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Effect of riboflavin on the photo-oxidative stability of vegetable oil in salad dressing

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ABSTRACT

Differential scanning calorimetry (DSC), headspace oxygen and solid phase microextraction gas chromatography and peroxide value were used to evaluate the effect of riboflavin on the photo-oxidation of vegetable oil in salad dressing. Salad dressings with 0, 5, 20, 50 and 100 ppm added riboflavin were stored under light (2500 lux) at 25 °C for 5 days. Crystallisation peaks in the DSC thermograms of the oil samples shifted to lower temperatures and enthalpies decreased as the storage time increased. As the riboflavin concentrations increased from 0 to 100 ppm, the crystallisation enthalpies increased from 27 to 31 J/g and the maximum crystallisation temperature increased from −64 to −62 °C during the 5 day storage. Headspace oxygen depletion rates, the formation of volatile compounds and peroxide values of the salad dressing samples simultaneously decreased with the addition of riboflavin, showing that riboflavin protected the oil in salad dressing from photo-oxidation.

1. Introduction

The United States Food and Drug Administration (FDA) regulations state that salad dressing (oil-in-water emulsion) must contain a minimum of 30% vegetable oil (FDA, 2011, title 21 of the *Code of Federal Regulations*, part 169, section 150). Soybean oil, the major oil ingredient in salad dressing, contains large amounts of polyunsaturated fatty acids, such as linoleic, linolenic, and oleic acids. These fatty acids are readily peroxidised by light, heat, metal ions and dissolved oxygen in food products (Jung, Oh, Kim, Kim, & Min, 2007). It is believed that the peroxidisation of fatty acid is mainly responsible for the formation of undesirable volatile compounds. When in salad dressings, these volatiles cause the product to become less acceptable to consumers. Therefore, the incorporation of an antioxidant into salad dressing can help to minimise the oxidation and decomposition of fats, and thus extend the shelf life of salad dressings.

Riboflavin is a natural part of vitamin B_2 and is present in some the product. Riboflavin tetrabutylate, a fat-soluble derivative of riboflavin, is used as a food additive to enrich the vitamin B_2 level in many foods. Riboflavin is an essential nutrient in the human body because it is an active part of the coenzymes of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). However, the main barriers that prevent commercial application of riboflavin in the food industry are its high light sensitivity and limited information about its effect on edible fats. In general, riboflavin is also considered to have a pro-oxidation effect on certain foods. Riboflavin has a complex photochemistry when affected by light. This is due to its ability to easily degrade and be oxidised by accepting and donating hydrogen or an electron (Choe, Huang, & Min, 2005). In an aqueous system, riboflavin produces singlet oxygen from ordinary triplet oxygen when exposed to light. This takes place via the excited triplet riboflavin and triplet oxygen annihilation mechanism. Consequently, lipid oxidation photosensitised by riboflavin has been implicated as the causative factor in the off-flavour of many foods (Choe et al., 2005; Lee, Jung, & Kim, 1998). However, Ohama and Yagi (1969) reported that riboflavin in linoleic acid suppresses hydroperoxide formation under light. Also, Toyosaki, Yamamoto, and Mineshita (1987) showed that riboflavin tetrabutylate has an antioxidant effect on emulsions in the dark. To effectively evaluate the role of riboflavin in emulsion systems, the degradation of riboflavin and the stability of food lipids during storage should be further investigated.

Differential scanning calorimetry (DSC) is a thermal analysis technique that monitors thermally induced conformational and phase transitions when components in samples are heated or cooled (Hohne, Hemmingger, & Flammersheim, 1996). The ability to determine peak temperatures and enthalpies of transitions makes DSC an invaluable tool in producing phase diagrams for various food component systems. Several studies have shown that DSC can reveal the crystallisation transition of oils when samples are cooled under a controlled rate and environmental condition (Aguilera & Gloria, 1997; Kaiserberger, 1989). Several publications have reported that the thermal oxidation of olive oil can be

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evaluated using changes in parameters associated with crystallisation transition data observed from DSC (Tan, Che Man, Selamat, & Yusoff, 2002a, 2002b; Vittadini, Lee, Frega, Min, & Vodovotz, 2003). This indicates that a DSC method can be used as a technique to detect light-induced lipid oxidation in food samples, with a shorter preparation time when compared with other traditional analytical methods.

The objective of this study was to examine the effect of riboflavin on salad dressing under light and in dark storage conditions over a five day period. The oxidation stability of vegetable oil in salad dressing was determined by DSC and other chemical analytical methods.

2. Materials and methods

2.1. Salad dressing

Salad dressing was purchased from a local grocery store in Columbus, Ohio. Ingredients in the salad dressing included soybean oil, water, vinegar, high fructose corn syrup, salt, xanthan gum, spices, natural flavours, calcium disodium EDTA, lemon juice concentrate, caramel colour and annatto extract. The salad dressing contained a small amount of riboflavin (<2 ppm); however, it was low enough to be ignored compared to the lowest concentration of our addition level. The fatty acid composition of the oil in salad dressing was determined by gas chromatography (GC), using AOCS method Ch 2a-94 (AOCS, 2004). Fatty acid methyl esters (FAMEs) of the samples were prepared by saponification and esterification, and analysed by GC (Agilent 6890, Palo Alto, California, USA), equipped with a flame ionisation detector (FID). The column was a SP-2580 (Supelco Inc.) capillary column (100 m × 0.25 mm with 0.2 μm film thickness) and the carrier gas was helium at 1 ml/min. The oven temperature of the GC was programmed at 140 °C for 5 min, increased to 220 °C at 4 °C/min and maintained at 220 °C for 15 min. The sample injection volume was 1 μl and the split ratio was 100:1 at 220 °C. The detector temperature was 250 °C. The fatty acids were determined by comparing the relative retention times of the reference fatty acids.

