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To cite this version:

Tayse da Silveira, Claire Bourlieu-Lacanal, Jérôme J. Lecomte, Erwann Durand, Maria-Cruz Figueroa, et al.. Effect of the mode of incorporation of antioxidants on the oxidative stability of oil-in-water emulsions. 17. Euro Fed Lipid Congress, Oct 2019, Seville, Spain. hal-02735216

HAL Id: hal-02735216

https://hal.inrae.fr/hal-02735216

Submitted on 2 Jun 2020

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Effect of the mode of incorporation of antioxidants on the oxidative stability of oil-in-water emulsions

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**Background**

- **Polar Paradox**
  - Porter et al., 1989

- **Polar antioxidants**
- **Non polar antioxidants**

**Antioxidants (Aox)**

- Chemical:
  - Reactivity
  - Number and OH position
  - OH Dissociation energy
- Physico-chemical:
  - Hydrophobicity
  - Partitioning
  - Location

**Which antioxidant?**

- Phenolic compounds and Phenolipids

**Chemical Equations**

\[ ROO^\cdot = \text{Fatty Acid Peroxide} \]

\[ \text{RO}^\cdot = \text{Alkoxy Radical} \]

\[ \text{ROOH} = \text{Fatty Acid Peroxide} \]
**PHENOLIPIDS: ADJUSTING AND OPTIMIZING AOX EFFICIENCY OF PHENOLIC COMPOUNDS**

**CHLOROGENIC ACID**

(A) ENZYMATIC ESTERIFICATION

\[
\text{HO-(CH}_2\text{)}_n\text{-CH}_3 \xrightarrow{\text{Lipase 5\%, 55°C}} \text{Lipophilic group}
\]

- \( n = 0 \) (methyl)
- \( n = 3 \) (butyl)
- \( n = 7 \) (octyl)
- \( n = 11 \) (dodecyl)
- \( n = 15 \) (hexadecyl)
- \( n = 17 \) (octadecyl)
- \( n = 19 \) (eicosyl)

Lopez Giraldo et al., Enz. Microb. Tech., 2009

**ROSMARINIC ACID**

(B) CHEMICAL ESTERIFICATION

\[
\text{HO-(CH}_2\text{)}_n\text{-CH}_3 \xrightarrow{\text{H}_2\text{SO}_4} \text{AOxs with varying polarities can be synthesized}
\]

- \( n = 0 \) (methyl)
- \( n = 3 \) (butyl)
- \( n = 7 \) (octyl)
- \( n = 11 \) (dodecyl)
- \( n = 15 \) (hexadecyl)
- \( n = 17 \) (octadecyl)
- \( n = 19 \) (eicosyl)

Lecomte et al., J. Am. Oil Chem. Soc., 2010

**LIPOPHILIZATION:** chemical or enzymatic linkage of phenolic compounds with lipophilic groups (e.g. alkyl alcohols or fatty acids)
Contradiction of the Polar Paradox
The cut-off effect (COE)
(Laguerre et al., 2009)

Maximum Antioxidant activity
Antioxidant capacity collapses with highly hydrophobic molecules

Below CMC
Above CMC
Critical micellar concentration

ROLE OF THE EMULSIFIER: interactions

- Modify AOX location by solubilization in micelles, modulating AOX efficiency
- Micelles could act as carriers of highly hydrophobic AOXs

From Laguerre et al., Annual R.F.S.Tech, 2017
Rosmarinates (160 μM) in low moisture foods (crackers)

Incorporation of phenolipids in the lipid phase prior to dough formation

Barden et al., JAFC, 2015

Incorporation of phenolipids by mixing in the aqueous phase prior to dough formation

EFFECT OF MODE OF INCORPORATION

Mode of incorporation modified C12 efficiency

RC20 > RC12 > RC0

Figure 2. (A) Lipid hydroperoxides and (B) headspace hexanal in crackers with rosmarinic and its esters (chain lengths = 0, 12, or 20 carbons) incorporated into the lipid prior to dough formation. Crackers were stored in the dark at 55 °C. Standard error bars are smaller than data points in some instances.

