Available online at http://journal-of-agroalimentary.ro

Journal of Agroalimentary Processes and Technologies 2025, 31 (4), pp 438-444

Journal of Agroalimentary Processes and Technologies

Development of an Olive Oil-Based Spread Functionalized with Bioactive Compounds from Olive Pomace

Diana Melo Ferreira[1*], M. Antónia Nunes[1], Susana Machado[1], M. Beatriz P. P. Oliveira[1], Rita C. Alves[1]

[1]REQUIMTE/LAQV, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Jorge de Viterbo Ferreira Street 228, 4050-313 Porto, Portugal

**Corresponding author: dimelo@ff.up.pt*

Received 5 November, 2025; Revised 18 November, 2025, Published 22 November, 2025

Abstract

The growing production of olive oil generates large amounts of olive pomace, a phytotoxic by-product that requires sustainable strategies for valorization. This study developed an olive oil-based spread functionalized with a functional ingredient obtained from fresh olive pomace from Portugal through mechanical pressing. Two formulations (A and B), composed mainly of 70% olive oil, were prepared with or without coconut oil. Their fatty acid profiles, phenolic content, hydroxytyrosol content, and antioxidant activity were compared with three commercial vegetable spreads. Formulations A and B showed a predominance of oleic acid (62% and 61%) and significantly higher total phenolics, hydroxytyrosol, and antioxidant capacity ($p < 0.05$) than commercial samples. These results demonstrate that olive pomace extract is an effective natural ingredient to improve the nutritional and antioxidant quality of vegetable spreads while supporting sustainable valorization of olive industry by-products and reducing the environmental impact of olive oil production.

Keywords: novel food, by-product, food innovation, sustainability, circular economy, fatty acids, antioxidants.

1. Introduction

The olive tree (*Olea europaea* L.) is a cornerstone of agricultural economies in Mediterranean countries, such as Spain, Italy, and Portugal [1], particularly regarding olive oil production (Figure 1).

In the 2024/2025 season, Portugal produced approximately 170,000 tons of olive oil [2, 3].

Around 4 to 5 kg of olives are required to produce 1 L of olive oil. The extraction yield ranges from 15% to 20%. Thus, the remaining residue corresponds to olive pomace (80-85%) [4]. This translates to approximately 675,000 to 960,000 tons of olive pomace, depending on the extraction yield.

Olive pomace is a phytotoxic by-product that presents several disposal challenges. The large quantities produced every year highlight the need for sustainable strategies, such as green extraction methods to recover bioactive compounds and minimize the environmental impact of olive oil production [5, 6].

Figure 1. Scheme of olive oil production.

This study aimed to extract a functional ingredient from olive pomace using an environmentally friendly methodology and to develop an olive oil-based spread enriched with this ingredient. The effects of its incorporation were evaluated through

DOI: 10.59463/JAPT.2025.4.6

the analysis of fatty acid profiles and antioxidant composition.

2. Materials and methods

2.1. Olive pomace sample

A mixture of fresh olive pomace from two-phase olive mills from the two major Portuguese olive oil-producing regions (Trás-os-Montes and Alentejo, Figure 2) was subjected to mechanical pressing, according to a patented process (EP3723491 (2024) and US11,918,007 B2 (2024)) to obtain an olive pomace functional ingredient [5].

Figure 2. Major Portuguese olive oil-producing regions.

2.2. Olive oil-based spread formulations

The olive pomace functional ingredient was incorporated into two formulations (A and B) composed primarily of 70% olive oil, water, and technological agents, with or without the addition of coconut oil (20%).

The functional ingredient was mixed with the water-soluble ingredients. Then, they were was combined with the lipid phase, and after emulsion formation, the mixture was cooled.

Three commercial vegetable spreads (C1, C2, and C3) available on the market were also evaluated, aiming to compare and explore the potential nutritional advantages of the developed formulations (Table 1).

Table 1. Samples used in this study.

Code	Sample
A	Formulation developed in the lab with the olive pomace functional ingredient
B	Formulation developed in the lab with the functional ingredient + coconut oil
C1	Commercial spread 1
C2	Commercial spread 2
C3	Commercial spread 3

2.3. Fatty acid (FA) profile

To obtain the fat, 10 g of sample were put in an oven at 60°C for 60 min, centrifuged at 2000 rpm for 5 min, and the lipid phase collected and filtered through anhydrous sodium sulfate at the same temperature.