2.2. Sample preparation and storage

Salad dressings containing 0, 5, 20, 50, and 100 ppm added riboflavin (98% purity, Sigma–Aldrich, St. Louis, MO) were prepared. An 8.0 g aliquot of each sample was transferred to 20-ml serum bottles (Supelco Inc., Bellefonte, PA) and sealed airtight with Teflon coated septum and aluminium caps. The sample bottles were placed in a light (2500 lux) and a dark box for up to 5 days at 25 °C. The oxidative stability of the salad dressing samples were determined by evaluating: (i) the headspace oxygen contents and volatile compounds in the sample bottles; and by analysing (ii) the DSC parameters and peroxide values of the extracted oil samples.

2.3. Oil extraction from salad dressing

Oil was extracted from the salad dressing by Carrez I and II solutions. Carrez I solution was prepared by dissolving 15 g of potassium ferrocianide (Fisher Scientific Inc., Pittsburgh, PA) in 100 ml of water (HPLC grade). Carrez II was prepared by dissolving 30 g of zinc acetate (Sigma–Aldrich, St. Louis, MO) in 100 ml of the water. In order to extract the aqueous portion, 1 ml of each Carrez I and II solutions was added to the salad dressing samples (8.0 g). Each mixture was centrifuged (8000 g/10 min/4 °C). In this way, three layers were obtained: a fat layer at the top, the aqueous layer in the middle and the lower precipitate layer. The samples were filtered with a 0.2 μm membrane filter (Corning Inc., Corning, NY). The top layer (extracted oil portion) from the Carrez extraction of each sample was used to determine the DSC parameters and peroxide values.

2.4. Differential scanning calorimetric analysis

A Model 2920 Modulated Differential Scanning Calorimeter (TA Instrument, New Castle, DE) was used to evaluate the effect of riboflavin on the oil in the salad dressing. The extracted oil from each sample was weighed (10.0 ± 0.1 mg wet weight) and carefully transferred into large volume stainless steel sample pans. An empty pan was used as a reference. The sample and reference pans were placed in the DSC and equilibrated at 10 °C using a liquid nitrogen cooling system. During the DSC analysis, the samples were cooled at a rate of 1.5 °C min^{-1} from 10 to −75 °C. Thermal parameters, such as crystallisation temperature (T_c) and heat of fusion (ΔH) corresponding to the exothermic peak areas between −55 and −70 °C were determined by integrating the temperature vs. heat flow curves using the software provided by the instrument manufacturer.

2.5. Headspace oxygen analysis

Headspace oxygen in the sample bottles was analysed by injecting 100 μl headspace gas into a HP 5890 GC, equipped with a stainless-steel molecular sieve column (13×, 80:100; Alltech, Deerfield, Il, USA) and a thermal conductivity detector (King & Min, 1998). High-purity helium (99.99%) was used as the carrier gas. The flow rate was 40 ml/min. The GC oven temperature was maintained at 40 °C. The temperature at the injector port and detector were maintained at 120 and 150 °C, respectively. Three sample bottles were used for each experiment and duplicate injections were performed for each sample bottle. Electronic counts were integrated by an HP 3396A integrator (King & Min, 1998). The depleted headspace oxygen content was expressed as micromoles of oxygen per millilitre of headspace gas. One millilitre of air contains 9.35 μmol of oxygen. The GC peak area of 9.35 μmol of oxygen was measured in electronic counts by injecting 1 ml of air into the GC and the electronic counts of 1 μmol oxygen were calculated (Bradley, 1991).

2.6. Peroxide value analysis

The hydroperoxides (LOOH) in the oil extracted from the salad dressing by the Carrez solution was determined spectrophotometrically, using a modified method by Shantha and Decker (1994), with cumin hydroperoxide as a standard. Briefly, a $Fe(II)Cl_2$ solution was prepared by dissolving 0.47 g/10 ml in 3.6% HCl. Ammonium thiocyanate solution was prepared by dissolving 30 g of ammonium thiocyanate in 100 ml distilled water. Twenty μl of the extracted oil and 4 ml of methanol (Sigma–Aldrich, St. Louis, MO) were placed in a vial. One hundred microlitres of Fe(II) sulphate solution was added to the vial and vortexed for 3 s. This was followed by the addition of 100 μl of ammonium thiocyanate solution and vortexed for 3 s. Within 10 s after addition of ammonium thiocyanate solution, the absorbance at 500 nm was measured using a Hewlett–Packard 8453 spectrophotometer system (Hewlett–Packard, Wilmington, DE).

2.7. Headspace volatile compounds analysis

The headspace volatile compounds were analysed using a solid phase microextraction (SPME) and gas chromatographic method. Headspace volatiles were isolated by SPME with a 65 μm polydimethylsiloxane/divinylbenzene (PDMS–DVB) fiber. The fiber