Euro Fed Lipid

european federation for the science and technology of lipids

3rd International Symposium on Lipid Oxidation and Antioxidants

eBook of Abstracts

23 – 24 November 2020

Web Meeting

Introduction

We would like to invite you to the 3rd International Symposium on Lipid Oxidation and Antioxidants (ISOLA) as a virtual meeting on November 23-24, 2020, organized by Euro Fed Lipid.

As you probably know, the meeting was originally planned to be held in Vigo, Spain, from 16-18 June 2018. Unfortunately, we have had to postpone it to 2022 because of the coronavirus crisis. Science is important, but, certainly, it is much more important to keep ourselves healthy.

The coronavirus crisis make us develop new academic skills and learn new technological techniques to communicate between us, with students and co-workers. These communication technologies can be employed to provide a “virtual” appropriate environment for showing the progress of our research, exchange ideas, establishing new collaborations, and try to solve common problems and challenges.

Bearing in mind this positive side, we have decided to fill the gap created by the coronavirus crises and organize a virtual meeting on November this year. Sure that it will not be the same as that previously organized – nothing can change a nice face to face talk enjoying the view of the sea – but will help us to keep in close contact creating a great opportunity for discussion of theoretical and practical aspects of both lipid oxidation and its control in different environments and discuss about new ideas to develop during the 2022 meeting at Vigo.

Topics to be covered during the next virtual meeting will be mostly the same as before, including among others, the following:

- Antioxidant and lipid oxidation evaluation methods
- Elucidation of lipid oxidation and antioxidant mechanisms
- Lipid oxidation in multiphase and complex systems
- Control of lipid oxidation
- Protein and lipid co-oxidation
- Lipid oxidation and antioxidants in nutrition and disease

We look forward to welcoming you to this virtual but exciting 3rd International Symposium on Lipid Oxidation and Antioxidants from 23-24 November 2020.

Carlos Bravo-Díaz	Fatima Paiva-Martins	Charlotte Jacobsen	Karin Schwarz
University Vigo	University Porto	Technical Uni. Denmark	University Kiel

Scientific Programme

Monday, 23 November 2020

09.30 a.m. Opening –Welcome

Session 1 - Proteins and Lipid Oxidation

Chair: Carlos Bravo + Charlotte Jacobsen

09.45 a.m. **Protein and Lipid Oxidation in Pressurized Meat: A Possible Link?**
C. Guyon, Nantes/FR, A. Meynier, Nantes/FR, M. de Lamballerie, Nantes/FR

10.00 a.m. **Influence of Oregano or Beer Addition on Lipid and Protein Oxidation during Cooking and Digestion of Chicken Meat**
M.M.C. Sobral, Porto/PT, S. Casal, Porto/PT, M.A. Faria, Porto/PT, S.C. Cunha, Porto/PT, I. M. P. L. V. O. Ferreira, Porto/PT

10.15 a.m. **Quantitative Spatiotemporal Mapping of Lipid and Protein Oxidation in Mayonnaise**
S. Yang, Wageningen/NL , A.A. Verhoeff, Wageningen/NL, D.M. Merkx, Wageningen/NL, J.v. Duynhoven, Wageningen/NL, J. Hohlbein, Wageningen/NL

10.30 a.m. **Identifying Peptides Derived from Seaweed, Single Cell and Potato Protein with Emulsifying Properties and Investigating their Functionality in 5% Fish Oil-in-Water Emulsions**
B. Yesiltas, Kgs. Lyngby/DK , P.J. García-Moreno, Granada/ES, A.M. Soria Caindec, Kgs. Lyngby/DK, L. Lægsgaard, Kgs. Lyngby/DK, M.L. Brinch, Kgs. Lyngby/DK, P. Marcatili, Kgs. Lyngby/DK, T.H. Olsen, Kgs. Lyngby/DK, S. Gregersen, Aalborg/DK, C. Jacobsen, Kgs. Lyngby/DK

10.45 a.m. Coffee Break

Session 2 - Antioxidant and Lipid Oxidation Evaluation Methods

Chair: Charlotte Jacobsen + Fatima Paiva-Martins

11.45 a.m. **Application of an Electronic Tongue as a Single-run Tool for Olive Oils' Total Phenols and Oxidative Stability Estimation**
Í. Marx, Porto/PT , N. Rodrigues, Bragança/PT, A. Veloso, Coimbra/PT, S. Casal, Porto/PT, J. Pereira, Bragança/PT, A. Peres, Bragança/PT

12.00 p.m. **Monitoring of Lipid Oxidation Fate of Different Lipid Classes by 2DxLC**
E. Lazaridi, Wageningen/NL , M. Hennebelle, Wageningen /NL, H.G Janssen, Wageningen /NL, J.P Vincken, Wageningen /AN

12.15 p.m. **Simple and Direct Analysis of Oils by HPLC-UV for Quantitative Evaluation of Major Oxidation Products.**
J. Velasco, Sevilla/ES , M.V. Ruiz-Méndez, Sevilla/ES

12.30 p.m. **Quantitative and Predictive Modelling of Lipid Oxidation in Mayonnaise**
D. Merkx, Wageningen/NL , A. Swager, Wageningen/NL, J. van Duynhoven, Wageningen/NL, M. Hennebelle, Wageningen/NL

12.45 p.m. **Centrifugal Ultrafiltration for the Determination of Antioxidant Partitioning in Emulsions – Pitfalls and Considerations**
K. Oehlke, Karlsruhe/DE

01.00 p.m. Lunch Break

Session 3 - Control of Lipid Oxidation

Chair: Fatima Paiva-Martins + Karin Schwarz

02.00 p.m. **Enhancement of Rancidity Stability of Canned Mackerel by Including an Aqueous Extract of Different Marine Algae in the Packaging Medium**
S.P. Aubourg, Vigo/ES , R.G. Barbosa, Florianópolis/BR, M. Trigo, Vigo/ES, B. Martínez, Ribeira/ES

02.15 p.m. **Effect of Feeding Acid Oils on Lipid Composition and Oxidative Stability of European Seabass Fillets**
P Albendea, Santa Coloma de Gramenet/ES , A Tres, Santa Coloma de Gramenet/ES, E Varona, Santa Coloma de Gramenet/ES, R Sala, Bellaterra/ES, S Vichi, Santa Coloma de Gramenet/ES, M Rafecas, Barcelona/ES, F Guardiola, Santa Coloma de Gramenet/ES

02.30 p.m. **Effect of Condensed Tannins on Cholesterol Oxidation in Enriched egg Pasta**
V. Cardenia, Turin/IT , C. Cantele , Turin/IT, A. Bonciolini , Turin/IT, N.I. Salgarella, Turin/IT, M. Bertolino, Turin/IT, G. Zeppa, Turin/IT

02.45 p.m. **Development of an Effective Dipping Strategy to Prevent Lipid Oxidation and Prolong Microbial Shelf Life of herring (*Clupea harengus*) Filleting Co-products**
H.H. Wu, Göteborg/SE , Bita Forghani, Gothenburg/SE, Mursalin Sajib, Gothenburg/SE, Ingrid Undeland, Gothenburg/SE

03.00 p.m. Coffee Break

Poster Session 1

03.30 p.m. **Poster Session 1**

Tuesday, 24 November 2020

09.30 a.m. Opening

Session 4 - Antioxidants and Lipid Oxidation in Nutrition and Disease

Chair: Charlotte Jacobsen and Fatima Paiva-Martins

09.45 a.m. **Crosstalk between Natural Antioxidants and Omega 3 against Oxidative Stress in Diet Induced Prediabetes**
L. Méndez, Vigo/ES , G. Dasilva, Vigo/ES, S. Lois, Vigo/ES, M.J. González, Vigo/ES, L. Barros, Vigo/ES, B. Miralles, Reus/ES, M.R. Nogués, Reus/ES, S. Ramos-Romero, Barcelona/ES, J.L. Torres, Barcelona/ES, I. Medina, Vigo/ES

10.00 a.m. **How far should we go Analytically to Support the Health Claim on "Olive Oil Polyphenols" (EC Regulation 432/2012) for Commercial Purposes?**
N. Nenadis, Thessaloniki/GR , A. Mastralexi, Thessaloniki/GR, M.Z. Tsimidou, Thessaloniki/GR, D.L. García-González , Seville/ES, T. Gallina-Toschi , Bologna/IT

10.15 a.m. **Oxidised Omega 3 PUFA and the Intestinal Barrier. Effect of Natural Antioxidants**
I. Medina, Vigo/ES , G. Dasilva, Vigo/ES, R. Maestre, Vigo/ES, J.D. Douglass, New Brunswick/US, M. Boller, New Brunswick/US, J. Storch, New Brunswick/US

10.30 a.m. **Stress during Slaughtering affects Oxidative Stability and Long Term Quality more than Dietary Antioxidant Supplementation in Cultured Trout**
M.J. Gonzalez, Vigo/ES , L. Mendez, Vigo/ES , G. Dasilva, Vigo/ES , G Secci, Firenze/IT, G. Parisi, Firenze/IT, H.M.C. Santos, Maringa/BR, I. Medina, Vigo/ES

10.45 a.m. Coffee Break

Session 5. Lipid Oxidation in Multiphase and Complex Systems

Chair: Karin Schwarz + Carlos Bravo

11.45 a.m. **Ingredient-dependent Extent of Lipid Oxidation in Margarine**
M. Pignitter, Vienna/AT , S. Fruehwirth, Vienna/AT, S. Egger, Vienna/AT, M. Ehrnhoefer-Ressler, Graz/AT, T. Flecker, Graz/AT, D. Kurzbach, Vienna/AT, J. Windisch, Vienna/AT, F. Jirsa, Vienna/AT, N. Firat, Vienna/AT

12.00 p.m. **Alkyl chain length of antioxidants modulates their activity in spray-dried emulsions**
S. ten Klooster, Wageningen/NL , P. Villeneuve, Montpellier/FR, C. Bourlieu-Lacanal, Montpellier/FR, E. Durand, Montpellier/FR, K. Schroën, Wageningen/NL, C. Berton-Carabin, Nantes/FR

12.15 p.m. **A new family of hydroxytyrosol phenolipids for the antioxidant protection of liposomal systems**
F. Paiva-Martins, Porto/PT , R. Lopes, Porto/PT, M. Costa, Porto/PT, M. Ferreira, Porto/PT, P. Gameiro, Porto/PT

12.30 p.m. **Influence of the temperature on the distribution of antioxidants in oil-in-water emulsions**
S. Losada-Barreiro, Vigo/ES , M. Costa, Porto/PT, F. Paiva-Martins, Porto/PT, C. Bravo-Díaz, Vigo/ES

12.45 p.m. **The mechanism of the antioxidant action of polyphenols during the oxidation of methyl linoleate in micelles**
I. Tikhonov, Yaroslavl/RU , L. Borodin, Yaroslavl/RU, V. Ryabkova, Yaroslavl/RU, E. Pliss, Yaroslavl/RU

01.00 p.m. Lunch Break

Poster Session 2

02.00 p.m. **Poster Session 2**

Ring Trial on Oxidative Stability of Oils

Chair: Karin Schwarz

03.00 p.m. **Ring Trial on Oxidative Stability of Oils**

Closing Remarks

Lectures

Protein and Lipid Oxidation in Pressurized Meat: A Possible Link?

Guyon C.[1], Meynier A.[2], de Lamballerie M.[1]

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While treatments using high pressure (HP) are effective on microbial contamination, the impact on oxidation reactions is still not widely known. The objective of this study was to evaluate the extent of protein and lipid oxidation after HP treatments in ground beef, and subsequent refrigerated storage. In order to ascertain the sequence of reactions, lipid and protein oxidation markers were determined as well as different meat iron species.

