Lipid oxidation and related protein modifications in oil-in-water emulsions

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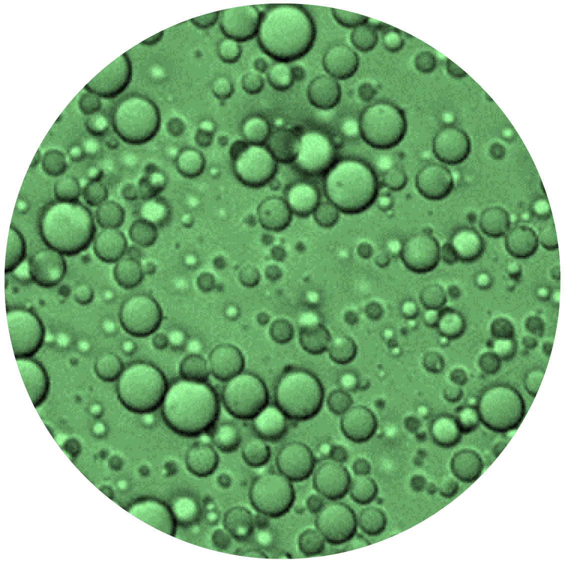

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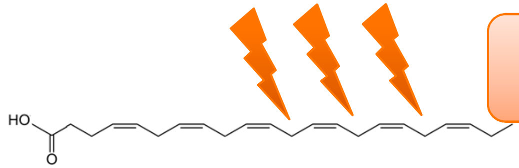
Context of the study

- Lipid oxidation: Major cause of degradation of food products containing unsaturated fatty acids



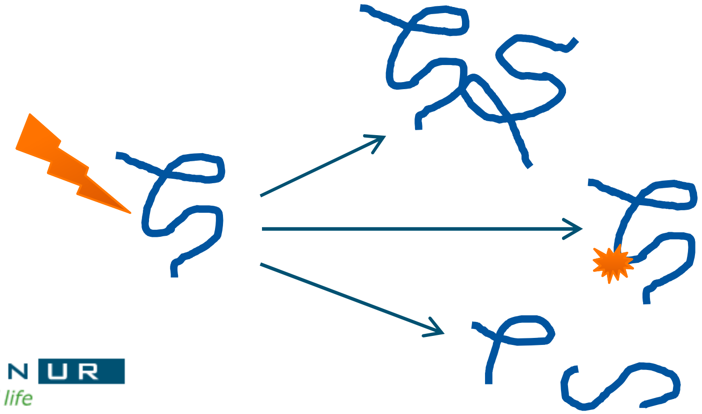
Degradation of sensory quality

Degradation of nutritional quality

Degradation of health-value

- In complex food products, oxidation targets other components than UFA

e.g., protein oxidation



Degradation of nutritional quality

Degradation of functional properties

Degradation of sensory quality

Schaich 2008. in. Lipid Oxidation Pathways. AOCS Press.

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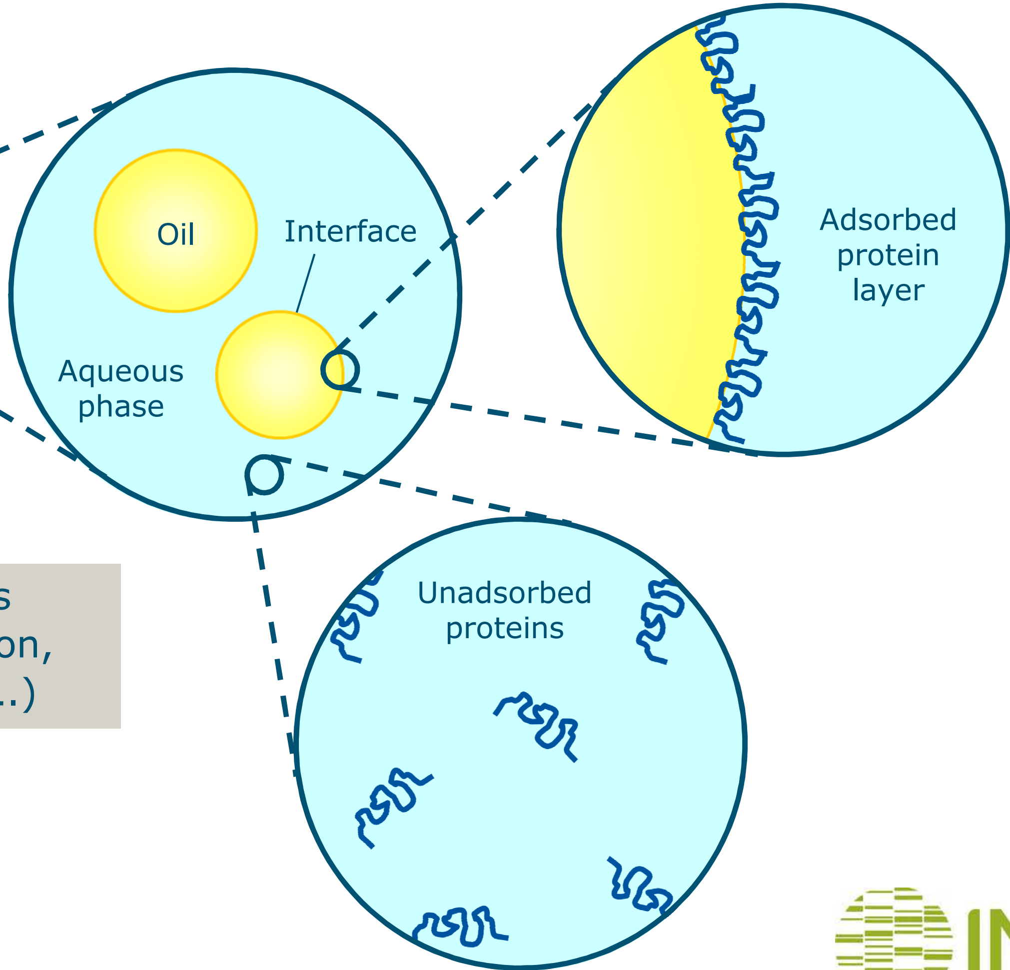
Context of the study

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



Control of various parameters (*i.e.* oil phase, oil volume fraction, emulsifiers, droplet size, pH, ….)

