出國報告（出國類別：考察/開會）

赴義大利波隆那出席「The 5th International Symposium on Lipid Oxidation and Antioxidants」及參訪波隆那大學心得報告

服務機關：國立臺灣大學醫學院附設醫院

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派赴國家：義大利

出國期間：113 年 7 月 4 日至 113 年 7 月 14 日

報告日期：113 年 8 月 23 日

壹、摘要

脂肪氧化及抗氧化國際研討會(International Symposium on Lipid Oxidation and Antioxidants, ISLOA)是 European Federation for the Science and Technology of Lipids (Euro Fed Lipid) 組織下的一個學術研討會。第 5 屆 ISLOA 在義大利波隆那大學舉行，為期三天，會期由 7 月 8 日至 7 月 10 日。本次大會針對脂肪氧化以及抗氧化等重要議題進行探討與交流。有世界各國數百位學者及研究人員參與，題目聚焦在脂肪氧化對食物及健康之影響及如何使用抗氧化物質來對食物進行保鮮的作用。因為本次會議在歐洲最古老的大學城，亦即義大利的波隆那舉行，所以同時也利用此開會的機會參訪已有近千年歷史的波隆那大學。

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一、目的

學習有關脂肪氧化及抗氧化物質之新知並參訪歐洲最古老的波隆那大學。

二、過程

第 5 屆脂肪氧化及抗氧化國際研討會在歐洲最古老的義大利波隆那大學舉行，本次大會節目包括主題演講(Plenary Lectures)、Keynote Lectures、研討會(Symposia)、Flash Communications、Round Table Discussion、口頭報告、壁報展示及廠商展示等。來自世界各國的學者共計數百人參加。大會流程順暢、時間掌控非常好、節目豐富緊湊、場場皆滿，至最後一天聽眾仍然出席踴躍。本人能有此機會參與盛會並能深入探索此近千年的古老大學實在難得，特別記錄所見、所聞與所學心得於下。

三、心得

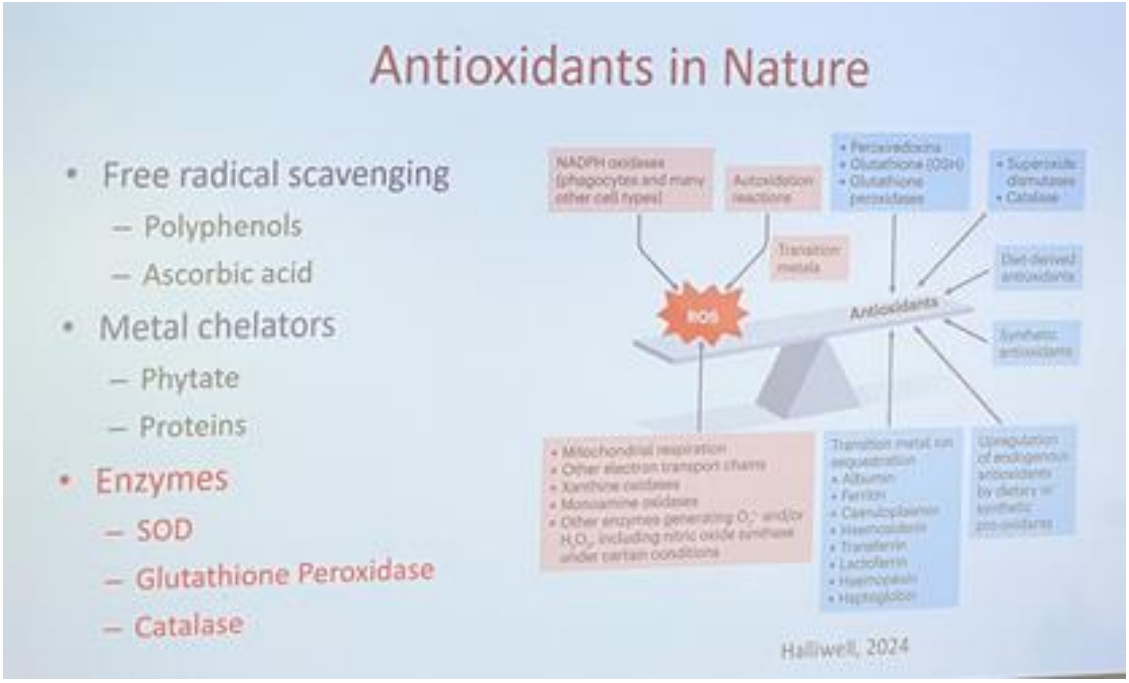
(一)、開會心得與研究新知：

以下分享一些心得與研究新知：

1. 食物中的脂肪氧化會導致油脂的酸敗(rancidity)，引起食物的味道改變及令人作嘔的氣味。降低脂肪氧化的方式可採取幾種方式：
1) 改變脂肪酸的組成(high oleic oils);
2) 避光；
3) 降低溫度；
4) 降低氧氣含量(阻斷與空氣的接觸)。

