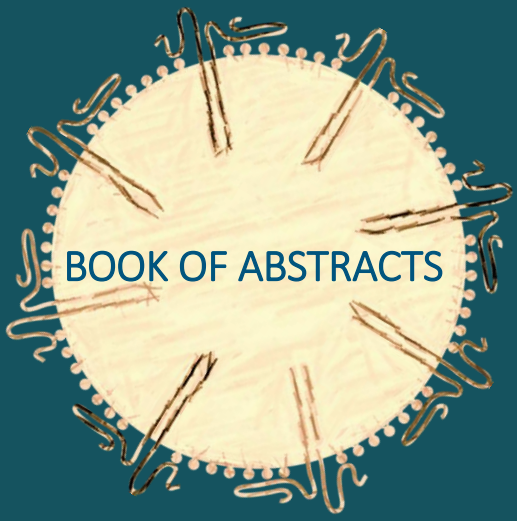
1st International Conference on Oil Bodies

Natural Oil Droplets
Ingredients for Industry



15 & 16 October 2018

Hotel de Wereld, Wageningen, The Netherlands

http://www.oilbodyconference2018.org

Organizers

Dr. Costas Nikiforidis
Biobased Chemistry and Technology, Wageningen UR, Wageningen, 6708 WG, The Netherlands

Dr. Costas Nikiforidis, has a background in Chemistry and a PhD on Chemistry and Physical Chemistry of Foods. He is currently Assistant Professor at the Biobased Chemistry and Technology Group at Wageningen University with a focus on "Bioderived and Bioinspired Functional Materials". His research involves fundamental understanding of the interactions in natural functional complexes in order to use them as such or mimic them. The properties of oil bodies/oleosomes is his main focus.

Dr. David Gray
Division of Food Science, University of Nottingham, Loughborough, LE12 5RD, UK

Dr. David Gray's research is summarised as follows: 'A Biomaterials Approach to Sustainable Nutrition and Ingredients'; much of this work involves characterising the properties of selected lipid-rich organelles ex-vivo. For a number of years he, and his group, have established certain physico-chemical and properties of oil bodies/oleosomes from a range of seeds. Funding for this work has included BBSRC, EPSRC and collaborations with industry through LINK-type projects.

Scientific committee

Dr. Costas Nikiforidis
Wageningen University, The Netherlands

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Dr. Thierry Chardot
INRA, Versailles, France

Dr. Thierry Chardot is in charge of the "Dynamic and Structure of Lipid Droplets" team at INRA Versailles (France). We are internationally recognized for our expertise in the purification and characterization of lipid droplets (LDs) from plants and microorganisms. Our main objective is to increase the knowledge of LD biology (structure, organization, function and dynamics). We use a wide range of techniques and developp tools to explore LDs dynamics (microscopies, specific antibodies, lipid analysis, proteomics…), identify associated proteins during life cycle (proteomics…) and understand their function and structure (protein solubilisation, dynamic light scattering, spectroscopies, structural proteomics, …).

Dr. Gustav Waschatko
Cargill R&D Centre Europe, Vilvoorde, Belgium

Dr. Gustav Waschatko received his Diploma in Biomedical Chemistry from the University of Mainz in 2009, followed by a PhD on "Oil Bodies – natural emulsifiers and their behavior at food relevant interfaces" at the Max Planck Institute for Polymer Research (2013). After Postdocs with

MPIP, AMOLF (Amsterdam) and Unilever R&D, he joined Cargill as "Oil Seed and Lipid Research Scientist" and is performing ingredient research and product development at the Cargill R&D Centre Europe in Vilvoorde (Belgium).

Prof. Remko Boom

Wageningen University, The Netherlands

Prof. Remko Boom obtained his PhD degree (highest honours) at Twente University. In 1993 he received the Royal Dutch Shell Study Tour Award for his PhD work. From 1992 to 1998 he worked for Unilever Research. In 1998, he was appointed as full professor of Food Process Engineering at Wageningen University. His research interests concentrate around the exploration of new principles to more sustainably produce healthier products: New ways to isolate ingredients, new ways to structure foods, and thermodynamic assessment of complex production systems using thermodynamics. He has currently (co-)published almost 300 peer-reviewed publications

Prof. Harry Bitter

Wageningen University, The Netherlands

Prof. Harry Bitter has a background in organic chemistry (Msc) and heterogeneous catalysis (PhD). He is the chair of the biobased chemistry and technology group at Wageningen University. His group focusses on the production of chemical building blocks and functional materials by extraction from and/or conversion of biobased feedstocks.

Prof. Vassilis Kiosseoglou

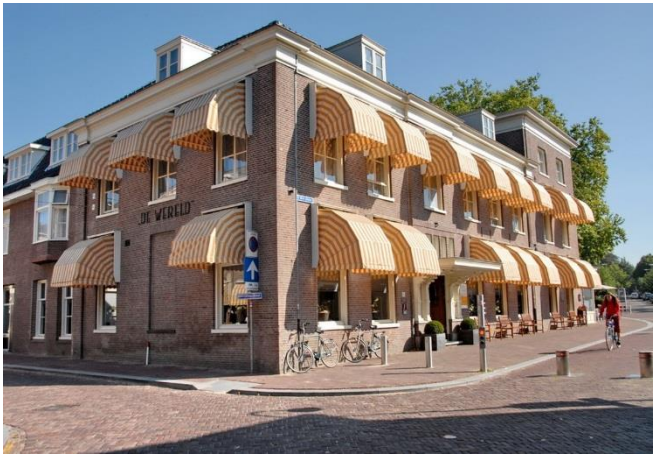
Aristotle University of Thessaloniki, Greece

Prof. Kiosseoglou has extensive experience in the study of physicochemical properties of food emulsifiers and biomacromolecules as well as their interaction and effect on food structure and/or physicochemical stability. He has been involved in research related to exploitation of protein in food emulsions, gel systems, edible films and recently in research in oil bodies extraction, investigation of their properties and their potential applications in food systems.

The venue

Wageningen is a city in the centre of the Netherlands, situated at the banks of the river Rhine.

The conference will take place in Hotel de Wereld. The historic hotel, located in the heart of the city, was the site of the capitulation of the German troops in the Netherlands, and the end of German occupation during World War II.



Hotel de Wereld, 5 Mei Plein 1, 6703 CD, Wageningen, The Netherlands

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11.10 – 11.30	Molecular microscopy of oil body and lipid droplet chemistry in situ with physiologically-relevant readouts *Alexandra Paul, Max Planck Institute for Polymer Research, DE*
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1 Keynote lectures

1.1 Seed oil-body (oleosome) proteins and their biotechnological applications

Jason T.C. Tzen

Graduate Institute of Biotechnology, National Chung-Hsing University, 40227, Taichung, Taiwan

*Email: TCTZEN@dragon.nchu.edu.tw

Three classes of oil-body integral proteins termed oleosin, caleosin and steroleosin were firstly identified in oil bodies of angiosperm seeds. So far, oleosin and caleosin, but not steroleosin, were detected in oil bodies of angiosperm pollens. Oleosins or caleosins in angiosperm pollens are not the same proteins found in seeds, and they are encoded by different genes. Comparably, oleosin, caleosin and steroleosin were also identified in oil bodies of gymnosperm seeds (pine megagametophytes); oleosin and caleosin, but not steroleosin, were detected in oil bodies of angiosperm pollens. In more primitive species, oleosin has been observed in most while caleosins have been observed in algae and fungi. Artificial oil bodies composed of triacylglycerols and phospholipids could be stabilized by oleoisn or caleosin but not steroleosin. Recombinant oleosin or caleosin expressed in *Escherichia coli* could also encapsulate artificial oil bodies with size, topology and stability comparable to those encapsulated with native oleosin or caleosin. Several application platforms have been developed on the basis of artificial oil bodies, including a protein expression system, an oral delivery system for hydrophobic drugs, a new enzyme fixation technique, a hapten presentation system for producing monospecific antibodies against small molecules, and an *in vitro* assay system to estimate tea astringency.