Waraho et al 2011 Trends Food Sci Technol 22:3-13.

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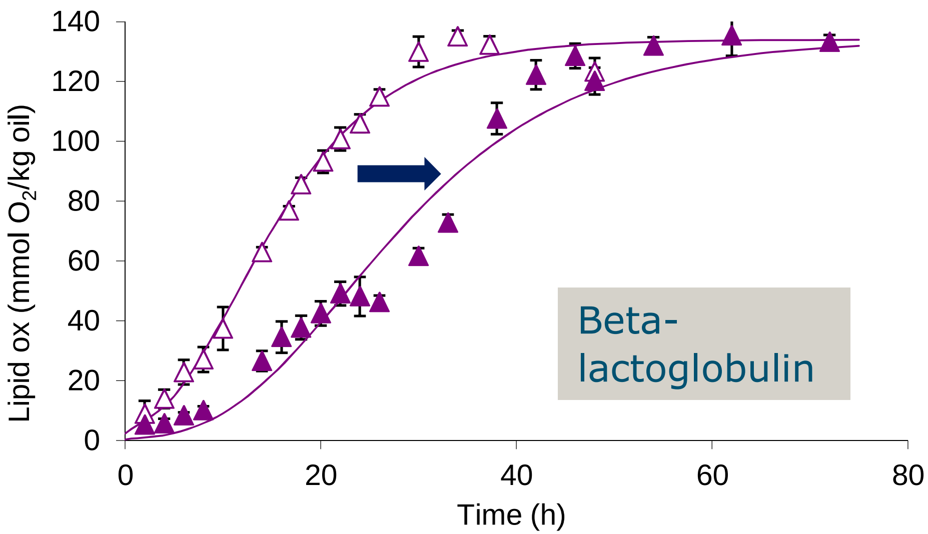
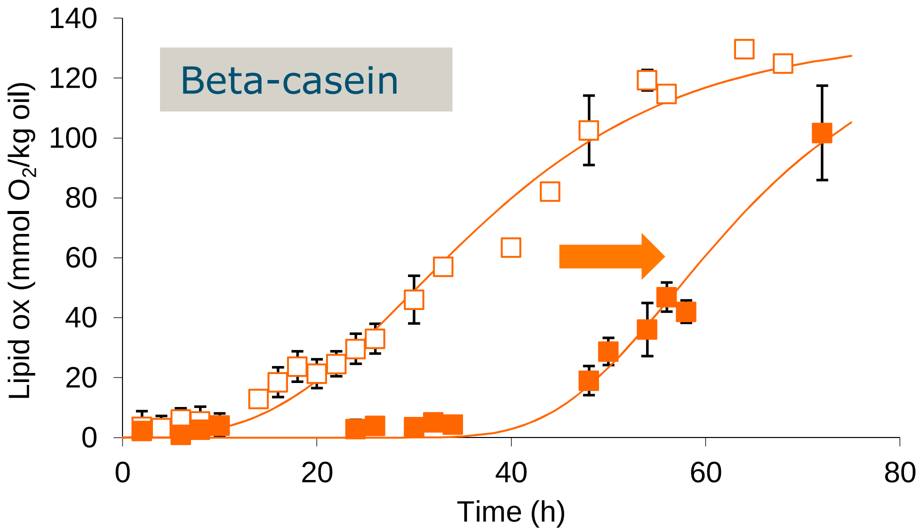
Context of the study: oxidation in O/W emulsions

Protective effect of effect of excess proteins in the aqueous phase





	% adsorbed BLG	[aqueous phase BLG] (g/L)
△ Regular BLG-stabilized emulsion	70%	1.5
▲ BLG-stabilized emulsion + excess BLG	39%	6.1

	% adsorbed BCN	[aqueous phase BCN] (g/L)
□ Regular BCN-stabilized emulsion	91%	0.45
■ BCN-stabilized emulsion + excess BCN	64%	3.6

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

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Context of the study: oxidation in O/W emulsions

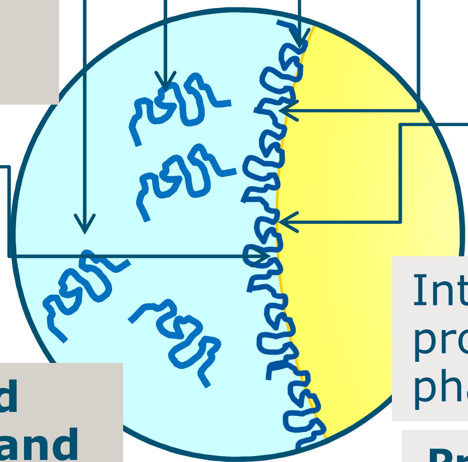
Mechanisms possibly involved in the protective effects of protein emulsifiers

- Non-covalent binding (metal ions, hydroperoxides, …)
- Scavenging of free radicals; reducing properties (SH, …)
- Covalent binding (reaction products

Higher interface thickness and cohesiveness ?

Locating antioxidants at interface

Repelling/attracting metal ions depending on pH ?