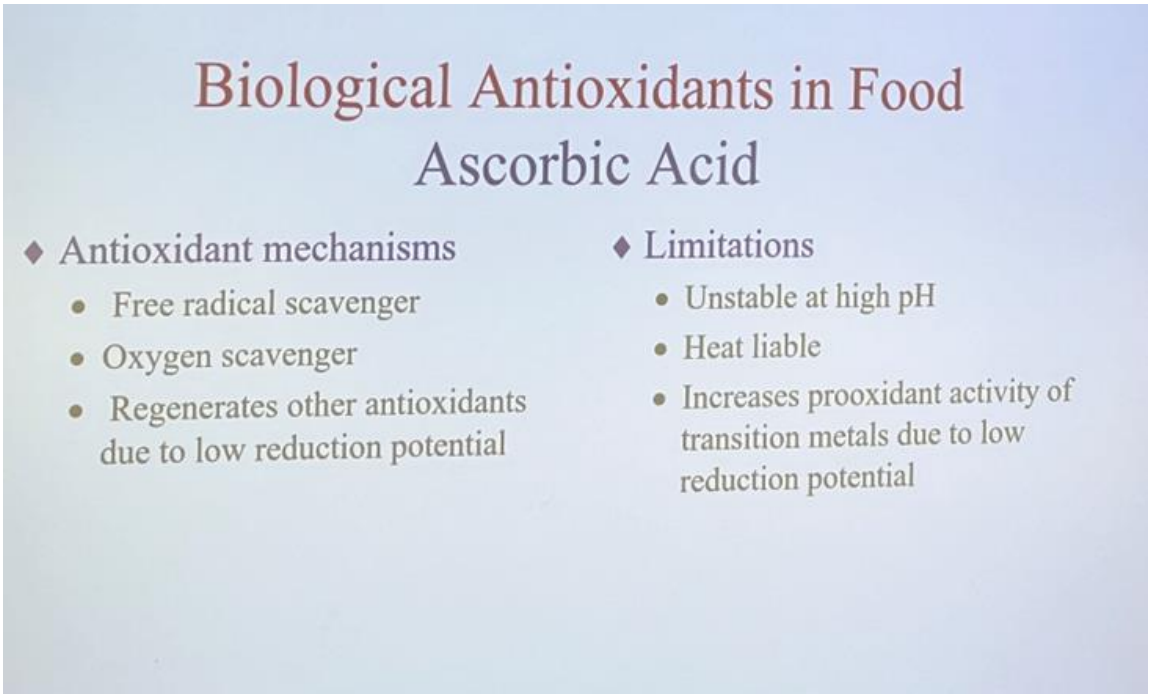
2. 在酸性環境之下，如胃酸之低 pH 環境中，也會促進氧化作用的進行。

3. 自然界中存在許多抗氧化物質，列示於下圖：

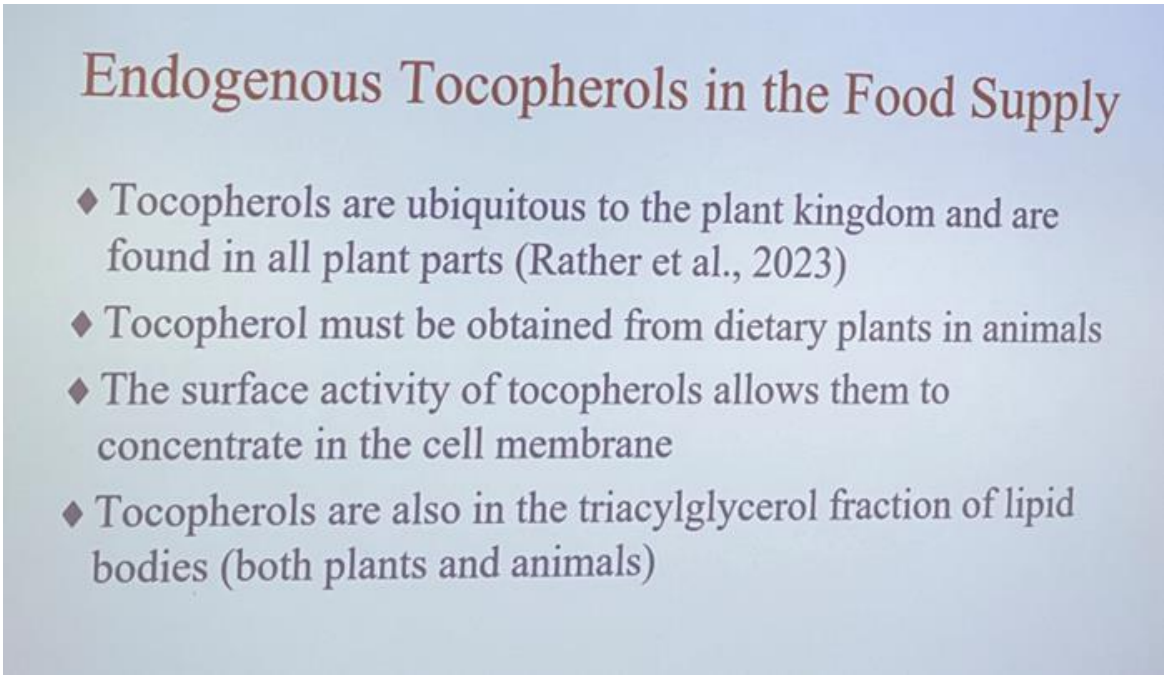


4. Vitamin C (ascorbic acid) 及 vitamin E (tocopherol) 具有抗氧化的作用。其中 tocopherol 經常被用來作為減緩脂肪氧化的抗氧化劑。

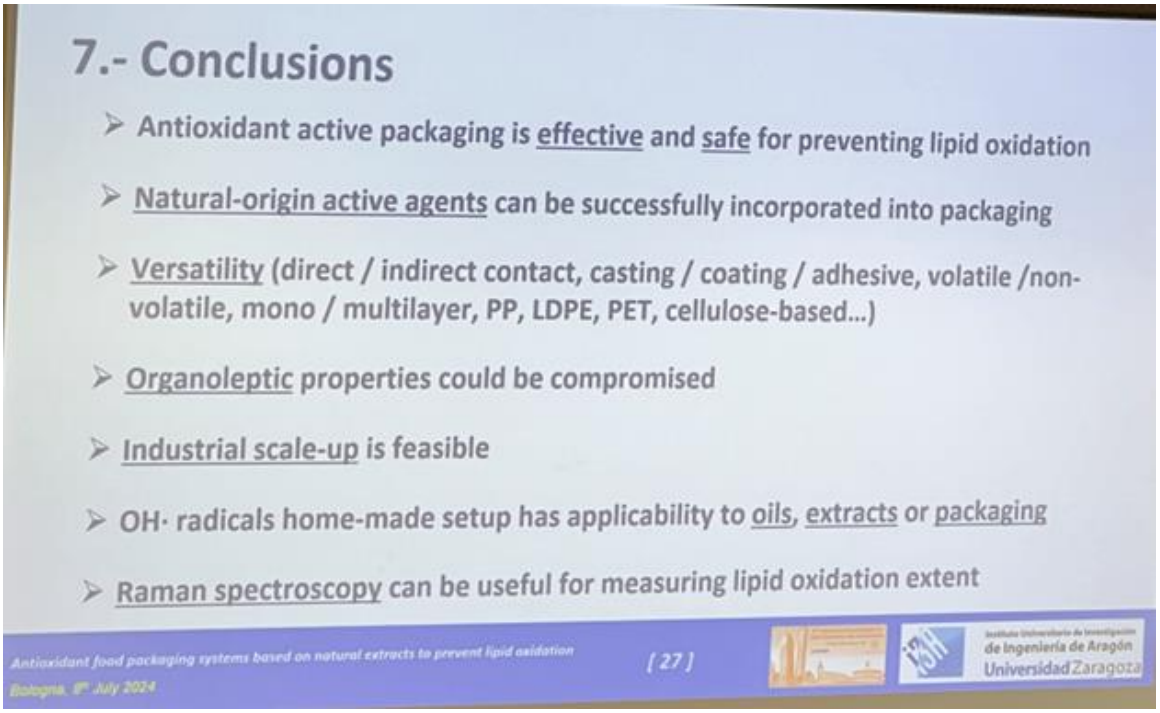
1) 食物中的 vitamin C 的抗氧化作用機轉及限制提示於下圖：



2) 食物中的 tocopherol 主要來自植物：



5. Active packaging 是減緩食物脂肪氧化的一些新的包裝處理方式。



6. Selenoperoxidase GPx4 (alias PHGPX, PIP): discovered in 1982, plays a unique and crucial role in inhibiting lipid peroxidation and preventing ferroptosis, a regulated cell death. Ferroptosis is implicated in both pathological and physiological conditions, including embryogenesis, cancer suppression, neurodegenerations, inflammatory disorders, metabolic syndrome, heart and kidney diseases and most recently COVID-19 pulmonary disease.
下圖比較 ferroptosis, apoptosis 及 necrosis 的不同點：

	Ferroptosis	Apoptosis	Necrosis
cell membrane	rounded, detached cells	rounded cells, membrane blebbing	rupture of plasma membrane
cytoplasm	small mitochondria, increased mitochondrial membrane density, loss of cristae, rupture of outer mitochondrial membrane	cell shrinkage, fragmentation into apoptotic bodies, little or no ultrastructural modification of cytoplasmic organelles	swelling of cytoplasmatic organelles, loss of intracellular contents
nucleus	normal	reduction of nuclear volume; nuclear fragmentation; chromatin condensation	chromatin condensation

7. Defect of selenoperoxidase GPx4 links to Sedaghatian-type Spondylometaphyseal Dysplasia (SSMD).

In humans, *GPX4* gene defects cause a sporadic, deadly neonatal disease named **Sedaghatian -type Spondylometaphyseal Dysplasia (SSMD).** SSMD was first described in 1980 as a neonatal condition displaying an unusual pattern of skeletal dysplasia combined with abnormalities of the central nervous system and the heart, which led to mortality due to respiratory failure in the early post-natal days. in 2020 when two additional cases bearing newly described truncations were described [27]. All together the literature reports twenty-five SSMD-affected children since 1980, all dead but three, one female and two males, apparently still alive in 2021 at the age of 6, 11, and 2 years, respectively. These patients share the same missense point mutation in GPX4, i.e., **the homozygous substitution of Arg152 with His (GPX4-R152H),** and exhibit only some of the skeletal, nervous, and cardiac abnormalities typical of the deadly forms of SSMD, together with severe additional symptoms.

8. Ferroptosis inhibitor: Ferrostatin-1 (Fer-1)

9. A study presented by RE Ward from Utah suggested that baked rodent diets that caused insulin resistance might be attributed to the lipid oxidation and not to the advanced glycation end product (AGE).