1.2 Identifying the functional properties of oleosomes – The journey so far

David Gray
Division of Food Science, University of Nottingham, Loughborough, LE12 5RD, UK

Email: david.gray@nottingham.ac.uk

Dr David Gray read Biochemistry at the University of Aberdeen, then gained a PhD in Plant Lipid Biochemistry (investigating the enzymes involved in the synthesis of polyunsaturated fatty acids on sunflower seeds) from the University of Birmingham (UK). Appointed to a lectureship in Food Chemistry at the University of Nottingham in 1993 then promoted to Associate Professor in 2006. 'A Biomaterials Approach to Sustainable Nutrition' captures the essence of much of his current work.

In addition to presenting at 10 international conferences by invitation, Dr Gray was invited to share his work (funded through a DEFRA-LINK program) on 'Sustainable Emulsion Ingredients through Bio-Innovation' at a symposium on 'Sustainable sourcing of functional ingredients from plants' (November 2012). This event was coordinated by Unilever and the BBSRC, and included a workshop to help shape funding priorities in this area, which led directly to the formation of the IBB network. His work on oleosomes has been funded for 15 years, and is currently supported by two projects with industry, and through the EPSRC-Funded 'Centre for Innovative Manufacturing of Food'.

Dr Gray's work on oleosomes has been a mix of curiosity-driven research and pragmatic experimentation, and his talk will capture the essence of his discoveries, some published and others best left unpublished. To finish, he will consider the way forward for oleosome research, and, if time allows, introduce some other organelles with functional properties that he is testing as functional ingredients for food/feed.

1.3 Oil bodies, a dynamic organelle, a smart emulsion

Thierry Chardot, [1]Sabine d'André, [1] Yann Goho, [1]Thibaud Loiselleux, [2]Marc Anton [2]*

[1]INRA, AgroParisTech, Institut Jean-Pierre Bourgin ,78000, Versailles, France
[2]UR1268 BIA, INRA, 44300, Nantes, France

[*] Email: thierry.chardot@inra.fr

In addition to their central role in the life cycle of higher plants, seeds from oil crops are major sources of oil for food, feed, and to a lesser extent for green chemistry. Seed oil is stored in specialized intracellular structures, with a size close to µm, called Lipid Droplets (LDs), Oleosomes or Oil Bodies. LDs consist of a core of neutral lipids (mainly triacylglycerols and sterol esters) surrounded by a half membrane in which various proteins, mostly structural, are embedded. These proteins have a role in controlling lipid mobilization. In water, LDs form a very stable emulsion. LDs contain minor compounds (vitamins, sterols) of interest for food and health. LDs are destroyed during the oil extraction process and the refined oil lacks minor compounds.

Results from a detailed study of LDs from seeds of several *B. napus* accessions with contrasted oil extractability suggest that the protein and lipid compositions of the half membrane of LDs affect their rigidity. We have deciphered the topology of structural proteins at the surface of LDs (3). We followed the digestibility of LDs using an *in vitro* model digestor mimicking different compartments. While seed LDs are quiescent in dry seeds, their structure and composition rapidly evolve during seed germination. We have identified a novel mechanism controlling LD dynamics and required to remove the oleosin coat that stabilizes LDs in dry seeds. It seems to us necessary to consider new methods of seed fractionation and extraction of oil taking into account the living nature of seeds. Water based extraction is an alternative to existing processes using a lot of energy and organic solvents.

1.4 Watching oil bodies work: molecular microscopy of morphology and chemistry *in situ*

Sapun H. Parekh

Department of Molecular Spectroscopy, Max Planck Institute for Polymer Research, D-55128, Mainz, Germany

*Email: parekh@mpip-mainz.mpg.de

Plant oil bodies (OBs), which are uniquely emulsified oil droplets, serve as the densest energy source during seed germination in all plants. In analogy, mammalian lipid droplets (LDs) are lipid storage organelles found in all cells and play a crucial role in energy homeostasis and metabolic disorders. Both OB and LD physiology and metabolism are regulated by coat proteins, whose identity varies depending on tissue type. The dynamics and chemistry of these lipid inclusions is related to development and disease in both plants and mammals. Historically, few probes exist to interrogate OBs and LDs *in situ*, and quantifying chemical changes in the lipid core of these inclusions is very difficult at the individual droplet level. In this talk, I will give an overview of the chemical imaging methods that we have developed to quantify OB and LD chemistry and dynamics. We have used this technology to map lipolysis in LDs by lipases in real time, quantitative lipid uptake in white blood cells, and measure lipid chemical changes in OBs and LDs. The blending of chemical imaging technology with recent advancements in fluorescence microscopy together offer an unprecedented combination of chemical and spatial resolution to study OBs dynamics in the future.

2 Oral presentations - Day 1

2.1 Molecular microscopy of oil body and lipid droplet chemistry in situ with physiologically-relevant readouts

Alexandra Paul, [1, 2]Mischa Bonn, [2]Sapun H. Parekh [2]*

[1]Chemical Biology, Chalmers University of Technology, SE-412 58, Göteborg, Sweden
[2]Department of Molecular Spectroscopy, Max Planck Institute for Polymer Research, D-55128, Mainz, Germany

[*]Email: alexandra.paul@chalmers.se

Spatial heterogeneity at the molecular scale is a ubiquitous feature of all biological tissues, which is fundamentally linked to their native functions and to pathology. Probing the local chemistry of complex biological tissues requires the development and application of imaging tools that can identify the intrinsic molecular features in a sample without sacrificing high spatial resolution.

In this talk, I will describe our efforts to tackle this challenge for measuring lipid inclusion chemistry and morphology *in situ*. We have developed nonlinear label-free microscopy and associated analytical tools to determine the biochemistry of lipid droplets and oil bodies with high spatial resolution in a variety of samples. Importantly, our method yields physiologically-relevant quantities (chain length and saturation) as opposed to physical chemical ratios. I will show how we have used this ability to map changes in oil bodies in germinating soy beans and how lipid droplet chemical composition in brown and white adipose tissue adapt to high fat dietary intervention. Going forward, we want to expand the types of molecules we can quantify to include, e.g. sterol esters and ceramides in order to quantify all major components and perceived pathological intermediates in lipid inclusions.

