

# Lipid and protein oxidation in interface-dominated food systems: Interrelation and functional consequences

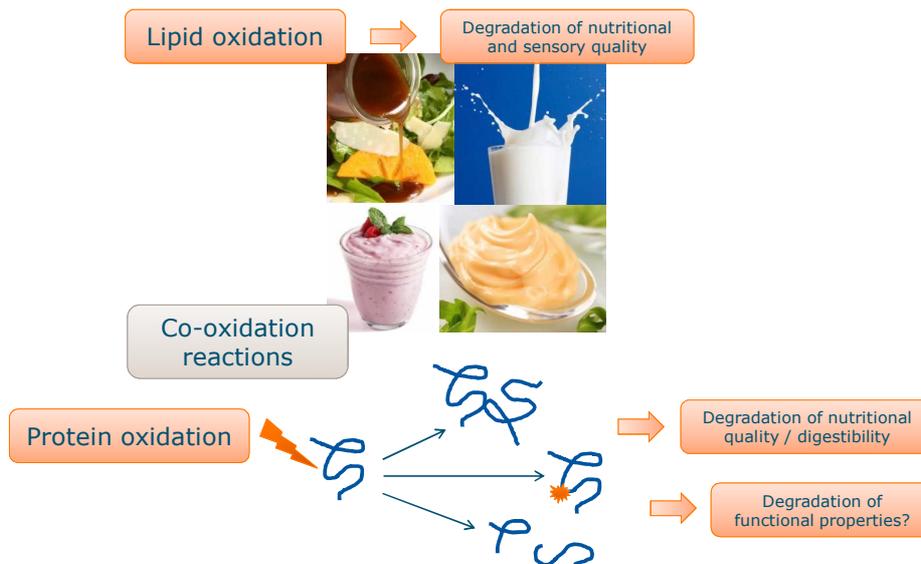
Claire Berton-Carabin

Assistant professor, Wageningen University



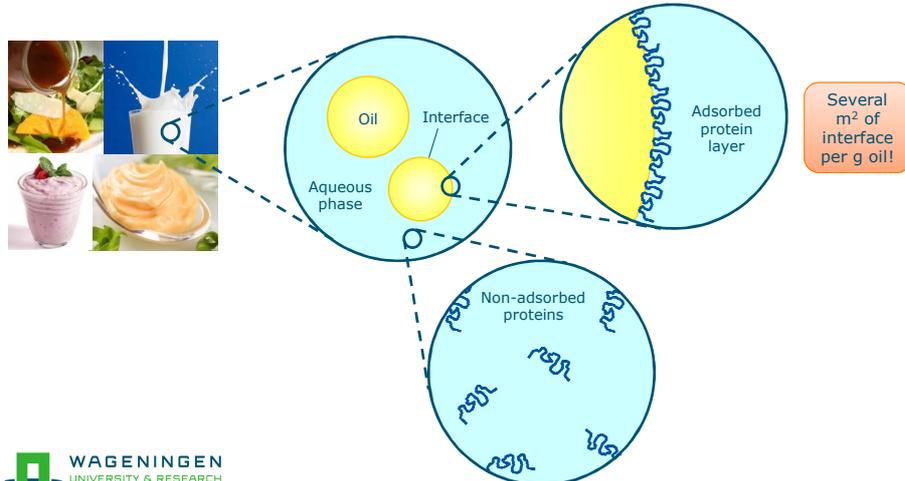
Agrotechnology and Food Sciences, Food Process Engineering group

## Context



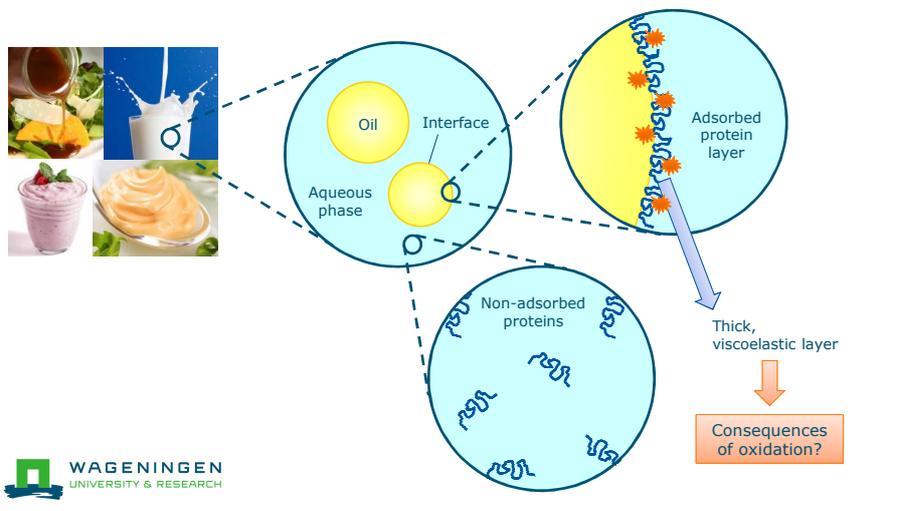
## Context

- O/W emulsions: suitable model systems to investigate oxidative phenomena



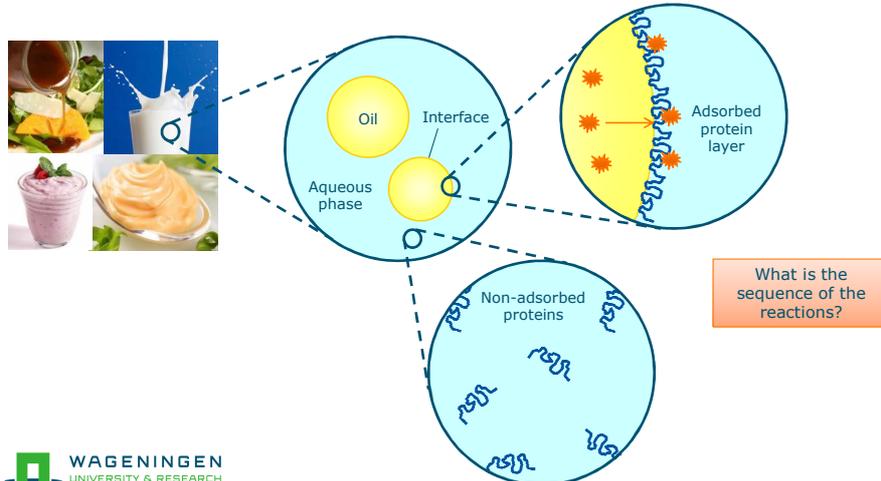
## Research questions

- Consequences of oxidation on the physical properties of emulsions?



