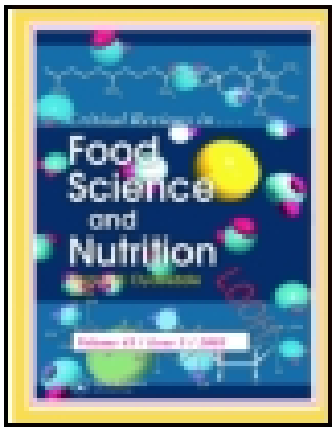

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Effects of Composition and Processing Variables on the Oxidative Stability of Protein-based and Oil-in-water Food Emulsions

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'EFFECTS OF COMPOSITION AND PROCESSING VARIABLES ON THE OXIDATIVE STABILITY OF PROTEIN- BASED AND OIL-IN-WATER FOOD EMULSIONS

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ABSTRACT

Because many common foods are emulsions (mayonnaise, coffee creamers, salad dressing,etc.), a better understanding of lipid oxidation mechanisms in these systems is crucial for the formulation, production, and storage of the relevant consumer products. A research body has focused on the microstructural and oxidative stability of protein stabilized oil-in-water emulsions that are structurally similar to innovative products which have been recently developed by the food industry (e.g., non-dairy creams or vegetable fat spreads, etc.) This review presents recent findings about the factors that determine the development of lipid oxidation in emulsions where proteins constitute the stabilising interface. Emphasis is given to “endogenous” factors, such as those of compositional (e.g. protein/lipid phases, pH, presence of transition metals) or processing

(e.g. temperature, droplet size) nature. Improved knowledge of the conditions that favour the oxidative protection of protein in emulsions can lead to their optimised use as food ingredients and thereby improve the organoleptic and nutritional value of the related products.

(1) INTRODUCTION TO LIPID OXIDATION IN FOOD EMULSIONS

The oxidation of edible oils rich in polyunsaturated fatty acids is a major concern of the food industry because it is directly related to economic, nutritional, flavor, safety, and storage problems (Akoh and Min, 1997). Oil-in-water emulsions (such as milk, infant formula, salad dressing, mayonnaise, cream, etc.) include the most common forms of lipids in foods (McClements, 2005). Moreover, emulsion systems generally mimic the amphiphilic nature and the basic structural characteristics of important biological membranes (e.g., with phospholipid components), which are also prone to oxidative degradation when attacked by singlet oxygen and free radicals (Halliwell and Gutteridge, 1995). In that respect, *in vitro* research on the oxidative stability and antioxidant effects in model emulsions could provide useful information of nutritional interest that thereby background knowledge to support *in vivo* clinical trials. (Kiokias and Gordon, 2003).

Emulsions are thermodynamically unstable because of the energy required to increase the interfacial area between the oil and water phases. Generally, the stability of food emulsions is complex because it depends on a large number of phenomena, including flocculation, coalescence, creaming, and final phase separation (Dickinson, 2001; Kiokias et al., 2008).

Oil-in-water (o/w) emulsions consist of three different components: water (the continuous phase), oil (the dispersed phase), and emulsifiers at the interface (Venditti et al., 2010). The mechanism of lipid oxidation in o/w emulsions differs from that in bulk lipids because emulsions have an oil water interface with a large interfacial area that has an effect on distribution of and interactions between prooxidants and antioxidants (Ambrosone, et al., 2007; Warahao, et al., 2011). In such a system, the rate of oxidation is influenced by the emulsion composition (relative

concentrations of substrate and emulsifier) and especially by the partition of the emulsifier between the interface and the water phase (Kiokias et al., 2009[a]). It has been reported that several physical characteristics of the emulsion droplets (particle size, charge etc.) as well as the packing properties of the surface-active molecules may considerably affect the oxidation kinetics in o/w emulsions (Osborn and Akoh, 2004). Furthermore, physicochemical properties (pH, presence of metal catalysts) and processing parameters (e.g. storage temperature, homogenisation pressure) have also been found to impact in various ways on the oxidative deterioration of oil-in-water emulsions (Gharsallaoui et al., 2010; Kiokias et al., 2007; McClements and Decker, 2000).

Gelation of food proteins and particularly of globular proteins (e.g. whey, soybean) has attracted much attention over the years because of its physicochemical and industrial significance given their ability to facilitate both the formation and stability of food emulsions (Chen at al., 2006; Clark et al., 2001). In particular, milk proteins are increasingly used as emulsifiers in a variety of industrial novel food products (Kim et al., 2005; Kiokias et al., 2009[b]; Yoshie-Stark et al., 2008). More specifically, caseins and whey proteins are well known for their ability to stabilize emulsions, by adsorbing at the interface, and creating thick stabilizing layers that protect oil droplets against flocculation and coalescence (Euston and Hirst, 1999; Kiokias et al., 2004[b]). The image of such a protein-based emulsion has been visualized by the use of Confocal Laser Scanning Microscopy (CSLM) (figure-1) (Kiokias and Varzakas, 2014). It is obvious that an elucidation of the mechanisms that govern both microstructural and oxidative stability of protein-based food emulsion products (e.g. dairy cream, fresh-cheese) during storage and consumer use, are important issues for the food industry.

A few years ago we published a review in this journal discussing the mechanism of action of various natural antioxidants which are added to bulk and emulsified oil-based systems to inhibit their oxidative degradation (Kiokias et al., 2008). In the current paper we primarily review the literature evidence of the last few years concerning the mechanisms through which various "endogenous" factors (e.g. emulsifier, droplet properties, pH, presence of metals etc.) affect the chemistry of lipid oxidation of oil in-water emulsions. The paper does not examine the effect of "exogenous" (added in the system) antioxidant compounds and particularly focuses on protein stabilised dispersions which are highly relevant to real food systems. A better knowledge of how the monitoring of certain important compositional or processing parameters could slow down the oxidative deterioration of lipids in these interfacial systems would be of significant technological importance.

(2) EFFECT OF THE TYPE OF EMULSIFIER ON LIPID OXIDATION

The size and conformation of the emulsifier has an impact on the thickness of the emulsion droplet interface through the formation of biopolymer layers (Djordjevic et al., 2007; Shaw et al., 2007). A few researchers have reported that emulsifiers with large molecular size could form a barrier that decreases interactions between lipids and aqueous phase prooxidants resulting in slower lipid oxidation rates (Silvestre et al., 2000; Waraho et al., 2011). This theory has been confirmed by a number of studies reporting that emulsions stabilised by lower molecular weight (LMW) emulsifiers (e.g. Tween, SDS, Brij 76) were oxidised much faster than the emulsions prepared under the same conditions with larger molecular weight emulsifiers such as Brij 700 (Chaiyasit et al., 2007) or certain proteins (Kiokias et al., 2006).

Other studies have further examined the inhibitory effect of various proteins against the oxidation of emulsions which was found to be dependent on the nature of the proteins as well as on their interaction with other antioxidants e.g, the synergistic effect of albumin with phenolic antioxidants (Almahano and Gordon, 2004; Fomuso et al., 2002; Villiere et al., 2005).

Kiokias et al. (2006) reported that 10% cottonseed o/w emulsions stabilised by whey-protein concentrates were oxidised more slowly at 40°C than emulsions containing sodium caseinate. Similarly, Osborn and Akoh (2004) reported that whey protein isolates had a better antioxidant effect than sugar esters in retarding formation of both primary and secondary oxidation products when used as emulsifiers to stabilise 10-30% canola o/w emulsions. In contrast, Hu et al. (2003[a]) found that the oxidative stability of 5% corn o/w emulsions based on different proteins was in the order of casein>whey protein isolates>soya protein isolates, as determined by monitoring both lipid hydroperoxides and headspace hexanal formation. According to Dagleish (1990), caseins can form an interfacial layer around the dispersed oil droplets up to 10 nm thick (compared to a 1-2 nm layer formed by whey proteins) and this could explain their enhanced antioxidant capacity. Apart from the thickness of the interfacial layer, the metal binding capacities of the different protein emulsifiers have a significant role in their protective activity, which will be discussed further.