2.2 Cryo-milling as novel processing approach for oil body recovery

*Vincenzo di Bari, *Simone De Chirico, Filippo Bramante, and David Gray*
Division of Food Science, University of Nottingham, LE12 5RD, Loughborough, UK

*Email: Vincenzo.Dibari@nottingham.ac.uk

Oil body (OB) emulsions are an attractive natural ingredient for the food and pharma industry. The process used to prepare these emulsions consists of three main steps: (I) seeds soaking in an aqueous medium; (II) wet milling to release the OBs from the seed matrix; (III) repeated washing cycles to remove exogenous material from the OB preparation. Although common in literature, this approach requires long incubation times and extensive water usage, which may limit the large scale upgrade of OB emulsions. The aim of this work was to evaluate for the first time the functionality of cryo-milling to recover intact OBs. This approach requires to cool seeds to sub-zero temperatures prior to their dry milling into a particulate material from which OBs are recovered using an aqueous medium. Various processing parameters (cooling regime, milling time and extraction conditions) were systematically investigated for OB recovery from rapeseeds. Three cooling regimes (-20˚C, -80˚C, and liquid nitrogen cooling) were assessed and compared to room temperature milling (control). Intact OBs were recovered only from seeds cooled with liquid nitrogen, which was attributed to the ability to maintain a deep cooling (below -70 ˚C) on milling. Increasing the cryo-milling time from 20 to 60 seconds resulted in a more finely comminuted material with 40% and 95% (wt%) of seed particles below 425 µm for 20 and 60s, respectively, and a higher recovery yield but induced OBs mechanical damage. Recovery conditions were optimised to 20s of milling followed by 1h of stirring in a 0.1M $NaHCO_3$ buffer for the seed particulate material. The produced OB emulsion displayed the same microstructure, isoelectric point, and particle size as the one obtained using a conventional wet-milling approach. This study demonstrate that cryo-milling could be used as novel approach to reduce processing time and the water usage to recover OBs.

2.3 Towards a simple extraction of oil bodies at neutral pH - The effect of salts

Juliana M. Romero-Guzmán,[1]Vasileios Petris,[1]Remko M. Boom,[1]Constantinos V. Nikiforidis[1, 2]*

[1]Food Process Engineering, Wageningen UR, 6708 WG Wageningen, The Netherlands
[2] Biobased Chemistry and Technology, Wageningen UR, 6708 WG Wageningen, The Netherlands

[*]Email: juliana.romeroguzman@wur.nl

The physiology of oil bodies allows their extraction in aqueous media, with highest yields achieved at alkaline conditions. However, these conditions at industrial scale are not always feasible, and thus we investigated the oil body aqueous extraction mechanism at neutral pH using edible salts: NaCl, KCl or $MgCl_2$ at 0.2 M. The extraction yield was strongly enhanced by the use of salts. KCl had a yield similar to that obtained at pH 9.5 (~53%). The overall protein extraction followed the Hofmeister series. However, the oil bodies' properties and recovery varied, depending on the salt used for the extraction. $MgCl_2$ enhanced oil bodies' coalescence after concentration and re-dispersion; with KCl and NaCl, the oil bodies showed some coalescence after re-dispersion, however they maintained stable structures afterwards. Overall, extraction of oil bodies at pH 7 seems feasible, having no impact on the stability of the recovered oil bodies.

2.4 Minimal separation processing steps for functional rapeseed protein-oil body mixtures

Eleni Ntone,[1, 2] Harry J. Bitter [2], Constantinos V. Nikiforidis [2]*

[1]TiFN, Wageningen, 6700 AN, The Netherlands
[2]Biobased Chemistry and Technology, Wageningen UR, Wageningen, 6708 WG, The Netherlands

[*] Email: eleni.ntone@wur.nl

Proteins are broadly used by industry for many applications, such as interface stabilization in foams and emulsions. Currently, animal proteins are mostly used, however due to sustainability aspects, plant protein sources have started to be exploited as well. Besides proteins, plants contain oil bodies which are surrounded by highly interfacial active proteins and phospholipids that lead to the formation of natural emulsions. However, little knowledge is available on the interfacial properties of these molecular mixtures. Additionally, intensive extraction conditions are needed in order to obtain them in relatively pure form. In this study we propose to apply minimal process steps that lead to native protein-oil body mixtures with high interfacial activity on oil-in-water systems.

Following this approach, we extracted rapeseed protein-oil body mixtures and study the factors that influence their composition and interfacial activity. The interfacial activity of these mixtures was related to compositional complexity and molecular properties. The results showed that oil body-rich mixtures can stabilize oil-in-water emulsions similarly to protein-rich mixtures. The interfacial activity of both mixtures was not hindered by the presence of other molecules that might also exhibit a synergistic effect on the interfaces.

This research proposes that native protein-oil body mixtures have an interfacial stabilization ability similar or even better than the pure protein systems. This will lead towards the application of simple extraction steps, to obtain interfacial active multicomponent systems.

2.5 Physical stability of oleosomes dispersions extracted from oleoproteaginous seeds

*Jean-François Fabre, *Eric Lacroux, Audrey Cassen, Muriel Cerny, Romain Valentin, Zéphirin Mouloungui*

Laboratoire de Chimie Agro-industrielle, Université de Toulouse, UMR 1010, INRA-INPT/ENSIACET, 31030, Toulouse, France

*Email: jeanfrancois.fabre@ensiacet.fr

Oleosomes can be extracted from different oleoproteaginous seeds through a simple integrated process combining an aqueous crushing of the seeds at high shear rate and a phase separation through centrifugation. The obtained cream is composed of oleosomes naturally stabilized by native surfactants, mainly oleosins and phospholipids. It shows a majorly elastic rheological behavior and a good resistance to coalescence. This process preserves the size of oleosomes, minor compounds as sterols and tocopherols and the fatty acid composition of triglycerides. However, according to the seeds, phospholipids may be altered, as revealed by mass spectroscopy and gas chromatography. Stabilizing proteins are not only oleosins carried initially by the membrane of oleosomes but also other hydrosoluble proteins extracted from the seeds. They all play an important role on the stabilization of oil droplets, acting both on flocculation (as evaluated by zeta potential measurements at different pHs) and coalescence (as assessed by freeze/thaw cycles and scanning electron microscopy after spray-drying and freeze/drying). Use of oleosomes for a wide range of applications must then focus on the preservation of phospholipids and the good environmental conditions for the expression of protein surface properties.

2.6 Blending oleosomes. Playing with natural plant emulsions to influence texture

Gustav Waschatko
Cargill R&D Centre Europe, 1800, Vilvoorde, Belgium

*Email: gustav_waschatko@cargill.com

For long time emulsion science and lipid droplet cell biology were two different worlds until creative scientists found the linker between both disciplines: food. Oil bodies in seeds and LDL particles in egg yolk are good examples for intracellular lipid droplets, both playing an important role in biology as organelle (e.g., germination) and for the stability of food emulsions (i.e., dressings).

Milk fat globules and lipoproteins in blood are extracellular lipid droplets equally intensively investigated by nutritionist and physicians. Compartmentalization of neutral lipids as energy storage inside a cell and solubilization for transport are the driving forces for evolution to develop these different native structures, working with the limited amount of natural emulsifiers, i.e., phospholipids, cholesterol and proteins.

Of all lipid droplet proteins, oleosins on the surface of oil bodies are the most remarkable emulsifiers with their unique 72 hydrophobic amino acid central domain penetrating into the triglyceride core and N-and C-termini covering the complete surface of an oil body to provide an electrostatic and steric barrier against coalescence. Representing up to 10% of the total protein content of seeds, oleosins are available in large quantities in oil seed crops like soybean, rapeseed or sunflower.