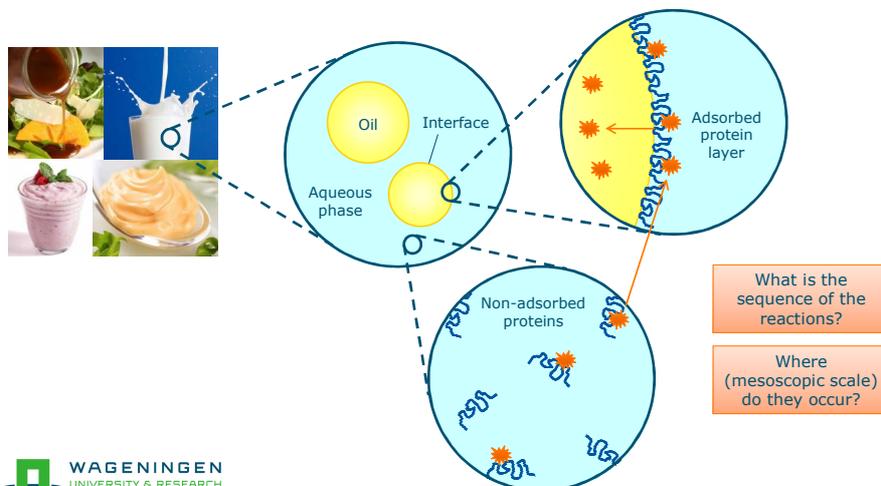
## Research questions

- How are lipid and protein oxidation interrelated?

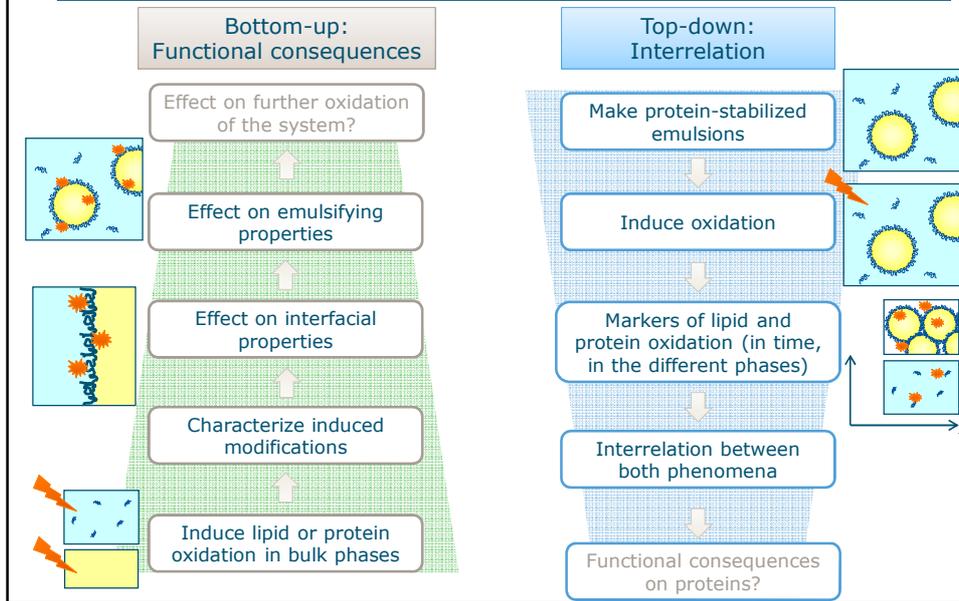


## Research questions

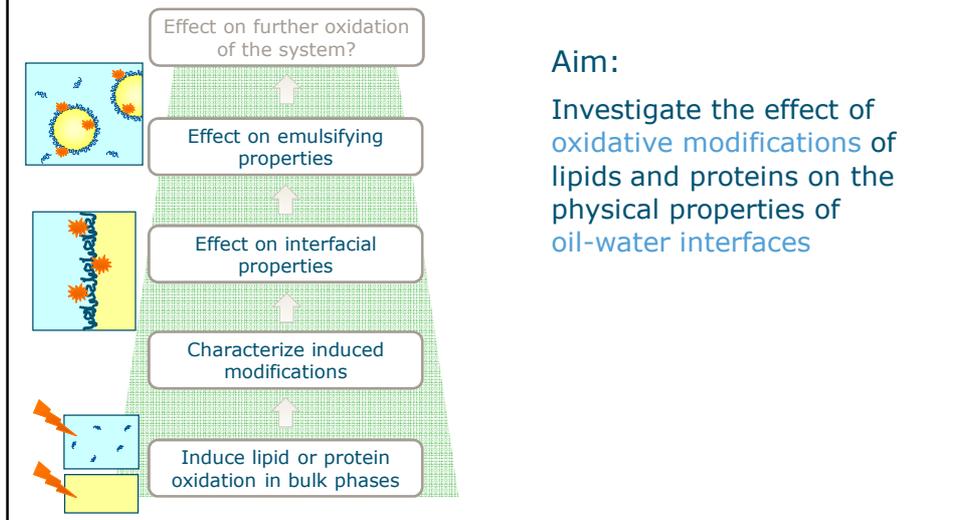
- How are lipid and protein oxidation interrelated?