The selected markers of lipid and protein oxidation highlighted that HP treatments act differently on the oxidation of lipids and proteins. Indeed, just after HP treatment, the markers of protein oxidation increased but remained steady during storage, while the markers of lipid oxidation were rather stable and low after treatment, but increased during storage. Only a treatment at 500 MPa modified the content of conjugated dienes and hexanal. The results showed a strong correlation between the compounds resulting from oxidation (conjugated dienes, hexanal and carbonylated proteins), the indicators directly linked to protein modifications (color, texture, extractability, free amino acids) and pressure. On the other hand, TBARS are negatively correlated with pH and strongly correlated with extractability of proteins, which supports the hypothesis of the formation of adducts under HP. These results showed that HP treatments directly affect the structure of proteins. Irreversible structural changes occurred above 300 MPa (pH, formation of disulfide bridges, modification of the heme, hydrolysis of amino acids, oxidation of thiol groups, formation of adducts). Conversely, the impact of HP on lipid oxidation is indirect and strongly related once to interactions with proteins and twice to the pro-oxidant action of the released ferric ions. In this study, heme iron content is negatively correlated with pH, supporting that the pH modification after meat treatment could influence the relative distribution of iron between heme and non heme compartment. Thus, lipid oxidation would be accelerated during storage, except after treatment at 500 MPa where the content of compounds resulting from lipid oxidation is higher as soon as the treatment by HP is completed. After treatment with HP, the acceleration of lipid oxidation during storage could be due to a change in accessibility of the oxygen-reacting substances.

Influence of Oregano or Beer Addition on Lipid and Protein Oxidation during Cooking and Digestion of Chicken Meat

M.M.C. Sobral, S. Casal, M.A. Faria, S.C. Cunha, I.M.P.L.V.O. Ferreira,
LAQV/REQUIMTE, Departamento de Ciências Químicas, Laboratório de Bromatologia e Hidrologia, Faculdade de Farmácia – Universidade do Porto
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Chicken meat is a valuable source of nutrients, such as high-quality proteins, micronutrients and polyunsaturated fatty acids (PUFAs), but the cooking process may considerably change its nutritional value. PUFAs can be oxidized to reactive aldehydes (LOPs) like malondialdehyde (MDA), hexanal (HEX), and 4-hydroxy-2-nonenal (HNE), and also increase carbonylation and Schiff bases (SB) formation as result from protein oxidation. Additionally, the acidic environment of the gastric phase, described as a bioreactor of lipid peroxidation, will likely increase the formation of these oxidized products. Adding spices, herbs or red wine before cooking prevents oxidation of lipids, however the influence of beer on protein and lipid oxidation has not been explored.

This work investigated the impact of six cooking practices – oven/microwave with/without seasoning with oregano/beer – and *in vitro* digestion on the formation of MDA, HNE, HEX, carbonyls, and SB as well as total fatty acid and free amino acids content [1].

Cooking increased all oxidation markers, except carbonyls. The oxidation observed was confirmed by the loss of ω-6 PUFAs. Oregano decreased LOPs (p<0.05), SB and preserved ω-6 PUFAs, while beer had no influence on oxidation but decreased PUFAs and some free amino acids. The *in vitro* digestion increased MDA, carbonyls and SB in all cooking practices, while HNE and HEX contents only increased in samples with oregano. Despite this increase, the lowest contents of all oxidation markers were observed after cooking and *in vitro* digestion of samples with oregano, suggesting this herb as a mitigation strategy to reduce the oxidation of lipid and proteins, preserve the nutritional quality of meat, as well as reduce humans' exposure to these hazardous compounds after ingestion of cooked meat.

Acknowledgments: FCT is thanked by researcher (MAF) and IF/01616/2015 (SCC) contracts and to project PTDC/SAU-NUT/30322/2017/POCI-01-0145-FEDER-030322.

References: [1] Sobral, et al. Food and Chemical Toxicology. 141 (2020), 111401

Quantitative Spatiotemporal Mapping of Lipid and Protein Oxidation in Mayonnaise

Suyeon Yang (S.Y.)[1], Aletta A. Verhoeff (A.A.V)[2], Donny W.H. Merkx (D.M.)[1, 2, 3], John P.M. van Duynhoven (J.v.D)[1, 2, *] and Johannes Hohlbein (J.H.)[1, 4, *]

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Lipid oxidation in food emulsions is mediated by emulsifiers in the water phase and at the oil-water interface. To unravel the physico-chemical mechanisms and to obtain local lipid and protein oxidation rates, we used confocal laser scanning microscopy (CLSM) thereby monitoring changes in both the fluorescence emission of a lipophilic dye BODIPY 665/676 and protein auto-fluorescence. Our data show that the removal of lipid soluble antioxidants from mayonnaises promotes lipid oxidation within oil droplets as well as protein oxidation at the oil-water interface. Furthermore, we demonstrate that ascorbic acid acts as either lipid anti-oxidant or pro-oxidant depending on the presence of lipid-soluble antioxidants. The observed protein oxidation at the oil-water interface was spatially heterogenous, which is in line with heterogeneous distribution of lipoprotein granules from the egg yolk used for emulsification. The impact of droplet size on local lipid and protein oxidation rates was minor compared to the effects of ascorbic acid addition and lipid-soluble antioxidant depletion. The presented results demonstrate that CLSM can be applied for unravelling the roles of colloidal structure and transport in mediating lipid oxidation in complex food emulsions.

Identifying Peptides Derived from Seaweed, Single Cell and Potato Protein with Emulsifying Properties and Investigating their Functionality in 5% Fish Oil-in-Water Emulsions

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There is an increasing demand for plant and microbial based natural ingredients with emulsifying activity from food industry and consumers. Within some of these sources (potato, seaweed, and single cell proteins), we have used bioinformatics to identify embedded peptides with good, predicted radical scavenging activity. The objectives of this study were i) screening the emulsifying activity of 28 synthetic peptides, which were predicted by bioinformatics, in 5% fish oil-in-water emulsions on small scale (5 ml) and ii) investigating the physical and oxidative stability of 5% fish oil-in-water emulsions produced on large scale (200 ml) with selected peptides based on the small scale study.

Twenty eight peptides were predicted as good emulsifiers by bioinformatics using developed algorithms based on their amphiphilicity. Synthetic peptides were used for the evaluation of the predicted peptides' emulsifying activity in 5% fish oil-in-water emulsions produced on small scale (homogenization by sonication) during storage. Based on zeta potential of the emulsions, interfacial tension of the peptides at the oil-water interface, changes measured in droplet size distribution, and creaming stability of the emulsions during storage, 9 peptides were selected as good emulsifiers.

These nine peptides were subjected to a large scale study (homogenization by high pressure), where both physical and oxidative stability of the 5% fish oil-in-water emulsions produced using these peptides as emulsifiers were investigated. Zeta potential, droplet size distribution, instrumental color test, and creaming stability were measured for the assessment of physical stability. Emulsions were also subjected to the analyses of peroxide value, tocopherol consumption and volatile oxidation products development to evaluate the effects of these peptides on the oxidative stability of the emulsions. The next step for this project is to extract the peptides with good emulsifying activities by enzymatic hydrolysis of the side streams from potato, seaweed and single cell production followed by purification of the peptides.

Application of an Electronic Tongue as a Single-run Tool for Olive Oils' Total Phenols and Oxidative Stability Estimation

Ítala M.G. Marx[a,b*], Nuno Rodrigues[a], Ana C.A. Veloso[c,d], Susana Casal[b], José A. Pereira[a], António M. Peres[a,d]

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Olive oil quality can be enhanced during olive oil extraction, particularly by promoting the extraction of phenolic componds extraction leading to the increase of the oxidative stability (OS). However, both total phenols content (TPC) and OS measurements (Folin-Ciocalteau spectrophotometric method and Rancimat, respectively) are time-consuming and expensive tasks, of difficult implementation in production lines. Thus, the present work studied the feasibility of using a potentiometric lab-made electronic tongue (E-tongue, Figure 1A), comprising non-specific lipid polymeric sensor membranes, coupled with multiple linear regression (MLR) models to predict TPC and an estimative of the OS of *cv*. Cobrançosa oils extracted at different malaxation temperatures (22 to 34 °C). For the potentiometric analysis, the oils' polar phenol fraction was extracted with *n*-hexane and an aqueous methanolic solution (MeOH/H_2O 80:20 v/v). The E-tongue-MLR models, based on sub-sets of 11 non-redundant sensors selected uisng the simulated annealing algorithm, allowed predicting (repeated K-folds-CV) the TPC (175<TPC<240 mg GAE/kg; RMSE=2.0±0.8 mg GAE/kg; R^2=0.99±0.01) and, indirectly, to estimate the OS (11.5<OS<14.5 h; RMSE=0.08±0.07 h; R^2=0.99±0.01), which values are directly related with the TPC. The satisfactory quantitative performance can be visualized in Figure 1B. In conclusion, the results showed that the E-tongue could be applied for quantifying TCP and predicting OS, both abovementioned shelf-life related parameters.

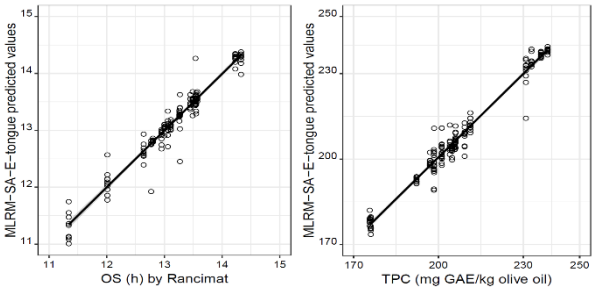

Figure 1. (A) E-tongue sensors arrays **(B)** E-tongue predictive performance

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Monitoring of Lipid Oxidation Fate of Different Lipid Classes by 2DxLC

Eleni Lazaridi[1, 2], Marie Hennebelle[1], Hans-Gerd Janssen[1, 2, 3], Jean-Paul Vincken[1]

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Lipid oxidation in food products is a crucial problem that causes undesirable changes in the food's flavor, texture, nutritional value and consequently reduces shelf life. Even though lipid oxidation has been examined extensively in bulk oils and fats, the processes behind it in more complex systems like emulsified foods are not fully understood. Oxidation reactions are believed to progress from the oil/water interface to the core of the oil droplets with different products being formed depending on the composition and location of the lipid ingredient. Novel analytical tools are needed to study the resulting, highly complex mixture of oxidized species.

A comprehensive two-dimensional liquid chromatography (2DxLC) method coupled with evaporative light scattering detection (ELSD) was set up. The 2D-ELSD method employs size exclusion chromatography (SEC) as the 1st dimension separation mechanism and separates the oil phase into the different lipid classes. In the 2nd dimension, each lipid class is subsequently separated to their oxidised and non-oxidised lipids by normal phase chromatography (NP). To develop the method, rapeseed oil spiked with DAG and MAG standards was subjected to an accelerated auto-oxidative protocol at 140^{o}C for 7 days in order to produce samples concentrated in lipid oxidation products. The online coupling of SEC with NP successfully allows the separation of non-oxidised TAG from their oxidised species as well as the isolation of non-oxidised DAGs and MAGs that usually interfere with the detection of a variety of oxidised products of similar polarity. This method will facilitate elucidating how interfacial composition affects oxidation kinetics in emulsified foods.