Imagine your only homogenizer are plant cells! What would you do to influence viscosity, creaminess, cloudiness and composition of your food emulsions? We carefully extracted oil bodies from 9 different botanical sources and showed ways to influence rheology, tribology and turbidity by blending chosen oil body droplet sizes.

2.7 Lipolytic activity: a new approach for the prediction of rapeseed oil body physicochemical stability

Simone De Chirico, Vincenzo di Bari, David Gray*

Division of Food Science, University of Nottingham, LE12 5RD, Loughborough, UK

*Email: simone.dechirico@nottingham.ac.uk

Seeds are the most common site for the accumulation of oil bodies (also called oleosomes) in plants. During the aqueous recovery of oil bodies, other water-soluble seed material is solubilised and carried-over in the final product. The use of alkaline pH solutions (>8.5) have been demonstrated to be more effective against the contamination of oil body material from seed proteins, compared to neutral pHs. However, despite the use of a $NaHCO_3$ solution (0.1 M, pH 9.5) recovered oil bodies from rapeseed with similar composition to the preparation washed in urea (9 M), the physical stability over storage was compromised, probably due to the presence of hydrolytic enzymes.

In this study, lipolytic activity has been used as a marker of oil body purity (in terms of carried over enzymes) and effectiveness of the thermal processing to enhance storage stability of the emulsion. The inactivation of enzymes was tested over storage looking at chemical (hydrolysis of lipids and integral proteins) and physical (particle size and zeta potential) stability.
The carry-over of lipolytic enzymes was dependent on the recovery and/or washing solution used. The optimal heating time at 95°C for storage stability experiments was of 6 minutes, based on >90% enzyme inactivation. In the optimized condition, oil bodies recovered in NaHCO3 (0.1 M, pH 9.5) were stable against the thermal treatment applied, having a D [4,3] of 1.4 µm and a zeta potential that changed from around +60 mV to -60 mV as the pH was increased from 3 to 10 (pI of 6.5). As a consequence of the reduced enzymatic activity, thermal treatment significantly enhanced the physicochemical stability of the emulsion over storage.

2.8 Pushing oxidative stability of sunflower oil bodies to the extreme

Gustav Waschatko
Cargill R&D Centre Europe, 1800, Vilvoorde, Belgium

* Email: gustav_waschatko@cargill.com

The superior oxidative stability of oil bodies compared to model emulsions, personal care and food emulsions is an intrinsic benefit that was studied in most groups working on a deeper understanding of seed oleosomes. Past literature results are usually based on peroxide value and secondary product formation (like Hexanal) with prior organic solvent extraction. In order to target the onset of oxidation of sunflower oil body containing model applications we developed a ML OxipresTM protocol. Our test successfully resolves differences in oxidation onset caused by minor changes in the continuous phase of oil body formulations. Among others, changes in pH-value, protein content, iron containing egg yolk and pectin addition and short heat treatment (pasteurization) showed a clear impact on the induction period of accelerated oil body oxidation (5 bar oxygen, 70°C) in the ML OxipresTM apparatus. These trends will help to push shelf life of oil body containing mayonnaises and dressings to commercial requirements.

The longest induction period (IP) of more than 300h (5 bar oxygen, 70°C) was measured using high oleic sunflower oil bodies.

2.9 Oat lipids: Bioaccessibility and digestibility

*Myriam Grundy,[1] *Peter Wilde [2]*

[1]School of Agriculture, Policy and Development, Sustainable Agriculture and Food Systems Division, University of Reading, RG6 6AR, Reading, , UK
[2]Quadram Institute Bioscience, NR4 7UA, Norwich, UK

* Email: m.m.grundy@reading.ac.uk

Oat consumption has been associated with various health effects, in particular the reduction of blood cholesterol levels and risk of cardiovascular diseases. Oats are rich in a wide range of healthy nutrients and bioactive phytochemicals (i.e. unsaturated fatty acids, phospholipids, galactolipids, phytosterols, tocopherols and saponins) with various physico-chemical, colloidal and interfacial properties. Compared with other cereals, oat contains higher amount of lipids and lipid soluble compounds. These characteristics are likely to have an impact on lipid metabolism.

The aim of this work was to investigate the impact of oat materials varying in complexity on the lipolysis process. The composition and overall structure of our systems (i.e., oat flakes, flour, bran, oil bodies and oils) were determined by GC-MS, ATR-FTIR and confocal microscopy. We then monitored the lipid released (bioaccessibility) from the complex oat matrices and the lipid digestibility of oat oil emulsions, oat oil bodies and sunflower oil enriched in phytosterols using the pH-stat. Finally, the surface activities of phytosterols were examined using the pendant drop technique.

Differences in lipid digestibility between the oat materials were clearly seen, the degree of complexity or purity of the oat material having an impact on lipid hydrolysis (sunflower oil > oat oils > oat oil bodies). In addition, the presence of lipid soluble molecules in the oil decreased the extent of lipolysis. Also, the production of free fatty acid during duodenal digestion of sunflower oil was reduced proportionally to the concentration of phytosterols present. This may be due to the interfacial properties of the phytosterols as demonstrated by the pendant drop experiments.

This work also highlights the importance of considering the overall structural complexity of the system studied and not only its composition.

3 Oral presentations - Day 2

3.1 Oil bodies, self-assembling microreactor systems

Romain Valentin

Laboratoire de Chimie Agro-industrielle, Université de Toulouse, UMR 1010, INRA-INPT/ENSIACET, 31030, Toulouse, France

*Email: romain.valentin@ensiacet.fr

In plants, lipids are stored in spherical organelles named oil bodies or oleosomes, which consist of small triglyceride droplets surrounded and stabilized by a layer of phospholipids associated with unique proteins named oleosins. The ability of oleosins to aid in the emulsification and stabilization of oil bodies is well known in the plant. An integrated process gives the opportunity, when introducing *Candida rugosa* enzyme, to liberate oil from seeds. Oil bodies are in the form of fatty acids in complex emulsions. In the presence of ROH additives (ROH = H_2O, alcohols) fatty acids and fatty esters are obtained by hydrolysis (ROH = H_2O), esterification and transesterification. These reactions are competitive in these enzymatically catalyzed and stabilized oil body microreactor.

To understand the mechanism of the stabilization of interfaces of oil/water interfaces of oil bodies, it is necessary to study the molecular interactions between oleosins and phospholipids. Thermodynamic and kinetic of adsorption analysis, based on interfacial measurements and reconstitution experiments were carried out. Effects of pH and relative phospholipid/oleosins ratio were investigated.

The relationships between possible structures adopted by oleosins at the interface and phospholipids have been discussed. The relation and the reactivity between acyl donor and acceptor were investigated. Phospholipids are needed to stabilize oil droplets, and oleosins are mandatory to avoid coalescence, both having the role of contacting lipophilic and hydrophilic phases. The thirth, *Candida rugosa* lipase, near interfacial enzyme, was necessary and active as acyl transfer catalyst.

3.2 Application of soybean oil bodies as an emulsifier toward various food emulsion systems

*Ishii Toya,*Kentaro Matsumiya, Yasuki Matsumura*

Laboratory of Quality Analysis and Assessment, Division of Agronomy and Horticultural Science, Graduate School of Agriculture, Kyoto University, 611-0011, Kyoto, Japan

*Email: ishii.toya.54u@kyoto-u.jp

Many food emulsions like dressings and beverages are prepared using animal proteins such as egg yolk and milk protein as an essential emulsifier although the use of plant proteins is economically and environmentally more beneficial. The excellent emulsifying properties of egg yolk can be attributed to lipoproteins, the structure of which is interestingly similar to that of plant oil bodies. We thus aimed to investigate interfacial and emulsifying properties of soybean seed oil bodies under various conditions toward the development of oil body-stabilized food emulsion products.