## Approach: Bottom-up vs top-down

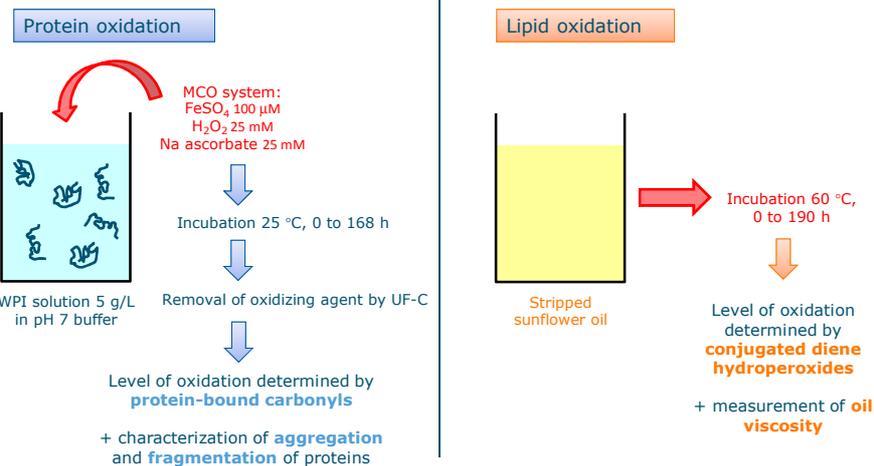


## Functional consequences of lipid and protein oxidation



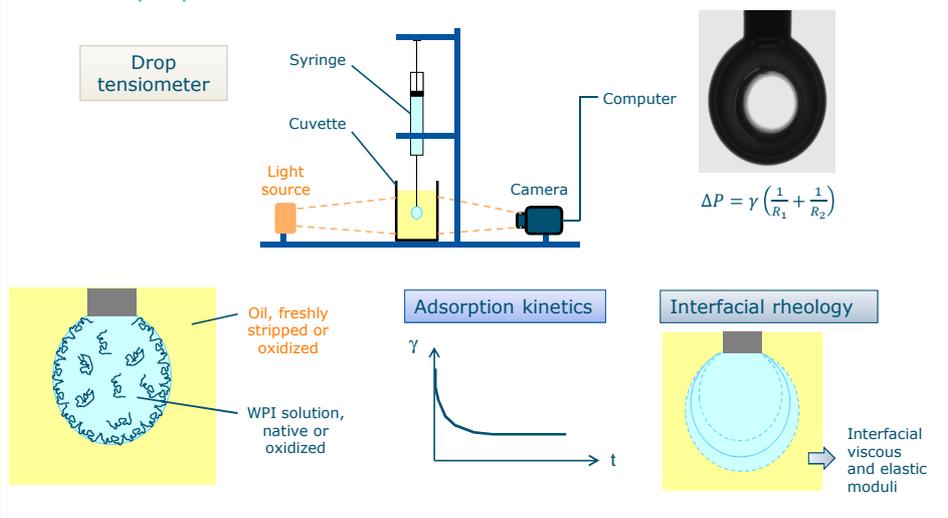
## Experimental

- 1/ Induce oxidation of dietary proteins and lipids, to various and controlled levels



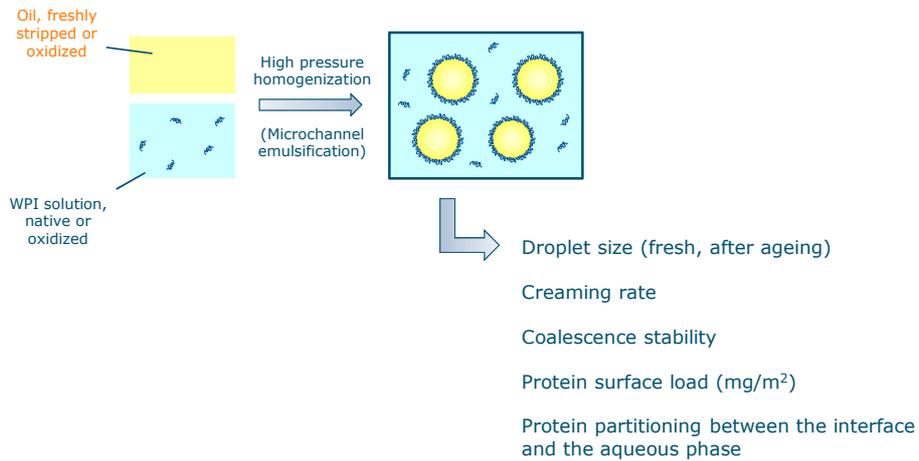
## Experimental

- 2/ Study the effect of these modifications on the formation and properties of the oil-water interface



## Experimental

- 3/ Study the effect of these modifications on the formation and stability of O/W emulsions

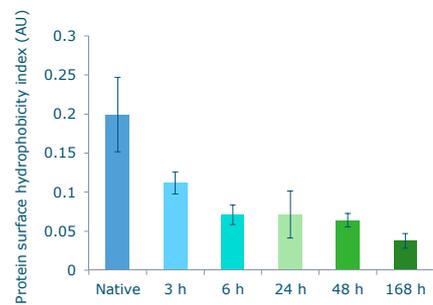
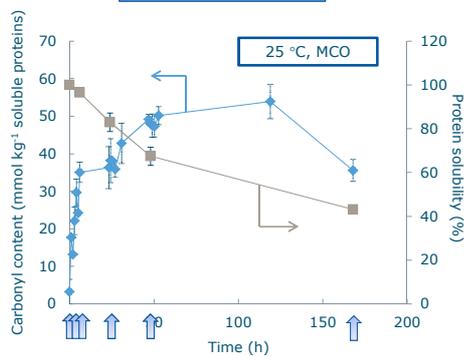


## Characterization of oxidized proteins

Formation of protein-bound carbonyls:

Protein surface hydrophobicity:

Protein solubility:

