OIL-IN-WATER EMULSIONS AS A DELIVERY SYSTEM FOR ω-3 FATTY ACIDS IN PORK SAUSAGES

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***Abstract*— Progress in interfacial engineering has led to the fabrication of physicochemically stable fish oil emulsions containing high concentrations of *ω*-3 fatty acids. It has been hypothesized that addition of such emulsions to foods may lead to the development of functional foods that have physiological benefits for consumers. The addition of fish oil-in-water emulsions to meat products on their oxidative and physical stability was investigated. Fish oil-in-water emulsions were prepared at pH 3 and 6. Fish oil (25% wt) and whey protein isolate solutions (2.5% wt) were homogenized using a high-pressure homogenizer and incorporated into a pork sausage. Emulsions were characterized for their particle size, charge and physical and oxidative stability. Oxidative stability and microstructure of meat with added fish oil emulsions were determined. The physical stability of fish oil emulsions at both pH 3 and pH 6 was excellent and neither coalescence nor aggregation occurred during 45 days of storage. At pH 3 and pH 6 the particle sizes and charges of fish oil emulsions were 0.47 ± 0.01μm and +47 ± 4mV, and 0.64 ± 0.01μm and -15.3 ± 0.8mV, respectively. Oxidative stability (measured as formation of lipid hydroperoxides and headspace propanal) was better at pH 6 compared to pH 3 likely due to the antioxidant effect of the protein. Incorporation of fish oil emulsions into pork sausages led to an increase in oxidation compared to sausages without the added fish emulsion and compared to the fish oil emulsion. Confocal microscopy of meat products with fish emulsions revealed that emulsions had destabilized in the meat matrix which may have contributed to the decreased oxidative stability. Results demonstrate that although encapsulation of ω-3 fatty acids in oil-in-water emulsions provides physical and oxidative stability of the base-emulsion, their incorporation in complex meat matrices may be non-trivial and may lead to destabilization.**

***Index Terms*—emulsions, ω-3 fatty acids, oxidative stability, physical stability**

I. INTRODUCTION

In the food industry, there is a growing need to fortify foods with lipophilic bioactive ingredients such as oil-soluble vitamins, antioxidants, phytosterols, lutein, lycopene, and polyunsaturated oils. Incorporation of bioactive compounds into food systems is a key step towards the development of novel functional foods that have clear physiological benefits to the consumer upon ingestion of the food such as reduced risk of attracting chronic diseases such as cancer, diabetes, and cardiovascular diseases that often lead to premature death (Kris-Etherton, Harris and Appel, 2002, Kris-Etherton, Hecker, Bonanome, Coval, Binkoski, Hilpert, Griel and Etherton, 2002). However, adding bioactive (lipophilic) ingredients successfully to foods presents many challenges, such as their low water solubility, susceptibility to chemical degradation and instability under processing and storage conditions. For instance, *ω*-3-fatty acids are extremely prone to oxidation. In many natural and processed foods containing *ω*-3 polyunsaturated fatty acids lipid oxidation has been shown to be the major cause of quality deterioration (McClements and Decker, 2000). The effectiveness of the bioactive material will depend on preserving the active ingredients. In addition, it is desirable that the addition of bioactive ingredients should not affect food sensory qualities.

Progress in interfacial engineering has led to the fabrication of physicochemically stable fish oil emulsions containing high concentrations of *ω*-3 fatty acids. The problem with incorporating emulsion delivery systems into food products is that most food matrixes differ entirely on their physical and chemical conditions compared to the incorporated emulsion. For protein stabilized emulsions pH and ionic strength are major factors dictating their stability due to electrostatic interactions. Various other factors that can affect oxidative and physical stability in oil-in-water emulsions include ingredient purity, ingredient partitioning, droplet and interfacial characteristics, chelating agents, antioxidants, and ingredient interactions (McClements and Decker, 2000). The aim of this study was encapsulate bioactive ω-3 fatty acids in oil-in-water emulsions and test their physical and oxidative stability. Subsequently, the encapsulated material was incorporated into a meat product, in this case a pork sausage. The impacts of processing and storage and their effects on structural integrity of encapsulated ω-3 fatty acids in the pork sausages were investigated.