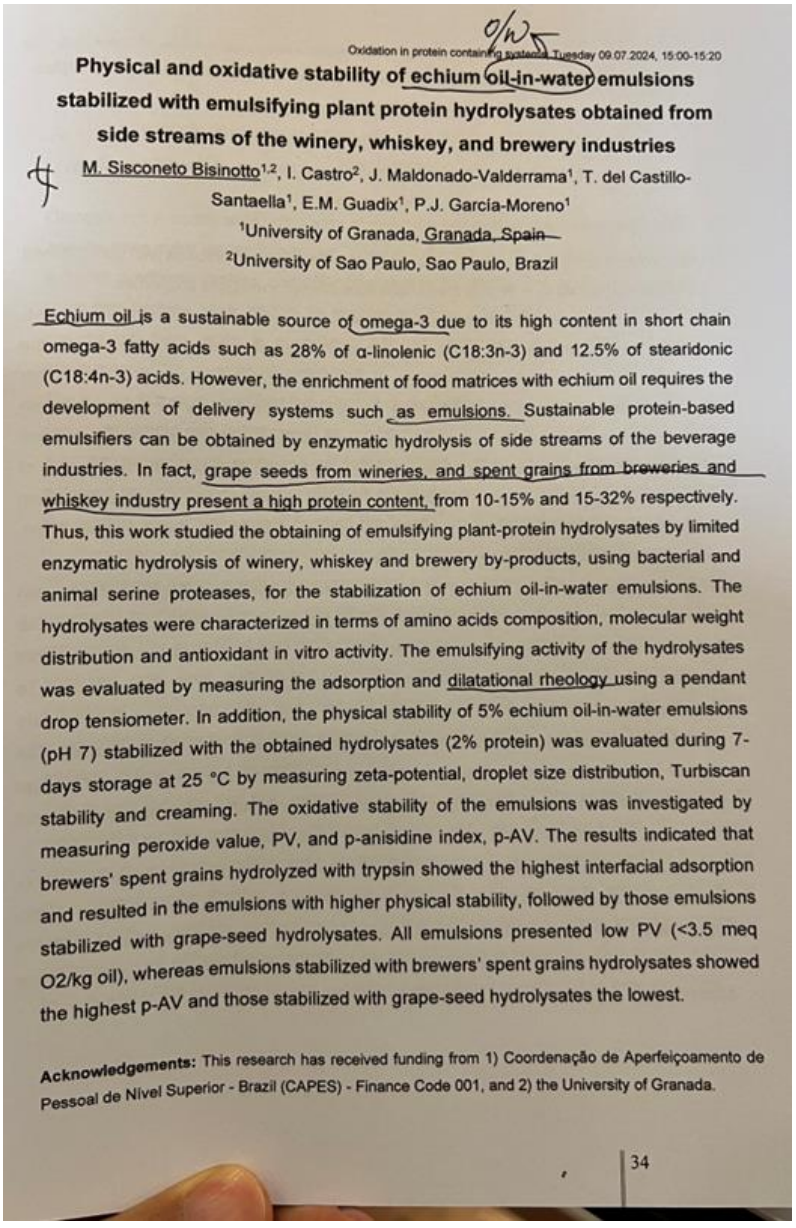
10. 奇亞籽油(chia oil)：富含脂肪酸 omega-3，是受到美膚人士喜愛的主因。

11. Microencapsulation of polyphenol extract: novel, effective antioxidant to prevent food oxidation.

12. Alkylferulates 具有抗氧化的作用。

13. 胺基酸也會進行氧化作用。最易發生氧化作用的是Hydrophobic and sulfur-containing amino acids. Protein oxidation products detected by fluorescence spectroscopy. When polyphenols are oxidized into quinones, they react with nucleophilic groups on proteins, such as cysteine, lysine, histidine and arginine residues.

14. Echium oil (藍薊)：一種植物，被發現富含 Omega 3 脂肪酸，可做為替代魚油的 omega-3 脂肪酸.



Oxidation in protein containing systems Tuesday 09.07.2024, 15:00-15:20

Physical and oxidative stability of echium oil-in-water emulsions stabilized with emulsifying plant protein hydrolysates obtained from side streams of the winery, whiskey, and brewery industries

M. Sisconeto Bisinotto[1,2], I. Castro[2], J. Maldonado-Valderrama[1], T. del Castillo-Santaella[1], E.M. Guadix[1], P.J. García-Moreno[1]