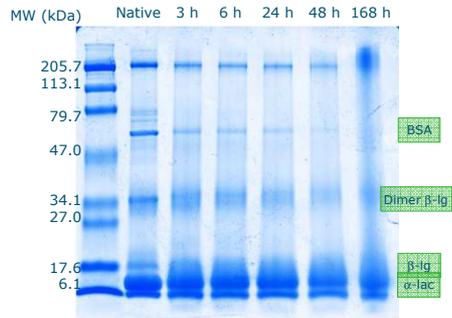


- Rapid increase in carbonyl content
- Loss in solubility

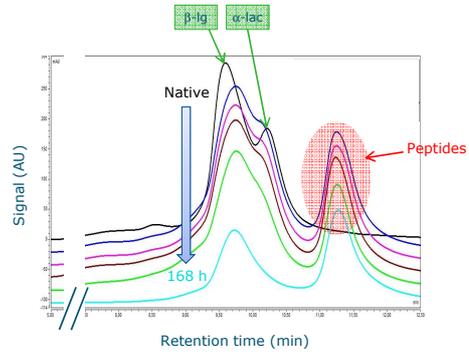
- Loss in protein surface hydrophobicity

## Characterization of oxidized proteins

SDS-PAGE (non-reducing):



HP-SEC:

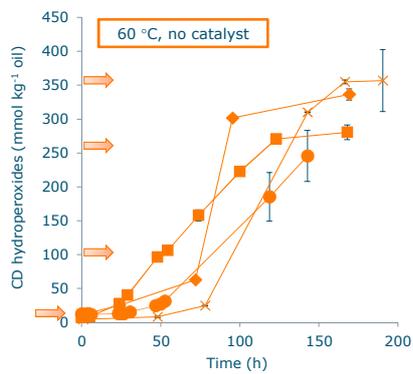


- Protein aggregation
- Protein fragmentation

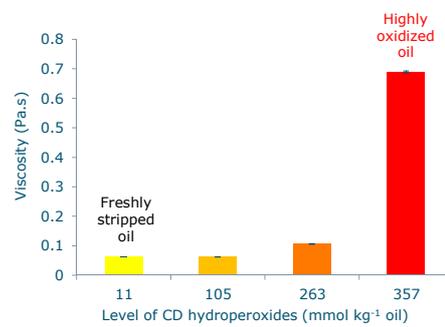


## Characterization of oxidized oil

Conjugated diene hydroperoxides:

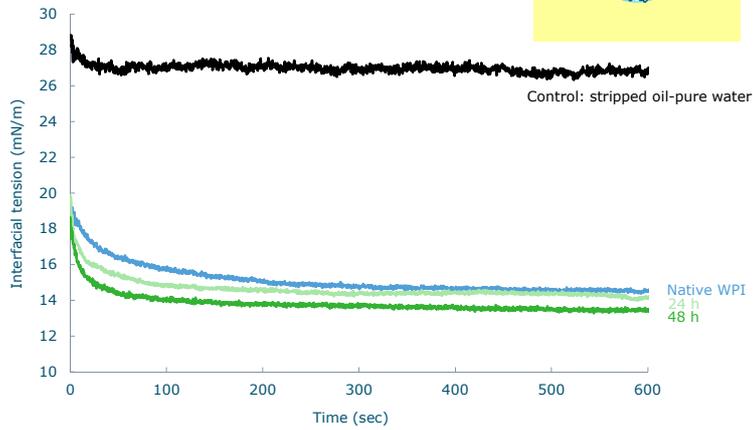
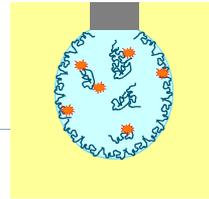


Viscosity:



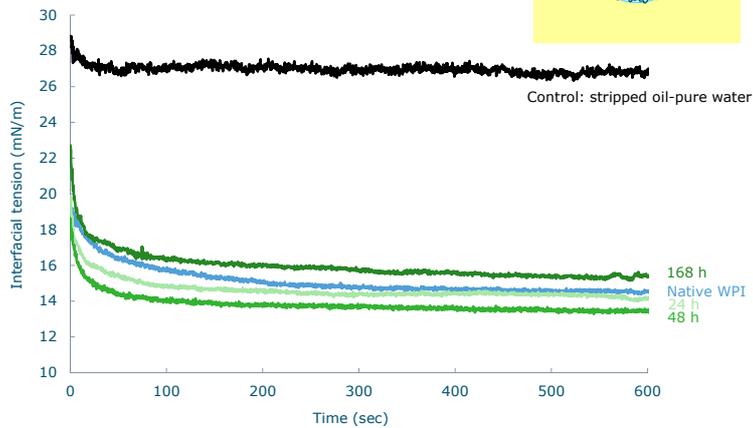
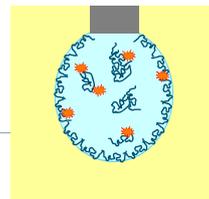
## Adsorption kinetics

Oxidized proteins – Freshly stripped oil



## Adsorption kinetics

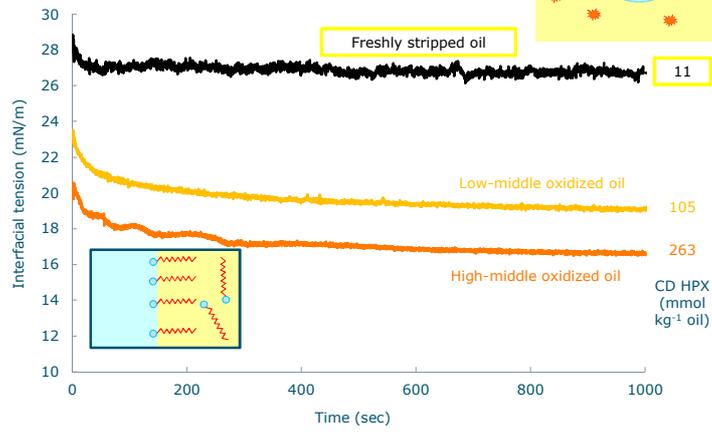
Oxidized proteins – Freshly stripped oil



- Interfacial tension decreases faster and more for oxidized proteins (up to a certain oxidation level)