The structure of the protein is reported as a critical factor to explain their different effect on lipid oxidation (Dickinson and McClements, 1995; Kato et al., 1992). Sodium caseinate is a mixture of disordered and flexible caseins (αs_1, αs_2, β- and κ- caseins), which adsorb at the oil/water interface through their hydrophobic domain (Euston et al., 1996). They can prevent lipid oxidation essentially by chelating metal ions but other mechanisms such as free radical

scavenging can be involved (Dickinson et al., 1988; Elias et al., 2008). The structural characteristics of globular whey proteins (e.g. β–lactoglobulin) are governed by disulphide bonding, and hydrophobic interactions, and these proteins act as antioxidants via the scavenging of free radicals (Peng et al., 2009). Besides milk proteins, other types of protein preparations have been found to exert antioxidant activity. Yoshie-Stark et al. (2008) reported the DPPH radical-scavenging capacity of precipitated rapeseed protein isolates as a basis for food applications. Tapal and Tiku (2012) concluded that complexes of soya protein isolates with curcumin exhibited enhanced antioxidant activity and are capable of forming foams and emulsions, indicating their possible utilisation in food product formulations. Faraji et al. (2010) reported that soya protein isolates (SPI) exerted a stronger protective effect against the oxidative deterioration of emulsions compared to milk proteins (whey proteins or caseins), as determined by formation of both hydroperoxides and headspace propanal. It is likely that sulfhydryl groups and amino acids (e.g. tyrptophan, methionine, and tyrosine) that can scavenge free radicals could also be involved in the antioxidant activity of the continuous phase proteins in the emulsion systems (Djordjevic et al., 2007; Hu et al., 2003[b]).

In contrast to the above mentioned findings, a cross-linked casein layer at the interface of menhaden o/w emulsions had no antioxidant effect (Kellerby et al. 2006). The same authors reported that disulfide crosslinked β–lactoglobulin did not have an effect on the decomposition of lipid hydroperoxides (Kellerby et al., 2006). Furthermore, Almajano et al. (2007) reported that bovine serum albumin (BSA) had very little antioxidant activity in the absence of phenolic antioxidants.

Several researchers have observed the phenomenon of competitive adsorption when proteins and LMW emulsifiers are used in combinations to stabilise food emulsions (Dickinson and Iveson, 1993; Euston et al., 2001). Most commonly this procedure leads to conformational changes in the adsorbing protein layer and limited protein displacement at the interface, which is induced more efficiently by water-soluble emulsifiers such as the currently used- Tween-20. (Dickinson, 2001; Euston, 1997). If time allows, extensive protein interfacial replacement can lead to coalescence and emulsion destabilisation. Moreover, competitive adsorption is further complicated by the binding of LMW emulsifiers to the protein molecule, which can change their adsorption behaviour at surfaces (Berger, 1990). To further investigate this issue, Kiokias et al (2006) examined combinations of Tween and sodium caseinate preparations in emulsions (20% o/w, 2% emulsifier mixture) during autoxidation at 60°C. An increase of Tween proportion in the emulsifier was found to decrease the oxidative stability of the emulsions, as evaluated by the determination of both primary (conjugated diene and lipid hydroperoxides) and secondary [thiobarbituric acid-reactive substances (TBARS)] oxidation products (as indicated in Figure-2). It could be that by increasing the proportion of Tween, protein is increasingly displaced leading to the reduction in thickness and weakening of functionality of the protein interfacial layer. Therefore, various prooxidant agents, (metals, free radicals etc.), which are commonly entering from the aqueous phase (Frankel, 1998), could more easily penetrate the protective protein layer at the interface and thereby attack the lipid core of the droplets, accelerating the oxidative deterioration of the emulsion (Kiokias et al., 2005). Besides the size and structural characteristics of proteins, other properties that affect the lipid oxidation rates in emulsions are discussed in the next sections.

(3) EFFECT OF PROPERTIES OF THE EMULSION DROPLETS ON LIPID OXIDATION

Typical food emulsions contain particle sizes ranging from 0.2 to 100 μm (McClements and Decker, 2000; Dickinson, 2008). The influence of the size distribution of oil droplets and the interfacial area of emulsions on lipid oxidation kinetics varies considerably in the tested food model systems (Lethaut et al., 2002). Smaller particle sizes result in larger surface area and thus greater possibility for lipid –aqueous prooxidant interactions (Mei et al., 1999; Osborn and Akoh, 2004). Gohtani et al. (1999) observed that droplets of docosahexaenoic o/w emulsion system with larger diameter were less oxidisable than smaller droplets. Jacobsen et al. (2001) similarly reported that in the initial stage of storage, an increase in the size of droplets was positively correlated with decreased oxidation rate in fish oil enriched mayonnaise. Other researchers claim that the emulsion droplet interfacial area has a minimal impact on the oxidation rates (Imai et al., 2008; Nakaya et al., 2005; Shimada, et al., 1992; Sun and Gunasekaran, 2009).

In particular for protein-stabilized o/w emulsions, the oil or fat droplet size distribution is an important characteristic because it reflects the quality of the initial emulsification process, and its increase reduces emulsion stability over time with respect to (partial) coalescence (Bot et al., 2003; Tesch and Schubert, 2002). The emulsifying ability of a protein can be quantified by measuring the surface area or the particle size of oil droplets generated by a particular concentration of the protein. (Dickinson, 2001; Euston, and Hirst, 1999; Kiokias et al. 2004[a]). As long as sufficient concentration is present for protein molecules to fully cover the droplet surface, fine milk protein-stabilised emulsions normally retain good microstructural stability (Dickinson, 2001; Segall, and Goff, 2002). Studies on emulsions stabilised by both casein and

whey protein-preparations led to reports that increasing protein concentration (in the range 0.5-2% w/w) resulted in smaller oil droplets ($d_{3,2}$ values) via more efficient protein adsorption and thicker layers at the interface (Kiokias et al., 2006; Kiokias and Bot, 2005). Similarly, Hu et al. (2003[a]) reported that the initial particle size decreased with increasing protein concentration in corn oil-in-water emulsions. According to Vilasaua et al. (2011), the particle size decreases with increasing ionic/nonionic surfactant ratio, up to a certain ratio above which emulsions aggregate. Kiokias at al., 2006 reported that in contrast to emulsions stabilised by low molecular weight emulsifiers (Tween 20, SDS), smaller oil droplets, obtained in protein emulsions through the increase of protein concentration with no change in processing conditions, were associated with enhanced protection from oxidative changes. An increase of protein concentration resulted in thicker layers at the interface due to more efficient protein adsorption. The stronger protein network acted as a more effective interfacial barrier against the attack of pro-oxidants, offering a better protection against oxidative deterioration. Coupland et al. (1996) and Mosca et al. (2013) also claimed that the thickness and composition of the surface layer of droplets in oil-in-water emulsions is crucial in controlling the oxidation of the oil because it may act as a physical barrier towards the entrance of pro-oxidants coming from the water phase.

Besides the emulsifier concentration, the homogenisation pressure of the emulsification process has a major effect on the droplet size. For emulsions prepared with the same concentration of emulsifier, the droplet size has been reported to decrease with an increase of homogenization pressure according to the equation: $d_{3,2} = p^{-0.4}$ (Kiokias et al., 2004[b]). In another study, it was reported that an increase of homogenization pressure (30-900 bars) resulted in a decrease of oil droplet size but did not affect the oxidative stability of 20% sunflower o/w emulsions,

independently of the type of emulsifier (Kiokias et al., 2007). Kuhn and Cunha (2012) reported that an increase of homogenization pressure from 20 to 80 MPa, decreased the mean droplet size in flaxseed oil emulsions stabilised by whey proteins, leading to a higher formation of primary oxidation products. This was explained by the increase in the surface area of the droplets.

These results are contradictory because of different experimental conditions. Indeed, when oxygen or other oxidising factors in the aqueous phase are limited, oxidation may be controlled by diffusion phenomena and, therefore, the droplet size has a significant effect. Otherwise, the rate of oxidation is limited by chemical reaction rates rather than by diffusion phenomena (Dimakou et al., 2007). Another significant factor is the identity and concentration of the emulsifier, and this is especially important for proteins. At low concentrations, as droplet size decreases and interfacial area increases there may be not enough protein to form a monolayer around the oil droplet that could act as a protective barrier against the attack of oxidising species.

The significance of the droplet charge for lipid oxidation in protein stabilised emulsions has also been examined in the literature (Hu et al., 2003[a]). As this is an issue closely linked to the pH of the aqueous phase this will be further discussed in the next section.