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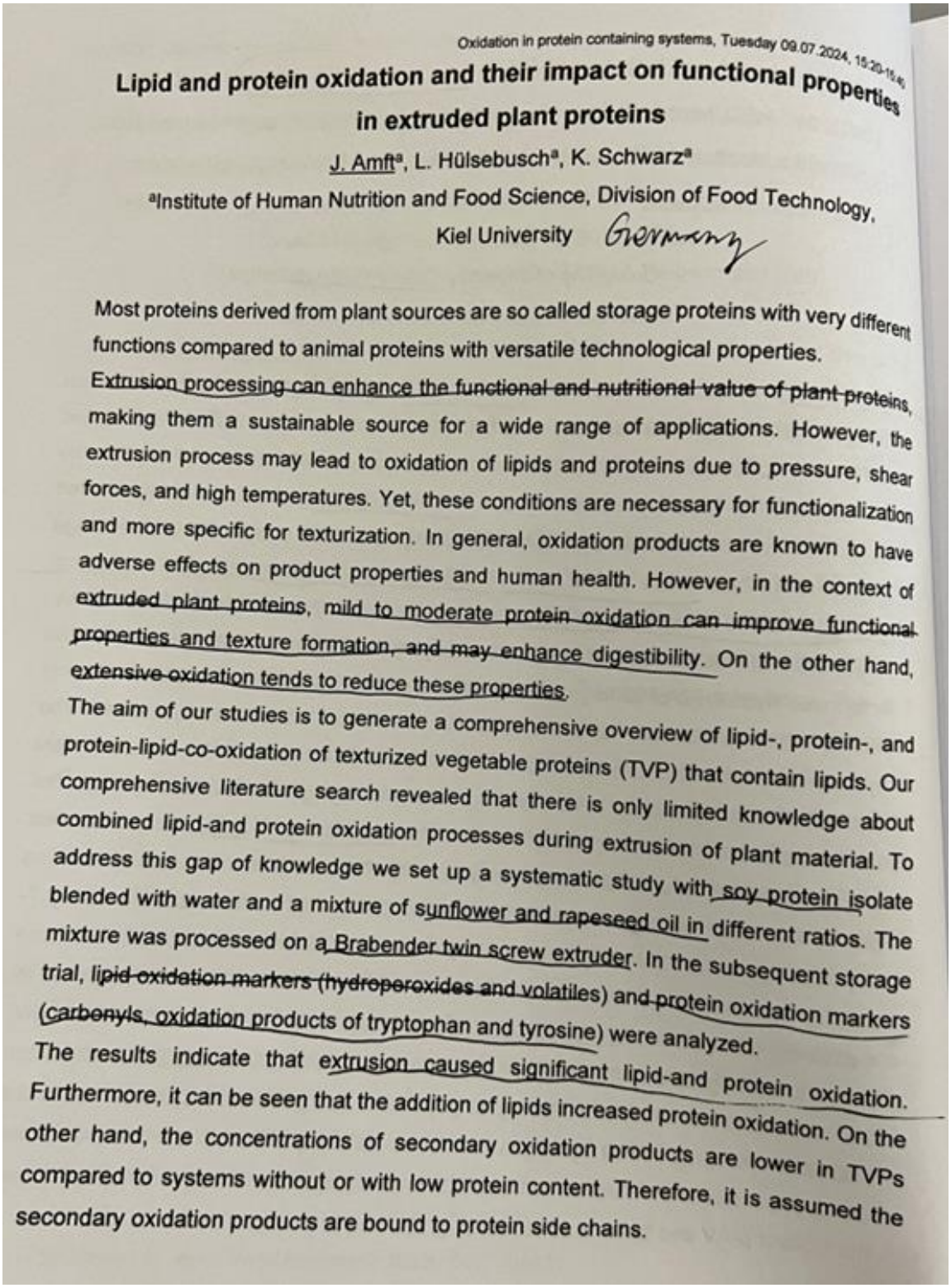
Echium oil is a sustainable source of omega-3 due to its high content in short chain omega-3 fatty acids such as 28% of α-linolenic (C18:3n-3) and 12.5% of stearidonic (C18:4n-3) acids. However, the enrichment of food matrices with echium oil requires the development of delivery systems such as emulsions. Sustainable protein-based emulsifiers can be obtained by enzymatic hydrolysis of side streams of the beverage industries. In fact, grape seeds from wineries, and spent grains from breweries and whiskey industry present a high protein content, from 10-15% and 15-32% respectively. Thus, this work studied the obtaining of emulsifying plant-protein hydrolysates by limited enzymatic hydrolysis of winery, whiskey and brewery by-products, using bacterial and animal serine proteases, for the stabilization of echium oil-in-water emulsions. The hydrolysates were characterized in terms of amino acids composition, molecular weight distribution and antioxidant in vitro activity. The emulsifying activity of the hydrolysates was evaluated by measuring the adsorption and dilatational rheology using a pendant drop tensiometer. In addition, the physical stability of 5% echium oil-in-water emulsions (pH 7) stabilized with the obtained hydrolysates (2% protein) was evaluated during 7-days storage at 25 °C by measuring zeta-potential, droplet size distribution, Turbiscan stability and creaming. The oxidative stability of the emulsions was investigated by measuring peroxide value, PV, and p-anisidine index, p-AV. The results indicated that brewers' spent grains hydrolyzed with trypsin showed the highest interfacial adsorption and resulted in the emulsions with higher physical stability, followed by those emulsions stabilized with grape-seed hydrolysates. All emulsions presented low PV (<3.5 meq O2/kg oil), whereas emulsions stabilized with brewers' spent grains hydrolysates showed the highest p-AV and those stabilized with grape-seed hydrolysates the lowest.

Acknowledgements: This research has received funding from 1) Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil (CAPES) - Finance Code 001, and 2) the University of Granada.

15. Lipid oxidation markers: hydroperoxides and volatiles. Protein oxidation markers: carbonyls, oxidation products of tryptophan and tyrosine.