Figure 3. (A) Lipid hydroperoxide and (B) headspace hexanal formation in crackers made by incorporating rosmarinic ester antioxidants (chain lengths = 0, 12, or 20 carbons) into the aqueous phase prior to dough formation. Crackers were stored in the dark at 55 °C. Standard error bars are smaller than data points in some instances.
The mode of incorporation of antioxidants in o/w emulsions affects their dynamic equilibrium in the emulsion system and reshape their efficiency.

An excess of emulsifier in the aqueous phase could interfere in the AOx behavior, by, for example, favoring its transfer from one droplet to another.

**Effect of mode of incorporation on the AOx**

**Effect of micelles on these systems above**

Emulsion System I - one type of oil
- Emulsions constituted of a single droplet of unsaturated oil

Emulsion System II – two types of oil
- Emulsions constituted of two populations of unsaturated oil
- Investigate the effect of the presence of micelles in these systems
All studied emulsions were physically stable over oxidation time.
Oxidative stability

All the applied AOxs exhibited an antioxidant effect compared to the control, regardless of the emulsifier concentration and mode of incorporation.
Emulsion system I: single lipid droplets emulsion system

1% Rapeseed, non stripped

AOxs added in the oil phase
**BEFORE** emulsification

AOx 50µM

Aqueous phase pH 7.0

**Emulsifier:** Sodium dodecyl sulfate (SDS)

Below CMC

Above CMC

CMC: presence of micelles

Pre-emulsion/Homogenization

Oxidation 40°C/15 days
Peroxide value (PV), TBARS

Control: No antioxidant
Emulsion system I: single lipid droplets emulsion system

- Experimental design

Gallic acid and Gallate esters C3-C16
**RESULTS:** Oxidative stability **below CMC**

**BEFORE**

- **PV (mmol/kg oil):**
  - C0, C3, C8, C12, C16 vs. days 0-15

- **TBARS (mg MDA/kg oil):**
  - C0, C3, C8, C12, C16 vs. days 0-15

**AFTER**

- **PV (mmol/kg oil):**
  - C0, C3, C8, C12, C16 vs. days 0-15

- **TBARS (mg MDA/kg oil):**
  - C0, C3, C8, C12, C16 vs. days 0-15

- **Rank of protection:**
  - Before: C16 > C12 > C8 > C3 > C0
  - After: C16 > C12 > C8 > C3 > C0

- **No cut-off effect (both modes of incorporation) – agreement with the Polar Paradox**
- **Rank of AOxs:** best C16 and C12, with slight effect of the mode of incorporation
**RESULTS: Oxidative stability below CMC**

- **Individual effect of mode of incorporation:** affected mainly C8 and C16, with lower oxidation when AOx was incorporated before.
- **In general,** mode of incorporation slightly affected the others AOxs.
RESULTS: Oxidative stability Above CMC

BEFORE

AFTER

Rank of protection

C16~C12~C8>C0~C3

✓ Rank of AOxs: Same as bellow CMC
✓ In presence of micelles, Rank of Aoxs was not affected by mode of incorporation
✓ Higher formation of TBARS for long chain compounds (C12 and C16)
RESULTS: Oxidative stability Above CMC

Individual effect of mode of incorporation: C16 and C8 were less affected by the mode of incorporation.

In general, effect of mode of incorporation slightly or no affected the others AOxs.
Possible effect of micelles on long chain compounds for formation of secondary compounds
Emulsion system II: mixed lipid droplets emulsion system

Experimental design

- **Control (no AOX)**
  - 1% RP
  - 1% MCT
  - AMNV

- **AOX in RP oil**
  - AOX
  - 1% RP
  - AMNV
  - 1% MCT

- **AOX in MCT oil**
  - 1% RP
  - AMNV
  - 1% MCT

**Pre-emulsion / Homogenization**

- **Below CMC**
  - AOX present in both lipid populations

- **Above CMC**
  - AOX present in only one of the lipid populations

- **Mix 1:1**
  - Mixed lipid droplets

**Oxidation 40°C/16 hours**

- Peroxide value (PV)
- TBARS

**RP:** Unsaturated Rapeseed, non-stripped

**MCT:** Saturated medium chain triglycerides

**AMVN:** Lipophilic radical initiator
2,2'-azobis (2,4-dimethylvaleronitrile)
Emulsion system I I: mixed lipid droplets emulsion system