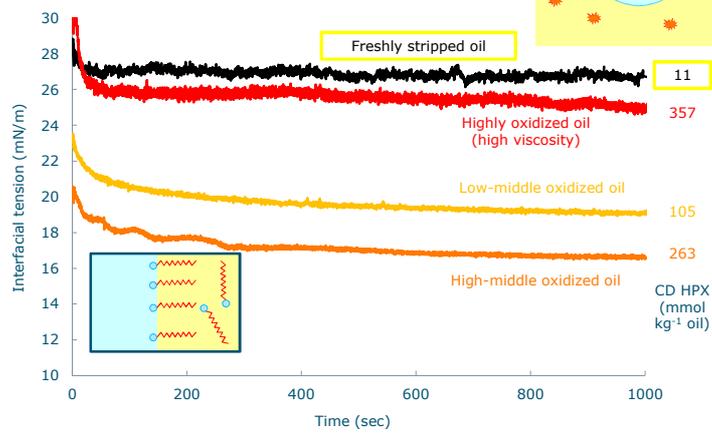
## Adsorption kinetics

Pure water – Oxidized oil



## Adsorption kinetics

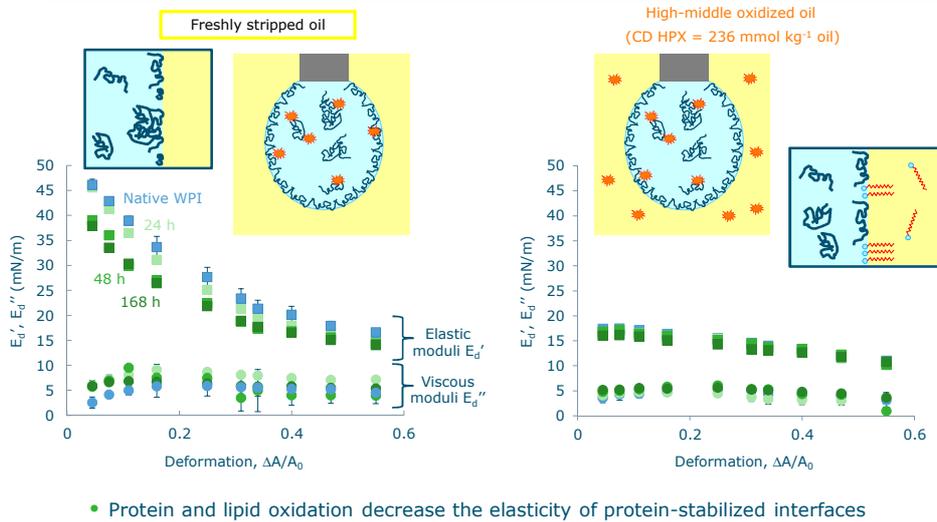
Pure water – Oxidized oil



- Lipid oxidation forms surface-active compounds (up to a certain oxidation level, after which oil viscosity rises and diffusion is restrained)

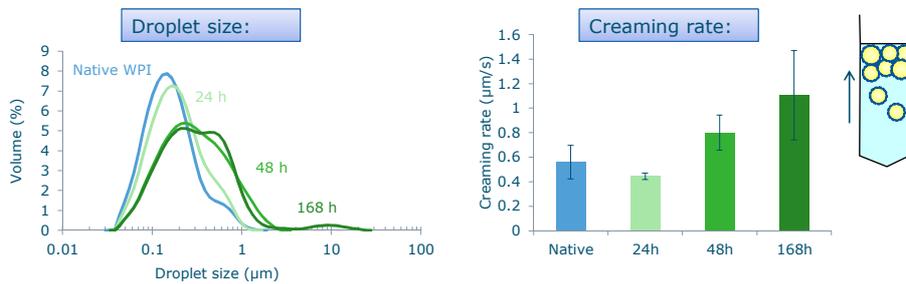
## Interfacial rheology – Amplitude sweeps

Oxidized proteins – Freshly stripped or oxidized oil



## Emulsifying properties

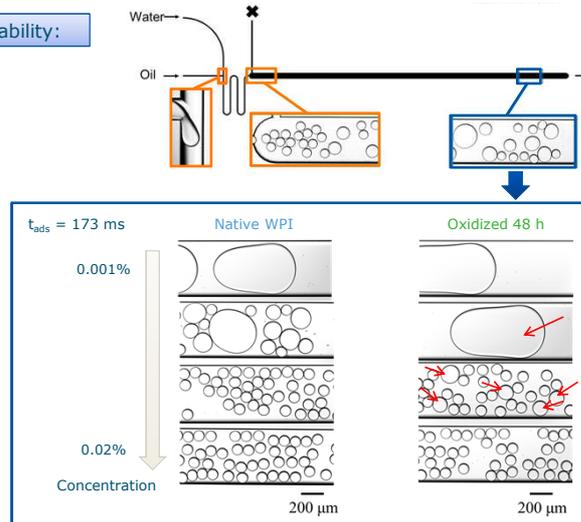
Oxidized proteins – Freshly stripped oil



## Emulsifying properties

Oxidized proteins – hexadecane

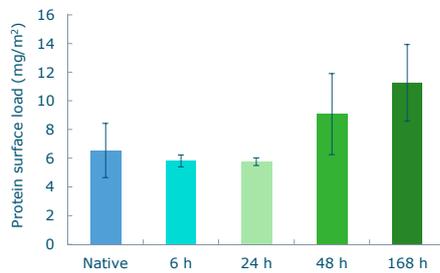
Coalescence stability:



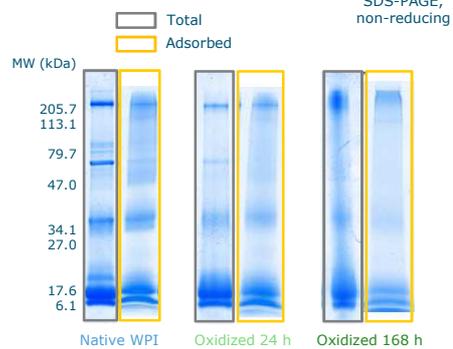
## Emulsifying properties

Oxidized proteins – Freshly stripped oil

Protein surface load :