Aqueous extraction was carried out at neutral and alkali pH conditions to obtain storage protein-coated oil bodies (OBC) and purified oil bodies (OB) from soybeans, respectively. OBC and OB were compared at pH 7.0 by measuring their colloidal and interfacial properties and evaluating characteristics of model OBC/OB-stabilized emulsions. These tests were further performed for OB under various pH (4.0-5.5) and salt concentration (0-100 mM NaCl) conditions. Preparation and rheological analysis of concentrated OB-stabilized emulsions were also conducted. Particle size analysis showed that OB were finer than OBC that partially aggregated. The interfacial tension at the oil-water (O/W) interface was lower for OB than OBC, indicating that the well-dispersed OB adsorbed to the O/W interface and probably packed more closely, leading to the formation of an elastic film at the interface that is helpful to prevent oil droplet coalescence. Lowering the pH value of the OB dispersions caused an increase of zeta-potential and a decrease of adsorption rate to the O/W interface, whereas the addition of NaCl suppressed such pH dependent changes probably due to the screening effect, suggesting that electrostatic potential generated by adsorbed OB toward non-adsorbed OB critically affects the adsorption kinetics at the interface. Rheological properties of the concentrated emulsions will be further discussed.

3.3 Sunflower oil bodies and their behaviour on oil in water interface

*Dimitris Karefyllakis,[1] *Atze Jan van der Goot,[1] Constantinos V. Nikiforidis [2]*

[1]Food Process Engineering, Wageningen UR, 6708 WG, Wageningen, The Netherlands
[2] Biobased Chemistry and Technology, Wageningen UR, 6708 WG, Wageningen, The Netherlands

*Email: dimitris.karefyllakis@wur.nl

The protection of oil against oxidation and its arduous mixing with hydrophilic systems has been resolved by nature with organelles called oil bodies. Oil bodies are equipped with a sophisticated membrane. The membrane consists of continuous monolayer of phospholipids to which a number of hydrophobic proteins are embedded. When oil bodies are extracted, the aforementioned noteworthy qualities of physical and chemical stability are conveyed to the resulting "natural" oil-in-water emulsions. What has not been clarified regarding the intricate oil body structure is what happens when external oil is added and an extra emulsification step is applied.

In order to provide more information about the specific role of oil body structure, intact oil bodies were isolated from sunflower seeds and their behaviour on the oil/water interface was investigated. The aim was to decipher what happens to the structure of the oil bodies and how their membrane behaves once they reach the interface. After the aqueous extraction, oil bodies were characterised and the interfacial activity of their dispersions was measured. External oil was added and emulsions were created using oil body dispersions as emulsifiers. Several microscopic techniques were employed in order to illustrate oil bodies and the way they existed on the interface. The experiments proved that the phospholipid membrane was playing a vital role for the stability of the interface. In addition, it was shown that oil bodies did not survive the emulsification process rather than merged with the oil phase when O/W emulsions were examined. Surprisingly, when images of W/O emulsions were analysed it was shown that the hydrophobic core did not merge with the bulk oil and that their membrane survived on the interface intact acting as Pickering particles. This is an intriguing bottom line regarding the future applications of oil bodies either as natural emulsions or as carriers of bioactive compounds and the importance of their sophisticated membrane.

3.4 Tracking molecules on interfaces

Jack Yang, [1]Constantinos V. Nikiforidis, [2]Erik Van der Linden [1]Leonard M. C. Sagis*

[1]Laboratory of Physics and Physical Chemistry of Foods, Wageningen UR, 6708 WG, Wageningen, The Netherlands

[2] Biobased Chemistry Technology, Wageningen UR, 6708 WG, Wageningen, The Netherlands

[*]Email: jack.yang@wur.nl

In recent years, oleosomes have been of considerable interest as a potential interface stabilizer in emulsion systems. The ability to stabilize oil droplets has been described by many, with the aim to understand interfacial microstructure formation and stabilization mechanisms. On the other hand, the interfacial rheological properties of oleosome-stabilized interfaces have not been studied extensively. A more extensive characterization of the interfacial behaviour of oleosome-stabilized interfaces could help us better understand emulsion properties. In this talk, we will discuss potential methods to study the interfacial properties of oleosome-stabilized interfaces, which include techniques such as interfacial rheology and imaging of the interface.

3.5 Characterisation of rapeseed oil bodies in natural dispersions and dried powders

Christos Fryganas, [1,]Vincenzo di Bari, [2]Lakshminarasimhan Sridharan, [1]Harry J. Bitter, [1]Constantinos V. Nikiforidis [1]*

[1]Biobased Chemistry and Technology,Wageningen UR, 6708 WG, Wageningen, The Netherlands
[2]Division of Food Science, University of Nottingham, LE12 5RD, Loughborough, UK

[*] Email: christos.fryganas@wur.nl

Natural oil-body (OB) based emulsions can be recovered from oleaginous seeds with mild aqueous extraction steps. They do not require homogenisation and they possess advantageous properties, such as innate physical stability, resistance to oxidation and gelation abilities. In addition, spray-drying of OB emulsions could prevent microbial spoilage and improve compound encapsulation. Therefore, they could be beneficial to novel food, pharma and cosmetic appplications. However, the impact of drying on OB surface morphology is still unknown.

A crude natural OB emulsion from rapeseed was extracted with water and dried using a benchtop spray drier. Surface mean values that did not differ between the emulsion (D[3,2]=2.8) and the powder (D[3,2]=2.7) however small OB aggregation was observed in the emulsion. Atomic Force Microscopy (AFM), allowed two-dimensional visualisation of OBs with a vertical height up to 0.6 μm and 0.9 μm and a width up to 1.2 μm and 6 μm for the natural emulsions and the dried powders respectively. Transmission electron microscopy (TEM) verified the particle size ranges and the shape variability from AFM analysis and confirmed the presence of smaller particles and protrusions on the OB surface, together with the extent of clustering. Confocal Laser Scanning Microscopy (CLSM) proved that the lipid/protein distributions were very similar between OB emulsions pre- and post-drying. Moreover, spray-drying did not have an effect on the protein content (19% vs. 23%) and composition. As a result the isoelectic points of the OB emulsion (4.52) and the powder (4.63) samples were also comparable.

This preliminary data suggests that rehydrated OB powders retain the properties of crude OB based emulsions and could be used as a future functional ingredient.

