values, acid values, and iodine values at time intervals for 10 weeks. The antioxidative effect of the mayonnaise containing PCHE was higher than that of mayonnaise with chemical antioxidants BHT and EDTA as positive control. The mayonnaise containing 0.4 g/kg PCHE showed the strongest antioxidative performance during storage. This study suggests that PCHE could be used as natural antioxidant in high fat food and as a substitute to chemical antioxidant with its purplish colour marking its difference from ordinary mayonnaise. Such colour difference will tell consumers that their food contains natural antioxidants.

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# 1. Introduction

Mayonnaise is the most widely used sauces in the world today. Traditional mayonnaise is an oil-in-water (O/W) emulsion despite containing 70-80% fat and egg yolk (Dupree & Savage, 2001; Worrasinchai, Suphantharika, Pinjai, & Jamnong, 2006). As with all high fat foods, mayonnaise is susceptible to deterioration due to autooxidation. Lipid oxidation of mayonnaise leads to reduction of storage generated by rancid order. Moreover, free radicals derived from lipid oxidation reactions are easily transferred to other molecules such as proteins, carbohydrates, and vitamins, especially in the presence of metals (Schaich, Kamal-Eldin, & Min, 2008). These oxidative attacks on food macromolecules contribute to the deterioration of flavour, aroma, colour, and nutritive value of food. In order to reduce and control lipid oxidation, antioxidants are added to foods. Synthetic antioxidants such as butylated hydroxy toluene (BHT), butylated hydroxy anisole (BHA), and ethylene diamine tetraacetic acid (EDTA) have been used in the food industry to prevent the oxidation of food fat. These products are more economical than natural antioxidant but also these products get a negative impression for being a chemical product. Plant materials rich in phenolic compounds have gained much attention because they exhibit a wide range of activities such as antioxidative, antimicrobial, antimutagenic, and anti inflammatory activities (Kong, Chia, Goh, Chia, & Brouillard, 2003). Phenolic compounds act as antioxidants by donating electrons and terminating radical chain reactions (Tsuda, Shiga, Ohshima, & Kawakishi, 1996), as well as chelators by binding metal ions (Kähkönen, Hopia, & Heinonen, 2001). The antioxidative effects of natural plant materials rich in phenolics, such as extracts from berries (Heinonen, 2007), green tea (Almajano, Delgado, & Gordon, 2007), raisins (Williamson & Carughi, 2010), olives (Mattia, Sacchetti, Mastrocola, & Pittia, 2009), and grape seeds (Brannan & Mah, 2007), have been tested in a variety of O/W emulsions. Research by Heinonen (2007) reported that anthocyanins isolated from black currants, raspberries, and lingonberries, as well as raspberry and blackberry juices, showed protection from lipid oxidation in O/W emulsions. In another study, olive polyphenols in O/W emulsions (Mattia et al., 2009), raisin extracts in O/W emulsions (Williamson & Carughi, 2010), and proanthocyanidin rich grape extracts in O/W emulsions (Brannan & Mah, 2007) exhibited antioxidant activity toward lipid oxidation. The anthocyanins used as a food colourant in commercial production are mostly extracted from purple corn kernels, purple corn cobs, purple sweet potatos, and blueberries (Kähkönen et al., 2001). Recently, we reported that purple corn husk contains







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approximately 10 times more anthocyanins (Li et al., 2008) than other anthocyanin producing plants (Cevallos-Casals & Cisneros-Zevallos, 2003; Jing, Noriega, Schwartz, & Giusti, 2007; Moreno, Hernández, & Velázquez, 2005). We can anticipate the industrial production of anthocyanin because purple corn husk contains 10% anthocyanin.

High-fat foods usually have been produced as O/W emulsion foods and frying foods. This study further investigated the antioxidative effects in stored mayonnaise as O/W emulsion food of anthocyanins extracted from purple corn husk.

# 2. Materials and methods

#### 2.1. Materials

Purple corn husk extract (PCHE) (containing 20 g anthocyanin/ 100 g purple corn husk extract) was prepared and extracted from purple corn husk (2 kg) by using 75% ethanol for 16 h at room temperature in darkness and evaporating at 50 °C. The extracts were redissolved in 1% HCl/MeOH solvents, and their absorbance at 535 nm was measured to detect anthocyanin. Refined soybean oil, eggs, cider vinegar, and salt were purchased from a local market in Chuncheon, Korea. BHT, EDTA, and *p*-anisidine were purchased from Sigma (St., Louis, MO, USA). All other chemicals used were of analytical grade.

