OXIDATIVE STABILITY AND MICROSTRUCTURE OF GRANOLA BARS ENRICHED WITH FISH OIL AND ALGAL ANTIOXIDANTS

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Abstract- In this study, the ability of Icelandic brown algae (*Fucus vesiculosus*) extracts inhibiting lipid oxidation in granola bars fortified with fish oil-in-water emulsion was investigated. It was also explored whether addition of the algal extracts affected the physical microstructure of the fish oil droplets in granola bars. The oxidative stability of the bars stored at 20°C was evaluated over a period of 10 week by measuring the development of peroxides, the change in fatty acid composition and volatile compounds by GC-FID and DHS GC-MS. The physical microstructure was visualized by microscopy including, SEM, ESEM and CLSM. All extracts, except water extract at low concentration, reduced lipid oxidation storage when added at a concentration of 0.5 or 1 g extract/100 g fish oil emulsion. Ethanol (EE) and acetone extracts (AE) were found to be most efficient antioxidants. The concentration dependent antioxidant efficacy of these two extracts was among others related to an improved incorporation of the fish oil-in-water emulsions in the bars, high total phenolic content, high radical scavenging activity together with high interfacial affinity of phenolic compounds and probably regeneration of tocopherol. Furthermore, the microstructure of granola bars prepared with EE and AE showed fewer oil pools and more ordered oil droplets. This study showed the application potential of F. vesiculosus extracts as a natural antioxidant in low-moisture foods such as granola bars.

Keywords: *emulsion, fish oil, Fucus vesiculosus, microscopy*

1. INTRODUCTION

Lipid oxidation in foods has been counted as a major problem due to its detrimental effects on the quality of food including formation of a wide range of unpleasant odors and rancid taste. The rate of lipid oxidation is highly dependent on the level on unsaturated fatty acids present in the food, and can be accelerated with the presence of n-3 long chain polyunsaturated acid (LC PUFA) of marine origin such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). Attempts to control lipid oxidation in food product added fish oil (FO) have been made by addition of antioxidants. Monomolecular antioxidants such as butylated hydroxytolulene and ethylenediaminetetra acetic acid, tocopherol, and ascorbic acid are in many cases not able to prevent oxidative flavor deterioration in FO-enriched foods. Hence, there are emerging interests in finding multi-functional antioxidants preferably of natural origin.

Brown algae, *Fucus vesiculosus*, are rich in the bioactive polyphenolic secondary metabolites, phlorotannins. Previous studies of extracts from F. vesiculosus found excellent antioxidant efficacy of these extracts in food emulsion systems, such as FO-enriched milk and mayonnaise [1, 2]. However, it can be difficult to extrapolate this antioxidant efficacy from one food system to another, the application of these extracts need to be evaluated in other food, such as low-moisture foods. In a study by Nielsen and Jacobsen [3], it was found that the granola bars enriched with emulsions were more stable to lipid oxidation than the bars enriched with neat FO.

The aims of this study were: (i) to study the antioxidant capacity of Icelandic brown alga *F. vesiculosus* extracts in an accelerated low-moisture food system, granola bars added 70% FO-in-water emulsions; (ii) to elucidate which extract are most important for efficient oxidative protection of the *F. vesiculosus*extracts in the low moisture systems; and lastly (iii) to investigate whether addition of extracts affected the physical microstructure of the oil droplets when incorporated into the bars.

2. EXPERIMENTAL

The water (WE), acetone (AE), and ethanol (EE) extracts were produced [4]. The 70% (w⁄w) FO-in-water emulsions were prepared mixing FO to a 10% (w/v) Na-cas solution with an ultra-turrax at 20 000 rpm. The seaweed extracts were dissolved in Na-cas solution having 0.5 or 1 g extract in 100 g emulsion. These two concentrations are indicated as sample codes of 1 or 2, respectively. 5% FO-enriched granola bars was produced according to Horn et al. [5]. The samples were packed in sealed plastic bags at atmospheric conditions and stored at room temperatures (21°C) in the dark up to 10 week. Dry matter of bars (wk 0) was~15%. The Fe content of samples ranged from 17.2±1.9 to 22.7±1.7mg Fe/g and was higher than the Cu content, which ranged from 1.7±0.2 to 3.9±1.3mg Cu/g granola bar. No significant differences were found in either Fe or Cu content among samples

3. RESULTS AND DISCUSSION

3.1. Droplet size distribution, fatty acid compositions, and tocopherol content

The average D [2, 3] value of control (Con), WE2, AE1 and 2, EE1 and 2 showed mainly a distribution of droplets from 0.8 to 1.2µm. However, WE1 showed a distribution of larger oil droplets of 2.4µm.

The changes in the fatty acid compositions in FO-enriched granola bars during 10wk of storage, was calculated by determining the relative decreases in area% (RDA%) of some n-3 LCPUFA (EPA, DHA and 18:4 n-3) and the total PUFAs from weeks 0 to 10. The RDA% of EPA, DHA, and 18:4 (n-3) was found to be significant ($p<0.05$) between Con and AE1, which the reduction is more pronounced in control samples.