3.6 Hypersterolemic lipid bodies of plants

*Anne Berna, [1, 2]Pierre Mercier, [1] Christine Schaeffer, [3]Jean-Marc Strub, [3]Dimitri Heintz, [1]Thomas J. Bach, [1]Andrea Hemmerlin, [1, 2]Sylvain Darnet, [2, 4]Hubert Schaller [1, 2 *]*

[1]Institut de biologie moléculaire des plantes (IBMP-CNRS), Université de Strasbourg, F-67084, Strasbourg, France
[2]International Associated Laboratory PALMHEAT, IBMP-CNRS/ UFPA
[3]Laboratoire de spectrométrie de masse bioorganique (LSMBO CNRS UMR7178), Institut Pluridisciplinaire Hubert Curien, Université de Strasbourg, F-67087 Strasbourg, France
[4]Institute of biological sciences, Federal University of Pará (UFPA), Belém, Pará, Brazil

[*]Email: hubert.schaller@ibmp-cnrs.unistra.fr

Plant sterol homeostasis is an essential process in development. In contrast to the welldocumented mechanisms of cholesterol homeostasis in mammals, knowledge about the regulation of phytosterol production is limited. A common aspect between plant and mammal sterol metabolism is a crucial role of some upstream enzymes implied in the mevalonate pathway. Up-regulation of the biosynthetic flux in this metabolic segment results in a strong and steady accumulation of sterols in the form of sterol esters that are deposited in cytoplasmic lipid droplets, particularly in leaf ontogeny. The exact nature and composition of such organelles are investigated with proteomic and lipidomic approaches to better understand the mechanisms of sterol ester formation and mobilization in lipid droplets in interaction with other cell compartments.

3.7 A story of a European funded project

*Stavroula Salakou, *Marielle Ramaekers*
The European Federation of Food Science and Technology, 6709PA, Wageningen, The Netherlands

*Email: r.salakou@effost.org

The H2020 EU funded OLEUM project (www.oleumproject.eu) seeks to better guarantee olive oil quality and authenticity by improving the detection and fostering the prevention of olive oil fraud. Twenty project partners are committed to developing advanced solutions, analytical tools and networking actions to enhance the body of knowledge to assure the authenticity and quality of olive oils at a global scale, with a particular focus on virgin olive oils. Partners are validating new and/or improved analytical methods and they are establishing an OLEUM Databank and OLEUM Network for stakeholders.

The OLEUM Network aims to enlarge the international body of expertise in the analysis of olive oils by congregating a wide user community of laboratories and related stakeholders active in the analysis and authentication of olive oil. The OLEUM databank is an online integrated quality assurance database of olive oil analytical methods and data related to chemical and organoleptic characteristics (e.g. related to the sensory experience such as taste, odour, texture).

4 Posters:

4.1 Flavour diffusion through oil body membranes

Lakshminarasimh Sridharan, [1] Juliana Romero Guzman, [1] Constantinos V. Nikiforidis [1]*

[1]Biobased Chemistry and Technology, Wageningen UR, 6708 WG, Wageningen, The Netherlands
[2] Food Process Engineering, Wageningen UR, 6708 WG, Wageningen, The Netherlands

[*]Email: lakshminarasimh.sridharan@wur.nl

Oil Bodies (OBs) are unique organelles with a stable bi-layer of proteins and phospholipids. This layer formation makes these organelles stable in solution. So, there is considerable interest in literature to use natural OB emulsion for food and pharma applications. An important function of such oil-in-water emulsion is to act as encapsulant of flavours or bio-actives. Therefore in this study, rapeseed OBs ability to encapsulate flavours was studied and compared with manufactured emulsions. Native OBs were extracted using an aqueous extraction process. The diffusion of limonene into the core of the OBs was studied based on the partition coefficient using chromatography and was compared to emulsions made of casein and Tween 20. The results showed OBs could encapsulate better the flavours under equilibrium condition than the other fabricated emulsions. Also, when dynamic release profile was studied, OBs had a sustained release of flavours through the test compared to other two emulsions. Total Internal Reflection microscopy was used to visualize in real time the diffusion of flavour molecule into the OBs with fluorescence. The obtained images showed the instantaneous movement of flavour molecule into the OBs through fluorescence.

4.2 Chia, Cameliae and Flaxseed seeds: oil, polysaccharides and proteins: Process of biorefinery

*Gwendoline Grave,*Jean-François Fabre, Muriel Cerny, Eric Lacroux, Romain Valentin, Zephirin Mouloungui*

Laboratoire de Chimie Agro-industrielle, LCA, Université de Toulouse, INRA, 31030, Toulouse, France

*Email: gwendoline.grave@ensiacet.fr

How to overcome the barrier of mucilage for extraction of oil?

Chia, Camelina and Flaxseed seed are oleoproteaginous seeds and they are also covered by mucilage. This layer of polysaccharide thickens the medium when seeds are immersed in water complicating oleosome extraction. This work aims to develop a process to obtain mucilages, oil bodies and fibers from mucilaginous seeds. The first step consists of separating polysaccharides of seed surface by sonication, in order to obtain mucilage, and also to increase the accessibility to oleosomes.

Once the polysaccharides layer removed oleosomes can be extracted grounding degumming seeds. Thanks to water and mechanical energy (high-shearing device and high pressure homogenizer) oil bodies as an emulsion are obtained. These emulsions triglycerides/water are stabilized by phospholipids and proteins. By the addition of an enzymatic lipolysis step, oleosomes become a source of fatty unsaturated acids. Besides oil bodies, this process gives access to fibers and an aqueous phase rich in minerals and proteins.

These oleosomes from Chia, Camelina or Flaxseed reveal the potential for the generation of new platform of unsaturated biomolecules, proteins, and monosaccharides. All these compounds have properties for a wide range of applications (alimentation, health, pharmaceutical and cosmetics).

4.3 One-step extraction of rapeseed oil bodies

Juliana M. Romero-Guzmán,[1]Vasileios Petris,[1]Remko M. Boom,[1]Constantinos V. Nikiforidis [1, 2]*

[1]Food Process Engineering, Wageningen UR, 6708 WG, Wageningen, The Netherlands
[2] Biobased Chemistry and Technology, Wageningen UR, 6708 WG, Wageningen, The Netherlands

[*]Email: juliana.romeroguzman@wur.nl

Twin-screw press is a continuous process that combines the disruption of seeds with a mixing area that allows a solid-liquid separation at the end of the screws, which are key steps for the aqueous extraction. Additionally its current application in the oil industry makes twin-screw press a good alternative to scale-up the oil bodies' aqueous extraction. Therefore, the aim of this work was to compare the performance of a small-scale twin-screw press to the performance of the conventional lab-scale process for oil bodies extraction, by evaluating the extraction yields and the quality of recovered oil bodies. The results showed that the quality of oil bodies extracted with the twin-screw extruder were similar to the obtained with the conventional lab-scale extraction. When pure water was used, it was noticed that mechanical forces are stronger in the twin-screw press than in the blender, as the extraction yield was higher. However, when salt solutions were used to perform the extraction, due to the lower liquid/solid ratio used in the twin-screw press, the yield was lower. Remarkable is the fact that by re-circulating the cake and/or de-hull the seeds, it was possible to increase the extracted oil body yield up to 90%, without compromising the integrity of the obtained emulsion. In conclusion, twin-screw extruder is a good option for scaling-up oil bodies' aqueous extraction as a continuous process.