# 2.2. Preparation and storage of mayonnaise

Mayonnaise samples were prepared following the procedure developed in our previous work (Worrasinchai et al., 2006). Mayonnaise recipe contained the following ingredients in weight ratio (w/w): soybean oil (850 g/kg), egg yolk (72 g/kg), 8% cider vinegar (6 g/kg), and salt (72 g/kg). PCHE was added to the mayonnaise at anthocyanin concentrations of 0.1, 0.2, and 0.4 g/kg mayonnaise, which were referred to as PCHE 0.1, PCHE 0.2, and PCHE 0.4, respectively. BHT was added at concentrations of 0.1 g and 0.2 g/kg mayonnaise; EDTA was added at a concentration of 0.075 g/kg mayonnaise. After preparation, the mayonnaise was filled in plastic commercial mayonnaise bags (700 ml) and sealed under vacuum. The bags of mayonnaise were stored at 4 °C and 25 °C in the dark, respectively, and sampling was performed at timed intervals.

#### 2.3. Lipid extraction from mayonnaise

The mayonnaise was gently mixed prior to sampling. Thirty gram portions of mayonnaise were poured into 50 ml polypropylene centrifuge tubes. According to the procedure of Lagunes-Galvez, Cuvelier, Ordonnaud, and Berset (2002), the samples were frozen at -20 °C for 24 h and thawed for 2 h at 4 °C to break the emulsion. Two milliliters of water were added and the mixtures were centrifuged at 4000 rpm for 20 min. The lipid phase separated from the emulsion residue was stored in closed glass flasks at -80 °C until analysed.

# 2.4. Oxidation experiments in mayonnaise

The progression of oxidation was monitored by determining peroxide values (POV), *p*-anisidine values (*p*-AV), total oxidation values (Totox V), acid values (AV), and iodine values (IV). AV and IV were determined using the method described by IUPAC (1987), and Totox V was calculated by a formula as reviewed by Rossell (1983): Totox V = 2 POV + *p*-AV. The pigment patterns of the PCHE-added mayonnaise were analysed with extracts from the mayonnaise stored for 10 weeks using a UV–Vis spectrophotometer.

## 2.5. Statistical analysis

The data are reported as means  $\pm$  SD for triplicate determinations. The analysis of variance and least significant difference tests (SPSS for Windows, Version Rel. 12.0, SPSS Inc., Chicago, IL) were conducted to identify differences among the means, while Pearson's correlation test was carried out to determine the correlations among means. Statistical significance was declared at p < 0.05.

### 3. Results and discussion

Mayonnaise, similar to all high fat foods, is susceptible to spoilage due to auto-oxidation. Lipid oxidation has long been classified as the major form of deterioration affecting both the sensory and nutritional quality of food. Hydroperoxides are the primary oxidation products and are measured by means of peroxide values. In this study, antioxidants (BHT and EDTA) had significant (p < 0.05) antioxidant effects on lipid oxidation in mayonnaise.

The POVs from the PCHE-added mayonnaise groups were significantly lower than those of the control, BHT 0.1, and EDTA groups from week 0 to 10 (Fig. 1). In all samples, POV increased throughout storage at 25 °C from 0.6-0.7 meg/kg in week 0 to 4.3-13.6 meq/kg in week 10 (Fig. 1A). When compared to the negative control sample, initial POV developed similarly in all mayonnaise samples in week 0, but the POVs of the PCHE-added samples were significantly (p < 0.05) lower than those in the negative control sample during the storage period. In week 10, the POV order of all mayonnaise samples was PCHE 0.4 < PCHE 0.2 < BHT 0.2 < PCHE 0.1 < BHT 0.1 < EDTA < control sample. With storage at 25 °C for 10 weeks, the PCHE 0.2 and PCHE 0.4 samples exhibited significantly (p < 0.05) lower POVs (5.7 meq/kg and 4.3 meq/kg) and the control sample presented the highest POV (13.6 meq/kg). In all samples, POV ranges increased throughout storage at 4 °C from 0.6–0.7 meq/kg in week 0 to 0.8–1.5 meq/kg in week 10 (Fig. 1B). This trend was also observed in the samples stored at 25 °C. In mayonnaise samples stored at 4 °C, the differences between POVs were quite small and not statistically significant (p < 0.05). In this study, antioxidants (BHT and EDTA) had significant (p < 0.05) antioxidant effects on lipid oxidation in mayonnaise. The POVs of the PCHE-added mayonnaise groups stored for 10 weeks at 25 °C were reduced compared to the antioxidant (BHT and EDTA)-added mayonnaise samples. PCHE exhibited a significant antioxidant effect against primary oxidation in the mayonnaise groups. Many studies suggest that interactions between lipid hydroperoxides, the first products formed by oxidation, located at the droplet surface and



**Fig. 1.** Peroxide values in the oil phase of mayonnaise stored in plastic bags for 10 weeks. Graphs represent mayonnaise samples stored at different temperatures: (A) at 25 °C; and (B) at 4 °C. ( $\bullet$ ): control, ( $\diamond$ ): PCHE 0.1 mg/kg, ( $\blacksquare$ ): PCHE 0.2 mg/kg, ( $\blacktriangle$ ): PCHE 0.4 mg/kg, ( $\diamondsuit$ ): BHT 0.1 mg/kg, ( $\Box$ ): BHT 0.2 mg/kg, ( $\bigtriangleup$ ): EDTA 0.075 mg/kg, Reported values are the mean ± 5.D. (N = 3).

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