(4) EFFECT OF pH ON THE OXIDATION OF THE EMULSIONS

The pH of the aqueous phase has been reported as a critical factor in controlling the microstructural stability of various model emulsion systems (Binks et al., 2006; Ese and Kilpatrick, 2004; Seo et al., 2012). However, there is contradictory evidence about the effect of pH on the oxidative deterioration of food emulsions (Jacobsen et al., 2001; Osborn and Akoh, 2003; Shimada et al., 1992).

Kiokias et al. (2007) concluded that the oxidation rates of sunflower o/w emulsions prepared with Tween 20 as emulsifier were faster as pH increased from 3.0 to 7.0. Other researchers (Huang et al., 1996; Ruth et al., 1999) similarly reported that oxidation of vegetable oil-in water emulsions, emulsified with Tween 20 or 60, proceeded faster at pH values ~6-7 than at lower values. The increased formation rate of oxidation products as pH increases may be associated with the partitioning of iron and other transition metals (that are naturally present in the oil, surfactant, and/or water), which is affected by pH. The increase of pH results in lowering the solubility of the transition metals in the continuous phase so that they precipitate to the droplets' surface and promote oxidation (Cho et al., 2002; Manusco et al., 1999).

The pH has been found to have a strong impact on the stability and rheological properties of protein-based emulsions (Dickinson, 2001; Laplante et al., 2005). Calero et al. (2013) reported an increased protein solubility and viscoelasticity in sunflower o/w emulsions stabilised by potato-protein as pH increased in the range 2.0 to 11.5. Demetriadis et al. (1997) examined the effect of pH (3-7) in corn oil-in-water emulsions stabilized by 2 % whey protein isolate and observed appreciable flocculation of droplets near the isoelectric point of whey protein (pH 4.6). Franko et al. (2012) observed that an increase in the pH of whey protein emulsions initially led to a decrease in the mean droplet size, up to a pH close to the protein isoelectric point, where a singular rheological behaviour was found. When the pH approaches the isoelectric point of the protein, the electrostatic repulsion of the protein adsorption layers decreases, which allows more compact packing and stronger attractive interfacial interactions so that coalescence and flocculation occur (Dickinson, 2008; Guzey and McClements, 2006).

Several authors have further examined the effect of pH on the oxidative deterioration of oil-in-water food emulsions. In protein stabilised emulsions, the change of pH to values above and below the pI of the protein, (a value ~ 4.5-5.0), results in a negative or positive charge on lipid droplets, which may induce changes in the rate of oxidative deterioration of emulsions. (Mancuso et al., 1999, 2000; Mei et al., 1998[a,b]). In general, the rate of lipid oxidation in protein-stabilised emulsions, has been reported to be faster when the pH is greater than the pI of the protein and the emulsion droplet is negatively charged (Djordjevic et al., 2007; Hu et al., 2004; Trunova, et al., 2007).

According to Hu et al. (2003[b]), at pH values below the pI where emulsion droplets are positively charged, lower oxidation rates would be expected because of the Coulombic repulsive forces between iron and the droplet interface. However, a slightly lower rate of formation of primary oxidation products at pH above the pI was reported in some studies (Osborn and Akoh, 2003) and this was attributed not to decreased rate of formation but to more rapid decomposition of the primary oxidation products by transition metals at higher pH. The effect of pH in metal-catalysed oxidation of emulsions is further discussed in the next section.

Dimakou et al., (2007) observed a decrease in pH of sunflower o/w emulsions as oxidation proceeds with time. This may be attributed to the formation of several short chain aliphatic acids (e.g. acetic, propanoic, 2-methylpropanoic, butanoic, pentanoic acids) formed as secondary products from lipid oxidation (Ruth et al., 1999). In particular for protein-stabilized emulsions, it has been reported that reaction of basic amino acids in proteins with primary or secondary products of oxidation may also be involved in the decrease of pH with aging (Villiere et al., 2005).

(5) PROTEINS AS METAL CHELATORS IN EMULSIONS

The aqueous matrix of food emulsions contains a variety of water-soluble components including transition metals that may occur naturally in the lipid phase (Alamed et al., 2009; Paiva-Martins and Gordon, 2002). Contamination of oils with specific metals (copper, iron, etc.) can also occur during the refining procedure (Mei, et al. 1998[a]). Metals can initiate fatty acid oxidation by reaction with oxygen and the relevant mechanisms in oil model systems have been well described in the literature (Akoh, 2002; Kiokias et al., 2009[a]). The rate of iron-mediated oxidation has been reported to be lower than that with the same concentration of copper, and most foods contain 3.1-31μM Cu^{2+} (Osborn and Akoh, 2003). Reduction of the prooxidant activity of transitions metals is a very effective method to decrease the rate of rancidity development in emulsions (Allen and Hamilton, 1994; Cho et al., 2003; Manusco et al., 1999). In relevant food emulsions such as mayonnaise and salad dressings, various chelating agents (e.g. citric acid, EDTA) have been found to inhibit lipid oxidation by decreasing metal reactivity or by partitioning the metal away from the lipid (Let et al., 2007; Nielsen et al., 2004).

As discussed in the previous section, Dimakou et al. (2007) suggested that the increased formation of primary oxidation products at higher pH may be associated with the partitioning of iron and other transition metals which is affected by pH. The increase of pH results in lowering the solubility of the transition metals in the continuous phase so that they precipitate at the droplets' surface and promote oxidation (Cho et al., 2002).

In iron-catalysed lipid oxidation of corn o/w emulsions stabilized with anionic, non-ionic or cationic surfactants, the oxidation rate was pH-dependent only for samples emulsified with an

anionic surfactant (Mei et al., 1998[a]). These results were attributed to iron association with negatively charged emulsion droplets (Mei et al., 1998[b]).

Diaz et al. (2003) noted that the use of proteins as metal chelators in foods could be problematic because protein denaturation can cause loss of protein solubility and thereby alter the metal-binding properties. However a few researchers have reported that caseinophosphopeptides could provide a more practical source of natural-based chelators having activity that is not influenced by denaturation (Hansen et al., 1997; Peres et al., 2001).

Caseins have been reported to bind iron acting as metal chelators via their phosphoseryl groups (Gaucheron et al., 1996; Hekmat and McMahon 1998). Thus, in oil-in-water emulsions the iron binding by phosphoseryl residues would most likely inhibit iron induced lipid oxidation by promoting the partitioning of iron away from the lipid droplet and by maintaining iron in the less reactive ferric state (Gaucheron et al., 1997). Indeed, Diaz et al. (2003) concluded that enriched phosphorylated peptides from casein effectively inhibited lipid oxidation in corn oil-in water emulsions. In addition, whey proteins were found capable of removing metals from the emulsion droplets at pH=7.0 at which point the proteins are non-ionic, an effect which is not expected at pH 3.0, when they are positively charged (Tong et al., 2000). In addition, Faraji et al. (2010), noted that the chelating ability of the proteins in o/w emulsions decreased in the order of CAS > SPI > WPI. Another dimension of milk protein-iron complexes suggested by Sugiarto et al (2010) was as a novel way of incorporating iron into fortified foods with high bioavailability, good flavour and no solubility problems.

(6) EFFECT OF THE TYPE AND CONTENT OF THE OIL PHASE

Fats play many functional roles in food emulsions as they contribute to the flavor, appearance, texture and shelf life of the related consumer products (Worrasinchai, et al., 2006). A wide variety of vegetable oils (e.g. soyabean, corn, olive, sunflower oils), have been traditionally used in food emulsions although their application as functional ingredients in the dispersion systems might be complex from a technological point of view (Jacobsen et al., 2001; Taherian et al., 2011). A trend nowadays is to replace traditional oils with more health promoting oils, such as fish and walnut oils, which are rich in polyunsaturated fatty acids (PUFAs) and various structured lipids (de Ciriano et al., 2010; Osborn and Akoh 2002). Recent research (Liu et al., 2007; Nikzade et al., 2012) has examined particular fat substitutes in specific applications in order to make novel emulsion products with a texture close to that of traditional foodstuffs (e.g. mayonnaise or dairy products).

The fatty acid profile of the lipid phase has been reported as one of the critical factors that affect the oxidative stability of protein based oil-in-water emulsions (McClements and Decker, 2000). Poyato et al. (2013) observed that olive oil emulsions stabilised with soy protein isolates were more susceptible to accelerated oxidation (3 days of storage at 48°C) than the same type of emulsions prepared with linseed oil (richest in PUFAs). Compared with other vegetable oils, walnut oil in water emulsions prepared with WPI or caseins as emulsifiers, were more rapidly oxidised because of their highest PUFA content.