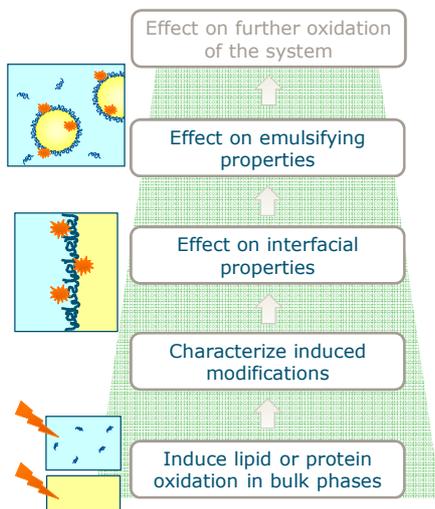


Adsorbed protein profile:



- Larger protein amounts at the interface when protein are (highly) oxidized
- All MWs found at the interface – yet less/no clear bands for high MWs

## Functional consequences of lipid and protein oxidation: Conclusions



- Protein oxidation → fragmentation, aggregation, side-chain modification
- Lipid oxidation → formation of surface-active species
- Both decrease the oil-water interface elasticity
- Protein oxidation promotes physical destabilization of emulsions

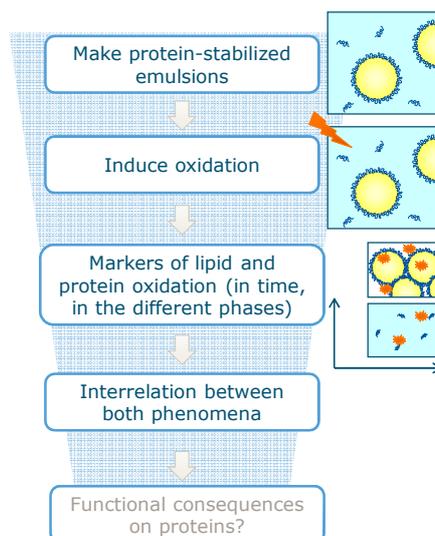
*Berton-Carabin et al., just accepted, Eur. J. Lipid Sci. Technol.*

## Interrelation between lipid and protein oxidation in emulsions

### Aim:

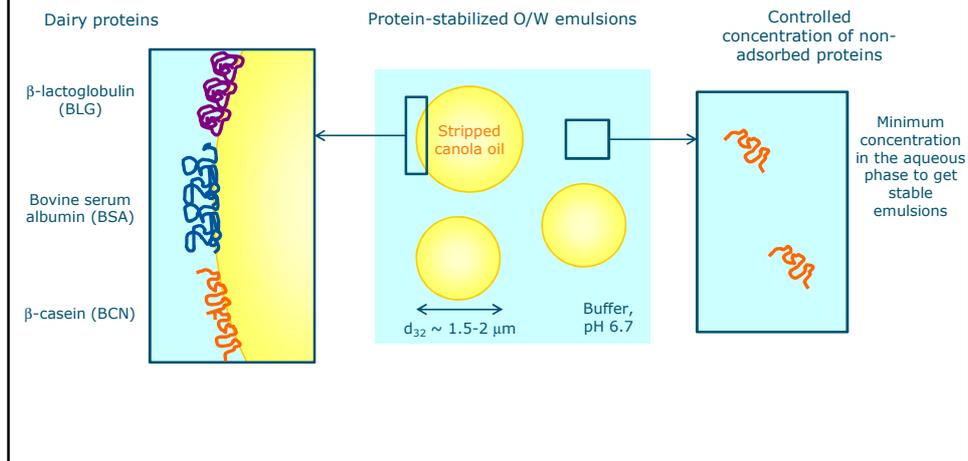
Getting insights on co-oxidation of lipids and proteins in O/W emulsions, regarding:

- Time (the sequence of the reactions)
- Location (where reactions occur)