The consumption of α-tocopherol (70%) was larger compared to γ-tocopherols (50%) at the end of storage. The fastest consumption of α-tocopherol was found in control. The α-tocopherol reduction in Con reached 65% after only 2 wk, whereas, at the same storage point no significant consumption of α-tocopherol was observed in granola bars with high or low concentration of AE added. After week 4, the consumption of α- and γ-tocopherols was significant in all samples and had reached its maximum as the tocopherol content reached a steady level in all samples.

3.2. Lipid hydroperoxides and volatile secondary oxidation products

Peroxide values (PV) ranged from 1.4 to 6–8 meq/kg in wk 0 and 27.6 to 35.4 meq/kg in wk 10. Development of PV in the granola bars was somewhat different when seaweed extracts were added into the formulation compared to the Con. A significant increase in PV was not observed in AE2 and EE2 before week 4, whereas the other samples only had a lag phase of 1 wk. Until week 4, PV was highest in the Con. After week 4, PV was generally higher in WE1 than in all other samples. In general EE1, EE2, and AE1 showed antioxidant activity as granola bars with these extracts added had lower PVs during storage (Fig.1).

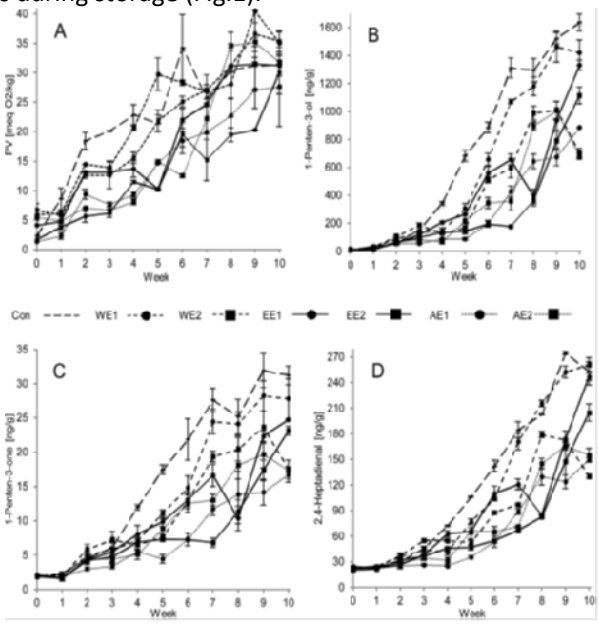

Figure 1.PV (meq/kg oil) (A) and development of volatile secondary oxidation products (ng/g granola bar) (B–D) in FO-enriched granola bars added F. vesiculosus WE, EE, or AE (0.5 (1) or 1% (2) in the FO emulsion) and a Con (without extract) stored dark for 10 wk at room temperature. The development of volatile secondary oxidation products exemplified by 1-penten-3-ol (B), 1-penten-3-one (C), and t,t-2,4-heptadienal (D). Error bars indicate SD of the measurements (n=2 for PV and n=3 for volatiles).

For determination of secondary oxidation products, 16 volatiles of 100 observed volatile compounds, were identified by MS and quantified: 1-penten-3-ol, 1-pentanol, 1-octen-3-ol, pentanal, t-2-pentenal, t-2-hexenal, hexanal, t,t-2,4-heptadienal, 4-heptenal, octanal, nonanal, decanal, 1-penten-3-one, 2-ethylfuran, 2-pentylfuran, and benzaldehyde. The identity of these volatiles was confirmed by comparison of retention times with those of external standards. Mainly, all volatile secondary oxidation products in the present study showed similar development patterns in volatile concentrations during the storage period, with a lag

phase followed by an increase in concentration particularly in the later part of the storage period. The only exceptions to this pattern were nonanal and decanal, for which the concentrations were initially high and at the same level forall samples, which could the result of heat-induced oxidation in the baking phase (data not shown).

In Fig. 1B–D, three representatives of oxidation products from EPA and DHA are shown, namely 1-penten-3-one, 1-penten-3-ol, and t,t-2,4-heptadienal, which have all been recognized as decomposition products of EPA and DHA [6]. In addition, 1-penten-3-one has been suggested as one of the markers for fishy and metallic off-flavors in fish-oil-enriched foods [7]. The length of the lag phase of the three representatives differed between samples and was depending on addition and concentration of the extracts. The Con had a short lag phase of 1–3wk, followed by a steep increase in concentration of secondary oxidation products in the subsequent storage weeks. Samples prepared with WE1 and WE2 had a lag phase up to 4 wk and hereafter had the same development as the Con. Granola bars containing the high amount of EE(EE2) had thelongest lag phase for volatiles up to 6–7wk followed by granola bars withAE (AE1 and AE2). The lag phases of 1-penten-3-one were generally shorter than for 1-penten-3-ol. A shorter lag phase of 1-penten-3-one than 1-penten-3-ol has been observed before in determination of lipid oxidation in FO-enriched foods. This has been found to the related to the reduction of 1-penten-3-one to 1-penten-3-ol.