Lipid and protein oxidation and their impact on functional properties in extruded plant proteins

J. Amft[a], L. Hülsebusch[a], K. Schwarz[a]

[a]Institute of Human Nutrition and Food Science, Division of Food Technology, Kiel University Germany

Most proteins derived from plant sources are so called storage proteins with very different functions compared to animal proteins with versatile technological properties. Extrusion processing can enhance the functional and nutritional value of plant proteins, making them a sustainable source for a wide range of applications. However, the extrusion process may lead to oxidation of lipids and proteins due to pressure, shear forces, and high temperatures. Yet, these conditions are necessary for functionalization and more specific for texturization. In general, oxidation products are known to have adverse effects on product properties and human health. However, in the context of extruded plant proteins, mild to moderate protein oxidation can improve functional properties and texture formation, and may enhance digestibility. On the other hand, extensive oxidation tends to reduce these properties.

The aim of our studies is to generate a comprehensive overview of lipid-, protein-, and protein-lipid-co-oxidation of texturized vegetable proteins (TVP) that contain lipids. Our comprehensive literature search revealed that there is only limited knowledge about combined lipid-and protein oxidation processes during extrusion of plant material. To address this gap of knowledge we set up a systematic study with soy protein isolate blended with water and a mixture of sunflower and rapeseed oil in different ratios. The mixture was processed on a Brabender twin screw extruder. In the subsequent storage trial, lipid oxidation markers (hydroperoxides and volatiles) and protein oxidation markers (carbonyls, oxidation products of tryptophan and tyrosine) were analyzed.

The results indicate that extrusion caused significant lipid-and protein oxidation. Furthermore, it can be seen that the addition of lipids increased protein oxidation. On the other hand, the concentrations of secondary oxidation products are lower in TVPs compared to systems without or with low protein content. Therefore, it is assumed the secondary oxidation products are bound to protein side chains.

16. Caffeic acid, sinapic acid and gallic acid slowed down the formation of hydroperoxides.

Oxidation and oil binding capacity of protein-based oleogels

M. Wittmüss, T. Heyn, J. Amft, K. Schwarz
Institute of Human Nutrition and Food Science, University of Kiel
Kiel, Germany

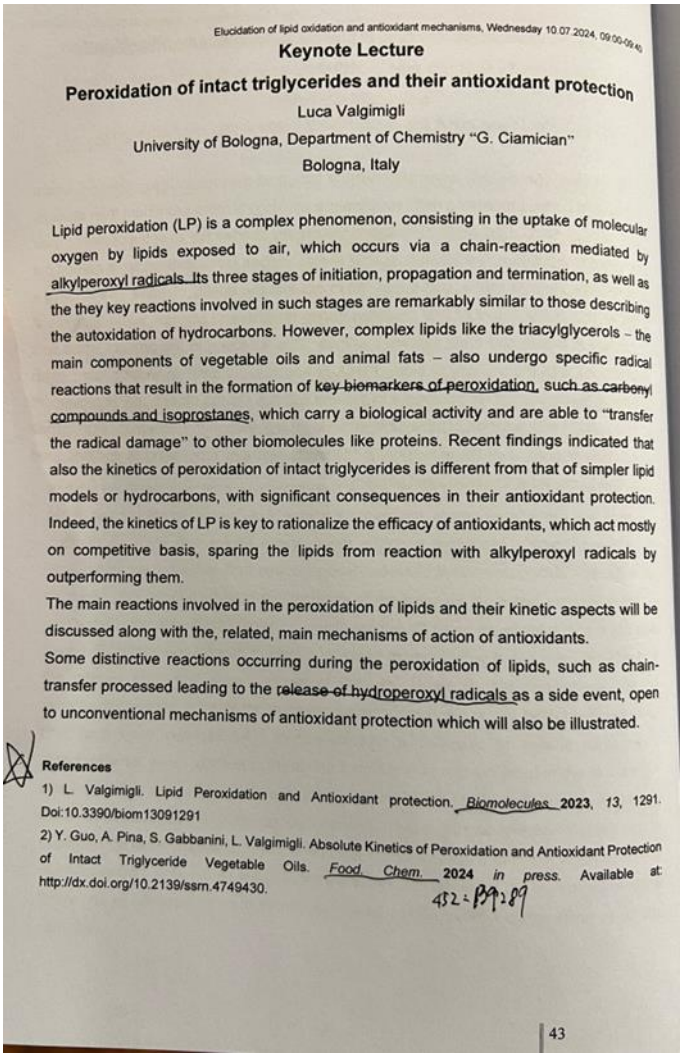
Oleogels are a rapidly expanding area of research due to their potential to address health and environmental issues in our diet. They are made from locally sourced liquid oil with superior nutritional profiles and offer comparable functional and structural properties to solid fats derived from animal or tropical sources. However, a higher proportion of unsaturated fatty acids is associated with an increasing susceptibility to oxidative processes, which must be counteracted.

The aim of this study is to test the effect of phenolic compounds (gallic acid, sinapic acid, chlorogenic acid and caffeic acid) on oxidation and functional properties of the oleogel, such as oil binding capacity and gel stability. We hypothesize that phenolic compounds will exert an antioxidant effect in oleogels which may be influenced by the interaction with the protein-based oleogelator. Further, the formation of the oleogelator may be impacted by interactions with phenolic compounds affecting its functional properties.