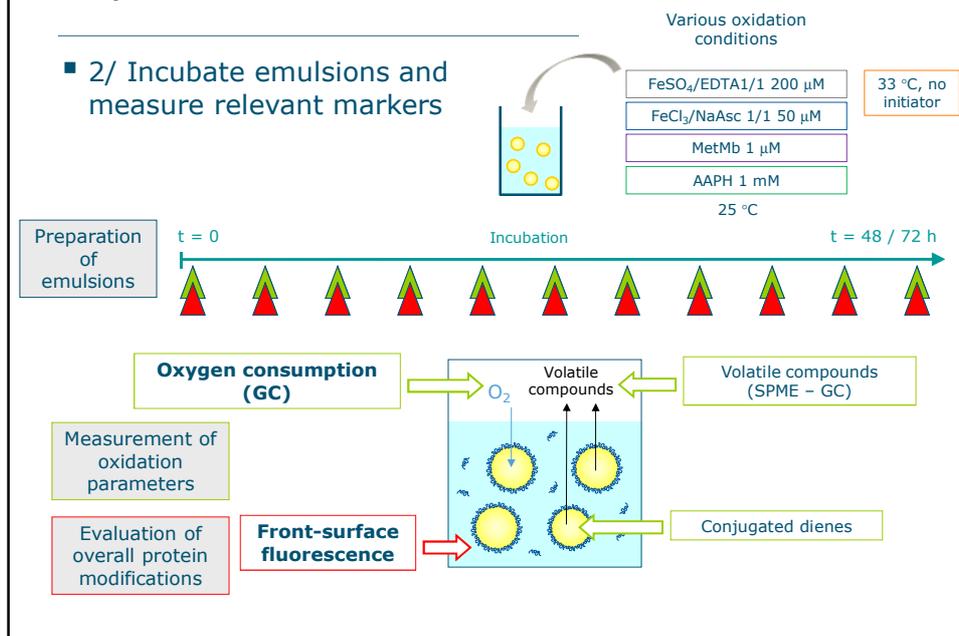
## Experimental

### 1/ Prepare protein-stabilized emulsions

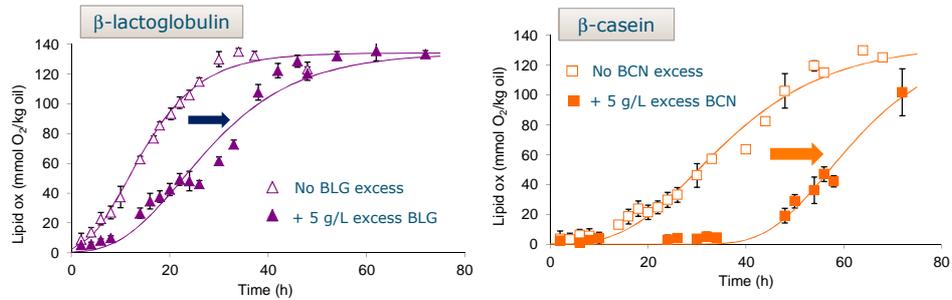


## Experimental

### 2/ Incubate emulsions and measure relevant markers



## Effect of aqueous phase proteins on lipid oxidation



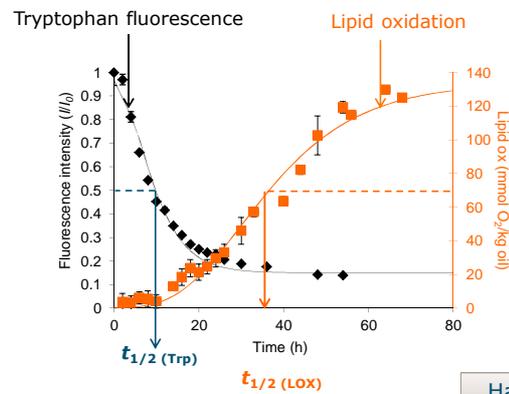
- Non-adsorbed (excess) proteins have a protective effect against lipid oxidation in emulsions

Berton et al., 2011 J. Colloid Interface Sci.  
Berton et al., 2011 J. Agric. Food Chem.

## Phasing of lipid oxidation and protein modifications

Ex: BCN-stabilized emulsion

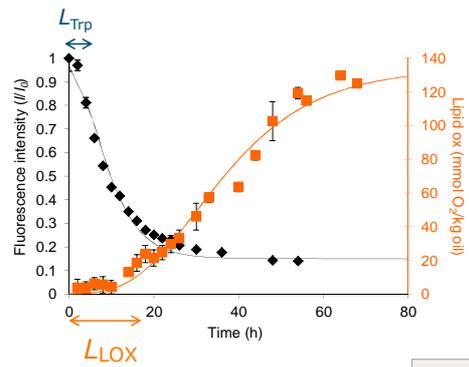
FeSO<sub>4</sub>/EDTA 1/1 200 μM 25 °C



## Phasing of lipid oxidation and protein modifications

Ex: BCN-stabilized emulsion

FeSO<sub>4</sub>/EDTA 1/1 200 μM 25 °C

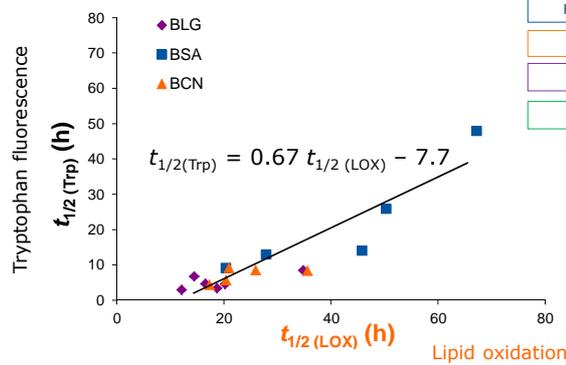


Lag-times of reactions



## Phasing of lipid oxidation and protein modifications

Half-times of reactions

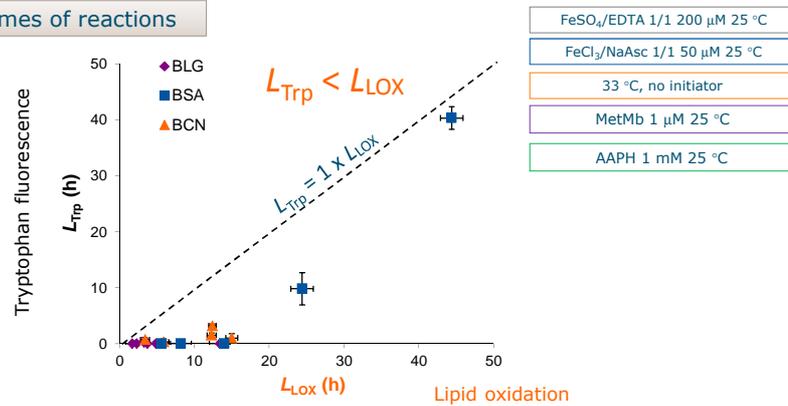


- Protein modifications and lipid oxidation are time-linked phenomena

Berton et al., 2012 J. Agric. Food Chem.  
Genot et al., 2013 Lipid Ox.: Challenges Food Syst.

## Phasing of lipid oxidation and protein modifications

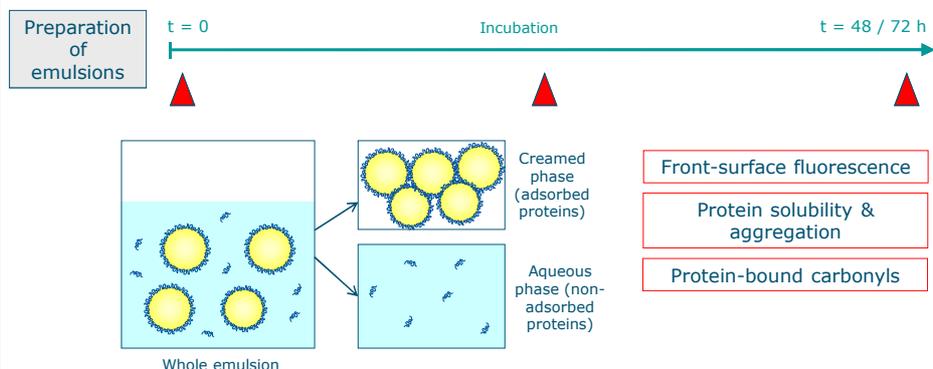
Lag times of reactions



- Protein modifications precede lipid oxidation in protein-stabilized emulsions

Berton et al., 2012 J. Agric. Food Chem.  
Genot et al., 2013 Lipid Ox.: Challenges Food Syst.