Kiokias et al. (2006) concluded that for caseinate-based emulsions prepared with various vegetable oils, changes of conjugated diene (CD) values at 233 nm (as indicator of primary oxidation products) were clearly associated with the fatty acid profile of the lipid phase. It must be stressed that the appearance of CD in oxidised lipids is attributed to an electronic shift due to

radical attack at a methylene-group separating double bonds (Kiokias and Oreopoulou 2006), which are mainly found in PUFA. Sunflower oil (containing ~70% 18:2) based emulsions were oxidised much faster than cottonseed and corn oil (both containing ~40% 18:2) based emulsions, whereas emulsions prepared with purified olive oil (~5%, 18:2) were the most stable against oxidative deterioration.

An increase of the oil content in protein stabilised emulsions was reported to decrease the average droplet size and influence the rheological and textural characteristics (Raymundo et al. 2002). A similar effect of oil content (in the range 5-10% w/v) was observed in emulsions stabilised by soya proteins (Achouri et al., 2012). Increasing the concentration of sunflower oil in the lipid phase (in the range 10-40% w/w) led to a reduction of the oxidative deterioration of sodium caseinate based o/w emulsions. (Kiokias et al., 2006). According to the authors, this effect may be due to an increased number of radicals generated per droplet as the oil concentration decreased. Similarly, Osborn-Barnes and Akoh (2004) reported that 10% canola oil-in-water emulsions contained significantly higher amounts of hydroperoxides compared to 30% o/w emulsions after 15 days of autoxidation at 50°C. McClements and Decker (2000) additionally proposed that at higher oil concentrations, more unsaturated fatty acids can move into the interior of the oil droplets and become less accessible to direct interaction with the prooxidants in the aqueous phase.

Overall, the amount and composition of the oil phase in an emulsion are important factors that influence the oxidative stability, the formation of volatiles, and the partition of the decomposition products, between the oil and water phases (Akoh 2002; Nikovska, 2010). Furthermore, it should be noted that lipid-protein adducts can form during the oxidation of

emulsions by a variety of mechanisms (Warahao et al., 2011) as interfacial proteins can interact with lipid oxidation products (Rampon et al., 2001; Shen et al., 2007). Given that adducts between lipid oxidation products, such as aldehydes, and protein will decrease the volatility of the lipid oxidation products that contribute to the sensory perception of rancidity (Zhou and Decker, 1999), a certain improvement of sensory properties of the emulsion product could be achieved.

(7) EFFECT OF TEMPERATURE AND PROTEIN DENATURATION ON EMULSION OXIDATION

The effect of temperature on the microstructural and oxidative stability of emulsion based foods is of great interest for food scientists. Heat treatment of protein-based emulsions at >60°C can occur for pasteurization or sterilisation of foods, and this may result in droplet flocculation because of the denaturation of proteins which may be important for the integrity of the droplets (Dickinson, 2001). Behavior at lower temperatures (such as <30°C) is also important, since these will represent conditions during storage of food emulsions at room or refrigeration temperatures . During isothermal lipid oxidation at relatively high temperatures, the concentration of hydroperoxides frequently peaks while at relatively low temperatures it only rises slowly. (Aragao et al., 2008). However, there are limited published data on the temperature dependence of the oxidation rate in oil-in-water emulsions, and these mostly involve sub-zero or low temperatures (Fomuso et al., 2002; Calligaris et al., 2007).

In a study by Dimakou et al. (2007), 20% sunflower oil-in-water emulsions prepared with Tween 20 were left to oxidize in incubators at 5, 15, 25, 40 or 60 °C, reflecting a range from chilling conditions to high temperatures used for storage and transport of processed foods. The results of

various oxidative indicators (CD, TBARs, lipid hydroperoxides) showed an increase of oxidative deterioration with increasing temperature. Moreover, a linear increase of CD with oxidation time was observed at all processing temperatures, following the equation indicated in Table-1. A similar pattern of linear increase of CD values has been also observed by other researchers in the accelerated oxidation of emulsions (Lethuaut, et al., 2002; Calligaris et al., 2007) or during storage of fried products (Houhoula and Oreopoulou, 2004). The Arrhenius plot of the rate constant (ln k) of CD formation versus reciprocal temperature (1/T) indicated a good correlation coefficient (R^2= 0.964) and an activation energy of 37.5 kJ mol^{-1}.

For protein based emulsions though, heat treatment may have a different effect on the oxidative process compared to emulsions prepared with LMW emulsifiers (Dissanayake and Vasiljevic, 2009). Heat treatment of protein-based emulsions at temperatures above 65°C can result in irreversible changes of protein structure that eventually have an effect on protein functionality, a process which sometimes is desirable and other times detrimental (Morgan et al., 2001; Raikos, 2010). Prolonged heating (15-30 min) of whey protein stabilised emulsions between 50-90°C led to an increased droplet size due to droplet flocculation induced by increased intermolecular interactions between the protein molecules at the interface (Keownmaneechai and McClements, 2006; Surh et al., 2006).

Kiokias et al. (2007) investigated whether thermally induced whey protein denaturation might correlate with oxidative changes in whey protein emulsions prepared with heat pre-treatment at varying temperatures (40–85°C). The results showed that an increase of whey protein denaturation (in particular at >60°C) was associated with a decrease of sensitivity of emulsion to oxidation, assessed by conjugated diene production. Similarly, Elias et al. (2007) reported that

thermally (50–95°C) denatured β-lactoglobulin provided the best protection against lipid oxidation of menhaden oil-in-water emulsions, after 7 days of storage. A partial unfolding of the whey proteins occurs upon heating and during homogenisation (Philips et al., 1994). This involves breaking of physical interactions (loss of secondary structure) so that hydrophobic parts of the molecule are exposed and they facilitate protein anchoring at the interface, enhancing its emulsifying capacity and leading to improved access of the protein to free radicals (Cheison et., 2010; Donato et al., 2007).

Furthermore, mixtures of native and denatured whey proteins have been found to act in combination in emulsified systems as native whey proteins move rapidly to the surface, whereas the denatured particles produce a thick membrane at the interface (Sliwinki et al., 2003). Kiokias and Bot (2006) revealed that the amount of whey protein associated with the fat phase roughly doubled over the temperature range of 65–85°C in whey protein stabilised emulsions. Therefore, the formation of thicker, viscoelastic protein films at the interface, as denaturation proceeds, may offer a stronger barrier to prooxidant agents present in the aqueous phase, preventing them from readily approaching the lipid core of the emulsion droplets. However, Adjonu et al. (2013) reported that heat pre-treatment of emulsions showed no apparent effect on the molecular weight distribution and antioxidant activity of the hydrolysates of WPI.

(8) CONCLUSION

After reviewing the literature about the effect of composition and processing factors, it can be concluded that it may be possible to design protein emulsions with greater oxidative stability;

1) by monitoring formation of a thick droplet membrane (e.g. through the selection of the appropriate type and concentration of protein) which acts like a physical barrier and limits the interactions between PUFA or lipid hydroperoxides and prooxidants;

2) by promoting the chelation of prooxidative metals with pH adjustment of the aqueous phase and formation of cationic charges on the surface of emulsions droplets which repel transition metals;

3) through inactivation of free radicals by different amino acids, a process that can be enhanced through thermally induced protein denaturation.

Overall, food proteins, apart from their excellent emulsifying properties, may represent a promising class of natural antioxidants that will enable food manufacturers to produce oxidatively stable emulsions. Further elucidation of the mechanisms that may affect oxidative deterioration of these dispersed systems would also be of significant technological importance for the development of various products of similar structure and formulation, (e.g. vegetable fat (non-dairy) spreads and creams).

REFERENCES

Achouri, A., Zamani, Y., and Boye, J. I. (2012). Stability and physical properties of emulsions prepared with and without soy proteins. *J. Food Res.* **1** : 254-267.

Adjonu, R., Doran, G., Torley, P., and Agboola, S. (2013). Screening of whey protein isolate hydrolysates for their dual functionality: Influence of heat pre-treatment and enzyme specificity. *Food Chem.* **136** : 1435-43.

Akoh, C. C., and Min, B. D. (1997). Food Lipid Chemistry, Nutrition and Biotechnology. Marcel Dekker, New York.