To investigate these questions, we prepared a whey-protein-based oleogel with purified rapeseed-sunflower oil mixture (50:50 v/v) and conducted a storage test at accelerated conditions. We quantified lipid oxidation by measuring the amount of hydroperoxide and analyzed both lipid and protein oxidation markers using ATR-FTIR measurement. Additional measurements, including oil binding capacity and texture analysis, were conducted to determine if these phenolic compounds affect the gel structure itself, in addition to their impact on oxidation.

The results indicate that lipid oxidation, i.e., lipid hydroperoxides, in the oil phase of an oleogel-like system was highly influenced by different phenolic compounds. The addition of higher concentrated caffeic acid, sinapic acid and gallic acid slowed down the formation of hydroperoxides. Further analyses by ATR-FTIR indicated some effects of phenolic compounds on lipid and protein oxidation in this system. No effect was found for phenolic compounds at different concentration on the oil binding capacity. This is an important finding which will ease the way to implement oleogel as constitutes in food products.

17. Lipid peroxidation is mediated by alkylperoxyl radicals, 3 stages of initiation, propagation and termination：



Elucidation of lipid oxidation and antioxidant mechanisms, Wednesday 10.07.2024, 09:00-09:40

Keynote Lecture

Peroxidation of intact triglycerides and their antioxidant protection

Luca Valgimigli

University of Bologna, Department of Chemistry "G. Ciamician"

Bologna, Italy

Lipid peroxidation (LP) is a complex phenomenon, consisting in the uptake of molecular oxygen by lipids exposed to air, which occurs via a chain-reaction mediated by alkylperoxyl radicals. Its three stages of initiation, propagation and termination, as well as the they key reactions involved in such stages are remarkably similar to those describing the autoxidation of hydrocarbons. However, complex lipids like the triacylglycerols – the main components of vegetable oils and animal fats – also undergo specific radical reactions that result in the formation of ~~key biomarkers of peroxidation~~, such as carbonyl compounds and isoprostanes, which carry a biological activity and are able to "transfer the radical damage" to other biomolecules like proteins. Recent findings indicated that also the kinetics of peroxidation of intact triglycerides is different from that of simpler lipid models or hydrocarbons, with significant consequences in their antioxidant protection. Indeed, the kinetics of LP is key to rationalize the efficacy of antioxidants, which act mostly on competitive basis, sparing the lipids from reaction with alkylperoxyl radicals by outperforming them.

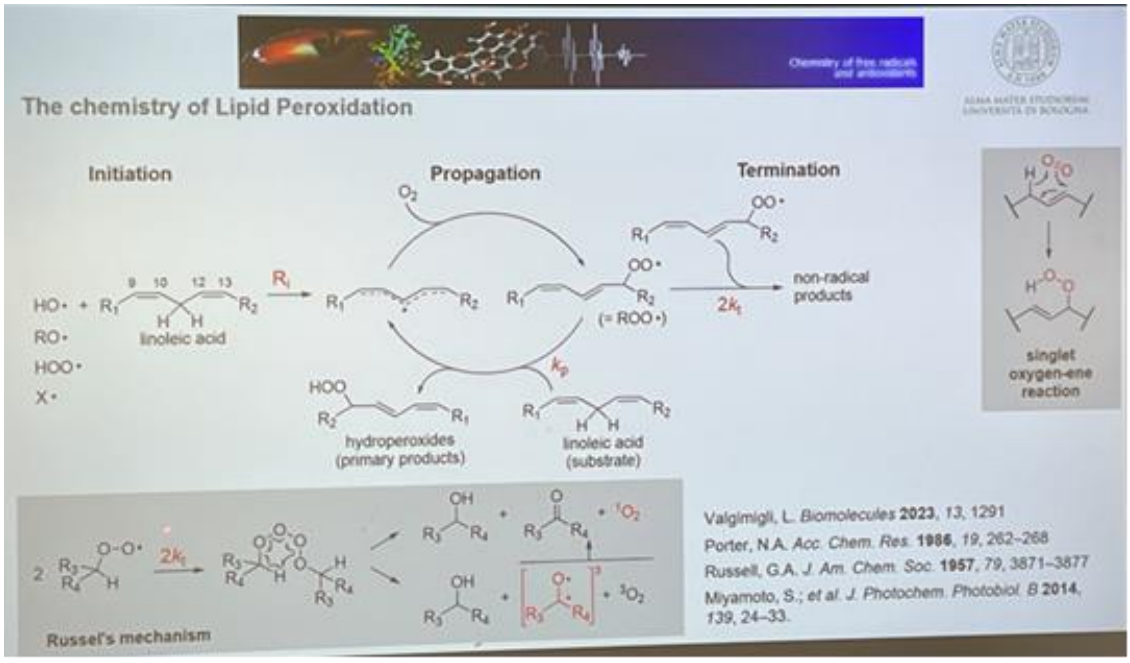
The main reactions involved in the peroxidation of lipids and their kinetic aspects will be discussed along with the, related, main mechanisms of action of antioxidants.

Some distinctive reactions occurring during the peroxidation of lipids, such as chain-transfer processed leading to the ~~release of hydroperoxyl radicals~~ as a side event, open to unconventional mechanisms of antioxidant protection which will also be illustrated.

