# Antioxidant activity of myofibrillar proteins in oil-in-water emulsions

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## Abstract

The antioxidant properties of proteins and derivatives (hydrolysates, peptides and amino acids) have been described in a wide range of food systems, including lipid dispersions. The purpose of the present study was to evaluate the protective role of myofibrillar proteins (MP) against lipid oxidation in 10% (w/w) oil-in-water emulsions. Emulsions containing increasing levels of MP (0.5%, 1% and 2% on the basis of lipid content) as well as 1% bovine serum albumin (BSA) were prepared and oxidized (3 $\mu$M copper acetate) for 10 days at 37ºC. Lipid oxidation was evaluated by measuring the increase of conjugated dienes (CD) and hexanal during the oxidation essay. MP acted as inhibitor of lipid oxidation because emulsions with higher MP content contained lower levels of CD and hexanal. BSA was not as effective as MP in inhibiting lipid oxidation. MP-emulsions had lower amounts of CD and hexanal than BSA-emulsions during the whole oxidation essay. The amino acid composition and three-dimensional structure of the proteins could have influenced their antioxidant potential. Certain amino acids from MP could act as inhibitors of lipid oxidation in food systems.

## Introduction

The oxidation of lipids and proteins is a hot topic in meat science and technology because of the critical consequences of oxidative reactions in muscle foods. The development of oxidative reactions in meat and meat products involves the loss of essential fatty acids, amino acids and vitamins and affects many quality characteristics such as flavor, color, texture and nutritive value (*1*). Although the effect of protein oxidation on processed meats is currently poorly understood, recent studies have related the oxidation of muscle proteins to texture changes in refrigerated meat (*2*) and frankfurters (*3*). Proteins and peptides play a double role on oxidative reactions since they are targets for oxidation while have been shown to inhibit the oxidative deterioration of lipids in a wide range of food systems including food lipid dispersions (e.g. oil-in-water emulsions) (*4, 5*). In fact, some particular amino acids such as methionine have been reported to act as endogenous antioxidants in proteins due to their recognized radical scavenging activity (*6*). In recent years interest in utilizing natural antioxidants has increased considerably (*7*). This has led to new investigations into assessing the antioxidant potential of biologically active peptides from protein hydrolysates. Whereas the antioxidant potential of myofibrillar proteins has been already reported (*8*), there is no information about the precise mechanisms and real impact of the antioxidant activity of MP against lipid oxidation. The present study is aimed to shed light on the aforementioned issue.

## Material and methods

All chemicals were supplied by J.T Baker (Deventer, Holland), Riedel de-häen, and Sigma Aldrich (Steinheim, Germany). Porcine *longissimus dorsi* muscle was purchased in a local supermarket in Helsinki.

*Extraction of MP*

MP were extracted from porcine *longissimus dorsi* muscle according to the procedure used by Park et al. (*9*). After the extraction, the myofibrillar protein isolated was stored in a tightly capped bottle, at 0ºC and used within 48 hours for emulsions preparation.

Preparation and oxidation of emulsions

Oil-in-water emulsions (10% w/w) were prepared following the procedure described by Viljanen et al. (*10*). Purified rapeseed oil (1 g) and 9 mL phosphate buffer (pH 6) were mixed to decreasing amounts of myofibrillar proteins (0.2, 0.1 and 0.05 g) leading to emulsions with 2%, 1% and 0.5% myofibrillar protein (calculated on the basis of lipid content. Emulsions containing 1% BSA were also prepared. The emulsions were placed in sealed vials and oxidized in the dark at 37 °C for 10 days. In order to promote the oxidation of the emulsions, 3 $\mu$M copper acetate solution was added to the vials. During the prooxidant storage, the emulsions were constantly stirred with magnets and sampling was carried at fixed times (days 1, 3, 5, 7 and 10)

*Lipid oxidation assessment*

Lipid oxidation was evaluated by assessing conjugated diene hydroperoxides (CD) and hexanal during storage of emulsions. CD were isolated and analyzed according to the method described by Viljanen et al. (*10*). Hexanal was measured using static headspace gas chromatography (HS-GC) (Autosystem XL gas chromatograph equipped with an HS40XL headspace sampler; Perkin-Elmer, Shelton, CT; column NB-54, Nordion) (*10*).

*Statistical analysis*

All types of emulsion were made three times and all analyses were made in duplicate (n=6). Data obtained from experimental analyses were used as variables and computed in a one-way analysis of variance (SPSS 12.05) to study the effect of MP content. Student-T tests were performed to compare means derived from emulsions with different MP content and from emulsions containing MP and BSA proteins. Statistical significance was set at $p<0.05$.

**Results and discussion**

The amount of CD increased in the emulsions over time until day 7, when the highest CD levels were detected. Emulsions with the highest MP level (2%) contained significantly smaller amounts of CD than those with low MP levels (0.5%) (Figure 1).