Simple and Direct Analysis of Oils by HPLC-UV for Quantitative Evaluation of Major Oxidation Products

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In previous studies we were able to undertake the quantitative analysis of the main oxidation products of linoleyl chains attached to TAG by transforming TAG into fatty acid methyl ester (FAME) derivatives. The isolation and quantification of linoleate hydroperoxy-, keto- and hydroxy-dienes was possible by normal-phase HPLC-UV. For quantitative purposes, response factors were obtained from the same analytes synthesized in the lab. The derivatization step caused losses of hydroperoxydienes that were lower than 10 wt% and formation of the main secondary oxidation products, i.e. keto- and hydroxy-dienes. In the present communication we present the analytical possibilities of the direct analysis of oils by HPLC-UV applying the same chromatographic conditions as used in the analysis of FAMEs. High-linoleic and high-oleic sunflower oils oxidized at 40 ºC to different oxidation extents were analyzed by both analytical procedures. The results showed that the direct analysis of oils permits to determine the concentrations of mono-hydroperoxydienes in edible oils without applying any isolation or derivatization step. It is direct, sensitive (LOQ 0.06 mmol/kg oil), precise (CV ≤ 5%) and also more accurate when compared to the FAME analysis. The method also enables to estimate the minor amounts of ketodienes, but not those of hydroxydienes, which present wide chromatographic bands and coelute with a number of different minor oxidation compounds.

In addition, the application of the proposed analytical method to evaluate the oxidation extent of oils and its principal advantages compared to the index of conjugated dienes will be discussed.

Quantitative and Predictive Modelling of Lipid Oxidation in Mayonnaise

Donny Merkx[a,b], Andries Swager[a], John van Duynhoven[a,b] and Marie Hennebelle[a]

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Lipid oxidation is a major concern in the food industry as it is one of the main contributors to the limited shelf life of lipid-based food products. This is a particular concern for food emulsions with high amounts of unsaturated fats, such as mayonnaise. In the food industry, accelerated shelf life tests are often applied to assess the oxidative stability of different formulations. One of the early oxidation products are the hydroperoxides, which can already be detected in the first days. These hydroperoxides are the precursors of the volatile aldehydes, responsible for off-flavour. The appearance of aldehydes at the so-called onset time is considered a measure for oxidative stability of a mayonnaise formulation.

Under accelerated shelf life conditions (50 ºC), the hydroperoxide concentration over time typically follows a sigmoidal curvature followed by an acceleration phase that occurs at a LOOH-concentration, between 35-50 mmol/kg, which we interpreted as a critical micelle concentration[1] (CC_{LOOH}). We hypothesized that the time at which CC_{LOOH} was reached, was related to the onset of aldehyde generation. We therefore hypothesized that if we can predict the LOOH-generation curvature based on kinetics in the first days, we can predict when the CC_{LOOH} will be reached and when the onset of the aldehyde generation will occur.

The aforementioned hypotheses were captured in a grey model comprising the semi-empirical Foubert[2] function to describe the autocatalytic character of hydroperoxide formation, in combination with a critical micelle concentration at which reaction rates accelerate and onset of aldehyde generation is imminent. This model could be used to predict onset of aldehyde generation from hydroperoxide generation kinetics in the first days. Furthermore, we found that defining parameters of the grey model could be used to group anti-oxidant mechanisms at play.

References:

1. Mickaël Laguerre; Mathieu Tenon; Antoine Bily; Birtic, S., Toward a Spatiotemporal Model of Oxidation in Lipid Dispersions: A Hypothesis-Driven Review. *Eur. J. Lipid Sci. Technol.* **2020,** *122*, 1900209.
2. Foubert, I.; Vanrolleghem, P. A.; Vanhoutte, B.; Dewettinck, K., Dynamic mathematical model of the crystallization kinetics of fats. *Food research international* **2002,** *35(10)*, 945-956.

Centrifugal Ultrafiltration for the Determination of Antioxidant Partitioning in Emulsions – Pitfalls and Considerations

Kathleen Oehlke, Max Rubner-Institut, Department of Food Technology and Bioprocess Engineering
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The partitioning of substances between (pseudo-)phases in emulsions and emulsion-based delivery systems is part of many studies considering e.g. antioxidant activity or stabilisation of bioactive compounds. Ultrafiltration (UF) has been commonly used in such studies. However, when the partitioning / distribution of a compound is governed by a dynamic equilibrium, and that is most often the case, the separation of the phases has to be avoided, since disturbing the equilibrium will cause distorted results. Furthermore, interactions between the analyte and the UF membrane must be kept to a minimum. Nevertheless, these prerequisites are often neglected or at least not reported in the current literature.

The aim of the present study was to experimentally demonstrate in which cases and under which circumstances UF can nevertheless yield reliable results. Therefore, the partitioning of ferulic acid (FA) in Tween 20 stabilized o/w emulsions of different compositions was studied by ultrafiltration using centrifugal filtration devices. The filtration conditions were varied in a way that different filtrate volumes were obtained. The impact of the filtration conditions and especially the impact of the filtrate volume on the apparent partitioning of FA in o/w emulsions was experimentally shown and could be integrated in an existing theoretical framework[1,2]. The proportion of FA could easily be overestimated. However, the data show that UF can yield reliable results with low centrifugal speed, short centrifugation times and proper choice of membrane, depending on emulsion composition.

While these findings are not surprising, their importance becomes most apparent when experimental conditions have to be chosen to study and compare the partitioning of a substance in different emulsions.

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Enhancement of Rancidity Stability of Canned Mackerel by including an Aqueous Extract of different Marine Algae in the Packaging Medium

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Canning represents one of the most important means of fish preservation, which can last for several years. However, most constituents of marine species are known to be particularly labile, lipid damage (oxidation and hydrolysis) development showing a marked negative effect on the nutritional and sensory values in the final canned product. On the other side, the use of marine algae as food ingredients in Western countries is receiving an increasing attention. Thus, seaweeds have been reported to include a wide variety of beneficial nutrients and chemical constituents with potential antioxidant and antibacterial activity.

This research focussed on the quality of canned fish. Its primary objective was the quality enhancement of canned mackerel (*Scomber scombrus* and *Scomber colias*) by including an aqueous extract of different algae (*Fucus spiralis*, *Bifurcaria bifurcata* and *Ulva lactuca*) in the packaging medium. For it, quality changes were analysed after a 3-month canned storage by evaluating lipid classes' modifications, development of lipid oxidation and hydrolysis, formation of volatile amines (total and trimethylamine) and muscle colour changes.

Phospholipids, sterols and α-tocopherol contents showed a marked loss as a result of canning, this loss being mostly inhibited when considering the canned fish packaged in the presence of the most concentrated algae extracts. Breakdown of free fatty acids and peroxides produced during the thermal treatment was inhibited by the presence of the algae extracts, a higher retention of such molecules being observed by increasing the algae extract concentration. Analysis of fluorescent compounds formation (tertiary lipid oxidation) and colour parameters (*L** and *b**) showed a marked increase after canning, this increase being partly inhibited by the presence of antioxidant compounds in the algae extracts.

A preservative effect on lipid constituents and rancidity development is concluded by the presence of aqueous extracts of algae in the packaging medium. This result is primarily linked to the presence of hydrophilic preservative molecules and constitutes a novel and promising strategy to enhance the quality of commercial canned fish products.

Effect of Feeding Acid Oils on Lipid Composition and Oxidative Stability of European Seabass Fillets

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Acid oils (AO) are fat by-products from edible oil refining, characterized by their high proportion of free fatty acids. For years, there has been an interest in using them in animal feeding because of their high energetic value. However, the effect of these fat by-products on fish flesh quality has been barely studied.

Specimens of European sea bass (*Dicentrarchus labrax*) were fed 5 dietary treatments with 16 % of added fat that was fish oil (F, control), or fish oil plus another fat source (at 25:75, p/p): olive pomace oil (OP), olive pomace acid oil (OPA), crude soybean oil (S) or soybean-sunflower acid oil (SA). After 3 months, fillets were sampled (5 analytical replicates per treatment). At 24h post-slaughter, fillets were stored under commercial refrigeration conditions ($CO_2/N_2/O_2$; 40/30/30; 2°C) for 0 or 6 days. The fatty acid (FA) composition, tocopherol (T) and tocotrienol (T3) content, lipid peroxide formation by the induced FOX assay (incubation for 96h), and TBA value of the fillets were determined.

The F fillets were the most unsaturated (much higher content in EPA and DHA). All treatments had α-; β-; γ- and δ-T, and β-T3, but their total level (T+T3) and profile differed: F showed the lowest level of total T+T3 and of α-T (the predominant tocol); S had the highest total tocols and γ-T levels, followed by SA. The refrigeration decreased the total T+T3 in all treatments. FOX results revealed that F had the lowest oxidative stability while OP and OPA were the most stable, and no influence of the refrigeration was observed. TBA values showed higher levels for F and for all refrigerated samples.

In conclusion, F had the lowest oxidative stability, while OP and OPA were the most stable treatments. S and SA had an intermediate stability because, although they were more unsaturated than OP and OPA, they had higher T+T3 content, which could have reduced secondary oxidation reactions. In addition, refrigeration clearly decreased oxidative stability in terms of TBA values.

Effect of Condensed Tannins on Cholesterol Oxidation in Enriched Egg Pasta

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The egg pasta represents the best of Italian tradition and it is consumed all over the world. Moreover, egg pasta naturally contains lipids and cholesterol, which can be oxidized leading to formation of hazard compounds such as cholesterol oxidation products (COPs). For these reasons, pasta could be fortified with antioxidants, such as phenolic compounds. The aim of this work was to evaluate the effect of two condensed tannins (ellagic acid esters of glucose (A); gallic acid esters of quinic acid (B)) used for the formulation of two egg pasta shapes (square spaghetti (S); fettuccine (F)) on cholesterol and COPs content. Tannins were added to egg pasta at three levels (0.25%, 0.5% and 1% w/w flour) and the total phenols content (TPC), radical scavenging activity (RSA), sterols composition and COPs content were determined. The tannins increased the TPC and RSA in egg pasta, in particular the B tannin reported greater value. Moreover, after cooking for 4 min, a decrease of TPC lower than 57% was observed. The sterols composition did not significantly (p > 0.05) change, the cholesterol resulted the main sterol (19.73-51.89 mg/g dry matter) followed by sitosterol, campesterol, campestanol and sitostanol. The COPs amount ranged from 0.16 to 0.74 µg/g dry matter, being the 7-ketocholesterol, 7α-hydroxycholesterol and 7β-hydroxycholesterol the main COPs; however, no significant differences (p > 0.05) were ascribed to cooking process. On the other hand, the B tannin was more able to counteract the cholesterol oxidation in F shape; while the A tannin displayed a prooxidant activity in both shapes. In conclusions, the two condensed tannins exhibited a different effect on cholesterol oxidation; in addition, due the capability of tannins to interact with cholesterol, the selection of appropriate tannins could be a promising hypocholesterolemic mechanism for reducing the bioaccessibility of both cholesterol and COPs.

* *The authors would like to thank Silvateam Spa for providing tannins.*

Development of an Effective Dipping Strategy to Prevent Lipid Oxidation and Prolong Microbial Shelf Life of Herring (*Clupea harengus*) Filleting Co-products

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Applying value-adding techniques to fish filleting co-products is rendered difficult due to their high susceptibility to lipid oxidation, microbial spoilage. In the first part of this study, seven components/formulas (water-soluble rosemary extract (RE-A), oil-soluble rosemary extract (RE-B), isoascorbic acid (IA), esculetin, α-tocopherol or two rosemary-containing commercial mixtures (Duralox MANC-213 and EVESA-C5) were directly added into minced herring co-products to screen their ability to prevent lipid oxidation during ice storage. The order by which the seven compounds delayed lipid oxidation was as follows: RE-B (0.25& 0.05%) = Duralox MANC (0.5%) > IA (0.5%) > RE-A (0.2%) > EVESA-C5 (0.1%) > esculetin (0.09%) > α-tocopherol (0.02%). The three most promising candidates based on both cost and efficiency were used in a second trial where herring co-products were dipped in antioxidant-containing solutions prior to subsequent cold-storage for 12 days in a minced or intact form. The effect of re-cycling the antioxidant solutions was also studied, and PV, TBARS and microbiological (total viable count) parameters were analyzed. Dipping of the co-products with a 0.2% RE-B solution, with and without 0.5% IA, remarkably increased the oxidation lag phase from < 1 day to >12 days during subsequent cold storage in minced or intact form. Even after re-use of the dipping solution up to 10 times, lipid oxidation was completely inhibited. Dipping in RE-B +/- IA also significantly ($p<0.05$) delayed total viable count, pointing at dual roles of the rosemary-derived phenolics. However, the antimicrobial ability decreased as solution re-cycling times increased. The presented dipping strategies could hereby facilitate more diversified end-use of herring co-products from current fish meal to high-quality minces, protein isolates or oils for the food industry.