Fatty acid methyl esters (FAME) were prepared by acid transesterification, according to ISO 12966-2:2017 [7] and analysed in a GC-FID system from Shimadzu (Tokyo, Japan) with a CP-Sil 88 silica capillary column (50 m × 0.25 mm, 0.20 μm) from Varian (Middelburg, Netherlands) according to the conditions described by Ferreira *et al*. [6].

FAME's retention times were compared with FAME 37 standard mix (Supelco, USA) for identification.

2.4. Extracts for antioxidant screening

The extracts for analysis were obtained according to Capannesi *et al*. [8] with slight modifications.

Briefly, 2.5 g of each sample was mixed with 5 mL of *n*-hexane and vortexed for 15 min. Then, 5 mL of a methanol/water (80/20, v/v) solution was added and vortexed for 30 min. The hydroalcoholic fraction was separated, and the process was repeated twice. The hydroalcoholic fractions were pooled and used for analysis.

2.4.1. Total phenolic content (TPC)

An aliquot of 30 μL of each extract was mixed with 150 μL of Folin-Ciocalteu reagent. After that, 120 μL of sodium carbonate solution was added. The mixture was incubated at 45°C for 15 min, and the absorbance measured at 765 nm as described by Ferreira *et al*. [6].

2.4.2. Hydroxytyrosol (HT) content

HT was analyzed using reversed-phase high-performance liquid chromatography (RP-HPLC) coupled with a diode-array detector (DAD) and fluorescence detector (FLD), according to the conditions described by Ferreira *et al*. [6].

Separation was performed on a Zorbax-SB-C18 column (250 × 4.6 mm, 5 μm particle size) at a flow rate of 1 mL/min and a column temperature of 20°C.

FLD was carried out with an excitation wavelength of 280 nm and an emission wavelength of 330 nm for quantification. The DAD recorded absorbance in the 200-600 nm range to confirm the identification of HT.

2.4.3. Ferric reducing antioxidant power (FRAP)

A 35 µL aliquot of each extract was mixed with 265 µL of freshly prepared FRAP reagent. The mixture was incubated at 37°C for 30 min. After incubation, the absorbance was measured at 595 nm as described by Ferreira *et al*. [6].

2.4.4. 2,2-Diphenyl-1-picrylhydrazyl radical (DPPH$^{\bullet}$) scavenging activity

A 30 µL aliquot of the sample extract was added to 270 µL of a 6 × 10^{-5} M DPPH$^{\bullet}$ solution freshly prepared in ethanol. The reaction mixture was monitored kinetically, with absorbance readings taken every 2 min and 40 min after mixing. The decrease in absorbance, corresponding to the reduction of the DPPH radical, was measured at 515 nm according to Ferreira *et al*. [6].

2.5. Statistical analysis

All experiments were performed in triplicate (n = 3). Significant differences between samples (p < 0.05) were assessed by ANOVA and Tukey's test, using IBM SPSS Statistics v. 26 (IBM Corp., USA).

3. Results and Discussion

3.1. FA profile

Spread A (Figure 3) was characterized by a high proportion of monounsaturated fatty acids (MUFA, 74.45%), with oleic acid (C18:1n9c) being the dominant component, accounting for 73.45% of the total FA.

Palmitic acid (C16:0) and linoleic acid (C18:2n6c) were also present in notable amounts (12.15% and 9.02%, respectively), followed by stearic acid (C18:0, 3.11%).

Minor FA, including C16:1 (palmitoleic acid), C18:3n3 (α-linolenic acid), C20:0 (arachidic acid), C20:1n9 (gadoleic acid), and C22:0 (behenic acid) were detected in small quantities (<0.85%).

Overall, the MUFA-rich composition of spread A reflects its olive oil basis, known for its oxidative stability and associated cardiovascular health benefits [9, 10].

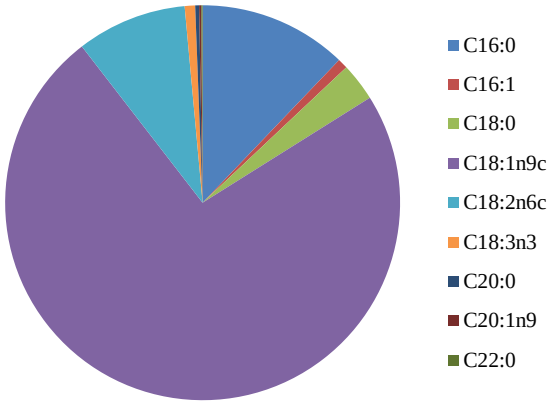

Figure 3. Fatty acid profile of spread A.