References

1) L. Valgimigli. Lipid Peroxidation and Antioxidant protection. *Biomolecules* **2023**, *13*, 1291. Doi:10.3390/biom13091291

2) Y. Guo, A. Pina, S. Gabbanini, L. Valgimigli. Absolute Kinetics of Peroxidation and Antioxidant Protection of Intact Triglyceride Vegetable Oils. *Food. Chem.* **2024** *in press*. Available at: http://dx.doi.org/10.2139/ssrn.4749430.



18. Catechols: quercetin, CAPE, have exceptional antioxidant activity.

(二)、波隆那大學參訪心得：

波隆那大學為歐洲最古老的大學，創立於 1088 年，首先出現的是世界上第一所法學院，再慢慢發展為一所綜合性公立大學。創立至今已有將近一千年的歷史，1988 年 9 月 18 日，在波隆那大學九百年校慶之際，歐洲 430 位大學校長共同簽署了歐洲大學憲章，宣布波隆那大學為「大學之母」（拉丁文：Alma Mater Studiorum），即世上所有大學的母校。目前波隆那大學的印章就寫著 Alma Mater Studiorum。

波隆那大學印章：

大學區主要分布在波隆那城的東北部，但大學也有許多建物散布在全城各地，許多學院都設立在古老的皇宮裡面。因其歷史悠久，所以有許多珍貴的資產，Sistema Museale di Ateneo (SMA)是波隆那大學將 15 個博物館和藏品以及數位博物館組成的網絡，有許多珍貴的收藏品，可由以下網址連結其網站：https://www.midaticket.it/eventi/universita-di-bologna/ 。有些博物館不收費，但也有一些是要收費的。如果要去參訪要注意每個博物館的開放日期及時間都不一定。最好事先上網查看。但目前是暑假時間，有些博物館正在整修，所以並沒有開放。

本人利用此次機會，造訪了以下幾個學院與博物館，但還是沒有辦法參觀完所有的博物館：

1. 動物學博物館(Museum of Zoology)
2. Comparative Anatomy Collection (比較解剖學收藏)
3. Anthropological Collection (人類學收藏)
4. Chemistry Collection at Giacomo Ciamician：適逢整修，九月後才開放。
5. 波吉宮博物館 (Museum of Pallazo Poggi)：科學與藝術收藏。
6. Johns Hopkins School of Advance International Studies (SAIS)，European Campus.
7. Palazzo dell’ Archiginnasio and Teatro Anatomico：

下圖是過去一整排連通的 10 間教室，目前已改為圖書館的藏書間，是不開放入內參觀的：

下圖是以前上大體解剖的教室，可付費參觀：

8. Palazzo Marescotti Brazzetti：以前的宮殿，目前是波隆那大學的 Department of Music and Drama Studies.
9. Arche dei Glossatori (Scholars' graves)：These tombs are tributes to some of the first and most important professors in the Middle Ages when the University of Bologna was renowned particularly for teaching law.
10. Palazzo Hercolani (Palazzo Ercolani): 目前是政治學院的所在地。

波隆那大學其實還有許多博物館值得參觀，可惜時間倉促，無法全部成行，這些包括：
1) 托雷德拉斯佩科拉 (Torre della Specola): 觀測行星及其運動的博物館；
2) Luigi Cattaneo：解剖學蠟像收藏館;
3) Giovanni Capellini Museum: Geological Collection;
4) 植物園與植物標本館；及
5) 礦物學收藏館等。

四、建議事項

1. 大會主要邀請來自歐美的重要學者參與，並給予 plenary lectures 及 keynote lectures，相當精彩，另外也舉辦各式的討論，如 symposia, flash communications, round table discussion 及 oral presentations 等。因為投稿數量甚多，有許多論文摘要則以壁報形式展現，讓與會者在午餐或 coffee breaks 時進行交流。會議採用模式讓與會者都有時間針對每個演講與論文發表與報告者或發表者當面進行溝通，值得學習。

2. 會議通常同時有兩位主持人，流程與時間的掌控都相當好，並沒有超時的現象，也不見有人舉牌告知講演者時間快到，顯示大會事先必定與講演者做了充分的溝通與要求才能做到的，是另一個值得學習的地方。

3. 受邀請演講的學者，都學有專精、表達能力也很好，並不是像在一些國際會議中時常出現講演者雖學養豐富，但表達能力不佳，以致於聽者不易理解其演講內容。想必主辦單位對講演者也是費了一番心力挑選出來的。此點對國內未來在舉辦國際會議時是很好的學習參考。

4. 在針對大學城進行參訪時，可以感受到這所近千年大學之所以偉大，並被譽為「大學之母」(拉丁文：Alma Mater Studiorum)，即世上所有大學的母校，實在不是浪得虛名。雖然大學各院所分布在整個波隆那城市的不同角落，但這麼多年來，主事者對各種文物的保留都不餘遺力，全校共擁有 15 個博物館收藏歷史藏品以及數位博物館組成的網絡，供後人參觀與了解。在動物學博物館(Museum of Zoology)中看到許多珍禽異獸的標本，連大象的整個消化系統都可以做成標本，供學生參考與學習。建議有百餘年歷史的臺灣大學的主事者應該及早思索如何維護臺灣大學的歷史建物以及其他歷史文物。