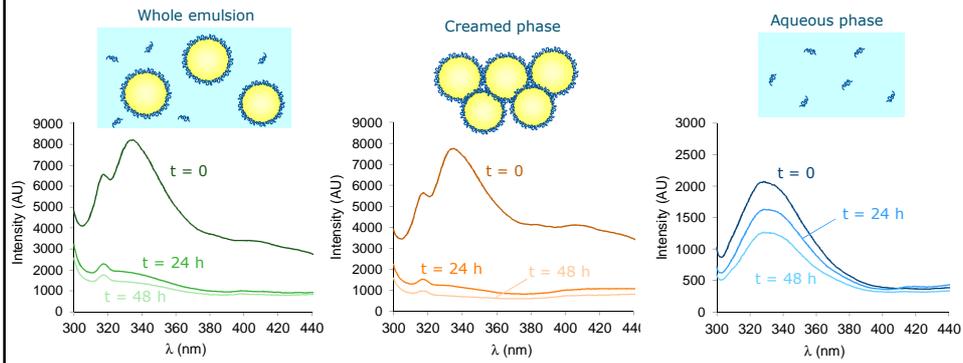
## Further characterization of protein oxidation



## Location of protein modifications

Comparison of Trp fluorescence quenching in adsorbed vs non-adsorbed proteins:

BLG-stabilized emulsion FeSO<sub>4</sub>/EDTA 1/1 200 μM 25 °C



- Adsorbed proteins undergo extensive modifications

*Berton et al., 2012 J. Agric. Food Chem.*  
*Genot et al., 2013 Lipid Ox.: Challenges Food Syst.*

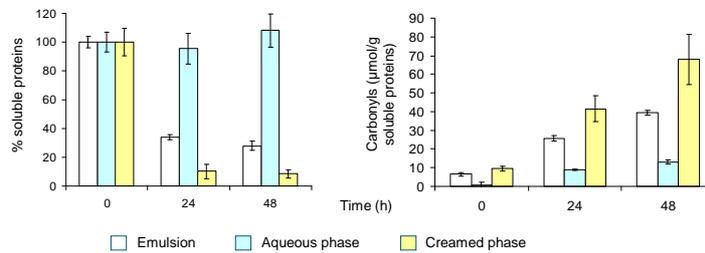
## Adsorbed proteins undergo extensive oxidation

Protein solubility in GuCl 6 M:

Protein-bound carbonyls (soluble proteins):

BLG-stabilized emulsion

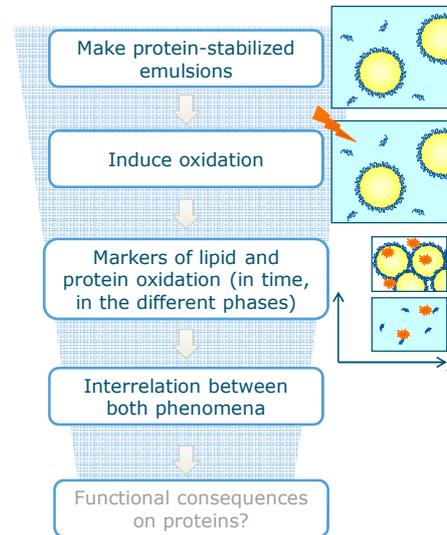
FeSO<sub>4</sub>/EDTA 1/1  
200 μM 25 °C



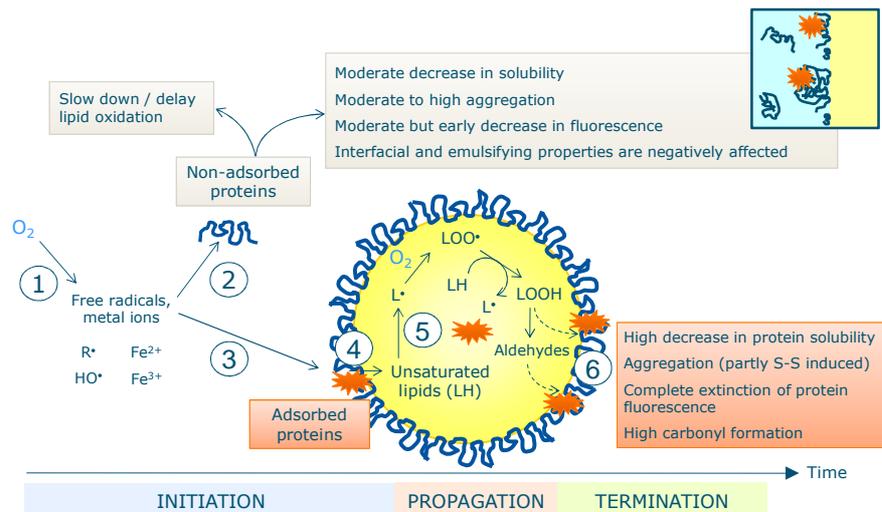
*Berton et al., 2012 J. Agric. Food Chem.*  
*Genot et al., 2013 Lipid Ox.: Challenges Food Syst.*

## Interrelation between lipid and protein oxidation in emulsions: Conclusions

- Protein modifications start prior to lipid oxidation
- Both reactions are timely linked
- Adsorbed proteins undergo extensive oxidation



## Towards a comprehensive scheme?



Thank you!

**WUR:**

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Ivanna Colijn

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