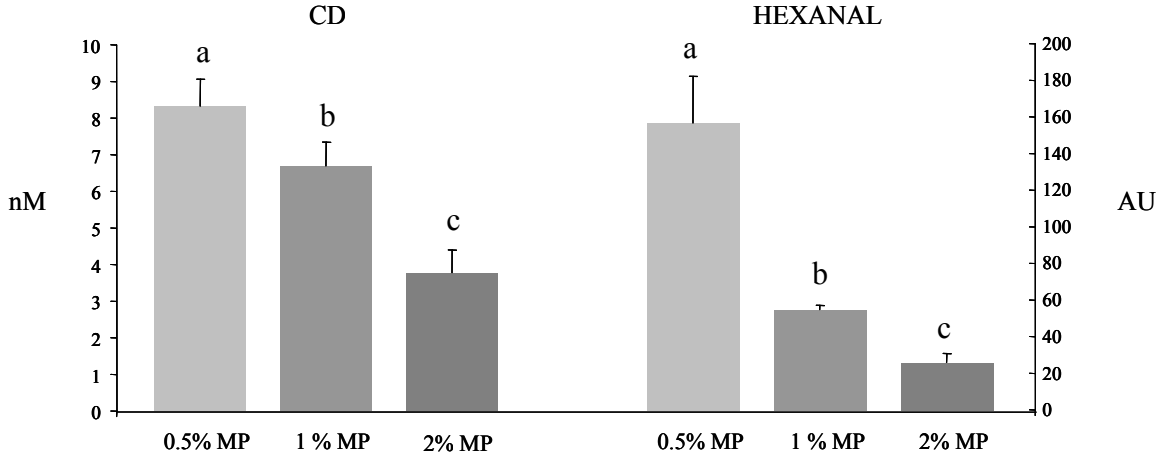


**Figure 1.** CD and hexanal counts (mean ± standard deviation) measured in oxidized oil-in-water emulsions (day 10) made with increasing amount of MP.

Emulsions with medium MP content (1%) showed intermediate values. Hexanal levels increased over time with the highest levels being measured at day 10. In agreement with CD values, emulsions with 2% MP had significantly lower hexanal counts than those with 0.5% MP whereas 1% MP-emulsions showed intermediate values (Figure 1). MP exhibited a clear protective role against lipid oxidation as emulsions with the highest MP content showed the lowest lipid oxidation. The antioxidant properties of MP can be attributed to the cooperative effect of a variety of properties including the ability of aromatic and sulfur-containing amino acids to scavenge free radicals, and the capacity to act as metal-ion chelators (*6, 10, 11*). Saigas et al. (*8*) demonstrated that some peptides from porcine MP containing glutamic and aspartic acids display an antioxidant potential equivalent to that of α-tocopherol. MP could also have inhibited the oxidation of lipids by preventing the penetration of lipid oxidation initiators within the emulsified oil droplet.

On the other hand, the influence of MP on lipid oxidation was different to that shown by BSA. MP-emulsions contained significantly lower amounts of CD than BSA emulsions at all sampling days except at day day 10. Consistently, the headspace (HS) in BSA-emulsions contained significantly higher levels of hexanal than that in MP-emulsions at all sampling days (Figure 2). The different ability of BSA and MP to protect lipids against oxidation might be derived from the different amino acid composition and their three-dimensional structures. In this sense, MP contain considerably high levels of amino acids with antioxidant potential such as aromatic and sulfur-containing amino acids (*11, 12*). Additionally, MP exhibit a recognized ability to interact

with lipids and form very stable emulsions that could enhance the oxidative stability of lipids by reducing, for instance, the interaction between lipid droplets and pro-oxidants from the aqueous phase.

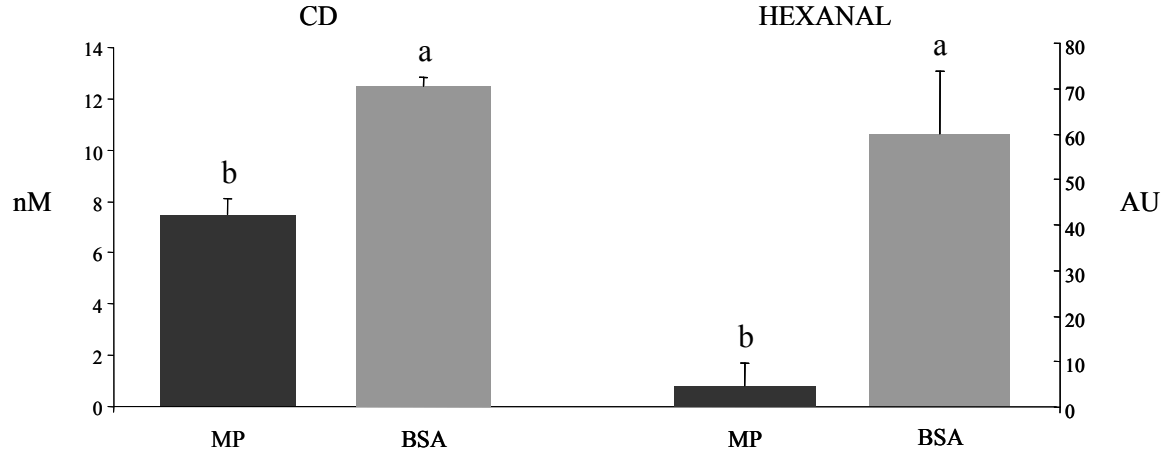


**Figure 2.** CD and hexanal counts (mean ± standard deviation) measured in oxidized oil-in-water emulsions (day 7) made with MP and BSA.

## Conclusions

In conclusion, MP exhibit a clear and intense antioxidant activity against lipid oxidation. Within a lipid-containing food, MP could inhibit lipid oxidation and enhance its technological and sensory value.


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