Assessment of Discontinuous Oxidation in Microencapsulated FAME based upon Analysis of Hydroperoxy-, Keto- and Hydroperoxydienes

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INTRODUCTION

Microencapsulation of lipids in proteins and/or carbohydrates from drying o/w emulsions is a technological process for preparation of functional food ingredients. The lipids constitute a disperse phase in the inner of solid particles [1]. Evaluation and control of lipid oxidation in these powdery food products are essential to avoid losses of quality and significant formation of compounds that may even be detrimental to health. Lipid droplets may oxidize at different rates and as a result complex mixtures of primary, secondary and even advanced oxidation compounds may be detected in quite different proportions to those found in the lipids in continuous phase [2].

An HPLC-UV method developed in our lab allows us to analyze the main oxidation products of linoleic acid, i.e. hydroperoxydienes and their principal secondary products, namely, keto- and hydroxy-dienes. Unlike common analytical indices, the method allows for quantitation of compounds and the simultaneous determination of both primary and secondary oxidation products [3].

OBJECTIVE

The aim of the present study was to assess if the HPLC analysis of hydroperoxy-, keto- and hydroxy-dienes is a suitable method to evaluate the complex lipid oxidation of dried microencapsulated lipids. Fatty acid methyl esters (FAME) obtained from sunflower oil were used as a lipid substrate. The microcapsules were obtained from freeze-drying of an o/w emulsion containing lactose and sodium caseinate. Formation of hydroperoxy-, keto- and hydroxy-dienes was studied during oxidation of the microcapsules and also in a sample of FAME in continuous phase.

SAMPLES

FAME were obtained by transmethylation of purified high-linoleic sunflower oil [3]. The microencapsulated FAME were made from freeze-drying of o/w emulsions containing 10 wt% FAME, 10 wt% sodium caseinate, 10 wt% lactose and 70 wt% water [2]. The emulsions were prepared by high-pressure homogenization with 70 MPa and 2 passes.

The samples were oxidized in an oven at 40ºC in the dark, under non-limiting oxygen conditions, and at constant relative humidity (23%).

METHODS

Quantitative analysis of hydroperoxy-, keto- and hydroxy-dienes. The FAME samples were analyzed by HPLC, under isocratic regime, using a silica column (LiChrospher® Si 60, 5 μm particle size) (Merck) and a UV detector at 234 and 268 nm. n-Heptane:diethyl ether (88:12, v/v) was the mobile phase [3].

Polymers. Polymers of FAME were analyzed by IUPAC standard method 2.508.

Lipid extractions from the microencapsulated samples. The free lipid fraction was extracted with n-hexane and then the microencapsulated fraction with dichloromethane-methanol (2:1, v/v) [4].

RESULTS AND DISCUSSION

The lipid extracts did not show chromatograms different from those of the neat sample in terms of occurrence of new peaks or changes in the distribution of isomers in each group of compounds (**Fig. 1**).

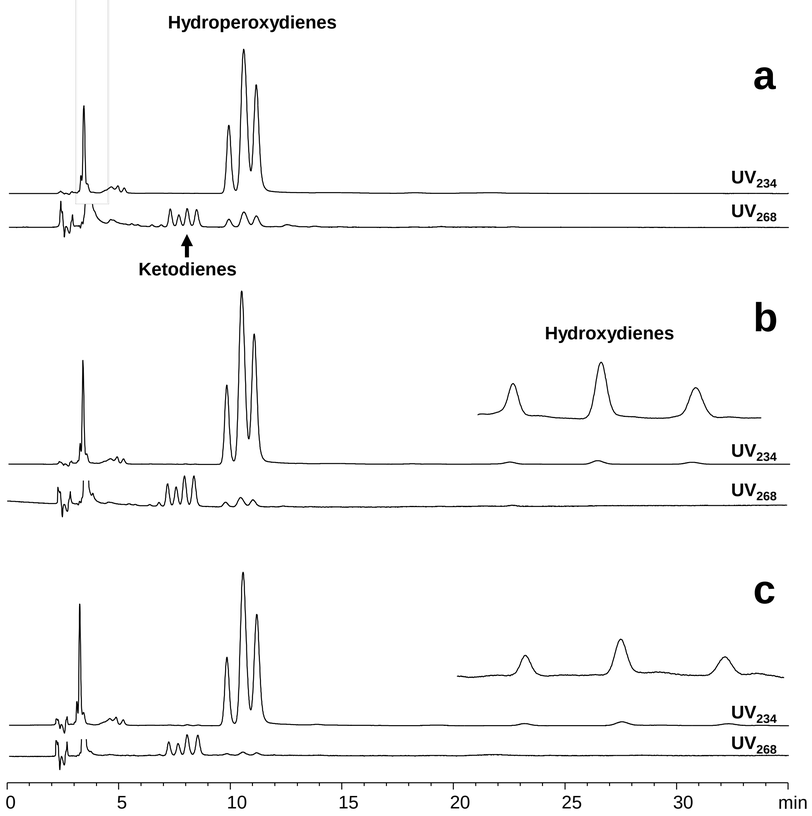

Fig. 1. HPLC chromatograms registered at 234 and 268 nm of oxidized neat FAME (**a**) and the free (**b**) and encapsulated (**c**) fractions of oxidized microencapsulated FAME.

