



Evaluation of processed cheese fortified with fish oil emulsion

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ABSTRACT

Processed cheese fortified with fish oil is an excellent food for the delivery of omega-3 long-chain polyunsaturated fatty acids (omega-3 LC PUFA). However, oxidation and the “fishy” flavour of fish oil limit the level of fortification. The physical properties, lipid oxidation, and sensory perception of model processed cheese slices fortified with a fish oil emulsion (encapsulated fish oil) were examined and were compared with those of samples fortified with straight fish oil. Peroxide values, the results of thiobarbituric acid reactive substances (TBARS) tests, and propanal values showed that cheese samples fortified with fish oil emulsion had lower levels of oxidation than cheese samples fortified with non-encapsulated fish oil. A sensory panel detected a “fishy” flavour at a higher level of fish oil addition in the samples fortified with fish oil emulsion. This suggests that a fish oil emulsion made with a milk protein complex is a useful carrier for elevating the fortification level of omega-3 LC PUFA in processed cheese products.

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1. Introduction

It has been recognized that omega-3 long-chain polyunsaturated fatty acids (omega-3 LC PUFA) provide extensive nutritional and health benefits in human health (Ruxton, Reed, Simpson, & Millington, 2004; Uauy-Dagach & Valenzuela, 1996). For example, omega-3 LC PUFA have been documented as contributing to the prevention of coronary heart disease, hypertension, Type 2 diabetes, rheumatoid arthritis, Crohn's disease, and obstructive pulmonary disease (Simopoulos, 1999).

Fish oil is a predominant dietary source of omega-3 LC PUFA. However, the average fish intake is currently far below the recommended 2–3 fish servings per week (Sanders, 2000). Fortification of various foods with fish oil is an innovative way of elevating the intake of omega-3 fatty acids without radical changes in eating habits (Kolanowski, Swiderski, & Berger, 1999; Kolanowski, Swiderski, Lis, & Berger, 2001).

Recognition of the potential benefits of omega-3 LC PUFA has stimulated interest in the fortification of foods with these lipids. However, the inclusion of omega-3 fatty acids in food products gives rise to major formulation challenges. Many lipids are sensitive to heat, light, and oxygen and undergo oxidative damage very quickly. Fatty acid oxidation is a major cause of food deterioration and can affect the flavour, aroma, texture, shelf life, and color of food, which limits the use of fish oil for food fortification (Kolanowski et al., 1999, 2001).

One way of reducing oxidative damage is to encapsulate the oxidizable lipid so as to reduce its contact with oxygen, trace met-

als, and other substances that attack its double bonds and other susceptible locations. To this end, oxidizable lipids have been combined with a number of other substances including other oils, polysaccharides, and proteins.

Milk proteins have been widely used as emulsifiers in the food industry. These proteins not only produce physically stable oil-in-water emulsions but also inhibit lipid oxidation (Djordjevic, McClements, & Decker, 2004; Faraji, McClements, & Decker, 2004; Hu, McClements, & Decker, 2003). Both casein and whey protein have exceptional water-binding capacity, fat emulsification properties, whipping ability, and a bland flavour. Fish oil emulsions stabilized by milk proteins have been characterized in terms of their physicochemical and oxidative properties (Day, Xu, Hoobin, Burgar, & Augustin, 2007; McClements, Decker, & Weiss, 2007); it has been demonstrated that casein and whey protein can efficiently stabilize fish oil emulsions and perform an antioxidative function in the emulsion system.

A patent cooperation treaty (PCT) publication (Singh, Zhu, & Ye, 2006) described a method, using an oil-in-water emulsion stabilized by a preheated complex of sodium caseinate and whey protein, to encapsulate fish oil to prevent the oxidation of omega-3 fatty acids. It was shown that a fish oil emulsion stabilized by the caseinate/whey protein complex had greater physical stability and lower oxidation levels during storage than an emulsion formed by the individual milk proteins.

Processed cheese/imitation cheese products are manufactured by blending various edible oils/fats, proteins, other ingredients, and water into a smooth homogeneous blend with the aid of heat (70–90 °C), mechanical shear, and emulsifying salts. Processed cheese has a relatively high pH, a high fat content, a solid consistency, and a high buffering capacity, which offers an attractive

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food-based delivery vehicle for omega-3 LC PUFA to prevent biological activity and for gastrointestinal transit (Hayes et al., 2006). However, oxidation of omega-3 LC PUFA during the processing and storage of processed cheese results in off-flavour development and hence products fortified with omega-3 LC PUFA have low acceptability (Jacobsen, 1999; Kolanowski & Weissbrodt, 2007).

The objective of this study was to evaluate the influence of fish oil fortification on the quality of rennet-casein-based model processed cheese slices. The model processed cheese slices, containing different levels of fish oil or fish oil emulsion, patented by Singh et al. (2006), were compared by examining their rheological properties, oxidation levels, and sensory properties.

2. Materials and methods

2.1. Materials

Whey protein isolate (WPI, ALACEN™ 895), sodium caseinate (ALANATE™ 180), and rennet casein (AlaREN™ 779) (containing 83% protein) powders were obtained from Fonterra Co-operative Group, Auckland, New Zealand. Tuna fish oil (RoPUFA '30' n-3 food oil) was obtained from Roche Vitamins (UK) Ltd. Soya oil was purchased from Davis Trading Company, Palmerston North, New Zealand. Tuna fish oil emulsion (Hi-Load 105; total fat, $29.7 \pm 1.6\%$; total n-3 PUFA, 99 mg g^{-1} ; total protein, 4%) was obtained from Speirs Nutritionals Ltd., New Zealand. Trisodium citrate (TSC) and citric acid were obtained from Jungbunzlauer, Basel, Switzerland. All of the chemicals used were of analytical grade and were obtained from either BDH Chemicals (BDH Ltd., Poole, England) or Sigma Chemical Co. (St. Louis, MO) unless otherwise specified.