Crosstalk between Natural Antioxidants and Omega 3 against Oxidative Stress in Diet Induced Prediabetes

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Prediabetes condition constitutes a high-risk state for developing type 2 diabetes (T2D) and is rising worldwide, with a projection of more than 470 million people suffering from it in 2030. The simultaneous presence of insulin resistance, increased body adiposity and β-cell dysfunction are often already present in prediabetes. A currently favoured hypothesis is that oxidative stress is the common pathogenic factor linked to the progression of these risk conditions. Given the marine omega-3 PUFA emerging evidence suggesting both positive anti-inflammatory and antioxidant modulatory effects on diet-induced metabolic diseases, we have investigated the influence of omega-3 PUFA supplementation on oxidative stress occurring in prediabetic subjects. However, the higher amount of PUFAs in the diet leads to greater amount of oxidized substrate that may deregulate redox homeostasis. For that, in the present study we have addressed the potential combined effect between a well-known family of natural antioxidants as grape seed proanthocyanidins and fish oils to act against the excessive oxidative stress underlying the first steps of T2D.

The effect of natural antioxidants and marine lipids on oxidative stress in prediabetic rats fed a high-fat high-sucrose (HFHS) diet were studied through a combined proteomics and lipidomics approach in the liver as target organ of redox imbalance. Our results demonstrated that the consumption of both supplements lead to significant decrease of oxidative stress by improving body antioxidant status. The antioxidant action exerted by fish oils was associated with the enhanced activity of the antioxidant system, especially by means of the stimulation of the GPX activity that leads a higher detoxification of hydroperoxides and the decrease of SOD activity and protein carbonylation. The possible mechanisms implicated in the down-regulation of oxidative stress due to polyphenols agree with the significant enhancement of the antioxidant system (increase of GPX and decrease of SOD), the amelioration of protein carbonylation and reduction of lipid peroxidation in liver. Nevertheless, the combination of both bioactive compounds was the one that rendered the most dramatic antioxidant effect. The double-supplemented group showed the least lipid peroxidation values (malondialdehyde and hydroperoxide formation) and protein carbonylation, the highest ORAC capacity and the most drastic regulation of antioxidant enzyme activities (highest up-regulation of GPX and down-regulation of SOD). Clinical biomarkers of oxidative stress, inflammation and insulin resistance determined in rats were in concordance with our lipidomics/proteomics data. Noteworthy, the combination fish oils and natural antioxidants significantly diminished insulin resistance in prediabetic subjects. To sum up, the regulation of oxidative stress occurring on prediabetic liver appears to have a pivotal role in both the development of metabolic alterations and the design of targeted-strategies for personalised nutrition.

How far Should we go Analytically to Support the Health Claim on "Olive Oil Polyphenols" (EC Regulation 432/2012) for Commercial Purposes?

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Launching of the EC Reg. 432/2012 authorized among other health claims one for olive oils that contain at least 5 mg of hydroxytyrosol and its derivatives (eg. oleuropein complex and tyrosol) per 20 g oil because these compounds, phenolic in nature, "protect blood lipids from oxidative stress" [1]. This health claim caused substantial awareness among interested parties for various reasons, a major one being the lack of an official or recommended method that could be unanimously used for the determination of the target compounds. Over the last decade various analytical approaches, more or less sophisticated, appeared in literature to address this gap. However, how far should we go analytically to support the particular claim for commercial purposes? Trying to answer this question a fit for the purpose UHPLC procedure has been developed within the OLEUM project frame, it was in-housed validated [2], compared with other procedures [3] and currently is subjected to an inter-laboratory testing. The procedure allows the quantification of the total hydroxytyrsol (Htyr) and tyrosol (Tyr) content in the olive oil polar fraction with detection at 280 nm, before and after acidic hydrolysis of their bound forms using commercially available standards. The present work pinpoints its pros/cons in comparison to those of other dedicated protocols from the perspective of the applicability for routine quality control. Moreover, where possible, data comparison is also presented in an effort to strengthen scientific evidence for its applicability.

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Oxidised omega-3 PUFA and the Intestinal Barrier: Effect of Natural Antioxidants

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Gastrointestinal digestion of marine oils has recently received huge attention because of gastric-derived lipid oxidation and the subsequent effects for intestinal uptake of oxidised PUFAs. Increasing concentrations of DHA in marine lipid supplements might enhance susceptibility to oxidation during gastric digestion, and, hence, limit the uptake and the resultant health benefits attributed to omega-3 PUFA consumption. In fact, high proportions of DHA in marine lipid supplements have demonstrated to reduce metabolic healthy effects compared with balanced EPA:DHA supplementations. Accordingly, we've tested the role of digestion on marine oils having increasing proportions of DHA by investigating their oxidation, the levels of bioaccessible lipids and the uptake efficiency. Several studies have also shown the inhibition of lipid oxidation in the stomach by natural polyphenols, which appear to arrive intact to the small intestine, because they are resistant to gastric acid hydrolysis. Therefore, our study has addressed the incorporation of antioxidant polyphenols in foods fortified with marine oils for inhibiting lipid oxidation in the gastrointestinal tract.

Gastric digestion was mimicked by the TNO dynamic gastrointestinal tract model and cultured small intestine enterocytes were used to study the absorption and metabolism of nutrients. To determine the influence of increasing DHA concentrations and oxidation status on the cellular uptake and metabolism of the lipids, enterocytes were incubated with radiolabeled oxidized and nonoxidized PUFA mixtures. The effect of an antioxidant grape extract added to marine oils during digestion and uptake was also studied.

Results of gastric stability, bioaccessibility and cell uptake supported an inverse correlation between gastric-derived lipid oxidation and further intestinal absorption. Furthermore, the results highlight different stability of marine oils with increasing DHA in the gastrointestinal tract, and how oxidation affects the intestinal assimilation of PUFAs. The addition of natural antioxidant grape polyphenols to food limited lipid oxidation during active digestion and lead to greater PUFA uptake into the enterocyte model. These results suggest that the correct design of diets and/or supplements containing natural antioxidants and marine lipids can strongly modulate the oxidative stability of PUFAs promoting intestinal uptake of the beneficial, unoxidized, n-3 PUFAs.

Stress during Slaughtering affects Oxidative Stability and Long-term-Quality more than Dietary Antioxidant Supplementation in Cultured Trout

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Dietary essential oils having natural antioxidants have been included in aquaculture feed for their potential healthy role on immune response and intestinal microbiota. Besides their use as antimicrobials, natural antioxidants added to fish feed have improved the redox balance of living fish and then promoted a higher oxidative stability of the corresponding final fishery products. Nevertheless, slaughter stress has demonstrated to affect the onset of post-mortem oxidation in cultured fish species. Hence, stress can provoke in short term the accumulation of lipid oxygenated products and reduce the commercial shelf-life, due to a higher susceptibility to develop rancidity.

Accordingly, in this work we have studied the potential synergism between the incorporation of a dietary antioxidant essential oil in the feed of rainbow trout (*O. mykiss*) and the use of an unstressed slaughter method to enhance the oxidative stability of trout flesh. For this purpose, the effects that two different slaughter methods, asphyxia and percussion, exerted on the post-mortem quality and stability of two groups of cultured trouts (control and essential oil supplemented feed), were investigated. A comprehensive redox Lipidomic/Proteomic combined study on muscle lipids and proteins was followed in the corresponding frozen trout fillets stored for 6 months at –10ºC. The study was completed with the organoleptic evaluation of the fish flesh and the degradation of nucleotides for the assessment of stress conditions.

Results demonstrated that frozen fish fillets from trout fed the antioxidant oil showed a significant protection of specific muscle proteins against oxidation and therefore, preserved muscle protein solubility and water holding capacity during frozen storage. Lipid oxidation and rancidity were also delayed in trout fed essential oils, resulting in frozen fish with higher organoleptic and sensory scores. However, stress during slaughter greatly influenced oxidative imbalance, thus promoting the earlier onset of biomarkers of instability as lipid mediators and protein carbonyls. As a consequence, fillets derived from non-stressed slaughtering method were more stable in terms of oxidative stability and showed longer shelf-life with no influence of the dietary antioxidant essential oil added to trout feed.

Ingredient-dependent Extent of Lipid Oxidation in Margarine

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Oil-in-water emulsions received much attention with regard to the factors influencing their oxidative stability, while water-in-oil emulsions have been investigated scarcely. Margarine, a widely consumed water-in-oil emulsion, is usually stored under sub-ambient conditions and thermally treated when used for baking. As different types of margarine contain different lipids and additives, the effect of these ingredients on the oxidative stability of margarine is of pressing importance.

Thus, the impact of different ingredients on the extent of lipid oxidation in four different types of margarine stored at 15°C for 99 days and at 80°C for 1 h was investigated by analyzing the generation of peroxides, conjugated dienes, epoxides and volatile compounds.

As expected, the progress of lipid deterioration depended on the lipid profile of the fat phase of the margarine. Besides hexanal, a commonly detected lipid oxidation marker, acetone was shown, for the first time, to be generated at concentration up to 0.8 µg/g in margarine during storage for 99 days by applying HS-SPME-GC-MS.

Among the tested additives, alpha-tocopheryl acetate promoted while rosemary and green tea extract decreased lipid oxidation in margarine treated at 80°C, whereby the latter showed the most prominent antioxidant effect. Combinations of green tea extract with citric acid, beta-carotene or NaCl increased lipid oxidation compared to the green tea extract alone. NMR revealed that the changes of the mobility of the emulsifier at the interface after addition of these additives seemed to be not the key factor in determining the extent of lipid oxidation in margarine.

The study reports the impact of margarine-representative ingredients on its oxidative stability identifying the generation of a novel lipid oxidation product and green tea extract as a promising antioxidant in margarine.

Alkyl Chain Length of Antioxidants Modulates their Activity in Spray-dried Emulsions

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Lipid oxidation is a well-recognized issue in dried food emulsions, such as infant milk formula. Research in this field has mainly focused on applied aspects, such as the influence of processing and ingredients on lipid oxidation. A few studies have dug deeper into the underlying mechanisms; for example, it was found that the encapsulated fat and surface free fat oxidize via different pathways.[1] For encapsulated fat, the degradation of antioxidants and formation of lipid oxidation products happen concurrently and in a linear fashion, whereas for surface free fat the degradation of antioxidants is followed by a rapid increase in lipid oxidation products. In wet oil-in-water (O/W) emulsions, lipid oxidation pathways have been studied more extensively, which has led to identification of matrix-related mechanisms. For example, antioxidants with an intermediate hydrophobicity tend to locate at the oil-water interface, where lipid oxidation initiation is supposed to occur. They are thus the most effective in delaying lipid oxidation, which is called the 'cut-off effect'.[2] Conversely, when antioxidants are too hydrophilic, they locate away from the oil-water interface, and are thus ineffective. For spray-dried powders, the situation could be rather different, because oil is entrapped as droplets in a glassy matrix, or present as free fat on the surface of the powder particles. In the present study, the hydrophobicity of the antioxidant gallic acid is systematically varied (gallic acid, propyl, octyl, lauryl and hexadecyl gallate), and these molecules are applied in O/W emulsions that are subsequently spray-dried. The obtained powders are incubated at 40 °C and lipid oxidation is measured over time with nuclear magnetic resonance, which allows measuring a range of primary and secondary products simultaneously.[3] We show that hydrophobic antioxidants are the most effective, and that depending on the antioxidant used, differences in oxidation levels between embedded and surface free fat are found. These insights do not just improve our basic understanding of lipid oxidation mechanisms in spray-dried emulsions, but also pave the way for more effective antioxidant strategies in related food products.