Akoh, C. C. (2002). Structured lipids. **In:** Food Lipids, pp. 877-908. Akoh, C. C., and Min, D. B., Eds., Marcel Dekker, New York.

Alamed, J., McClements, D. E., and Decker, E. A. (2009). Relationships between free radical scavenging and antioxidant activity in foods. *J. Agric. Food Chem*. **57** : 2969-2976.

Allen, J. C., and Hamilton, R. J. (1994). Rancidity in Foods, pp. 1–22, 3rd ed. Chapman & Hall, London.

Almajano, M. P., Delgado, E., and Gordon, M. H. (2007). Albumin causes a synergistic increase in the antioxidant activity of green tea catechins in oil-in-water emulsions. *Food Chem.* **102** : 1375–1382.

Ambrosone, L., Mosca, M., and Ceglie, A. (2007). Impact of edible surfactants on the oxidation of olive oil in water-in-oil emulsions. *Food Hydrocoll.* **21** : 1163–1171.

Aragao, G. M. F., Corradini, M. G., and Peleg, M. (2008). A phenomenological model of the peroxide value's rise and fall during lipid oxidation. *J. Am. Oil Chem. Soc*. **85** : 1143-1153.

Arvanitoyannis, I.S., Varzakas, T., Kiokias, S., and Labropoulos A. (2010). Lipids, Fats and Oils. **In** 'Advances in Food Biochemistry'. pp. 131-203, Yildiz (ed.), CRC Press, Taylor and Francis Group,

Berger K. G. (1990). Ice cream. **In :** Food Emulsions. pp. 367–444. Larsson, K., and Friesberg S., Eds., Marcel Dekker, New York.

Binks, B. P., Murakami, R., Armes, S. P., and Fujii S. (2006). Effects of pH and salt concentration on oil-in-water emulsions stabilized solely by nanocomposite microgel particles. *Langmuir*. **22** : 2050–2057.

Bot, A., Kiokias, S., van Maurik, E., Hoos, P. B., and Reszka, A. A. (2003). Droplet size measurements in heat-treated acidified protein stabilised oil-in-water emulsions using static light scattering and low-field NMR. *Proceed. 3rd Int. Symp. Food Rheol. Struct*, pp. 353–357. Zurich, Switzerland.

Calero, N., Muñoz, J., and Guerrero A. (2013). Effect of pH on o/w emulsions formulated with potato protein and chitosan. *Grasas y Aceites*. **64** : 15-21.

Calligaris, S., Manzocco L., and Nicoli, M. C. (2007). Modelling the temperature dependence of oxidation rate in water-in-oil emulsions stored at sub-zero temperatures. *Food Chem.* **101** : 1019–1024.

Chaiyasit, W., Elias, R., McClements, D. E., and Decker, E. A (2007). Role of physical structures in bulk oils on lipid oxidation. *Crit. Rev. Food Sci. and Nutr*. **47** : 299-317.

Cheison, S. C., Schmitt, M., Leeb, E., Letzel, T., and Kulozik, U. (2010). Influence of temperature and degree of hydrolysis on the peptide composition of trypsin hydrolysates of b-lactoglobulin: Analysis by LC-ESI-TOF/MS. *Food Chem.***121** : 457–467.

Chen, L., Remondetto, G. E., and Subirade, M. (2006). Food protein-based materials as nutraceutical delivery system. *Trends Food Sci. Technol.* **17** : 272–283.

Cho, Y.-J., McClements, D. J, and Decker, E. A. (2002). Ability of surfactant micelles to alter the physical location and reactivity of iron in oil in-water emulsions. *J. Agric. Food Chem.* **50** : 5704-5710.

Cho, Y.-J., Alamed, J., McClements, D.J, and Decker, E.A (2003). Ability of chelators to alter the physical location and prooxidant activity of iron in oil-in-water emulsions. *J. Food Sci*. **68** :1952-1957.

Clark, A. H., Kavanagh, G. M., and Ross-Murphy, S. B. (2001). Globular protein gelation-theory and experiment. *Food Hydrocoll.* **15** : 383–400.

Coupland, J., Zhu, Z., Wan, H., McClements, D. J, Nawar, W., and Chinachoti, P. (1996). Droplet composition affects the rate of oxidation of emulsified ethyl linoleate. *J. Am. Oil Chem. Soc*. **73** : 795-801.

Dagleish, D. G. (1990). Denaturation and aggregation of serum proteins and caseins in heated milk. *J. Agr. Food Chem.* **38**: 1995–1999.

de Ciriano, M. G., Rehecho, S. B., Calvo, M. I., Cavero, R. Y., Navarro, I., Astiasarán, I., and Ansorena, D. (2010). Effect of lyophilized water extracts of *Melissa officinalis* on the stability of algae and linseed oil-in-water emulsion to be used as a functional ingredient in meat products. *Meat Sci*. **85** : 373–377.

Diaz, M., McClements, D. J, and Decker, E. A. (2003). Use of casein phosphopeptides as natural metal chelators to inhibit oxidative reactions in oil-in-water emulsions. *J. Agric. Food Chem.* **51** : 2365-2370.

Dickinson, E., Rolfe S. E., and Dagleish, D. G. (1988). Competitive adsorption of αs1-casein and β-casein in oil-in-water emulsions. *Food Hydrocoll.* **265** : 397–405.

Dickinson, E., and Iveson, G. (1993). Adsorbed films of α-lactoglobulin +lecithin in the hydrocarbon water and triglyceride water interfaces. *Food Hydrocoll.* **6** : 533-541.

Dickinson, E., and McClements, D. J. (1995). Advances in Food Colloids, pp. 18–23

Blackie Academic and Professional, London.

Dickinson, E. (2001). Milk protein interfacial layers and the relationship to emulsion stability and rheology. *Coll. Surf. B: Biointerf.* **20** : 197-210.

Dickinson, E. (2008). Interfacial structure and stability of food emulsions as affected by protein–polysaccharide interactions. *Soft Matter*. **4** : 933-942.

Dimakou, C., Kiokias, S., Tsaprouni, I., and Oreopoulou,V. (2007). Effect of processing and storage parameters on oxidative deterioration of oil-in-water emulsions. *Food Biophysics*. **2** : 38-45.

Dissanayake, M., and Vasiljevic, T. (2009). Functional properties of whey proteins affected by heat treatment and hydrodynamic high-pressure shearing. *J. Dairy Sci*. **92** : 1387–1397.

Djordjevic, D., Cercaci, L., Alamed, J., McClements, D., and Decker, E. (2007). Chemical and physical stability of citral and limonene in sodium dodecyl sulfate chitosan and gum Arabic-stabilized oil-in water emulsions. *J. Agric. Food Chem.* **55** : 3585-3591.

Donato, L., Guyomarc'h, F., Amiot, S., and Dalgleish, D. G. (2007). Formation of whey protein/k-casein complexes in heated milk: preferential reaction of whey protein with κ-casein in the casein micelles. *Int. Dairy J*. **17** : 1161–1167.

Elias, R., McClements, D. J, and Decker, E. A (2007). Impact of thermal processing on the antioxidant mechanisms of continuous phase β-lactoglobulin in oil-in-water emulsions. *Food Chem*. **104** : 1402-1409.

Elias, R., Kellerby, S., and Decker, E. A. (2008). Antioxidant activity of proteins and peptides. *Crit. Rev. Food Sci. Nutr*. **48** : 430-441.

Ese, M. H., and Kilpatrick, P. K. (2004). Stabilization of water-in-oil emulsions by naphthenic acids and their salts: Model compounds, role of pH, and soap:acid ratio. *J. Dispers. Sci. Technol*. **25** : 253–261.

Euston, S. E., Singh, H., Munro, P. A., and Dalgleish, D. G. (1996). Oil-in-water emulsions stabilized by sodium caseinate or whey protein isolate as influenced by glycerol monostearate. *J. Food Sci*. **61** : 916-920.

Euston, S. R. (1997). Emulsifiers in dairy products and dairy substitutes. **In**: Food Emulsifiers and Their Applications, pp. 173–210. Hasenheuttl, G. L., and Hartel, R. W., Eds., Chapman and Hall, New York.

Euston, S. R., and Hirst, R. L. (1999). Comparison of the concentration-dependent emulsifying properties of protein products containing aggregated and non-aggregated milk protein. *Int. Dairy J.* **9** : 693-701.