- Experimental design

Enzymatic synthesis and purification of rutin ester C16
Lue et al., J Am Oil Chem Soc, 2010

Rutin + Palmitic acid (C16:0) → Rutin ester C16 + H₂O
Lipase
55°C/4 days

Rutin ester

RC0

RC16
**RESULTS:** Oxidative stability

Gallic acid (GC0) vs. Gallate ester C16 (GC16)

**Below CMC**

**Above CMC**

- GC16 was more effective than GC0
- For GC0, modes of incorporation slightly affect results whereas micelles did not
- For GC16, no difference between the modes of incorporation and micelles

Same trend for TBARS
### RESULTS: Oxidative stability

**Rutin (RC0) vs. Rutin ester C16 (RC16)**

**Below CMC**

**Above CMC**

- RC0 was more effective than RC16
- For RC0, no difference between modes of incorporation and micelles
- For RC16, mode of incorporation and micelles did affect

Same trend for TBARS
CONCLUDING REMARKS.

1. Hypothesis 1
   - Mode of incorporation
     - In single lipid droplet emulsions (System I), mode of incorporation affected mainly C8 and C16.

   - The effect of incorporating AOXs in different lipid droplet populations (System II) showed to be molecule-dependent, with significant effect only for very bulky compounds (RC16).

2. Hypothesis 2
   - Presence of micelles
     - In single lipid droplets emulsions (System I), the presence of micelles affected mainly long chain compounds (TBARS value).

   - In emulsions with different lipid droplet populations (System II), the effect of the presence of micelles also seems to be related with the molecule bulkiness.
THANK YOU FOR YOUR ATTENTION!
Concluding remarks and future studies

- The mode of incorporation affected the efficiency of the antioxidants in a single lipid emulsion system.

- In mixed lipid systems, the effect of the mode of incorporation depended on the compounds and on the chain length. No effect was observed for Gallate ester C16, while for Gallic acid (C0), it was more effective when added to MCT droplets.

- Rutin and Rutin ester C16 showed an opposite behavior to that of Gallic acid and Gallates.

- An excess of emulsifier reshaped the antioxidant activity of phenolipids in a single lipid emulsion system.

- For a mixed lipid emulsion system, an excess of emulsifier only affected Rutin ester C16 behavior.

We propose that:

- Future works:
  - Establish mechanisms involved in the observed systems
  - Future studies:
    - Investigate the behavior of Emulsion System II using a hydrophobic radical initiator
    - Investigate the mechanisms underlying our observations
ROLE OF THE EMULSIFIER: Interactions

Panya et al., JAFC, 2012

Below CMC

Above CMC

AOx location, distribution

Modulation of AOx efficiency by solubilization in micelles

Critical micellar concentration

ROLE OF THE EMULSIFIER

Could emulsifier micelles act as carriers of highly hydrophobic AOx?

ML = lipophilic molecule

Background

imeline in oil-in-water emulsions (o/w)

Interfacial phenomena in emulsion oxidation (Frankel et al., 1994)

What makes good an antioxidant in o/w emulsions?

Polar Paradox
Porter et al., 1989

Which antioxidant (AOx)?

Phenolic compounds and Phenolipids

BE STRONG AND BE AT THE RIGHT PLACE

- Chemical:
  - Reactivity
  - Number and OH position
  - OH Dissociation energy
- Physico-chemical:
  - Partitioning
  - Location
  - Hydrophobicity

Oil droplet

ROO
H
AOx

Polar antioxidants
Non polar antioxidants

RO• = Alkoxyl Radical
ROOH = Fatty Acid Peroxide