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A New Family of Hydroxytyrosol Phenolipids for the Antioxidant Protection of Liposomal Systems

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Hydroxytyrosol (HT) is a well-known olive oil polyphenol for its high antioxidant capacity and important cardio and neuroprotective effects. However, its use in lipidic systems is limited, due to its hydrophilic character. In this study, we approach the particular structure of xanthophylls and synthetize HT esters specially designed for the protection of liposomal systems. These HT esters contain two polyphenolic moieties separated by a lipophilic alkyl spacer of different length (12, 16 or 22 carbons). To evaluate the antioxidant activity of these compounds against the 2,2′-azobis(2-amidinopropane) hydrochloride induced oxidation, soybean phospholipid liposomes were used. Fluorescence quenching studies were used to assess the insertion of the compounds in the liposomes. The synthetized HT derivatives were able to protect liposomes from induced oxidation when added to the suspensions. The rank of activity was severely influenced by the alkyl chain length of the spacer molecule, being the C12 derivative the most active antioxidant, with an increase in the oxidative stability of liposomes of 2.2 times when compared with the control. The incorporation of compounds during liposome preparation improved the antioxidant capacity of all HT derivatives by about 2.8 times, when compared to the control. This is probably due to a similar transmembrane position with both polyphenolic rings located at the phospholipid polar heads. The synthesis of bis-ester derivatives seems to be a promising strategy to fine-tune antioxidant molecules at biomembranes, thus increasing the oxidative stability of liposomal systems by improving the antioxidant activity of hydrophilic phenolic compounds with high free radical scavenging activity.

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Influence of the Temperature on the Distribution of Antioxidants in Oil-in-Water Emulsions

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Addition of antioxidants (AOs) to food products is one of the strategies commonly used in the food industry to retard or inhibit lipid oxidation. Efficiency of AOs depends, among others, on their concentration in the interfacial region, where the lipid oxidation takes place. AOs may distribute between the oil, aqueous and interfacial regions of oil-in water emulsions and their percentage in each region depend on a number of parameters including the relative polarity of the AOs and oil, the nature of the emulsifier and environmental conditions such as acidity and temperature. As a consequence, there has been an upsurge in the efforts to understand how different parameters control the AO distribution in emulsified systems and how the AO partitioning affects the lipid oxidation reactions.

Here, we report on the effects of temperature and emulsifier volume fraction (Φ_l) on the distribution of representative AOs – gallic acid and its derivatives- in a model food emulsion. The partition constants are obtained in the intact emulsions from the kinetic analyses of the variation of the observed rate constant, k_{obs}, with the emulsifier volume fractions (Φ_l) for the reaction between a chemical probe and AOs. The results are interpreted on the basis of the pseudophase kinetic model.

Results show that the transfer of gallates to the interfacial region is spontaneous. An increase in temperature favors the incorporation of gallates into the interfacial region in an extent that depends on antioxidant hydrophobicity. Results are basic to get a deeper knowledge on the driving force that partitions the AOs in emulsions and to select the best AO to prevent or minimize the lipid oxidation in multiphasic systems.

The Mechanism of the Antioxidant Action of Polyphenols during the Oxidation of Methyl Linoleate in Micelles

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Polyphenols (QH_2) are one of the major classes of bioantioxidants. We have studied the antioxidant activity of a number of poly- and methoxyphenols of various classes (catechol and pyrogallol derivatives, hydroxy acids, flavonoids, catecholamines) during AAPH-initiated oxidation of methyl linoleate (LH) in Triton X-100 micelles (310 K, pH 7.4). This system was used as a simplified model of lipid membrane. It was found that phenols of various classes differ significantly in their antioxidant action. Thus, the inhibition coefficients (f) for mono- and methoxyphenols do not exceed two (theoretical value for phenols). At the same time, these values are significantly higher (f = 4 – 6) for catechol derivatives. The probable reason is the formation of phenolic structures from the conversion products of antioxidants (o-quinones) as a result of nucleophilic addition to the quinoid ring.

Pyrogallol derivatives and catecholamines do not inhibit LH oxidation under the studied conditions due to the side reactions of QH_2 or $QH^{\bullet}$ with oxygen. As a result, $HO_2^{\bullet}$ / $O_2^{\bullet-}$ radicals are formed, which further lead the oxidation chains. In the presence of the enzyme superoxide dismutase (SOD), these processes are suppressed, and such phenols show the antioxidant action. In the absence of SOD, pyrogallol derivatives and catecholamines inhibit LH oxidation at lower pH values (4 – 6). This confirms the participation of ionized forms of antioxidants (QH^-, $Q^{\bullet-}$) in side reactions.

For most polyphenols, a significant decrease in the effective values of the rate constant for the reaction of QH_2 with the peroxide radical (k_7) is observed on going from the oxidation of LH in a homogeneous medium to micelles. It is explained by the formation of hydrogen bonds between the OH group of phenol and a surfactant or water, as well as by the distribution of the antioxidant between the phases (water and the inner part of the micelle). The last factor seems to be determinal. Some antioxidants (in particular, vanillic acid) in micelles had higher k_7 values than in a homogeneous medium. It was found that with a decrease in pH, the antioxidant activity of such compounds significantly decreases. The reason for the higher k_7 values at pH 7.4 is the contribution of reactions of ionized forms of antioxidant to total inhibition.

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Interlaboratory study “lipid oxidation in bulk oils” - a first update on the results

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Lipid oxidation is a major cause of oxidative deterioration of lipid-containing foods and storage tests are an important measure to assess the stability food and related systems. Various methods for measurement of lipid oxidation are used and storage conditions used may vary between the individual research groups or laboratories. It is therefore an important concern to clarify the comparability of oxidation tests and the results derived.

At the “2nd International Symposium on Lipid Oxidation and Antioxidants” in 2018 in Graz the idea for an interlaboratory study “lipid oxidation in bulk oils” was born.

The objective of the present interlaboratory trial was to study the effect of the storage temperature on the lipid oxidation of two different bulk oils. The study was conducted to evaluate different methods for measurement of lipid oxidation and to compare measured values for conjugated dienes (CD) generated by different laboratories in an interlaboratory study.

To address this question, two commercial vegetable oils (sunflower oil (SFO) and rapeseed oil (RSO)) provided by Brökelmann & Co Oelmühle GmbH & Co (Germany) were shipped to the participants. The oils were stored at 20, 30, 40, and 60 °C for 9 weeks and lipid oxidation markers (CD, hydroperoxides, headspace-gas chromatography, etc.) were measured at 10 different points in time according to a detailed protocol concerning sampling, storage conditions, and analysis of CD. Sixteen participants contributed in the study and provided results. The oxidation data were aggregated in the activation energy (E_a) according to Arrhenius from reaction rates calculated by differential regression. For comparison, the data were statistically evaluated with a grand mean test.

The results showed that with increasing storage temperature an increase of the CD could be observed across all laboratories for both oils. The E_a for RSO ranged from 45.9-73.3 with a mean of 57.5 kJ mol^{-1} and the E_a for SFO ranged from 26.9-62.6 with a mean of 45.8 kJ mol^{-1}.

Posters

Hepatotoxicity of Exirel in Wistar Rats: Toxicology Study

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Our study aims to investigate the toxicity of a new insecticide "Exirel" that belongs to the family of diamids anthranilic and its effects on some parameters related to liver function.

This study was carried out by an experiment on 30 rats of the Wistar strain divided into 5 groups of 6 rats, our obtained results showed that the treatment with Exirel causes a hepatotoxicity which resulted in a modification of biochemical and enzymatic parameters; this is revealed by the increasing in protein levels, malondialdehyde (MDA) and GST activity. In addition, we have recorded a decrease in lipid and GSH levels, GPx activity. From this, exposure to the Exirel presents a real danger to environment generally and human health.

Keywords: insecticide, anthranilic diamides, Exirel, hepatotoxicity, rats, etc....

The Effect of *Azadirachta Indica Oil* on Hepatic Lipid-Oxidation Metabolism on Ochratoxin A-Induced Acure Mycotoxicity

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Ochratoxin A is a mycotoxin produced by the *Aspergillus and Penicillium* species, receiving increasing attention due to its hepatotoxic and nephrotoxic side effects and carcinogenic activity. The *Azadirachta Indica oil* is a plant antioxidant that inhibits the mycotoxins overproduction, subsequent lipid peroxidation levels and hepatic oxidative changes by scavenging and reduction of free radicals. The aim of this 14th day-long study was to investigate the *in vivo* protective properties of the oil against ochratoxin A -induced hepatic and lipid-oxidative damages leading to acute mycotoxicity. In the experiment 24 male irc/ni mice were used. The mice were divided in four groups: group 1: basal diet (bd) with no supplementation of *ochratoxin* and *Azadirachta Indica*; group 2: bd with 100mg/kg *Azadirachta Indica*; group 3: bd with 1.6 mg/kg ochratoxin; group 4: bd with 1.6 mg/kg ochratoxin + 100mg/kg *Azadirachta Indica*. Two weeks after oral treatment mice were sacrificed and the liver was investigated for the lipuid-oxidation levels by different methods. The lipid biochemical study in the hepatic cells was spectrophotometrically determined.

It was found significant increase in the malondialdehyde ($p<0.002$) and lipid oxidation ($p<0.05$) levels in the 3rd group compared to group 2 and 4. Based on the results it can be concluded that *Azadirachta Indica* co-administration partially ameliorated ota-induced liver damages and antioxidantly reduced the oxidative changes in the hepatic cells.

Keywords: oil, lipid oxidation, mycotoxicity

Acknowledgments: The study was supported by scientific project 3/2020 of Medical Faculty, Trakia University, Bulgaria.

Protein and Lipid Protection of Neem Oil on the Splenic Macrophages after Chronic Ochratoxicosis

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The antioxidant importance of neem oil seeds (*Azadirachta Indica)* was investigated for its possible protein and lipid oxidation protective effect on the splenic macrophages in 24 male icr/ni albino mice against chronic ochratoxicosis. The aim was to instigate the ochratoxin A oxidative changes on the proteins and lipids and the free radicals overproduction. Reactive oxygen species, proteins and consequent lipid-oxidative damages caused by mycotoxins were considered to be the main mechanisms leading to oxidative disorders in the splenic macrophages.

The animals were divided in four groups: **1)** basal diet (bd); **2)** bd with 100mg/kg *neem oil*; **3)** bd with 1.6 mg/kg ochratoxin A; **4)** bd with 1.6 mg/kg ochratoxin A + 100mg/kg *neem oil*. Neem oil oral administrations prevent the physiological status of mice and improve the biochemical parameters of the urine. Moreover, electron paramagnetic resonance analysis shows decreasements of lipid oxidation/ reactive oxygen species productions in splenic macrophages after neem oil administration, even after 27 days ochratoxin -pretreatment. The significant decrease in malondialdehyde formation in spleen and the increased expression of antioxidant enzymes in neem oil-treated ($p<0.004$) and neem oil+ ochratoxin groups ($p<0.004$), confirm the positive modulatory effect of the oil on the cellular antioxidant system. Neem oil ($p<0.002$) and neem oil+ ochratoxin groups ($p<0.05$) caused significant reduction of protein carbonyl content, compared to ota group. The results suggested that neem oil protect against chronic ochratoxicosis and other oxidative abnormalities. As typical antioxidant neem oil could protect splenic macrophages and regain intracellular antioxidant capacity.