Oxidative reactions and interactions of adsorbed and unadsorbed proteins before and during oxidation ?

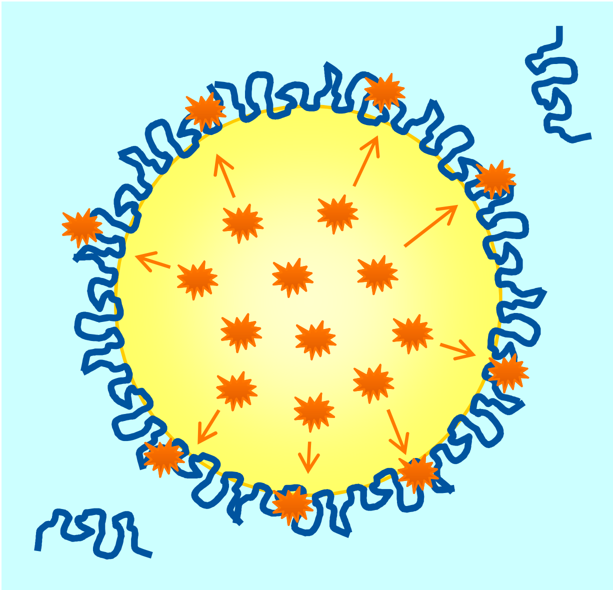
Interface stabilised by only proteins do not protect the lipid phase against oxidation

Protein-stabilized O/W interfaces are more heterogeneous than surfactant stabilized interfaces

Berton et al., 2012 J. Colloid Interface Sci.
Berton et al., 2012 Food Chem

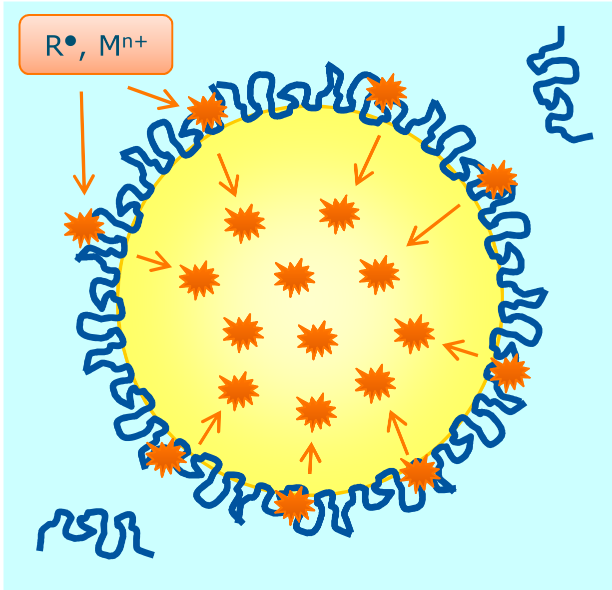
Context of the study

- In multiphase systems, which of both reactions (lipid *vs* protein oxidation) first starts?



Hyp 1: Lipid oxidation starts, then lipid radicals / lipid oxidation products (*e.g.*, aldehydes) attack interfacial proteins

Gardner, 1979 J. Agric. Food Chem.
Karel et al., 1975 J. Agric. Food Chem.
Dalsgaard et al., 2010 Dairy Sci. Technol.



Hyp 2: Interfacial proteins get oxidized, then the reaction propagates to the inner lipids

Ostdal et al., 2002 Free Radic. Biol. Med.
Salminen et al., 2010 J. Am. Oil Chem. Soc.

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Aim of the study

- Getting new insights on the chemical co-modifications (oxidation) of lipids and proteins in O/W emulsion systems...

... Regarding TIME
(the sequence of the reactions)

... Regarding LOCATION
(where – at a mesoscopic scale – reactions occur)

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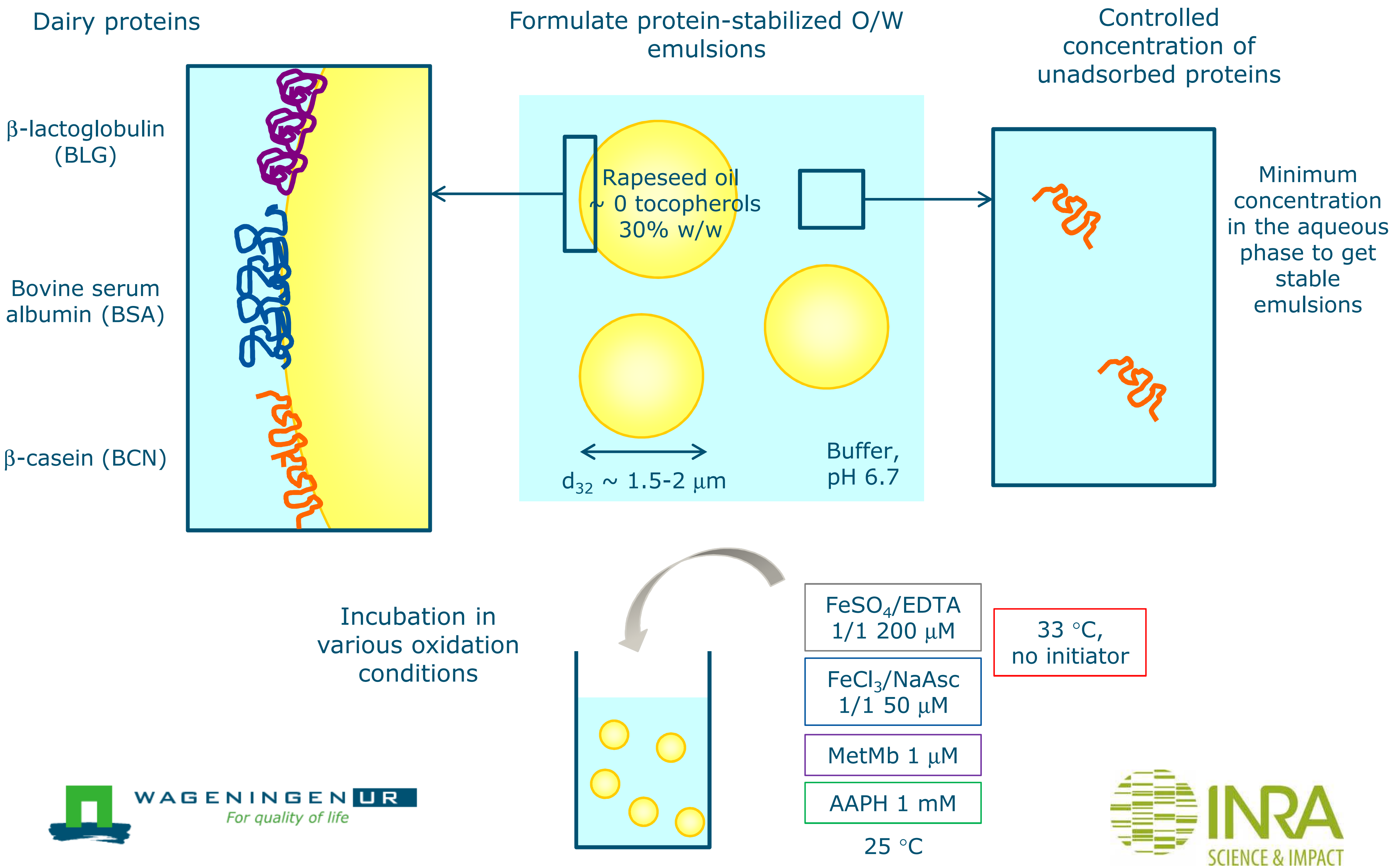
Experimental approach: 1. emulsion design