Euston, S. R., Finnigan S. R., and Hirst, R. L. (2001). Kinetics of droplet aggregation in heated whey protein-stabilized emulsions: Effect of polysaccharides. *Food Hydrocoll.*. **16** : 499–505.

Faraji, H., McClements, D. J, and Decker, E. A. (2010). Role of continuous phase protein on the oxidative stability of fish oil-in-water emulsions. *Dairy Sci. Technol*. **90** : 87-98 Frankel, E. (1998). Free radical oxidation. **In** : Lipid Oxidation, pp. 13-22. The Oil Press Dundee, Scotland.

Gaucheron, F., Le Graët, Y., Boyaval, S., and Piot, M. (1997). Binding of cations to casein molecules-Importance of physicochemical conditions. *Milchwissenschaft*. **52** : 322 – 327.

Gaucheron, F., Famelart, M. H., and Le Graët, Y. (1996). Iron-supplemented caseins: preparation, physicochemical characterization and stability. *J. Dairy Res*. **63** : 233-243.

Gharsallaoui, A., Saurel, R., Chambin, O., Cases, E., Voilley, A., and Cayot, P. (2010).

Utilisation of pectin coating to enhance spray-dry stability of pea protein-stabilised oil-in-water emulsions. *Food Chem*. **122** : 447-454. Gohtani, S., Sirendi, M., Yamamoto, N., Kajikawa, K., and Yamamo, Y. (1999). Effect of droplet size on oxidation of Docosahexaenoic acid in emulsion systems. *J. Dispersion Sci. Technol*. **20** : 1319-1325. Halliwell, B., and Gutteridge, J. (1995). Free Radicals in Biology and Medicine, 2nd ed., Clarendon Press, Oxford, U.K. Hansen, M., Sandström, B., Jensen, M., and Sørensen, S. S. (1997). Casein phosphopeptides improve zinc and calcium absorption from rice-based but not from whole-grain infant cereal. *J. Pediat. Gastr. Nutr.* **24** *:* 56-*62.* Hekmat, S., and McMahon, D. J. (1998). Distribution of iron between caseins and whey proteins in acidified milk. *LWT-Food Sci.Technol*. **31**: 632-638. Houhoula, D. P., and Oreopoulou, V. (2004). Predictive study for the extent of deterioration of potato chips during storage. *J. Food Eng.* **65** : 427-432.

Hu, M., McClements D. J., and Decker, E. A. (2003[a]). Lipid oxidation in corn oil-in-water emulsions stabilized by casein, whey protein isolate and soya protein isolate. *J. Agric. Food Chem.* **51** : 1435-1439.

Hu, M., McClements, D. J., and Decker, E. A. (2003[b]). Impact of whey protein emulsifiers on the oxidative stability of salmon oil-in-water emulsions. *J. Agric. Food Chem.* **51** : 1435-1439.

Hu, M., McClements, D. J., and Decker, E. A. (2004). Impact of chelators on the oxidative stability of whey protein isolate-stabilized oil-in-water emulsions containing u-3 fatty acid. *Food Chem.* **88** : 57-62.

Huang, S. W., Frankel, E., Schwarz, K., and German, J. (1996). Effect of pH on antioxidant activity of a-tocopherol and Trolox in oil-in water emulsions. *J. Agric. Food Chem*. **44** : 2496-2502.

Imai, H., Maeda, T., Shima, M., and Adachi, S. (2008). Oxidation of methyl linoleate in oil-in-water micro- and nanoemulsion systems. *J. Am. Oil Chem. Soc*. **85** : 809-815.

Jacobsen, C., Timm, M., and Meyer, A. (2001). Oxidation in fish oil enriched mayonnaise: ascorbic acid and low pH increase oxidative deterioration. *J. Agric. Food Chem*. **49** : 3947-3956.

Kato, A., Mifuru, R., Matsudomi, N., and Kobayashi, K. (1992). Functional casein–polysaccharide conjugates prepared by controlled dry heating. *Biosci. Biotechnol. Biochem*. **56** : 567–571.

Kellerby, S., Gu, Y., McClements, D. J., and Decker, E. A. (2006). Lipid oxidation in a menhaden oil-in-water emulsion stabilized by sodium caseinate cross-linked with transglutaminase. *J. Agric. Food Chem.* **54** : 10222-10227.

Keownmaneechai, E., and McClements, D. J. (2006). Influence of EDTA and citrate on thermal stability of whey protein stabilized oil-in-water emulsions containing calcium chloride. *Food Res. Int.* **39** : 230–239.

Kim, H. J., Decker, E. A., and McClements, D. J. (2005). Influence of droplet characteristics on the formation of oil-in-water emulsions stabilized by surfactant-chitosan layers. *Lagmuir*. **21** : 134-139.

Kiokias, S., and Bot, A. (2005). Effect of protein denaturation on temperature cycling stability of heat-treated acidified protein-stabilised o/w emulsions. *Food Hydrocoll.***19** : 493-501.

Kiokias, S., and Bot, A. (2006). Temperature cycling stability of pre-heated acidified whey protein-stabilised o/w emulsion gels in relation to the internal surface area of the emulsion. *Food Hydrocoll.* **20** : 246-252.

Kiokias, S., and Gordon, M. (2003). Dietary supplementation with a natural carotenoid mixture decreases oxidative stress. *Europ. J. Clin. Nutrit.* **57** : 1135-1140.

Kiokias, S., Dimakou, C., Tsaprouni, I., and Oreopoulou,V. (2006). Effect of compositional factors against the thermal oxidation of novel food emulsions. *Food Biophysics*. **1** : 115-123.

Kiokias, S., Dimakou, C., and Oreopoulou, V. (2007). Effect of heat treatment and droplet size on the oxidative stability of whey protein emulsions. *Food Chem.* **105** : 94-100.

Kiokias, S., Dimakou, C., and Oreopoulou,V. (2009[b]). In vitro antioxidant activity of synthetic beta-carotene and natural carotenoid extracts against the oxidatative degradation of food emulsions. **In** : beta-carotene, Dietary sources, Cancer and Cognition, p.231-262.. Haugen, L., and Bjornson T, Eds., Nova Science publisher. NY.

Kiokias, S., Lampa, K. Tsimogiannis, D. and Oreopoulou, V. (2005) Inhibition of oxidative deterioration in food emulsions. *Proceedings of INTRAFOOD-EFFoST Conference*. **2** : 1237–1240.

Kiokias, S., Reiffers-Magnani, C., and Bot, A. (2004[b]). Stability of whey-protein stabilized o/w emulsions during chilled storage and temperature cycling. *J. Agric. Food Chem.* **52** : 3823- 3830.

Kiokias, S., Reszka, A. A., and Bot, A. (2004[a]). The use of static light scattering and pulsed-field gradient NMR to measure droplet size changes in heat-treated acidified protein stabilised oil-in-water emulsion gels. *Int. Dairy J.* **14**: 287-295.

Kiokias, S., and Oreopoulou, V. (2006). Antioxidant properties of natural carotenoi preparations against the AAPH-oxidation of food emulsions. *Innov. Food Sci. Emerg. Technol.* ***7*** : 132-139.

Kiokias, S., Varzakas, T., and Oreopoulou,V. (2008). In vitro activity of vitamins, flavonoids, and natural phenolic antioxidants against the oxidative deterioration of oil-based systems. *Crit. Rev. Food Sci. and Nutr*. **48** : 78-93.

Kiokias, S., Varzakas, T., Arvanitoyannis, I., and Labropoulos, A. (2009[a]). Lipid oxidation and control of oxidation. **In** : Advances in Food Biochemistry, pp.384-403. Yildiz (ed.), CRC Press, New York.

Kiokias, S., and Varzakas, T. (2014). Activity of flavonoids and b-carotene during the auto-oxidative deterioration of model food oil-in water emulsions. *Food Chem*. 150 280–286

K. R., and Cunha, R. L. (2012). Flaxseed oil – whey protein isolate emulsions: Effect of high pressure homogenization. *J. Food Eng.* **2** : 449–457.

Laplante, S., Turgeon, S. L., and Paquin, P. (2005). Effect of pH, ionic strength, and composition on emulsion stabilising properties of chitosan in a model system containing whey protein isolate. *Food Hydrocoll*. **19**: 721-729.