Keywords: neem oil, reactive oxygen species, lipids, proteins, ochratoxicosis

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The Effect of *Lemna Minor L.* on Intracellular Lipid Oxidative Damages in Experimental Pulmonary Fibrosis

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Lemna minor extract (purity 85%) has previously been reported to alleviate appearance of lung alterations. The current study examined the antioxidant activity and therapeutic action of the *Lemna minor* extract to modulate and protects the lung alterations in experimental bleomycin-induced pulmonary fibrosis. The hypothesis was that *Lemna minor* would protect the lung alterations by inhibiting intracellular lipid peroxidation, lowering biochemical parameters and reducing oxidative stress levels.

Pulmonary toxicity was induced by intraperitoneal injection of mice once daily with bleomycin (0.069 U/mL; 0.29 U/kg bw) for a period of 21 days. The *Lemna minor* was administered once a day for 4 weeks, 2 h prior at dose (100 mg/mL; 0.370 mg/kg/day).

Bleomycin intoxication produced oxidative damages, in which the antioxidant system functioned incorrectly and ROS production significantly increased. The *Lemna minor* extract provided significant pulmonary protection against induced toxicity by improving superoxide dismutase ($p<0.05$), catalase ($p<0.04$), total cholesterol ($p<0.05$) levels and decreasing malondialdehyde ($p<0.002$) and reactive oxygen species ($p<0.002$) in the group receiving BLM ($p<0.05$), leading to reduced intracellular lipid peroxidation. In conclusion, the *Lemna minor* extract facilitated recovery from bleomycin-induced pulmonary injury by suppressing oxidative stress damages. Therefore, the *Lemna minor* stimulates antioxidant-scavenging activity and lipid peroxidation reduction in lungs. Our results make it appropriate to propose the use of the extract as a possible addition to the treatment of chronic pulmonary alterations associate with bleomycin-induced toxicity.

Keywords: antioxidant; lipid-scavenging imbalance; pulmonary fibrosis

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The Antioxidant Activity of *Lemna Minor* Extract on Protein-Carbonilization Levels in Progressive Pulmonary Fibrosis

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Idiopathic pulmonary fibrosis is a progressive and deadly lung disease, damaging alveolar epithelial cells, formatting the fibroblastic foci, and extracellular matrix deposition. The goal of this study was to use experimentally-induced lung fibrosis by repeated intraperitoneal injections with bleomycin to understand when protein carbonyl levels are elevated and lead to increased oxidative damages. In addition, the treatment of animals with Lemna minor (purity 85%) extract, immunomodulator and intracellular lipid-oxidation inhibitor demonstrated that the level of progressive fibrotic changes was significantly reduced. To examine progressive fibrosis, wild-type 12 irc/ni male mice were administered BLM (0.069 U/mL; 0.29 U/kg) trice a week for 21 days.The *Lemna minor* extract (200 mg/mL; 0.417 mg/kg), dissolved in corn oil was administered once (in 12 animals) a day for the next 4 weeks, 2 h prior. Mouse lung tissues were washed under ice, homogenized and investigated using a carbonyl-protein content and antioxidant enzymes Elisa kits. Our data suggest that bleomycin elevates protein carbonyl levels ($p<0.004$) and lower antioxidant enzymes ($p<0.004$) in the fibrotic lung and in so doing exacerbates pulmonary fibrosis, most likely through previously identified oxidative stress-mediated mechanisms. The *Lemna minor* extract effectively provided pulmonary protection against progressive toxicity by improving antioxidant enzymes ($p<0.03$) levels and significantly decreasing protein carbonylation ($p<0.002$) in the group receiving *Lemna minor*+bleomycin. In conclusion, was demonstrated that *Lemna minor* treatment inhibits bleomycin-induced protein oxidation as monitored by measuring the formation of protein reactive carbonyl contents in pulmonary fibrotic cells and provides further indication that the extract protects alveolar epithelial cells from oxidative stress via its antioxidant property.

Keywords: antioxidant; protein- carbonilation; progressive pulmonary fibrosis

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Acute Oxidative Stress and Protein Carbonyl Levels as Predictors during Pregnancy Complicated by Preterm Birth

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Acute oxidative stress is an inescapable component of pregnancy due to increasing oxygen demand from the fetus and increased redox imbalances in the cellular reactive oxygen species/ physiological stress in the maternal body. The elevated acute stress levels and the proteins structure injures are risk factors for the adverse pregnancies and can cause premature aging of fetal tissues, triggering pathological mechanisms which can lead to premature/ preterm activation of birth. The objective of the present study was to investigate plasmatic levels of protein carbonylation as marker of protein oxidation in preterm birth by competitive *in vivo* enzyme-linked Immunosorbent assay along with cellular reactive oxygen species measured by electron paramagnetic resonance spin-trapping as predictors to study the oxidative-stress damages.

The study included 30 non-pregnant (20-32 years) normotensive volunteers and 80 (17-38, Ist, IInd and IIIrd trimester) preterm birth women. Patients were examined after informed consent in initial medical view or obstetric admission at UMBAL, Stara Zagora, Bulgaria. Statistically significant increase in the levels of ascorbate ($p<0.03$), lipid peroxidation ($R = 0.977$, $p<0.005$), nitric oxide levels ($p<0.05$) along with protein carbonylation ($R = 0.932$, $p<0.05$) has been noted in preterm birth women in IIIrd trimester. In conclusion, the results provide evidence of increased acute oxidative stress in the form of elevated cellular redox imbalance and protein structure disturbances in preterm birth. This may be responsible for different complications in the fetus, fetal cell senescence and develop a supplementary inflammatory environment.

Keywords: acute oxidative stress, protein carbonylation, preterm birth

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Oxidative Stress as a Pathophysiological Mechanism of Spontaneous Idiopathic Preterm Birth

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Preterm birth is clinical-social problem, lead to 15 million premature babies born each year. The inflammation process is the first mechanism lead to preterm birth. Indeed, the inflammation signs could be found in the cervix, myometrium, amniotic membranes and intra-amniotic space. In the preterm spontaneous birth of a term, the tissues are infiltrated with neutrophilic granulocytes, the production of cytokines and chemokines is increased, but there are no paraclinical inflammatory changes in the mother's blood. The inflammation was localized in the membranes and myometrium and only present is pro-inflammatory response. This process is an integral part of the uterine component of childbirth. In different clinical cases, the nature and severity of the inflammatory process are different- sterile inflammation, subclinical intrauterine infection or extensive chirioamnionitis. The important question- is there other mechanisms that cause preterm birth? The main goal is the prevention and treatment of prematurity, as inflammation generates uterotonic signals that are stronger than the signals of fetal maturity. Inflammatory leukocyte activation, elevated inflammatory cytokines and chemokines, and collagenolysis of extracellular matrix metalloproteinase lead to activation of the myometrium. The investigation of oxidative stress damages in preterm birth aims to better (new-line of) understand of its pathophysiology. Presumably, the stress-elevated reactive oxygen species caused decreased antioxidant activity in mother's organism. The prooxidants/ antioxidants imbalance probably is the second pathophysiological mechanism of preterm birth. In conclusion, oxidative stress leads to damage in the maternal-fetal compartment on the fetal membranes, placenta and lead to "process of aging" of all fetal cells, generation of uterotonic molecular signals and triggering to the cascade of reactions.

Keywords: oxidative stress, mechanism, preterm birth

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Comparing Octanol-water, P_W^{OCT}, and Oil-water, P_W^O, Partition Constants in Binary Systems: P_W^O Values cannot be Predicted from P_W^{OCT} Values and need to be Evaluated for each Antioxidant.

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The octanol-water partition constants, P_W^{OCT}, are widely employed for a rational description of the partitioning of drugs in environmental and pharmaceutical studies. They are also important to assess the relative hydrophobicity of molecules. P_W^{OCT} values are determined by all intermolecular interactions, including electrostatic, hydrogen bonding, and dispersion forces between the solute and the two phases in which it is dissolved. Its value thus depends on the balance of all intermolecular forces involving a solute and the two phases between which it partitions.

We hypothesize that changes in the nature of the oil should lead to changes in P_w^{oil} values and to investigate if P_w^{oil} values can be predicted from P_W^{OCT}, we determined the partition coefficients, P_w^{oil}, of four series of potent, natural AOs of increasing lipophilicity between vegetable oils (olive, soybean, and corn) and water. Results indicate that the contribution of the −CH2 groups in the alkyl chains of the antioxidants to the overall lipophilicity of the AO is the same irrespective of the oil employed. Extrathermodynamic linear relationships between the P_w^{oil} and P_W^{OCT} could be established, but they are only valid for a given series of chmologous antioxidants, suggesting that, in general, P_w^{oil} values cannot be predicted from P_W^{OCT} values and need to be determined for each antioxidants or series of AOs.

When AOs contain ionizable groups (phenolic and ascorbic acids), the P_w^{oil} and P_W^{OCT} values depend on the actual value of the acidity, decreasing upon increasing pH following a sigmoidal-like curve, and their apparent values can differ by more than one order of magnitude, reflecting the changes in the hydrophobicity of the AO because of its ionization.

Extraction of *Fucus vesiculosus* using Pressurized Low Polarity Water: Influence of Extraction Temperature on Antioxidant Activity of the Extracts

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In recent years, the quest for new antioxidants from natural resources is intensified. This is partly due to the suspicion that synthetic antioxidants have undesired consequences and due to the growing consumer awareness towards natural antioxidants. Therefore, antioxidants from less explored sources such as from marine resources receive increasing interest from researchers and functional foods and nutraceuticals producers. *Fucus vesiculosus* is a brown marine alga, which is widely grown in the waters of Denmark and other Nordic countries. Conventional solid liquid extraction of this seaweed using organic solvents showed high levels of phenolic compounds such as phlorotannins, which are responsible for high antioxidant activity of this seaweed extracts. However, using organic solvent as extraction medium has several drawbacks including the fact that these solvents are toxic and environmentally unfriendly. In addition, long extraction time and difficulty to separate the extract from the solvent are other drawback. To address these problems, pressurized low polarity water extraction (PLPW) has received increasing attention in recent years. PLPW is water between its boiling point (100°C) and critical point (374°C) and sufficiently high pressure to maintain the water in liquid state. At this condition, water will have low viscosity, low surface tension and low dielectric constant. This temperature dependent property of water provides water with comparable properties as those of organic solvents such as ethanol and methanol. By tuning the extraction temperature, we can get different dielectric constant of water so that we can extract different compounds with varying degree of polarity. Therefore, in this study, *F. vesiculosus* was treated using PLPW at different temperatures and the influence of extraction temperature on the total phenolic content and antioxidant activity of the extract is presented.

An Approach to the Extraction of Bioactive Lipid Constituents from *Illex spp.* By-products

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Marine species are known to provide high contents of important constituents for the human diet. Among them, polyunsaturated fatty acids (PUFA) belonging to the ω3 series (i.e., eicosapentaenoic and docosahexaenoic acids; EPA and DHA, respectively) have been reported to lead to the inhibition of the development of different kinds of illnesses such as cardiac, circulatory and inflammatory. Interestingly, ω3-PUFA compounds have been signalled to be especially concentrated in marine phospholipid (PL) classes. On the other side, by-products of marine species are body parts that are usually removed to improve quality during the commercialisation process and distribution chain. By-products can include heads, blood, bellies, viscera, skin, etc. Among marine species, cephalopods commercialisation has notably increased in the latest decades, this leading to a high production of by-products, which in turn have originated great environmental inconveniences. As a matter of interest, a great accumulation of PL and ω3-PUFA has been observed in different kinds of cephalopod by-products such as ovary and gonads.