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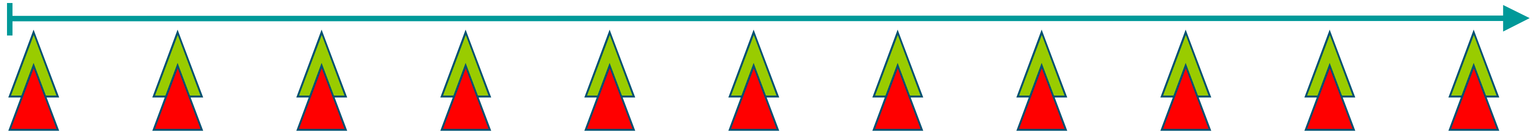
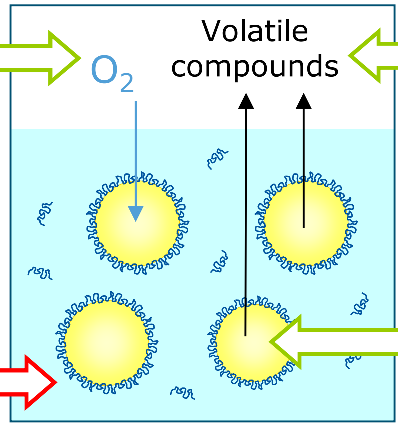
Experimental approach: 2. kinetics

Preparation of emulsions



Oxygen consumption (GC)

Volatile compounds (SPME – GC)



Measurement of oxidation parameters

Conjugated dienes

Evaluation of overall protein modifications

Front-surface fluorescence

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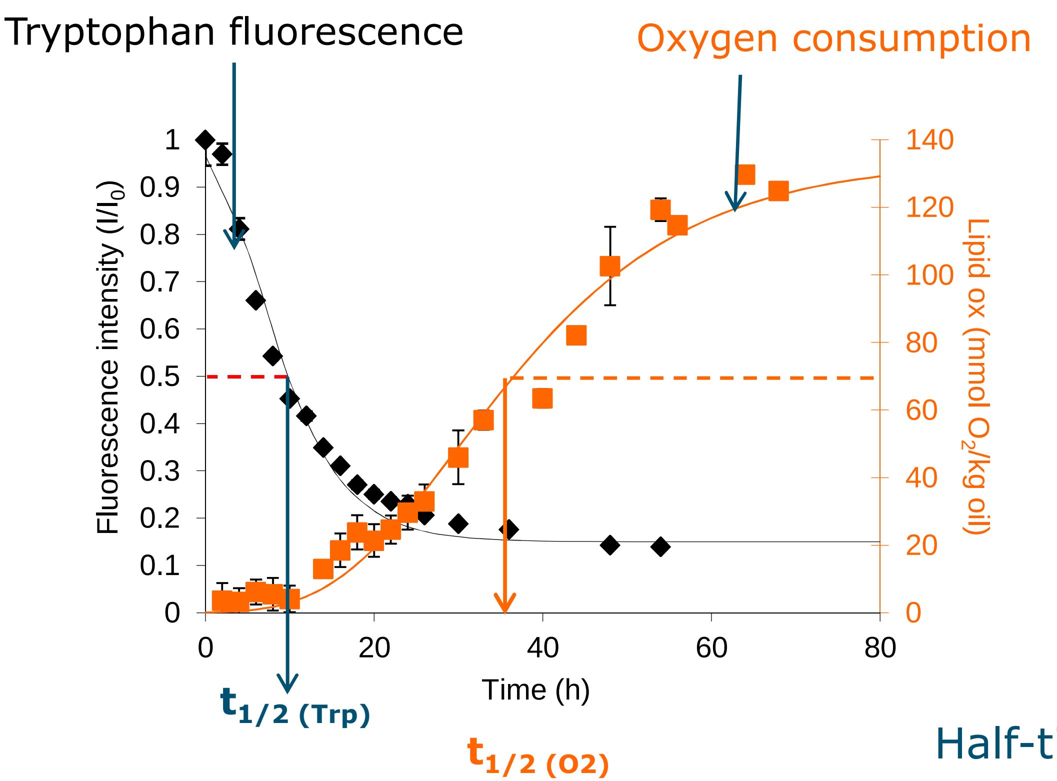
Results

1. Phasing of lipid oxidation and protein modifications

Ex : BCN-stabilized emulsion

$FeSO_4$/EDTA 1/1 200 µM 25 °C



Half-times of reactions

Berton et al., 2011 J. Agric. Food Chem.
Berton et al., 2012 Food Chem.