II. MATERIALS AND METHODS

A. Materials

Omega-3 fish oil (18% EPA and 12% DHA) was donated by Lysi (Reykjavik, Iceland). Medium chain triacylglycerides (chain length C_8 and C_{10}) Miglyol 829 oil was donated by Sasol GmbH (Witten, Germany). Food grade whey protein isolate (WPI) (DSE 9273, protein 93.9%) was donated by Fonterra GmbH (Hamburg, Germany). All other chemicals were all obtained from Sigma-Aldrich (Steinheim, Germany). All solvents were of analytical grade from Carl Roth GmbH + Co. KG (Karlsruhe, Germany). Distilled, deionized water was used throughout the study. Meat and fat from pork were obtained from a local butcher.

B. Preparation of emulsions

Whey protein isolate–stabilized fish oil-in-water emulsions were prepared at pH 3 and pH 6. The emulsifier solution was prepared by dispersing whey protein isolate into 50 mM sodium citrate buffer (pH 3.0 or pH 6.0). A primary emulsion was prepared by homogenizing 25 wt% fish oil with 75 wt% emulsifier solution (2.5 wt% whey protein isolate, pH 3.0 or pH 6.0) with a standard unit homogenizer for 2 min followed by a two-stage high pressure homogenizer. All emulsions were held in ice (0°C) during homogenization. All emulsions samples were stored in the dark at +4°C during the study.

C. Preparation of pork sausages

Pork sausages were prepared at meat pilot plant facilities. Pork sausages were prepared without added antioxidants or spices unless otherwise stated. Red meat from pork shoulder and fat from pork back, ice water and sodium chloride (or spice-antioxidant mixture when applicable) were homogenized in a bowl chopper. In the end, oil-in-water emulsions were added to the meat batter at the end of the homogenization process. For control sample ice water was added instead of the emulsion. The final meat batter was filled into non-permeable casings by using a piston filling machine and the casings were closed and heated up in a steam chamber with 100% steam to internal temperature of 72°C. The outside temperature in the chamber was set at 76°C. Then the sausages were cooled down immediately in the chamber, and stored at +4°C. The pH of all the sausages ranged between pH 5.7-5.8.

D. Physical stability of the emulsions

The particle size distribution of the emulsions was determined by a laser diffraction instrument. The particle charge (ζ-potential) was measured using an electrophoresis instrument (Zetasizer).

E. Oxidative stability of emulsions and meat products

Lipid oxidation was monitored by following the formation of lipid hydroperoxides and volatile propanal and hexanal. Lipid hydroperoxides were determined according to established methods (Shantha and Decker, 1994, Nuchi, McClements and Decker, 2001). The formation of secondary oxidation products was detected using a gas chromatography equipped with a headspace autosampler.

F. Confocal laser scanning microscopy

The microstructures of emulsion and meat samples were measured using a confocal laser scanning microscope with a 60x oil immersion objective lens. The samples were stained with Nile Red solution. Samples were excited with 543 nm laser line. The fluorescence emitted from the sample was monitored using a fluorescence detector (590-550 nm).

III. RESULTS AND DISCUSSION

A. Physical stability of emulsions

The droplet size and particle charge of whey protein isolate –stabilized oil-in-water emulsions at pH 3 and pH 6 were measured during 45 days of storage. The measured mean particle diameter (d_{32}) of fish oil-in-water emulsions at pH 3 (0.47 ± 0.01μm) and pH 6 (0.64 ± 0.01μm) did not change during the course of experiment, which indicated that the emulsions were stable to droplet coalescence. At pH 3.0, the particle charge (ζ-potential) of fish oil-in-water emulsions was positively charged (+47 ± 4mV). The relatively strong electrostatic repulsion between these droplets was thus sufficient to overcome the attractive van der Waals and hydrophobic interactions acting between the droplets (McClements, 1999), thereby preventing droplet aggregation. At pH 6.0, the particle charges were negative (-15.3 ± 0.8mV) for fish oil-in-water emulsions. The measured electrical charge of all emulsions did not change during the storage time. The ζ-potential of the protein-coated lipid droplets changed from positive to negative as the pH of the emulsions was increased from pH 3 to 6. This is because the electrical charge of absorbed whey protein molecules goes from positive to negative as the pH moves from below to above their isoelectric point. It has been shown that whey protein–stabilized emulsions tend to flocculate at pH values (~4-5.5) close to their isoelectric point (~5) (Demetriades, Coupland and McClements, 1997).