Taking all this into account, the current study is a first approach focused on the isolation of bioactive lipid compounds corresponding to processing wastes resulting from the commercialisation of different kinds of products from *Illex* spp. For it, frozen by-products provided by a seafood factory were thawed, heated (partial drying) and pressurised, the liquid phase being centrifuged and the lipid fraction separated. Different experimental conditions (thawing speed, heating time and temperature, pressure and centrifugation speed) were tested to enhance the lipid content yield, as well as the final level of PL and ω3-PUFA in the lipid fraction obtained. Highest yields on PL, EPA and DHA contents were obtained by previous heating at 80 ºC for 60 min when using a high-speed centrifugation. Results confirm the possibility of employing *Illex* spp. by-products as a source of valuable lipid constituents by applying a solvent-free methodology. Further research is on-going to optimise the yield of such bioactive compounds.

Statistical Treatment of Analytical Data Obtained using Protocols Developed or Applied within the OLEUM Project Framework for the Determination of the Total Hydroxytyrosol and Tyrosol Content Indicates the Need for Consensus Among Scientists for the Benefit of the Olive Industry

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Within the OLEUM framework an in house validated UHPLC protocol was developed for the determination of the total hydroxytyrosol and tyrosol content in virgin olive oil fit for the purpose of the health claim according to the EC Regulation 432/2012 for "Olive Oil Polyphenols [1]. The analytical results were compared with those produced in OLEUM consortium labs that declared expertise in the olive oil phenol analysis using other liquid chromatographic separation protocols or ^{1}H-NMR spectroscopy [2]. This work presents the analytical steps for each protocol and the statistical treatment of the data produced for a set of 30 virgin olive oils. Method comparison was carried out using the Passing-Bablok regression, a robust method free of bias characterizing the commonly used correlation analysis approaches [3]. Bland-Altman analyses, additionally used, provided information on the mean difference and possible limits of agreement [4]. Data analysis indicated slight or statistically significant differences and made clear the need for consensus among scientists regarding the method/s of choice for the determination of the target compounds for the benefit of the olive industry and society.

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Kinetic Features of the Oxidation Chain Termination by Nitroxyl Radicals in the Liquid Phase and Heterogeneous Systems

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The oxidation of methyl linoleate (LH) in micelles is used as a model system to study the antioxidant activity of nitroxyl radicals (>NO•) under conditions close to the cell of a living organism. It was found that >NO• terminate LH oxidation chains through a cycle of reactions with a hydroperoxyl radical (HO_2•): HO_2• + >NO• → >NOH + O_2 ($k_{>NO•}$) and HO_2• + >NOH → >NO• + H_2O_2 ($k_{>NOH}$), with a competing reaction L• + >NO• → >NOL. Such competition, as well as the distribution of reagents and the possibility of reactions in the organic and aqueous phases, complicate the analysis of the >NO• reactivity in this process. Reactions occurring in different phases can be investigated separately using simplified models. The oxidation of 1,2-substituted ethylenes and 1,4-substituted butadienes, inhibited by >NO•, is suitable for studying the regularities of the interaction of >NO• and HO_2• in an organic medium because HO_2• radicals propagate oxidation chains in this system. We have studied the kinetics of this process in solvents of different polarity. It is shown that the kinetic inhibition coefficient for >NO• exceeds 10^2. The rate-limiting step of the process is the reaction of >NO• and HO_2•. The $k_{>NO•}$ values are in the range $(2 – 40)\cdot10^5$ l·mol^{-1}·s^{-1} and depend on the >NO• structure and the solvent polarity. These values correlate well with the calculated values of Δ*E* for this reaction for low-polarity solvents. The effects of specific solvation of HO_2• and >NOH play an essential role in polar solvents. Oxidation of water-soluble organic substrates in an aqueous solution is a promising model to study possible chain termination reactions by >NO• radicals in an aqueous medium and the contribution of these processes to the general mechanism of inhibition in a heterogeneous medium.

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Determination of Lipid Hydroperoxides in Oil-in-Water Emulsions using by a Novel Technique

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Krill oil has accepted as a new source omega-3 fatty acids and these fatty acids have many health benefits. On the other hand, nanoemulsions have some important potential advantages over conventional emulsions due to the high physical stability, and ability to increase the bioavailability of lipophilic bioactive. Conventional lipid hydroperoxides methods (titrimetric, AOCS, thiocyanate) did not work to determine lipid hydroperoxides in this emulsion system. DPPP itself is not fluorescent, but when it reacts with lipid hydroperoxides, its oxide form was generated, and it has fluorescent properties. In this study, DPPP (diphenyl-1-pyrenylphosphine) was used a fluorescent probe to determine lipid hydroperoxide by a novel technique. Krill oil-in-water emulsions were prepared using by microfluidizer at 12000 psi pressure. The mean particle diameter was determined around 179 nm and the droplet charge (ζ-potential) was determined from -29 mV. Emulsion samples were incubated at 37^{o}C photo-oxidation. Lipid hydroperoxides were determined by fluorescens spectrophotometer using with DPPP probe. The results show that fluorescent technique can be used to determine lipid hydroperoxides at different environmental conditions and also this technique was more sensitive and selectivity than conventional techniques.

Influence of Water Content and Lipid Oxidation Products on Phosphatidylcholine Critical Micelle Concentration Changes in the Process of Rapeseed Oil Autoxidation

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Lipid oxidation takes place mainly at the oil-water interface in association colloids. Understanding of this phenomena mechanism is necessary to control the rate of lipid oxidation. Association colloids play an important role in the bulk oil autoxidation process. These are physical structures formed by surfactants (amphiphilic compounds) in a non-polar environment (bulk oil) with small content of water. At concentration above their critical micelle concentration (CMC) they self-aggregate and form reverse micelles. The presence of water and amphiphilic compounds are basic requirements for the formation of asssociation colloids in oils. The purpose of this study was to analyze changes of water content, increase of oxidation products concentration and their impact on changes in critical micelle concentration of DOPC phospholipid (1,2-dioleoyl-sn-glycero-3-phosphocholine) during rapeseed oil oxidation. This study describes purified rapeseed oil autoxidation experiment under accelerated oxidation conditions at 55˚C for 10 days. Samples were taken to control changes of water content and CMC. Simultaneously, the effect of water on oil autoxidation in the absence and presence of asssociation colloids was also determined by analyzing the content of hydroperoxides and hexanal. Presented studies confirm the prooxidant effect of phospholipid DOPC, also indicating that the water content affects the critical micelle concentration of DOPC. Similarly, the rate of the autoxidation process depends on the water content and CMC. In the initial phase of oil oxidation, the water content decreases, which is also associated with a decrease of CMC, while the oxidation products are at a similar low level. However, on the fourth day of experiment, the water content increases, as like the CMC value and the rate of oil autoxidation process. This confirms that the presence and changes in the structure of association colloids affect the process of rapeseed oil autoxidation.

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Analysis of Lipid Oxidation and Radical Formation in Corn Extrudates and Model Systems using Electron Paramagnetic Resonance Spectroscopy (EPR) and Conventional methods

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Understanding the oxidation process in complex low-moisture foods is essential to improve their shelf life. In this study, the formation of short-lived and stable radicals was investigated using electron paramagnetic resonance (EPR) spectroscopy in complex starch-protein-lipid model systems, as well as in corn extrudates. These results were compared with hydroperoxide and hexanal formation.

Short-lived lipid radicals were measured in ethyl acetate extracts of model systems and extrudates by the use of the spin trap PBN. Stable radicals were analyzed directly in ground samples without sample preparation.

The results showed significant PBN adduct formation after 30 min incubation at 50 °C. During storage, lipid radicals (PBN adducts) and conventional lipid oxidation markers increased in model systems. Simulation of the EPR spectra of bulk oil demonstrated that mainly alkoxyl radical adducts were detected. Furthermore, rapidly decomposing peroxyl radical adducts resulted in the significant amounts of alkoxyl radical adducts.

After extrusion the signal intensity of the stable radicals increased compared with the unextruded premix. During further storage their intensity remained constant. The g-value of 2.00467 was attributed to protein radicals based on a comparison with zein containing model systems exhibiting a g-value 2.00474. Superposition in the EPR-spectra indicated that these radicals are carbon-centered or nitrogen-centered.

In conclusion, the investigated model systems enable the annotation of the short-lived radicals and of the stable radicals by EPR in extrudates. This understanding of the oxidation processes in extrudates may in future allow a targeted use of antioxidants to inhibit radical formation in different environments (or phases) of complex matrices.

Inhibition of Lipid Autoxidation Reaction by Isothermal Calorimetry

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The work aimed to develop a method to study the inhibition of lipids autoxidation by isothermal calorimetry. The working principle consisted of following the heat evolved during the reaction between styrene and 2-2'-azobisisobutyronitrile (AIBN) as a radical initiator. The inhibition of the reaction was controlled by adding α-tocopherol, at concentrations from 50 to 500 µM. Through the analysis of the thermograms, the parameters related to the kinetics of the reaction were obtained and compared with data available in the literature. This approach was then applied to vegetable waxes extracted from apples and oranges by supercritical carbon dioxide. In this case, linseed oil was used as the oxidizable substrate instead of styrene, while other experimental conditions were maintained constant. The results showed that waxes were able to slow down linseed oil autoxidation, with apple waxes being more active than orange waxes. However, such activity was significant only at relatively high concentrations (> 1 % of waxes), greatly higher than the concentration typically used with radical chain breakers, like BHT (0.2 %). It was demonstrated that the apparent antioxidant effect of the waxes was related to the formation of a network of interconnected platelet-like crystals with a dimension of approximately 10-20 µm, which slowed down the rate of oxygen diffusion-controlled reactions, like lipid oxidation. Thus, although waxes cannot exert a classical chain breaking activity, their capacity to inhibit or retard lipid oxidation can still be of significance for food applications. This was demonstrated by testing their activity in combination with primary antioxidants such as BHA, ethoxyquin and α-tocopherol. When waxes were present with these antioxidants, the resulting rate of inhibition was significantly higher than the individual antioxidants alone. The ability to amplify the radical scavenging activity of primary antioxidants could be useful for the development of novel antioxidant systems. Thanks to these results, the study clearly showed the potential of isothermal calorimetry to follow the inhibition of lipids autoxidation reactions.

Characterization of Biscuits and Taralli Obtained with Different Lipidic Formulations and Determination of their Oxidative Stability

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Bakery products such as biscuits and taralli are among those products subjected to lipid oxidation because of their long shelf-life. In this study biscuits and taralli were formulated with different lipidic formulations in order to find out what resist oxidation the most. After a lipid fractions characterization, the oxidative stability of biscuits and taralli; was studied with Oxitest®. Taralli (T) were composed of wheat flour, white wine, salt and five different lipid fractions: olive oil (EVO, T0), high oleic sunflower oil (OSO, T1), EVO:sunflower oil 87.5:12.5 (EVO:SO, T2), EVO:rice oil 75:25 (EVO:RO, T3) and OSO:coconut oil 87.5:12.5 (OSO:CO, T4). Biscuits (B) were composed of wheat flour, sugar, eggs, lemon zest, baking powder and six different lipid fractions: palm oil (P, B0), butter (B, P1), B:EVO 50:50 (B2), B:OSO 50:50 (B3), OSO (B4) and CO:SO 87.5:12.5 (B5). In both samples of taralli and biscuits the fatty acid profile reflected that of the relative lipid fractions; prevalence of monounsaturated fatty acids (MUFA) was evident, with the exception of palm oil, butter and CO:SO 87.5:12.5 where saturated fatty acids (SFA) prevailed instead. Three tocopherols (α-tocopherol, β-tocopherol e γ-tocopherol) and four tocotrienols (α-tocotrienol, β-tocotrienol, γ-tocotrienol e δ-tocotrienol) were identified in the different lipid fractions. Palm oil showed the highest content and was the only which contains all of them. Samples T0 and T3, showed the highest oxidative stability; the latter showed the high importance of γ-orizanol which is naturally present in rice oil. Biscutis B1 and B5 showed the higher oxidative stability related to the combination of high presence of SFA content in butter and of tococromanol in SO. The oxidative stability depends on the oil/fat composition, but it is not exclude a contribution of the products from maillard reaction. In biscuits, in fact, reducing sugar and protein are relevant and the development of such reaction may contribute to oxidation resistance. For this reason, in fact, B4 and T1 samples had the same lipid fraction (OSO) but the oxidative stability of the second one was almost four time lower than first one; probably because of Maillard reaction that occurs in biscuits.