3.3. Microstructure and droplet size distribution

Starch grains were found by all microscopic methods (Fig. 2) and are marked. In the confocal images, the starch grains were clearly distinguished in the water phase as green grains with cracks in the middle. In the SEM and ESEM images, the starch grains were found as pointed spheres on the fracture surface of the bars. Some of the starch grains were polygons, most likely originating from the rice components. The starch grains were widely distributed in the matrix both as large and smaller grains. According to the confocal images, the oil was not highly associated with air (Fig 2D1). The oil droplets were not only found as small spherical oil droplets but also as large oil pools in the bars. The oil pools were most pronounced in the Con where no extract was added. Among samples with extracts, the oil pools were seen more often in granola bars prepared with WE, and more evenly distributed spherical oil droplets were observed in the bars prepared with EE and AE.

The confocal images, Fig. 2A1–D1, show that the bars contained few large spherical water droplets that could be a site for lipid oxidation. The images also showed that protein bands were formed (light green areas) and surrounded some of the oil droplets forming a physical barrier on the surface of the droplets, which could influence oxidation. This is typically seen in protein emulsified o/w emulsions.

3.4. Discussion

From the results, it was observed that the addition of WE (only high concentration), EE or AE to 5% FO-enriched granola bars improved the oxidative stability of the bars during storage at room temperature. The decrease in some n-3 PUFAs was reduced by the addition of these extracts and also the development in secondary volatile oxidation products related to oxidation of FO was reduced to a great extent. The order of the extracts and concentrations at wk 10 toward limiting formation of volatile secondary oxidation products in the bars was as follows; AE1>EE2>AE2>EE1>WE2>WE1>Con. A high radical scavenging capacity of *F. vesiculosus*extracts has been related to their high phlorotannin content, the major phenolic compound in the brown algae and thereby also the total phenolic content. AE and EE showed highest radical scavenging capacity of the three tested extracts. It is assumed that the phlorotannins are the main contributors to this property.

Polymerized and very polar phlorotannins will typically be poor radical scavengers compared to oligomer and less polar phlorotannins. This can be related to that polymerized phlorotannins more likely are branched and that the functional OH-groups are enclosed in the structure. Hence, when using less polar solvents such as acetone the extraction of smaller and more amphiphilic phenolic compounds are favored compared to when using water for extraction, which to a larger extent will extract the polymerized and highly polar phlorotannins. Moreover, the polarity of the smaller oligomers could determine their location in oil-in-water emulsions, such as the preemulsions added to the granola bars.

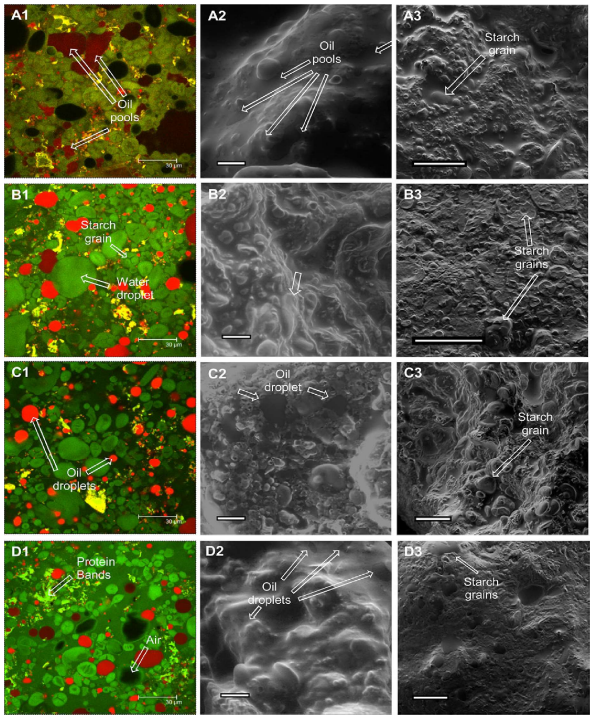

Figure 2. Microstructure of the granola bars at week 0 at 20°C. A: The Con without extract, B: granola bar added WE, C: granola bar added EE, D: granola bar added AE, all with 1% extract in the FO emulsion. CLSM (1) red is the fat phase and green is the water phase, scale bars are 30mm. ESEM (2) and SEM (3), scale bars are 20 and 50mm, respectively.

The more amphiphilic nature of phenolic compounds especially in AE, was confirmed by Hermund et al. [1] and Honold et al. [2], who studied the partitioning of AE, EE, and WE. They found that the phenolic compounds were mainly hydrophilic and mainly located in the water phase and not in the oil phase. However, high amounts of phenolic compounds were found at the interface indicating that some of the phenolic compounds are amphiphilic. This amphiphilic nature of the phenolic compounds in the extracts will increase their antioxidant efficacy in granola bars, as the phenolic compounds then are located at the surface of the oil droplets close to the site of lipid oxidation. The improved incorporation of the preemulsions when the extracts were added, as observed in the microscopy images, indicates more interfacial interaction when the extracts are added and is probably also due the more amphiphilic phlorotannins. Hence, when the dough was added the preemulsions and baked, the water content and mobility inside the bars decreased and the amphiphilic phlorotannins were presumably more or less locked on the surface of the oil droplets, close to the site of lipid oxidation where these can act as tocopherol-regenerators.

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