The FA profile of spread B (Figure 4) was also dominated by MUFA (61.74%), with oleic acid (C18:1n9c) as the major component (60.92%), which was expected given that olive oil was the major ingredient.

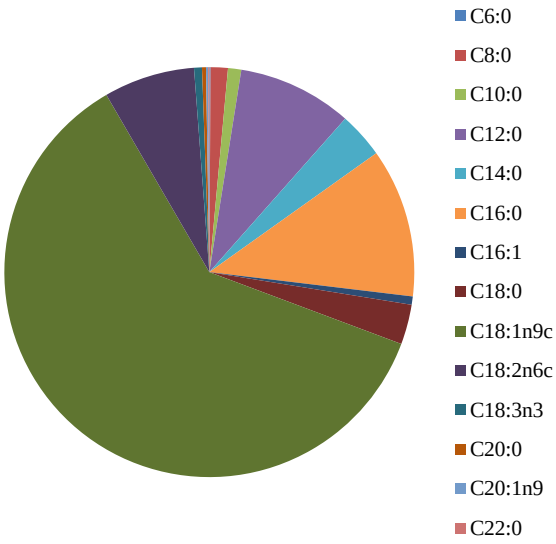

Figure 4. Fatty acid profile of spread B.

Saturated fatty acids (SFA, 30.45%) were also present in notable proportions, particularly palmitic acid (C16:0, 11.75%) and lauric acid (C12:0, 9.04%), along with smaller amounts of myristic acid (C14:0, 3.61%) and stearic acid (C18:0, 3.15%), reflecting the addition of coconut oil in this formulation.

Polyunsaturated fatty acids (PUFA, 7.81%) were present at moderate levels, mainly linoleic acid (C18:2n6c, 7.18%) and α-linolenic acid (C18:3n3, 0.62%).

Caproic (C6:0), caprylic (C8:0), and capric (C10:0)

acids were detected in low concentrations and are related to the presence of coconut oil.

Overall, spread B combines the MUFA benefits of olive oil with the structural firmness imparted by coconut oil [9-11].

Spread C1 (Figure 5) presented a balanced FA distribution, with SFA, MUFA, and PUFA accounting for 22.94%, 42.31%, and 34.75% of total FA, respectively.

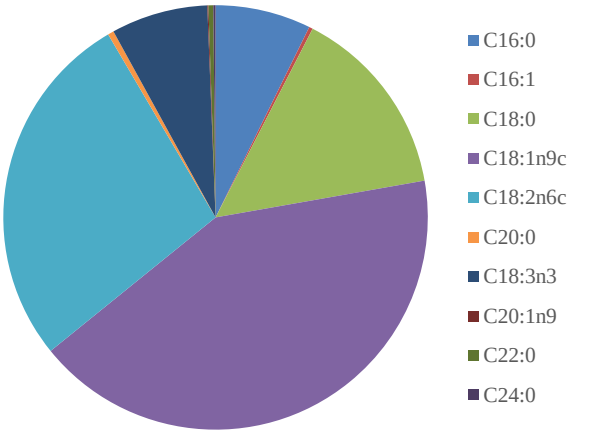

Figure 5. Fatty acid profile of spread C1.

Oleic acid (C18:1n9c) was the predominant component, accounting for 41.95% of total FA, followed by linoleic acid (C18:2n6c, 27.38%), stearic acid (C18:0, 14.66%), α-linolenic acid (C18:3n3, 7.37%), and palmitic acid (C16:0, 7.28%), reflecting the ingredients declared in the label (sunflower oil, extra-virgin olive oil, flaxseed oil, and shea fat).

Minor amounts of palmitoleic (C16:1), arachidic (C20:0), eicosenoic (C20:1n9), behenic (C22:0), and lignoceric (C24:0) acids were present below 0.5%, similar to spreads A and B, these FA were probably sourced by olive oil.

The combination of both n-6 and n-3 FA provides nutritional value, although the relatively high PUFA content may reduce oxidative stability [10, 12-14].

Spread C2 (Figure 6) was characterized by a predominance of unsaturated FA, particularly PUFA (39.10%) and MUFA (33.88%), while SFA were 27.03%.