Characterization of Oils Obtained from the Co-pressing of Olives and Oleaginous Matrices with High Nutritional Value and Determination of their Oxidative Stability

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Olive oil has always been one of the main components of the Mediterranean diet. Compared to other vegetable oils, extra virgin olive oil (EVO) has a unique chemical composition that also determines its beneficial effect on human health.

The aim of this work was emphasise and improve the functional and health quality of the EVO by co-pressing olives with other valuable oil matrices, like grape seeds (GS) and pomegranate seeds (PS) that are usually by-products. After a lipidic characterization of the oleaginous matrices, the seeds (GS and PS) were co-pressed with olives and the composition in fatty acids, antioxidants (tococromanols and total phenols), sterols and the oxidative stability of the final oils (OG and OP) were determined, compared to a control sample (olive oil, O).

The obtained data underline how the selected raw materials represent an important source of bioactive substances that can help make EVO oil an even more interesting and precious product. However, the results about co-pressed oils are partially positive because they still show a profile not so different from the control (O). So, it is necessary re-evaluate the percentage ratios between olives and the different oilseeds.

In particular, the OP oil showed the best profile in bioactive compounds, but the oxidative stability should be improved, suggesting protective and small packaging in order to guarantee its maximum quality.

In conclusion, these co-pressed oils are potentially very interesting for "noble" cold uses, finding the approval of the most demanding consumers, sensitive also to environmental issues.

Correlations between Antioxidant Activity, Distribution, and Chemical Properties of Phenolic Compounds in O/W Model Emulsions

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Oil-in-water emulsions are widely used not only in the food industry but also the cosmetics, pharmaceutical and medical industries to encapsulate, protect and release bioactive lipids. Therefore, it is important to understand the factors that influence the oxidative stability of these formulations and to increase their safety and shelf life.

In this work we evaluated the antioxidant activity of some phenolipids, which have similar alkyl chains (with 8 and 16 carbons) and different phenolic rings (derived from caffeic, 4-hydroxycinnamic, dihydrocaffeic, 4-hydroxyhydrocinamic, tyrosol and hydroxytyrosol) in 4:6 (oil / citrate buffer, 0.04M, pH = 3.65 / Tween 80) emulsions. In order to understand the importance of each property for the antioxidant activity of compounds, Pearson's correlation analysis was carried out between the chemical properties of the various antioxidants, namely first anodic potential, reducing capacity (FRAP value), DPPH radical scavenging activity (EC_{50}), concentration of antioxidant in each region of the emulsified system and antioxidant capacity. Results confirmed the importance of the effective concentration of AOs in the interfacial region and of the first anodic potential in predicting the antioxidant capacity of the compounds. It also showed the importance of AOs concentration in the aqueous region for the lower stability of emulsions. A stepwise linear regression analysis of the data showed that concentration of AO in the interfacial region, $[AO]_I$, explains more than 80% of the results of the antioxidant capacity.

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Olive Oil Oleogels as Strategy to Confer Nutritional Advantages to Burgers

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Due to low price, convenience, and high sensory quality, beef burgers are widely consumed worldwide. However, because of their excessive content in saturated fatty acids, they have been related to an increased incidence of cardiovascular diseases. To produce healthier burgers, it is necessary to reduce their fat content and modify their fatty acid profile. However, reducing and replacing saturated fats by unsaturated ones may decreases the oxidative stability and the sensory quality (taste, aroma and juiciness) of the product. Thus, the challenge of the meat industry is to find viable alternative to decrease the fat level and provide a healthier lipid profile in their products without damage their oxidative stability, and their technological and sensory quality.

The effect of bovine backfat replacement by oleogels containing pork skin and olive oil on the oxidative stability, physicochemical, technological, nutritional, and sensory parameters of burgers was evaluated. Three different hamburger batches were manufactured: HC with 90% of lean beef and 10% of bovine backfat, and HVOO and HSOO with the 10% of bovine backfat replaced by pork skin/water/virgin olive oil (VOO) or stripped olive oil (SOO) oleogels respectively, at 20 : 60 : 20 ratio.

A fat reduction to 30% and an improvement of the fatty acid profile were achieved in the reformulated burgers. Although some differences regarding appearance, colour and fat perception among raw burgers were observed, after processing at 180 ºC, hardness, chewiness, color, taste and odor and the overall acceptability were high and comparable to control. The three different batches were oxidative stable during 7 days at 4 ºC. However, after 45 days of storage at -20 ºC only HC and HVOO showed not to be oxidized. Apparently, the antioxidant content in the virgin olive oil used in the manufacture of oleogels was able to prevent oxidation in these HVOO samples.

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Improving the Oxidative Stability of Guts from Cod Filleting by Antioxidant Dipping – A Route to Better Seafood Side-stream Utilization

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There is a growing interest for sustainable food production and increased utilization of existing food raw materials. In the cod filleting industry up to 60% of the weight ends up as side-streams, e.g. guts, heads and frames. At the moment, the majority of these side-streams ends up as low value products for feed. To increase utilization for food applications, new preservation solutions are needed to maintain a high quality of the side-stream before further processing. The aim of this study was to evaluate the effect of antioxidant dipping of cod guts on the oxidative stability during subsequent storage. Selection of antioxidants was based on earlier findings with commercial formulations of rosemary extracts for preservation of herring solid side-streams (Wu et al., 2020). The following treatments were evaluated: no dipping, dipping in 0.9% NaCl, dipping in 2% Duralox MANC (commercial rosemary preparation fortified with ascorbic acid, α-tocopherol and citric acid), or dipping in 0.05% and 0.2% lipophilic rosemary extract having a carnosic acid content 15 times higher than that in Duralox-MANC. After antioxidant dipping, the guts were drained and minced to obtain homogeneous samples after which they were subjected to short- and long-term storage (5 ^{o}C for 7 days and -20 ^{o}C for 6 months). The effect of the antioxidant treatment was evaluated by regularly measuring peroxide value (PV) and TBA-reactive substances (TBARS) during storage. Results from the short term storage show a positive effect of the antioxidant dipping on the oxidative stability of cod guts. Duralox MANC inhibited the formation of PV throughout the storage and TBARS was inhibited for 4 days. Rosemary extract (0.2%) also inhibited the formation of PV for 7 days, however, the inhibition of TBARS was 2 days. For the control, PV and TBARS already increased after 1 day of storage. Duralox MANC was most efficient followed by rosemary extract applied in 0.2% (the high concentration evaluated).

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CROSS – A Novel Clean Label Concept for Preventing Lipid Oxidation of Fish Protein Isolates Produced with the pH-shift Process

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The pH-shift process is a promising tool to recover functional proteins from fish and shellfish co-products. However, lipid oxidation can be triggered during the process due to presence of polyunsaturated fatty acids (PUFA) and hemoglobin (Hb) combined with exposure to critical pH´s, affecting the quality of isolated proteins. Based on the wish for clean label within the food industry, we have earlier explored and shown good oxidation-inhibiting potential of lingonberry press cake, shrimp peels and brown seaweed (*Saccharina latissima*) when cross-processing these materials with herring and salmon co-products. We here aimed to further study the cross-processing concept by broadening the selection of supporting materials to also include apple press cake, oat fibre residue, barley-spent grain and green seaweed (*Ulva fenestrata*); underutilized materials which are all rich in natural antioxidants. We also wished to study the effects of cross-processing herring co-products with different supporting materials on the oxidative stability of produced protein isolates during extended storage on ice. Lipid oxidation was followed by analyzing the aldehyde levels (MDA, HHE and HNE) of protein isolates.

MDA, HHE and HNE levels revealed that all kinds of supporting materials prevented lipid oxidation both during the actual pH-shift processing of herring co-products and during the subsequent 16-day ice storage, although to different extents. Lingonberry press cake followed by apple press cake showed the strongest effect on reducing aldehyde formation compared to isolates produced from herring co-products alone. Other supporting materials delayed the production of aldehydes during ice storage but failed to reduce the maximum level of aldehydes. The results indicated beneficial effects from cross-processing fish co-products with antioxidant-rich non-fish materials to minimize lipid oxidation both during pH-shift processing and during subsequent ice storage of protein isolates. The combination with lingonberry press cake was the most promising approach and will be subject for further studies.

Measures to Prohibit *post mortem* Hemoglobin Mediated Lipid Oxidation in Herring and Rainbow Trout by Understanding RBC hemolysis

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Hemoglobin (Hb) is a major lipid pro-oxidant in fish muscle. In vivo the Hb molecules are highly concentrated in the red blood cells (RBCs). However, post-mortem biochemical changes and processing factors cause the RBCs to lyse, thus Hb molecules are released into the plasma and can subsequently contaminate the fish muscle when capillaries burst during various processing operations. In our previous paper (Ghirmai, Eriksson, Wu, Axelsson, & Undeland, 2020) we used fish blood or a RBC model system to identify and optimize some of the most important fish processing parameters such as salinity, temperature and mechanical stress, that affect hemolysis of the RBCs). We also identified certain plasma components that could minimize hemolysis. Currently, we are further deepening our knowledge on the protective effects of plasma components on hemolysis, such as different antioxidants. Furthermore, we are extending our blood/RBC model system to also comprise fish muscle, which will allow us to study the relation between hemolysis and lipid oxidation development. Previous studies of Hb-induced fish lipid oxidation have mainly focused on addition of pure Hb or hemolysate to fish muscle, why our research will provide a new level of understanding to this problem; namely how contact between Hb and the fish lipids is mediated.

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Synthesis and Evaluation of Lipophenols as New Therapeutics toward Retinal Degeneration

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Dry age-related macular degeneration (AMD) and Stargardt disease go through a known toxic mechanism caused by carbonyl and oxidative stresses (COS), which is responsible for the accumulation of toxic lipids called bis-retinoids. Both pathologies have currently no preventive treatment. The aim of this work is therefore to propose new lipophenols able to reduce the two stresses involved in photoreceptor degeneration for the development of therapeutics.

Preliminary *in vitro* studies have identified a leading isopropyl-phloroglucinol-DHA conjugate, called IP-DHA (DHA: docosahexaenoic acid, C22:6, n-3). This lipophenol was able to reduce carbonyl stress in retina cells. However, as its antioxidant properties were still weak, we focused on designing different families of lipophenol derivatives to conserve anti-carbonyl activity and improve antioxidant properties. We developed synthetic pathways to access various isopropyl-polyphenol derivatives (phloroglucinol, resveratrol and quercetin) linked to the poly-unsaturated fatty acid (PUFA) moiety. The strategies elaborated allowed the introduction of isopropyl and DHA moieties in specific positions for each polyphenol backbone. For all lipophenol derivatives, their corresponding polyphenol bearing only isopropyl function or only lipid moiety was produced, to evaluate the impact of those substituents on their *in vitro* properties.

Our results confirmed the necessity of the isopropyl group as well as the lipid part for anti-carbonyl stress activity. All new lipophenols showed antioxidant properties in two different tests targeting oxidative stress in AMD.

Rational design of lipophenols allowed the development of four synthetic pathways leading to a library of 14 derivatives. Among the families of lipophenol produced, the most promising is the quercetin lipophenol which shown best anti-carbonyl and antioxidant properties, and was the most efficient to protect retina cells against light induced toxicity of A2E (toxic *bis*-retinoid).

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