2.2. Preparation of model processed cheese slices

The formulation used for the model processed cheese slices was: 20% rennet casein, 25% soya oil, 2.8% TSC, 1.0% sodium chloride, about 1% citric acid, 0.1% potassium sorbate, and 50.5% water. The processed cheese slice samples were prepared using a Rapid Visco Analyzer (RVA-4; Newport Scientific Pty Ltd., Warriewood, NSW, Australia), which can mix and heat a 30 g sample in an aluminum cell fitted with a polycarbonate paddle. The dry ingredients and the oil were manually blended with water and hydrated for 1 h at room temperature. The mixture was then heated to 90 °C over 4 min and was held at 90 °C for 6 min while mixing with a shear speed of 800 rpm. The sample was cast into a slice with a uniform thickness of approximately 2.4 mm at 90 °C and was sealed in a plastic bag. The slice was immediately cooled to 4 °C and was stored at 4 °C for further analysis. The processed cheese samples fortified with fish oil were prepared by replacing some of the soya oil in the control formulation with the same amount of fish oil or fish oil emulsion on an equivalent weight basis. For example, a processed cheese slice with 5% fish oil contained 20% soya oil. For the samples with added fish oil emulsion, the rennet casein concentration was adjusted to compensate for the amount of protein present in the emulsion. The pH of all cheese slices was $\text{pH } 5.8 \pm 0.1$, and was controlled by adjusting the amount of citric acid added during processing.

2.3. Dynamic rheological analysis of processed cheeses

After storage of the processed cheese slices at 4 °C for 24 h, their rheological properties were measured using a stress-controlled rheometer (Physica MCR 301, Anton Paar, Austria) fitted with a 20 mm diameter parallel plate with a 2.0 mm gap. Cheese samples were carefully cut into 20 mm diameter disks with a cylindrical cutter. The cheese disks were glued to the surface of the lower

plate. The upper serrated plate was lowered until it reached a 2 mm gap distance and the sample was trimmed. The exposed edge of the sample was coated with a thin layer of mineral oil to minimize moisture loss during the measurement. A temperature sweep was performed at a constant frequency of 1 Hz and a constant strain amplitude of 0.5%, with the temperature being varied from 20 to 90 °C at $3 \text{ }^\circ\text{C min}^{-1}$ using a Peltier heating element. The storage modulus (G'), the loss modulus (G''), and the loss tangent ($\tan \delta$) were determined. Two samples from each slice of processed cheese were measured.

2.4. Determination of average droplet size

A Malvern MasterSizer 2000 (Malvern Instruments Ltd., Malvern, Worcestershire, England) was used to determine the average diameter of the oil droplets in the emulsions before adding into processed cheese. The parameters that were used to analyze the droplet size distribution were defined by the presentation of milk fat. The relative refractive index (N), i.e., the ratio of the refractive index of the oil droplets (1.456) to that of the dispersion medium (1.33), was 1.095. Droplet size measurements were reported as the Sauter-average diameter, $d_{32} (= \sum n_i d_i^3 / \sum n_i d_i^2)$, where n_i is the number of particles with diameter d_i . Mean particle diameters were calculated as the average of duplicate measurements.

2.5. Determination of lipid oxidation

2.5.1. Peroxide values

The extraction of lipid was based on the method described by Mortensen, Sorensen, and Stapelfeldt (2002). About 10 g of each cheese sample was transferred to a 500 mL plastic centrifuge tube and 200 mL of chloroform-methanol (7:3) was added. The sample was homogenized using a K Ultra Turrax® homogenizer (T25B, Kika Works (Asia), Malaysia); 50 mL of 1 mM CaCl_2 was added to the suspension, which was shaken for 10 s. The mixture was then centrifuged at 1400g at 20 °C for 30 min and the supernatant was transferred to a 500 mL separation funnel to collect the lower chloroform layer. The upper layer and the solids were combined and mixed with 150 mL of chloroform. The mixture was homogenized and then centrifuged under the same conditions above. The procedure was then repeated once more. The total of the three separated chloroform layers was poured into a 1000 mL conical flask. The chloroform layer was evaporated at 60 °C using a Buchi vacuum rotavapor R-215 (Buchi Labortechnik AG, Switzerland) to remove the chloroform. The remaining oil was then flushed with nitrogen and frozen for peroxide value (PV) analysis.

Lipid samples (0.02 g) were weighed into a 25 mL volumetric flask and then 15 mL of chloroform-methanol (7:3) was added to the flask. For each sample, 0.2 mL of 1% ferrous chloride solution and 0.2 mL of 4 M ammonium thiocyanate solution were added to the flask and the sample was then made up to 25 mL using chloroform-methanol (7:3). The sample was mixed and kept under dimmed light for 5 min for absorbance determination at 505 nm. The result was expressed as milliequivalents of oxygen per kilogram of lipid. A sample blank and a reagent blank were also measured. All the measurements were done in triplicate and were carried out under dimmed light. The standard curve was determined under the same conditions as for the samples using ammonium ferric sulfate (AR grade) as a standard.

2.5.2. Thiobarbituric acid reactive substances

TBARS were measured using a method described by Kristensen and Skibsted (1999). The thiobarbituric acid (TBA) reagent was prepared immediately before use by mixing equal volumes of freshly prepared 0.025 M TBA (brought into solution by neutralising with NaOH) and 2 M $\text{H}_3\text{PO}_4/2 \text{ M}$ citric acid. The combination of

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