4.4 Oil bodies of plant origin for the development of novel food products

Anthia Matsakidou, [1]Eleni Naziri, [1]Thomas Koupantsis, [1] Constantinos V. Nikiforidis, [2] Fani Mantzouridou, [1] Adamantini Paraskevopoulou, [1]Maria Z. Tsimidou, [1]Vassilios Kiosseoglou [1,]*

[1]Laboratory of Food Chemistry and Technology, School of Chemistry, Aristotle University of Thessaloniki, 54124, Thessaloniki, Greece
[2] Biobased Chemistry and Technology, Wageningen UR, 6708 WG, Wageningen, The Netherlands

[*]Email: kiosse@chem.auth.gr

The present work is the outcome of a three-year's research project focusing on the study of the properties of plant oil bodies aqueous extracts and aiming at their exploitation in developing novel food products. The extracts were evaluated in terms of physical, chemical and microbiological stability, following their submission to various treatments or stored under various storage conditions. The main target of the project was to develop popular food products based on oil bodies as potential alternatives to animal fat and containing natural constituents with superior nutritious value. Thus, oil bodies-rich creams from sesame seed and hazelnut were used as a natural o/w emulsion base for the preparation of salad dressings and mayonnaise. Moreover, the retentate obtained from the application of ultrafiltration to maize germ or to hazelnut extract were used for the total replacement of milk fat for the preparation of ice cream and yogurt-type products. The results, in general, revealed the potential that the oil bodies may have for future commercial applications, that could meet the present and future demand of the consumer for natural, healthy and green food palette choices.

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4.5 *In vitro* lipid digestion in raw and roasted hazelnut particles and oil bodies

Eleni Ntone, [1,2] Edoardo Capuano, [1]Nicoletta Pellegrini, [1,3]Constantinos V. Nikiforidis [2]*

[1]Food Quality and Design Group, Wageningen UR, 6708 WG, Wageningen, The Netherlands
[2]Biobased Chemistry and Technology, Wageningen UR, 6708 WG Wageningen, The Netherlands
[3]Human Nutrition Unit, Department of Food and Drug, University of Parma, Parco Area delle Scienze, 27/A 43124, Parma, Italy

[*]Email: eleni.ntone@wur.nl

Previous studies have proved that the physical encapsulation of nutrients by the cell walls of plant foods modulates macronutrient bioaccessibility during human digestion. In this study, we investigated structural factors that modulate lipid hydrolysis during in vitro digestion of raw and roasted hazelnut particles and isolated oil bodies. Isolated oil bodies exhibited a significantly higher lipid hydrolysis compared to hazelnut particles. Moreover, roasting had an impact on the structure of hazelnut cell walls implying a more efficient diffusion of digestive fluids and enzymes into the hazelnut cells. Heat treatment also caused destabilization of oil body interfacial protein membranes, facilitating their proteolysis under gastric conditions, altering the emulsion properties and enhancing fatty acid release during intestinal digestion. This study underlined the barrier role played by the plant cell wall as well as the impact of heat processing on lipid bioaccessibility in hazelnuts.

4.6 Metabolic engineering to increase the corn seed storage lipid quantity and change its compositional quality

*Ali Izadi-Darbandi, [1, 2] *Hussien Alameldin, [2]Scott A. Smith, [3]Venkatesh Balan, [2]A. Daniel Jones, [3]Mariam Sticklen[2]*

[1]Department of Agronomy and Plant Breeding Sciences , University of Tehran, Tehran, Iran
[2]Department of Plant, Soil and Microbial Sciences, Michigan State University, East Lansing, MI 48824, USA
[3]Mass Spectrometry and Metabolomics Core, Research Technology Support Facilities, Michigan State University, East Lansing, MI 48824, USA

*Email: aizady@ut.ac.ir

Given limited global food supplies and the fact that the global population is expected to double by 2050, there is an urgent need for the development of high-calory foods, including culinary oils. The seeds of oil crops contain high-energy density oil composed of triacylglycerides (TAGs) at up to 80% by dry mass. However, maize (*Zea mays* L.) seeds are relatively poor in calories and nutritional values. Therefore, in this report, we address this constraint via metabolic engineering to improve maize seed lipids including TAG and seed TAG nutritional values by overexpression of three major genes, including: (i) the *Arabidopsis thaliana* (L.) Heynh. diacylglycerol acyltransferase 1 (AtDGAT1), a gene that catalyzes the TAG biosynthesis final step prior to packaging of TAGs into oil bodies; (ii) the transcription factor WRINKLED 1 (WRI1), which promotes the regulation of the expression of genes involved in fatty acid biosynthesis; and (iii) the *A. thaliana* oleosin (AtOleosin) gene, a gene coding for a protein that protects TAGs from degradation. The overexpression of the above three genes, and probably certain unintentional *in vitro* culture genetic variabilities, resulted in 17% increase of seed TAGs and 25% increase in total oil contents when compared with the wild-type control corn seeds. In addition, the above genetic modifications led to major shifts in the fatty acid profiles in favor of human health.

4.7 The sensorial attributes of CapSol™ versus traditional emulsifiers in sunscreen formulations

Kristin Brancato, Zizi Kandil, John Chase *

Botaneco, NJ 08530, Lambertville,USA

*Email: jchase@botaneco.com

CapSol™ has the unique ability to load sunscreen filters in a way that dramatically reduces UV filter levels required for targeted SPF levels. Sunscreen emulsions tend to be heavy, greasy and generally not appealing. This is in part due to the chemical sunscreen actives themselves. The ability to lower the level of sunscreen actives would have a positive effect on the overall sensorial profile of the product.

Emulsions can be formulated over a broad viscosity range from flowing fluids to thick creams. CapSol™ can be used with polymeric thickening agents, natural gums and other viscosity modifiers and stabilizers. A key sensory attribute of CapSol™, when it is incorporated into an emulsion, is that it produces a unique improved feel during the initial application of the emulsion on the skin, as well as during the transitional and intermediate stages. The result is a light, and pleasant feeling that continues to deliver conditioning without any heaviness. The emulsions have a very fine texture with an elegant appearance. The sensory panel studies were conducted comparing the sensorial attributes of CapSol™ versus a traditional emulsifier blend, cetearyl alcohol and dicetyl phosphate and ceteth-10 phosphate.

4.8 Ultra-stable dried oil capsules from natural oil droplets (oleosomes) through a single step

Saskia Tersteg, [1]Constantinos V. Nikiforidis [2]

[1]Food Process Engineering, Wageningen UR, 6708 WG, Wageningen, The Netherlands
[2]Biobased Chemistry and Technology, Wageningen UR, 6708 WG Wageningen, The Netherlands

*Email: costas.nikiforidis@wur.nl

A new facile technique to produce highly stable oil capsules in powder is reported here. A natural rapeseed oil body (oleosome) extract was used, comprised by oil bodies and co-extracted proteins, with an oil/protein ratio of 1.3. Spray-drying the oil body dispersion as such, without the addition of any hydrocolloids which are typically used resulted to a fine powder. The obtained oil powder had similar oil/protein ratio to the initial extract and no caking or oil droplet coalescence was observed. The sophisticated oil body membrane, together with the co-extracted proteins provided sufficient elasticity to the capsule and protection to the encapsulated oil. Additionally, the oil capsule powder performed greatly regarding re-dispersibility, since it self-dispersed into individual oil capsules without any agitation. The ease and inexpensive production of naturally obtained dried oil capsules will open new routes for advanced applications in cosmetics, pharmaceutical, medical and food science.