Results

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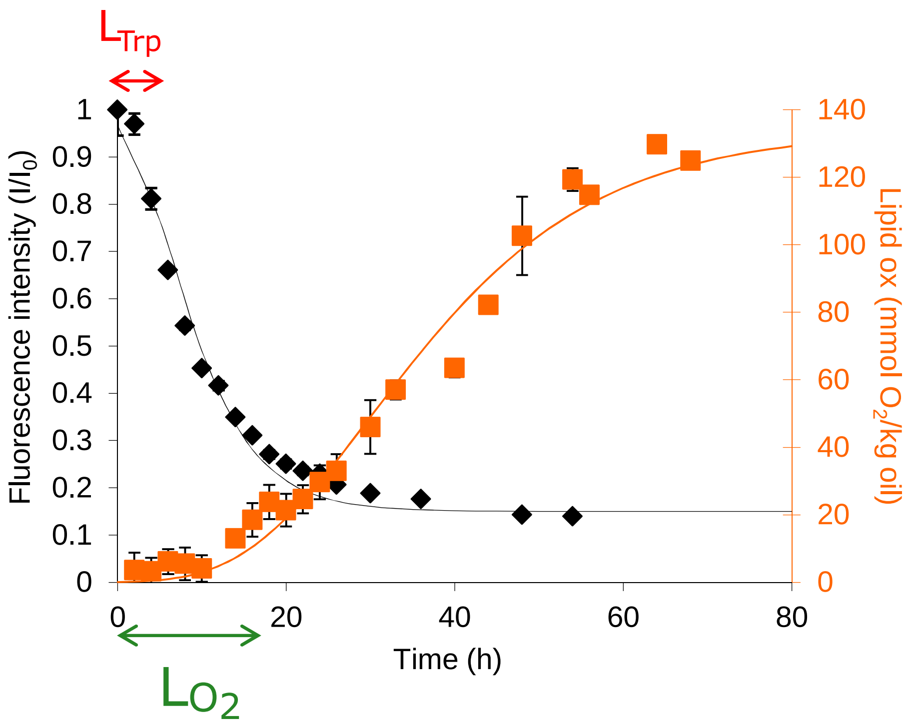

Lag-times of reactions

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

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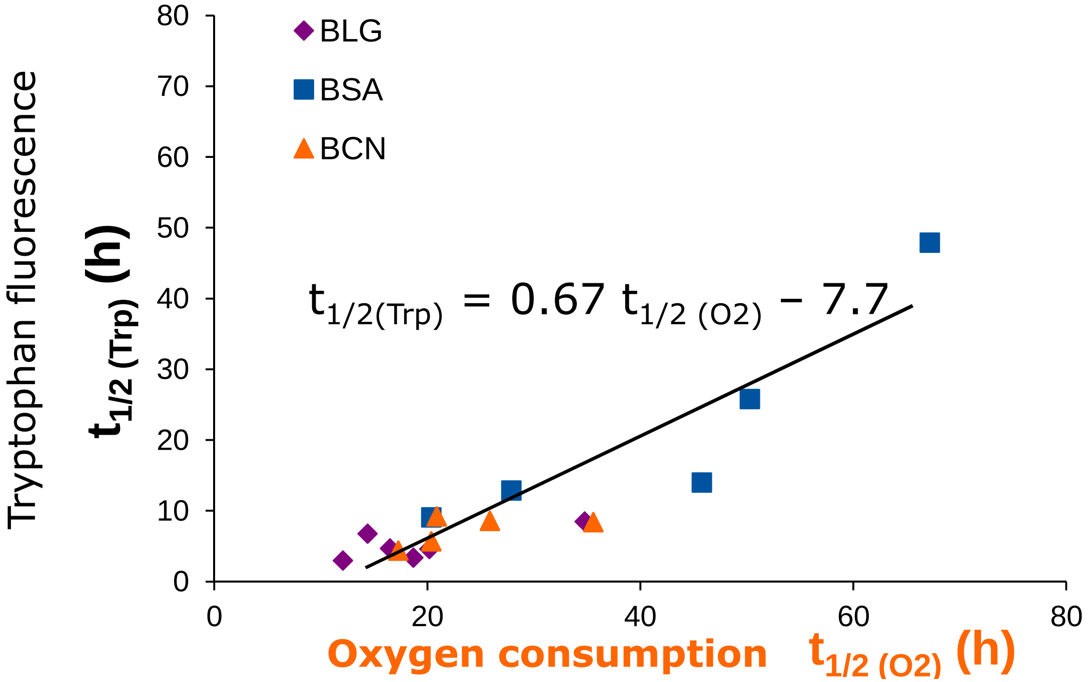
Results