Let, M., Jacobsen, C., and Meyer, A. (2007). Ascorbyl palmitate, gammatocopherol, and EDTA affect lipid oxidation in fish oil enriched salad dressing differently. *J. Agric. Food Chem.* **55** : 2369-2375.

Lethuaut, L., Metro, F., and Genot, C. (2002). Effect of droplet size on lipid oxidation rates of oil-in-water emulsions stabilized by protein. *J. Am. Oil Chem. Soc.* **79** : 425-430.

Liu, H., Xu, X. M., and Guo, Sh. D. (2007). Rheological, texture and sensory properties of low-fat mayonnaise with different fat mimetics. *LWT-Food Sci. Technol.* **40** : 946-954.

Mancuso, J., McClements, D. J, and Decker, E. A. (1999). The effects of surfactant type, pH, and chelators on the oxidant of salmon oil-in-water emulsions. *J. Agric. Food Chem.* **47** : 4112-4116.

Mancuso, J., McClements, D. J., and Decker, E. A. (2000). Iron accelerated cumene hydroperoxide decomposition in hexadecane and trilaurin emulsions. *J. Agric. Food Chem.* **48** : 213-219.

McClements, D. J., and Decker, E.A. (2000). Lipid oxidation in oil-in-water emulsions: impact of molecular environment on chemical reactions in heterogeneous food systems. *J. Food Sci.* **65** : 1270-1282.

McClements, D. J. (2005). Food Emulsions: Principles, Practice, and Techniques., 2nd ed. Boca Raton, CRC Press, NY.

Mei, L., Decker, E. A., and McClements, D. J. (1998[b]). Evidence of iron association with emulsion droplets and its impact on lipid oxidation. *J. Agric. Food Chem.* **46** : 5072-5077.

Mei, L., McClements, D. J., and Decker, E. A. (1999). Lipid oxidation in emulsions as affected by charge status of antioxidants and emulsion droplets. *J. Agric. Food Chem.* **47** : 2267-2273.

Mei, L., McClements, D. J., Wu, J., and Decker, E. A. (1998[a]). Iron-catalyzed lipid oxidation in emulsions as affected by surfactant, pH and NaCl. *Food Chem*. **61** : 307-312.

Morgan, F., Micault, S., and Fauquant, J. (2001). Combined effect of whey protein and a_{S1}-casein genotype on the heat stability of goat milk. *Int. J. Dairy Technol.* **54** : 64–68.

Mosca, M., Cuomo, F., Lopez, F., and Ceglie, A. (2013). Role of emulsifier layer, antioxidants and radical initiators in the oxidation of olive oil-in-water emulsions. *Food Res. Int.* **50** : 377–383.

Nakaya, K., Ushio, H., Matsukawa, S., Shimizu, M., and Ohshima, T. (2005). Effects of droplet size on the oxidative stability of oil-in water emulsions. *Lipids*. **40** : 501-507.

Nielsen, N., Petersen, A., Meyer, A., Timm-Heinrich, M., and Jacobsen, C. (2004). Effects of lactoferrin, phytic acid, and EDTA on oxidation in two food emulsions enriched with long-chain polyunsaturated fatty acids. *J. Agric. Food Chem.* **52** : 7690-7699.

Nikovska, N. (2010). Oxidative stability and rheological properties of oil-in-water emulsions with walnut oil. *Adv. J. Food Sci. Technol.* **2** : 172-177.

Nikzade, V., Tehrania, M., and Saadatmand-Tarzjan, M. (2012). Optimization of low-cholesterol low-fat mayonnaise formulation: Effect of using soy milk and some stabilizer by a mixture design approach. *Food Hydrocoll.* **28** : 344–352

Osborn, H. T., and Akoh, C. C. (2002). Structured lipids-novel fats with medical, nutraceutical, and food Applications. *Compreh. Rev. Food Sci. Food Safety***. 1 :** 110–120**.**

Osborn, H. T., and Akoh, C. C. (2003). Copper catalyzed oxidation of a structured lipid-based emulsion containing α-tocopherol and citric acid: Influence of pH and citric acid. *J. Agric. Food Chem.* **51**: 6581-6585. Osborn, H. T., and Akoh, C. C. (2004). Effect of emulsifier type, droplet size, and oil concentration on lipid oxidation in structured lipid-based oil-in-water emulsions. *Food Chem.* **84** : 451-456.

Paiva-Martins, F., and Gordon, M. (2002). Effects of pH and ferric ions on the antioxidant activity of olive polyphenols in oil-in-water emulsions. *J. Am. Oil Chem. Soc.* **79** : 571–576.

Peng, X., Xiong, Y., and Kong, B. (2009). Antioxidant activity of peptide fractions from whey protein hydrolysates as measured by electron spin resonance. *Food Chem.* **113** : 196-201.

Peres, J. M., Bureau, F., Neuville, D., Arhan, P., and Bougle, D. (2001). Inhibition of zinc

absorption by iron depends on their ratio. *J. Trace Elem. Med. Biol.* **15** : 237-241.

Poyato, C., Navarro-Blasco, I., Calvo, M. I., Cavero, R. Y., Astiasarán,Y., and Ansorena, D. (2013). Oxidative stability of o/w and w/o/w emulsions: Effect of lipid composition and antioxidant polarity. *Food Res. Int.* **51** : 132–140.

Raikos, V. (2010). Effect of heat treatment on milk protein functionality at emulsion interfaces. *Food Hydrocoll.* **24** : 259–265.

Rampon, V., Lethuaut, L., Mouhous-Riou, N., and Genot, C. (2001). Interface characterization and aging of bovine serum albumin stabilized oil-in-water emulsions as revealed by front-surface fluorescence. *J. Agric. Food Chem*. **49** : 4046-4051.

Raymundo, A., Francob, J. M., Empisc, J., and Sousad, I. (2002). Optimization of the composition of cow-fat oil-in-water emulsions stabilized by white lupin protein. *J. Am. Oil Chem. Soc*. **79** : 783-790.

Ruth, S. M., Roozen, J. P., Posthumus, M. A., and Jansen F. J. H. M. (1999). Volatile composition of sunflower oil-in-water emulsions during initial lipid oxidation: influence of pH. *J. Agric. Food Chem.* **47** : 4365-4369.

Segall, K. I., and Goff, H. D. (2002). Secondary adsorption of milk proteins from the continuous phase to the oil-water interface in dairy emulsions. *Int. Dairy J*. **12** : 889–897.

Seo, S. R., Lee, H. Y., and Kim, J. C. (2012). Thermo- and pH-responsiveness of emulsions stabilized with acidic thermosentive polymers. *J. Food Eng.* **111** : 449–457.

Shaw, L., McClements, D. J., and Decker, E. A. (2007). Spray-dried multi-layered emulsions as a delivery method for u-3 fatty acids into food systems. *J. Agric. Food Chem.* **55** : 3112-3119.

Shen, Z., Rusli, J., Sanguansri, L., and Augustin, M. (2007). Retention of propanal in protein-stabilised tuna oil-in-water emulsions. *Food Chem.* **101** : 746-752.

Shimada, K., Fujikawa, K., Yahara, K., and Nakamura, T. (1992). Antioxidative properties of xanthan on the sutoxidation of soybean oil in cyclodextrin emulsion. *J. Agric. Food Chem.* **40** : 945-948.

Silvestre, M., Chaiyasit, W., Brannan, R., McClements, D., and Decker, E. (2000). Ability of surfactant head group size to alter lipid and antioxidant oxidation in oil-in-water emulsions. *J. Agric. Food Chem.* **48** : 2057-2061.

Sliwinki, E. L., Roubos, P. J., Zoet, F. D., van Boekel, M. A. J. S., and Wouters, J. T. M. (2003). Effects of heat on physicochemical properties of whey protein-stabilised emulsions. *Coll. Surf. B: Biointerf.* **31** : 231–242.

Sugiarto, M., Ye, A., Taylor, M. W., and Singh, H. (2010). Milk protein-iron complexes: Inhibition of lipid oxidation in an emulsion. *Dairy Sci. Technol.* **90** : 87-98.

Sun, C., and Gunasekaran, S. (2009). Effect of protein concentration and oil-phase volume fraction on the stability and rheology of menhaden oil-in-water emulsions stabilized by whey protein isolate with xanthan gum. *Food Hydrocoll.* **23** : 165-174.