4.9 Encapsulation kinetics of hydrophobic compounds in oil bodies through molecular dynamic simulations

Benjamin Rosenbaum, Lakshminarasimh Sridharan, Harry Bitter, Constantinos V. Nikiforidis, Lars Kiewidt [*]

Biobased Chemistry and Technology, Wageningen UR, 6708 WG Wageningen, The Netherlands

[*]Email: lars.kiewidt@wur.nl

Oil bodies composed of glycerides surrounded by a layer of lipids and oil body proteins are promising alternatives for synthetic emulsions produced from pure compounds that require additional emulsifiers and consume a lot of energy for purification of the starting materials and for the emulsification process itself. Of particular interest are oil bodies as carrier for hydrophobic flavor systems to protect the flavor molecules. In contrast to encapsulation of flavors in synthetic emulsions, in which flavor molecules are dissolved in the oil phase prior to emulsification, the encapsulation of flavors in oil bodies requires the permeation of the flavor molecules through the lipid layer. The additional transport resistance might reduce the efficiency of the encapsulation and thus limit the application of oil bodies as carriers. The aim of this study is thus to investigate the transfer kinetics of hydrophobic compounds through lipid monolayers to assess the potential of native oil bodies as flavor carriers.

In this study, molecular dynamics (MD) simulations will be applied. The use of MD has already been successfully demonstrated by others to predict the state of lipid bilayers and the accumulation of small hydrophobic compounds and fullerene in lipid bilayers. Here, we will particularly investigate the influence of the temperature on the permeation of flavor molecules (e.g. lemonene). In addition, we will investigate the influence of the concentration of the flavor molecules in the surrounding aqueous phase to explore clustering of the flavor molecules which might further hinder transport through the lipid membrane.

The obtained results will allow to quantify the encapsulation kinetics in terms of diffusion coefficients of hydrophobic flavor molecules through the lipid layer and the attainable concentration within the oil bodies, and provide insights into the encapsulation mechanism.

4.10 Sensorial Performance of Sunflower Oleosomes in Dressings, Sauces and Mayonnaises (DSM): Approach for EDTA and Starch Replacement

Emine Donmez

Cargill R&D Centre Europe, Vilvoorde, Belgium

*Email: emine_donmez@cargill.com

The organoleptic attributes of sunflower oil bodies (SFOB) in DSM are underexplored with only very few examples in literature (1). We formulated 8 traditional and egg free (vegan) mayonnaise like products with 30 (Mayo30) and 50 % (Mayo50) oil content using SFOBs.

Decreasing the pH from neutral to 4 with vinegar in traditional mayonnaise iron ions (up to 27 mg/kg) are released from egg yolk proteins (2), especially from Phosvitin, carrying 95% of Fe3+ (3). Egg yolk derived iron ions need to be complexed by chelators like EDTA, to avoid oxidation initiation. Triangle sensory tests were used to find differences between 75 ppm EDTA added and blank Mayo30/50 directly and after storage. Our internal taste panel couldnâ€™t detect significant differences at 95% confidence level. The addition of egg yolk lowered the induction period (IP) in the ML Oxipresâ„¢ test by only 2% for Mayo50 and 3% for Mayo30. Both sensory and ML Oxipresâ„¢ test confirmed that in contrast to conventional mayonnaise the pro-oxidative effect of protein derived iron and cupper ions is intrinsically prohibited in SFOBs containing DSM applications.

Using SFOBS emulsification isnâ€™t needed. Nevertheless, for a smooth texture of starch containing products, blenders are needed, that can introduce more/less air. A clear difference in air inclusion using two different equipments was observed. Higher inclusion had a clear negative influence on oxidative stability (IP Mayo50: -20%) and sensory.

Starches are commonly used thickeners. However, an emerging trend is starch free DSM products, which can be achieved by combining oil bodies with other ingredients like egg yolk, dairy and vegetable proteins.

Matching the taste profile of traditional mayonnaise remains a challenge and different flavor descriptors for SFOB containing DSM products are needed. The original pleasant taste â€œcharacterâ€• of SFOBs gives opportunities for new product developments like dip sauces with fresh vegetables and herbs.

4.11 Oleosomes & Nutrition: Use of lipid droplets in food application for different dietary needs

Susen Gottwald

Cargill GmbH, Vilvoorde, Belgium

*Email: susen_gottwald@cargill.com

For the most food applications, where vegetable oils and fats are an essential part of the recipe, single ingredients are blended together and processed to the final product.

Since some years the food industry faces various trends and growing niche markets for â€œmodern foodâ€• answering the dietary needs of consumers. The interest about where and how the food is made is steadily increasing. Less processing and more natural derived ingredients are on the wish list of food companies. Are their lipid based alternatives to refined oils and emulsifiers? We think, yes!

Oleosomes: plantâ€™s instant and stable emulsions are ready to serve a future food market. The Oleosomes can be used for supporting product claims like:

- Clean label
- Free from â€¦
- Vegan
- Protein enriched
- Low carb

We have tested the oleosomes in our kitchen cabinet for:

- Dressings, Dips, Sauces
- Protein enriched Smoothies
- Bread, rolls, cakes, doughs & cookies
- Plant based yogurt and ice cream
- Chilled desserts
- Chocolate products and many more

The possibility to use oleosomes in food application is very versatile. We are just about to discover oleosomes potential in food.

Either you replace some ingredients, like emulsifiers, stabilizers and dairy, soy, egg derived ingredients or you create your own new recipe and combine with almond, oat & coconut products.

The emulsion droplets contain all the goodies of vegetable oils, like Tocopherols, Phytosterols and Phospholipids and on top you get an extra boost of vegetable proteins.

4.12 An insight on oil bodies and oleosins in Coffea arabica L. seeds: different extraction protocols and a preliminary investigation during germination steps

MARIN, Veronica[1,2], DEL TERRA, Lorenzo[1], CRISAFULLI, Paola[1], NAVARINI, Luciano[1]

[1]illycaffè S.p.A, Trieste, Italy; [2]AREA Science Park, Trieste, Italy.

Oleosins have been well described in oil-rich seeds, but little information is available in *Coffea arabica*, classified as semi-oleic (lipid fraction 12-16% w/w depending on botanical variety and geographical origin). This is surprising given the involvement of lipids in the physical (foam, aroma carrier), chemical (aroma, antioxidant) and physiological (health effects) properties of the coffee beverage. To shed light on this subject, two protocols have been adapted in order to purify oil bodies and oleosins from coffee.

Oil bodies from *C. arabica* seeds of various geographical origins were extracted according to two methods by Tzen et. al (1990, 1997). Oil body integrity was observed by an optical microscope, using differential interference-contrast technique. TAG content of oil bodies was analyzed by TLC. SDS-PAGE was used to evaluate the purified protein fraction. The selected protocol was employed to analyse oil bodies and oleosins during different germination stages of freshly harvested coffee seeds.

The "Tzen 1997" protocol substantially improved removal of lipid/protein debris non specifically associated to oil bodies. Major protein bands were observed between 15 and 20 kDa, matching the predicted molecular weight of oleosins. TLC analysis indicated a relevant TAG content as proof of oil body integrity. In these preliminary results, the coffee germination timeline revealed a marked initial increase in oleosin content, followed by a progressive decrease during the subsequent germination steps.

Oleosins are the main class of proteins in the oil body membrane and provide a high surface-to-volume ratio that facilitates lipase access during germination. The reported findings confirm that the oleosin protein family is abundant in coffee seeds, even if they are not classified as oil-rich seeds. A better understanding of the biology of oil body genesis and degradation in *Coffea arabica* will be of great help in elucidating the role of lipids in green coffee quality.

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