1. Phasing of lipid oxidation and protein modifications

Half-times of reactions



$FeSO_4$/EDTA 1/1 200 µM 25 °C

$FeCl_3$/NaAsc 1/1 50 µM 25 °C

33 °C, no initiator

MetMb 1 µM 25 °C

AAPH 1 mM 25 °C

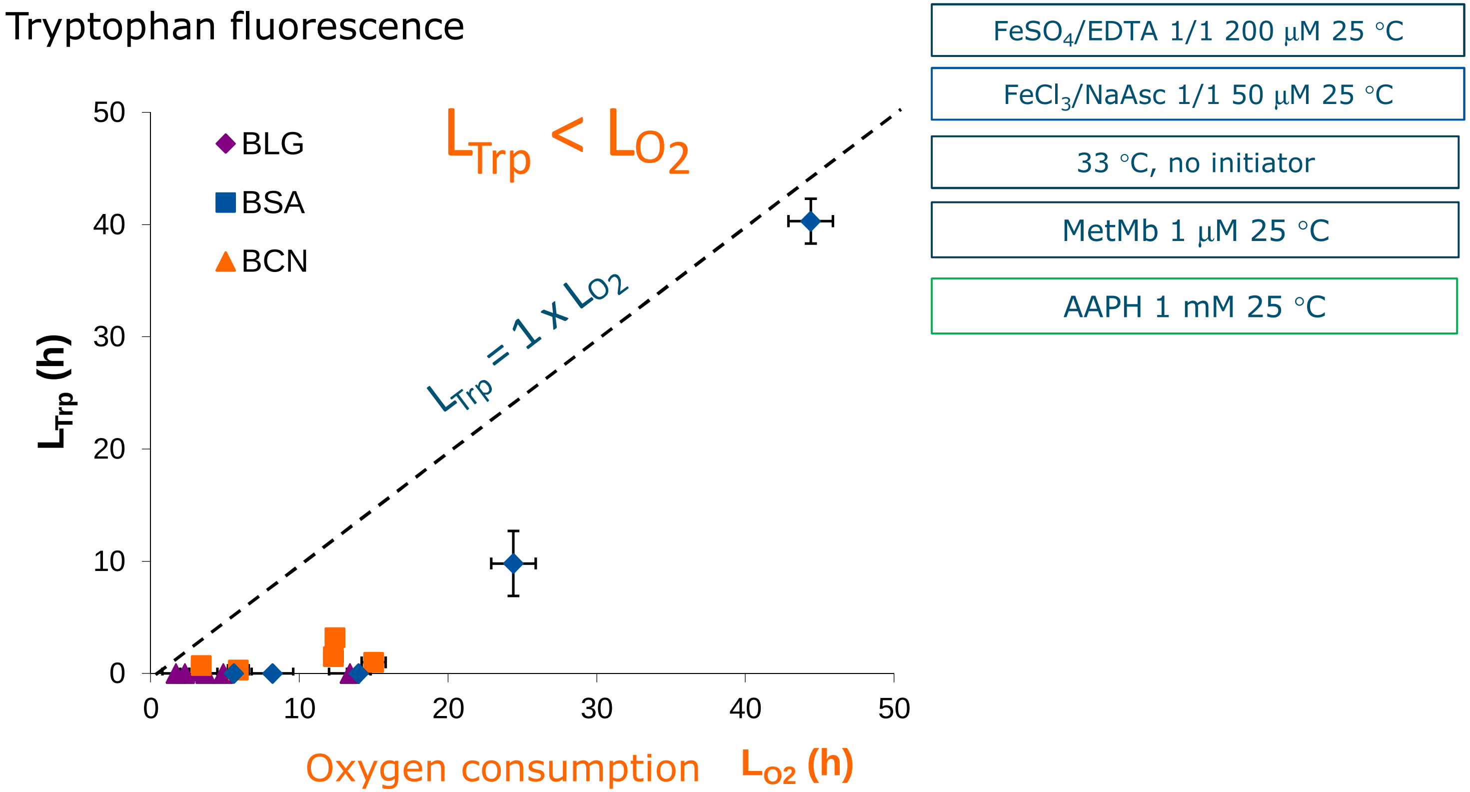
Protein modifications and lipid oxidation are time-linked phenomena

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

Results

1. Phasing of lipid oxidation and protein modifications

Lag-times of reactions



Protein modifications precede lipid oxidation in protein-stabilized emulsions

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

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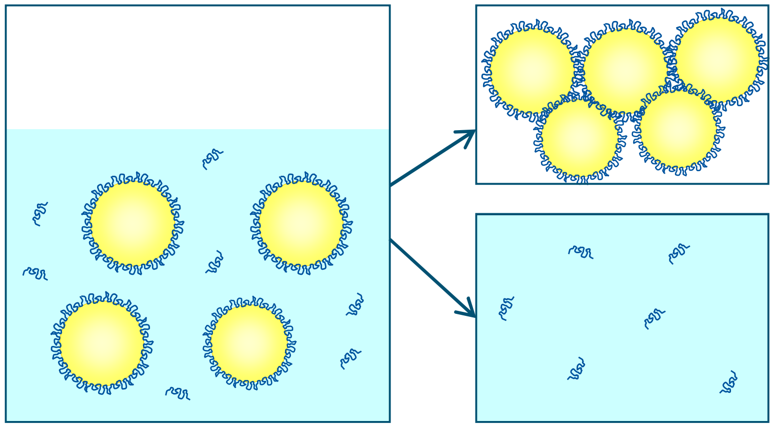
Experimental approach: 3. location

Evaluation of protein oxidation

Preparation of emulsions

t = 0 — Incubation — t = 48 / 72 h



Creamed phase (adsorbed proteins)

Aqueous phase (unadsorbed proteins)