Surh, J., Ward, L. S., and McClements, D. J. (2006). Ability of conventional and nutritionally-modified whey protein concentrates to stabilize oil-in-water emulsions. *Food Res. Int.* **39** : 761–771.

Taherian, A., Britten, M., Sabik, H., and Fustier, P. (2011). Ability of whey protein isolate and/or fish gelatin to inhibit physical separation and lipid oxidation in fish oil-in-water beverage emulsion. *Food Hydrocoll.* **25** : 868–878.

Tapal, A., and Tiku, P. K. (2012). Complexation of curcumin with soy protein isolate and its implications on solubility and stability of curcumin. *Food Chem.* **130** : 960-965.

Tesch, S., and Schubert, H. (2002). Influence of increasing viscosity of the aqueous phase on the short-term stability of protein stabilized emulsions. *J. Food Eng.* **52** : 305–312.

Tong, L., Sasaki, S., McClements, D., and Decker, E. (2000). Mechanisms of the antioxidant activity of a high molecular weight fraction of whey. *J. Agric. Food Chem.* **48** : 1473-1478.

Trunova, N., Kartasheva, Z., Maksimova, T., Bogdanova, Y., and Kasaikina, O. (2007). Decomposition of cumene hydroperoxide in the systems of normal and reverse micelles formed by cationic surfactants. *Colloid J*. **69** : 655-659.

Venditti, F., Cuomo, F., Ceglie, A., Ambrosone, L., and Lopez, F. (2010). Effects of sulphate ions and slightly acidic pH conditions on Cr(VI) adsorption onto silica gelatin composite. *J. Hazard. Mater.* **173** : 552–557.

Vilasaua, J., Solansa, C., Gómezb, M. J., Dabriob J., Mújika-Garaib, R., and Esquenaa J. (2011). Influence of a mixed ionic/nonionic surfactant system and the emulsification process on the properties of paraffin emulsions. *Coll. Surf. A*: *Physicochem. Engin. Aspects.* **392** : 38– 44.

Villiere, A., Vian, M., Brownec, I., Moreau N., and Genot. C. (2005) Oxidative stability of bovine serum albumin- and sodium caseinate-stabilized emulsions depends on metal availability. *J. Agric. Food Chem.* **53** : 1514–1520.

Waraho, T., McClements, D. J., and Decker, E. A. (2011). Mechanisms of lipid oxidation in food dispersions. *Trends Food Sci. Technol.* **22** : 3-13.

Worrasinchai, S., Suphantharika, M., Pinjai, S., and Jamnong, P. (2006). β-Glucan prepared from spent brewer's yeast as a fat replacer in mayonnaise. *Food Hydrocoll*. **20** : 68-78.

Yoshie-Stark, Y., Wada, Y., and Wasche, A. (2008). Chemical composition, functional properties, and bioactivities of rapeseed protein isolates. *Food Chem.* **107** : 32–39.

Zhou, S., and Decker, E. A. (1999). Ability of amino acids, dipeptides, polyamines and sulfhydryls to quench hexanal, a saturated aldehydic lipid oxidation product. *J. Agric. Food Chem*. **47** : 1932-1935.

Table 1. The rate constants (k) and correlation coefficients (R^2) for the rate of increase of various oxidation indicators including: thiobarbituric acid-reactive substances (TBARs-532nm); lipid hydroperoxides (LH-510nm) and conjugated diene hydroperoxides (CD-232 nm), during the oxidation of 20 % sunflower o/w emulsion following their storage at different temperatures (Dimakou et al., 2007).

Temperature	CD		TBARs	LH
	k		(mmol/kg oil h)	(mmol/kg oil h)
(^{o}C)	(g/kg oil h) (x 10^3)	R^2	(x 10^5)	(x 10^2)
5	3	1	3.9	3.11
15	3.9	0.995	4.14	3.5
25	10.4	0.912	5.45	3.7
40	23.9	0.986	11.56	10.3
60	40.1	0.977	14.1	11.46

In particular for CD, the following equation applied:

$$CD = CD_O + kt$$

where *CD* is the value of conjugated dienes after time *t* of oxidation

CD_o is the initial value in fresh emulsion

k is the rate constant of the reaction

t is the time of oxidation (h)

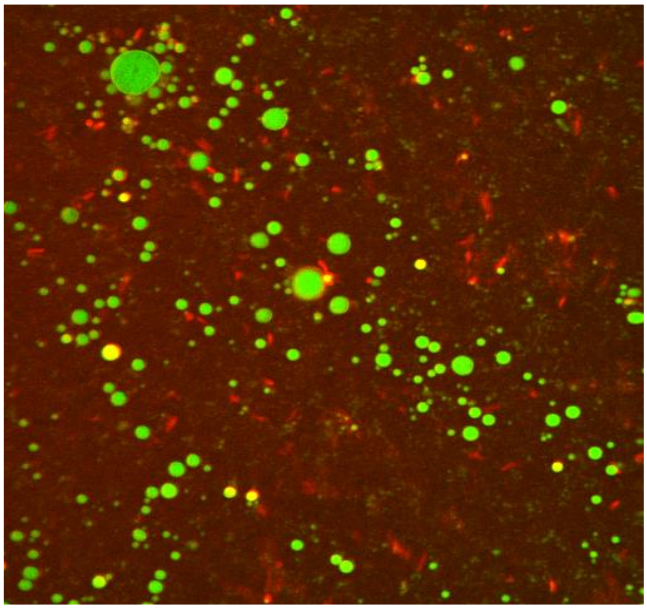

Figure-1 Image of a cottonseed o/w emulsion (10%) stabilized with 1% sodium-caseinate (spherical oil droplets stabilized in protein network). The picture was taken with Confocal Scanning Laser Microscopy (CSLM) (Image size: 131 x 131μm) (Kiokias & Varzakas, 2014).

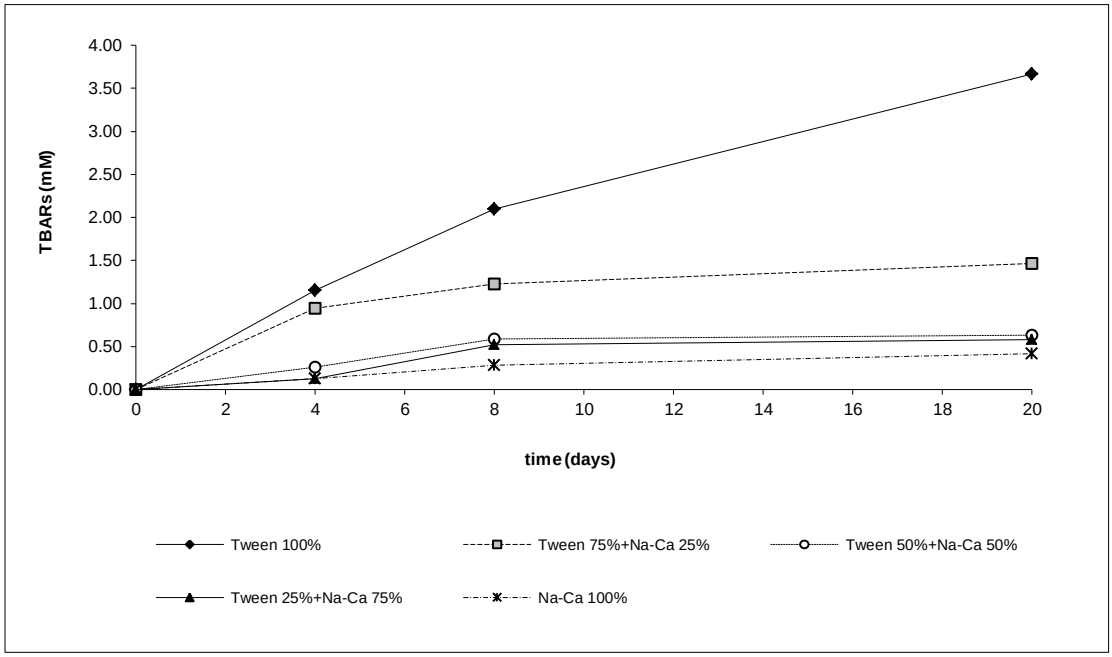

Figure 2 Oxidative changes as reflected by TBARs changes in 20% sunflower o/w emulsions stabilised with varying emulsifier mixtures (Tween + sodium caseinate : 2% w/w in the emulsion) after 20 days of thermal autoxidation at 60°C (Kiokias et al., 2006).