B. Oxidative stability of emulsions

The formation of primary oxidation products, i.e. lipid hydroperoxides, and secondary oxidation products, propanal and hexanal, was measured in whey protein isolate–stabilized oil-in-water emulsions at pH 3 and pH 6 at + 4°C over 45 days. At pH 3, fish oil-in-water emulsions oxidized faster than at pH 6 (**Figure 1**). Oxidation is mainly initiated by traces of metal catalysts in the presence of hydroperoxides. The ability of iron to break down hydroperoxides can depend largely on its physical location relative to the interface of the emulsion sample. The primary hydroperoxide products are highly susceptible to decomposition especially in the presence of metals. Decomposition of lipid hydroperoxides, via β-scission reactions, yields low molecular weight, volatile compounds that are responsible for off-flavours and aroma in foods (McClements and Decker, 2000). Consequently, the formation of propanal in fish oil-in-water emulsions proceeded faster at pH 3 than at pH 6. It was proposed that the better oxidative stability at pH 6 was due to the antioxidant effect of the whey proteins.

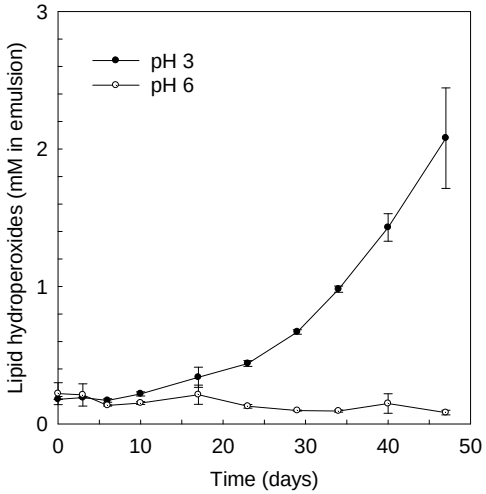

Figure 1. Formation of lipid hydroperoxides in fish oil-in-water emulsions in the dark at +4°C.

C. Oxidative stability and microstructure of meat products

Lipid oxidation, measured as the formation of secondary oxidation products propanal and hexanal, was determined in pork sausages with added whey protein isolate–stabilized oil-in-water emulsions at +1°C during 35 days of storage. The oxidation was monitored in model pork sausages without added antioxidants to assess the effects of encapsulation. Pork sausages were prepared with either (i) a 20% total fat content or (ii) without pork fat (i.e. the fat free sausage). In addition, the effect of antioxidants and spices in the pork sausages with added fish oil-in-water emulsions was determined. In meat, oxidation was accelerated after cooking in both types of pork sausages (with and without pork fat). This is because during cooking temperatures, iron is released from myoglobin. This nonheme iron is a known prooxidant of lipid hydroperoxides (Møller and Skibsted, 2006). In pork sausages with 20% fat content as well as in fat free pork sausages, the addition of fish oil-in-water emulsions increased the formation of propanal when compared to the control. It has been reported that fish oil-in-water emulsions incorporated into ground turkey patties, pork sausages and vacuum packed restructured cured hams without added antioxidant showed higher lipid oxidation rate compared to the control (Lee, Hernandez, Djordjevic, Faraji, Hollender, Faustman and Decker, 2006).

Pork sausages (with 20% fat content) with incorporated ω-3-fatty acids with added spices and antioxidants were prepared to assess their effect on oxidation status. According to our results no oxidation was detected when antioxidants were added to the pork sausages with added fish oil-in-water emulsions. This is in accordance with earlier studies reporting that addition of antioxidants into ground turkey patties, pork sausages and vacuum packed restructured cured hams decreased lipid oxidation (Lee, Hernandez, Djordjevic, Faraji, Hollender, Faustman and Decker, 2006). In addition, sensory tests revealed that the antioxidant-spice mixture was able to mask the fishy flavor and taste detected in the base emulsions. Thus, encapsulation in oil-in-water emulsions seems not to be sufficient to protect the oxidatively unstable ω-3 fatty acids but antioxidant strategies are still needed to overcome rancidity. The effectiveness of spices in controlling oxidation is due to their antioxidant and antimicrobial activities.

The microstructures of the sausages were evaluated by confocal laser scanning microscopy. The microstructures of fat free sausages revealed that the emulsion droplets aggregated during storage. Results demonstrate that although encapsulation of ω-3 fatty acids in oil-in-water emulsions provides physical and oxidative stability of the base-emulsion, their incorporation in complex meat matrices may be non-trivial and may lead to destabilization.

IV. CONCLUSION

This study describes results of incorporation of omega-3 fatty acids in meat products. It shows the difficulties encountered in terms of physical and oxidative stability when mixing omega-3 fatty acids into a complex food matrix such as meat sausages.

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