Whole emulsion

- Front-surface fluorescence
- Protein solubility & aggregation
- Protein-bound carbonyls

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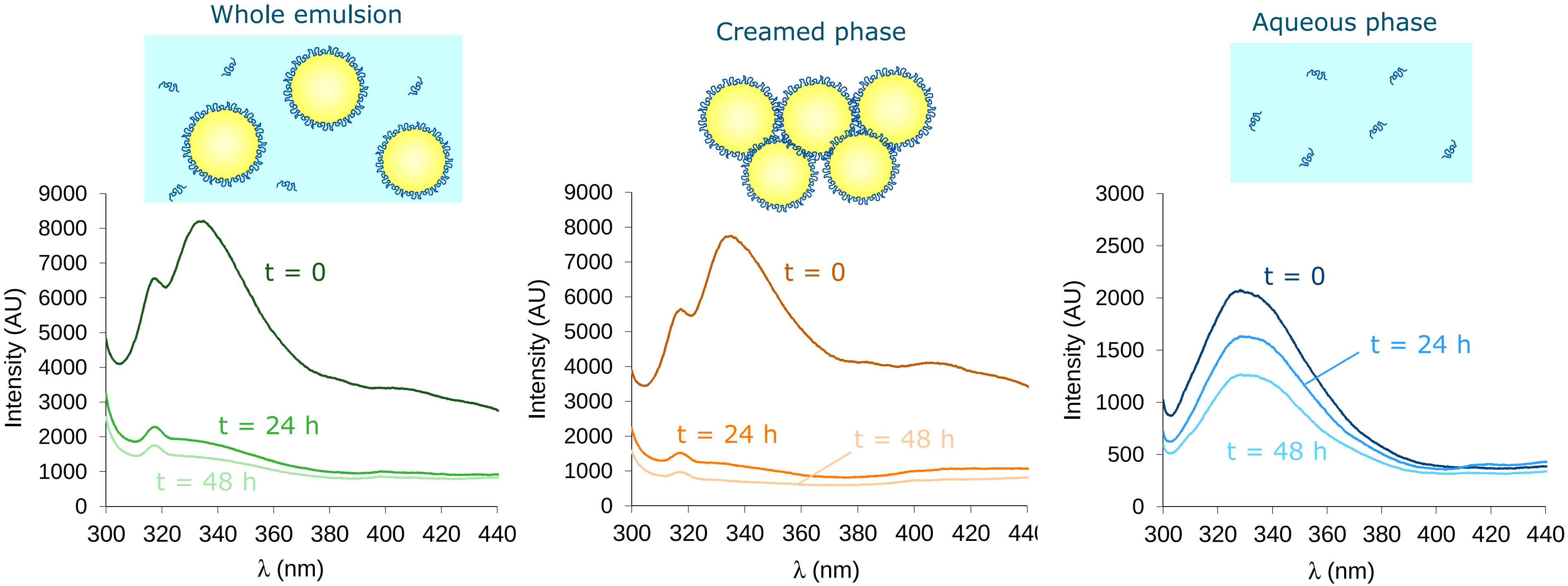
Results
2. Location of protein modifications

Comparison of Trp fluorescence quenching in adsorbed *vs* unadsorbed proteins:

BLG-stabilized emulsion | $FeSO_4$/EDTA 1/1 200 µM 25 °C



Adsorbed proteins undergo extensive modification

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

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Results

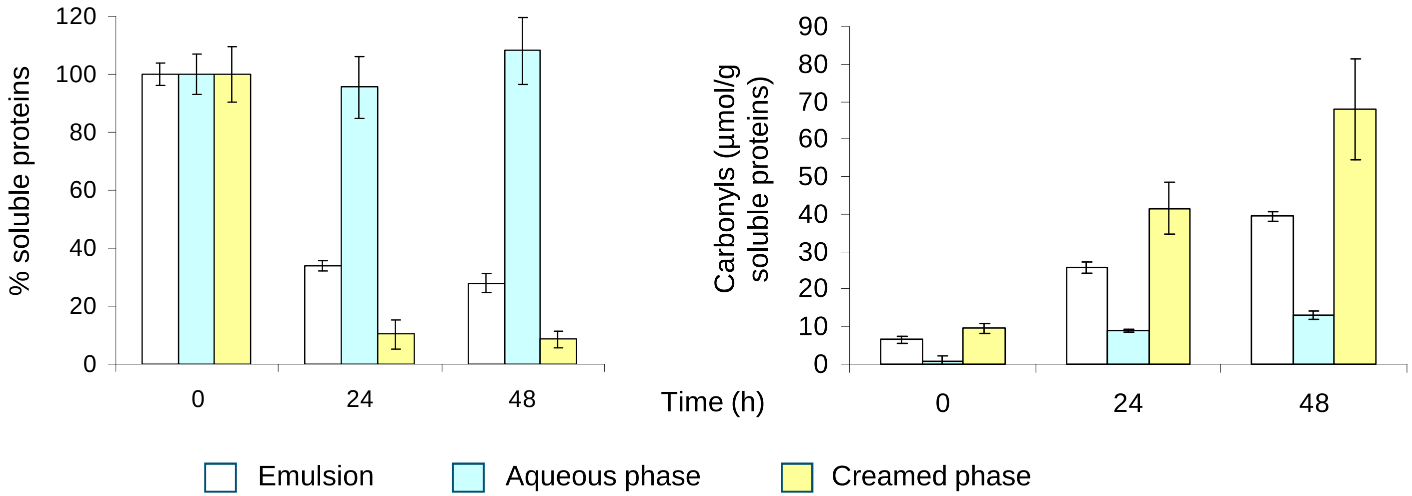
3. Adsorbed proteins undergo extensive oxidation