As expected, oxidation was quicker in the continuous system, showing a substantial increase of hydroperoxydienes from Day-4. This occurred from Day-8 in both the free and encapsulated fractions of the microencapsulated sample. The increase of hydroperoxides was slower in the encapsulated fraction, indicating clearly lower availability of oxygen (**Fig. 2**). Keto- and hydroxy-dienes displayed similar patterns to that of hydroperoxides (not shown).

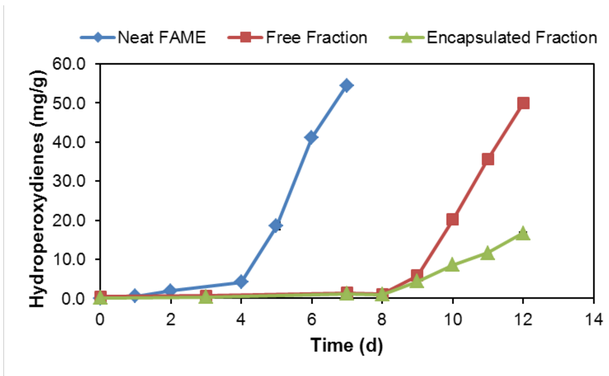

Fig. 2 Formation of hydroperoxydienes.

For a given content of hydroperoxydienes, much more elevated amounts of secondary products were detected in the microencapsulated sample compared to the neat sample of FAME (**Fig. 3**). The contents of keto- and hydroxy-dienes found in the microencapsulated FAME ranged as a whole between 6 and 31 wt% of the analyzed compounds, while the neat sample showed values lower than 1.5%.

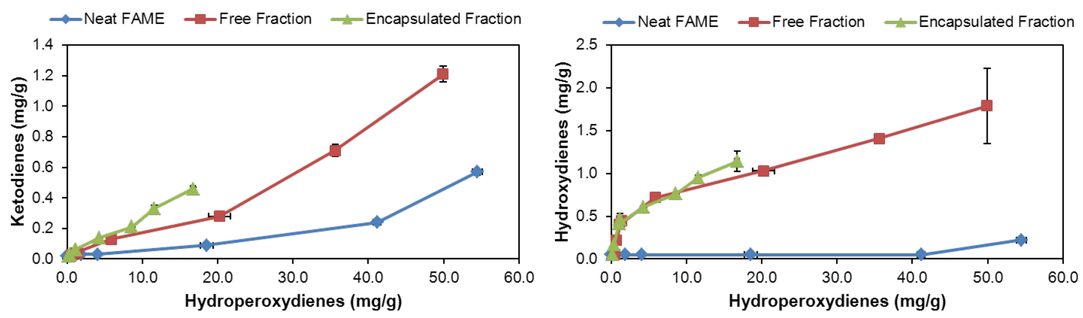

Fig. 3 Relationships of keto- and hydroxy- with hydroperoxy-dienes.

Similarly, higher contents of polymers were detected in the microencapsulated sample for a given content of hydroperoxydienes (**Fig. 4**).

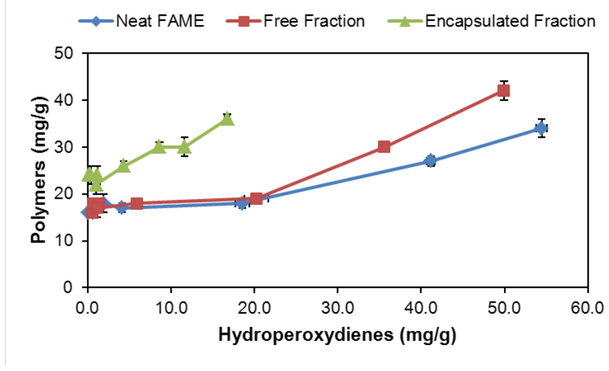

Fig. 4. Relationship between polymers and hydroperoxydienes.

The results of this study can be attributed to the discontinuous nature of oxidation in the microencapsulated FAME. Thus, lipid droplets oxidized at different rates and presented different oxidation states. On the one hand, the extract would be formed from droplets in early stages of oxidation, containing hydroperoxides and very low contents of secondary products, and, on the other, from droplets in advanced stages with decreased hydroperoxides and substantial contents of secondary products.

CONCLUSIONS

1 Analysis of hydroperoxy-, keto- and hydroxy-dienes allowed for the evaluation of discontinuous oxidation in microencapsulated lipids.

2 Applying complementary analytical methods to those assessing the primary oxidation products only is essential in the evaluation of oxidation in foods containing lipids in a disperse phase.

3 Substantial formation of secondary oxidation products and even polymerization compounds can occur in these products, even when the level of global oxidation is low.

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