BLG-stabilized emulsion

FeSO$_4$/EDTA 1/1
200 μM 25 °C

Protein solubility in GuCl 6 M:

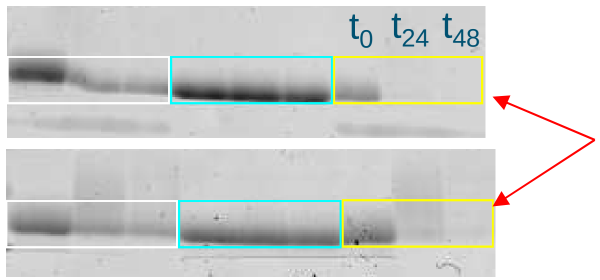
Protein-bound carbonyls in the soluble proteins:



SDS-PAGE:

Non-reducing conditions

Reducing conditions → S-S reduction



Non-soluble proteins?

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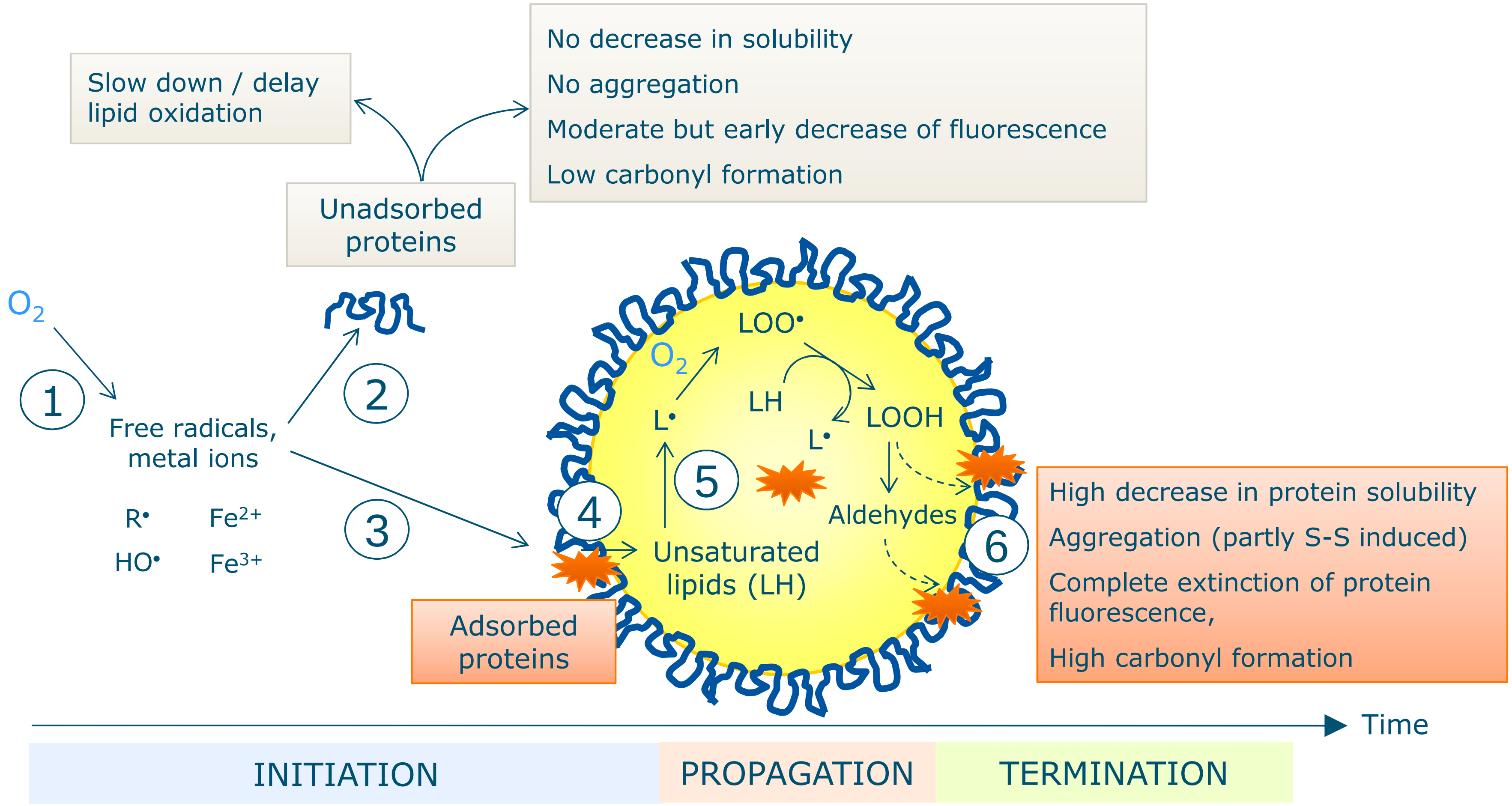
Conclusion

Towards a comprehensive scheme?



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Genot et al., 2013 Lipid Ox.: Challenges Food Syst.

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Further work… *to do list*

- Analyse and characterize the modifications of the unsoluble proteins
- Understand the mechanisms underlaying Trp fluorescence decrease
- Characterize the modifications of the proteins (nature & extent; preferred locations of modified amino acids; adsorbed *vs* unadsorbed protein)
- More rapid kinetic approach: characterize the first steps of unadsorbed and adsorbed protein modifications in relation to the initial steps of lipid oxidation
- Integrated chemical/mathematical models taking account the location of the reactants, their dynamics and complex / imbricated reaction schemes
- Effects on protein digestibility ; health effects ?

Keeping realistic food models or real foods

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Thank you for your attention

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Région
PAYS DE LA LOIRE

pH-D funding of CB

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