Linoleic acid (C18:2n6c) was the major component, contributing 39.10% of the total FA composition, followed by oleic acid (C18:1n9c) at 33.88%. This composition reflects the use of sunflower oil, as declared on the product label.

Among the SFA, palmitic acid (C16:0) was the most abundant (16.33%), with moderate amounts of lauric (C12:0, 4.07%) and stearic (C18:0, 3.51%) acids, and smaller quantities of myristic acid (C14:0, 1.61%), likely due to the presence of palm oil as an ingredient. Trace amounts of caprylic (C8:0) and capric (C10:0) acids were also detected, possibly due to the inclusion of buttermilk in the spread.

Interestingly, α-linolenic acid (C18:3n3) was not detected.

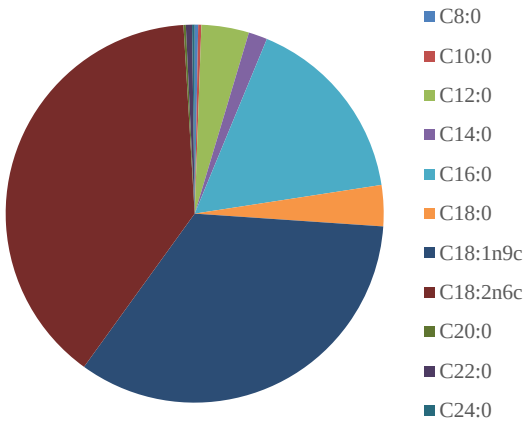

Figure 6. Fatty acid profile of spread C2.

The high degree of unsaturation (over 70%) suggests that spread C2 would exhibit good spreadability and a softer texture at room temperature. However, the elevated PUFA content may reduce oxidative stability, thereby necessitating the addition of antioxidants to prolong shelf life [10, 12, 14].

Spread C3 (Figure 7) exhibited the highest unsaturation level, with PUFA (44.62%) as the predominant fraction, followed by MUFA (31.64%) and SFA (23.74%).

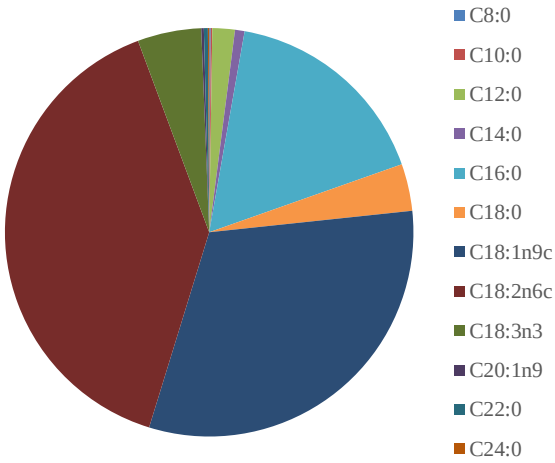

Figure 7. Fatty acid profile of spread C3.

Linoleic acid (C18:2n6c) was the main FA at 39.58%, accompanied by a notable amount of oleic acid (C18:1n9c, 31.43%), followed by palmitic acid (C16:0, 16.82%), α-linolenic acid (C18:3n3, 5.04%), and stearic acid (C18:0, 3.72%), with smaller amounts of lauric (C12:0, 1.78%) and myristic (C14:0, 0.76%) acids, reflecting the ingredients described in the label: soy, palm, and coconut oils.

Trace levels of caprylic (C8:0), capric (C10:0), eicosenoic acid (C20:1n9), behenic (C22:0), and lignoceric (C24:0) acids were detected below 0.32%. The spread has nutritional quality due to the presence of essential FA. However, the high PUFA content may reduce oxidative stability [10-12].

The FA profiles clearly differentiated the spreads. Spreads A and B were characterized by high oleic acid content (73% and 61%, respectively), typical of olive oil-based formulations.

From the commercial spreads, C1 presented the highest MUFA content (42%), resembling spreads A and B, due to the presence of olive oil in the formulation as well.

In contrast, commercial spreads C2 and C3 were more polyunsaturated, reflecting the use of seed oils such as sunflower and soybean.

Consequently, spreads A, B, and C1 exhibited greater oxidative stability and health-promoting potential due to their MUFA richness, while C2 and C3 emphasize PUFA content, offering nutritional benefits related to essential FA but potentially lower shelf-life stability.

3.2. TPC

The TPC of the samples, expressed as mg of gallic acid equivalents (GAE) per 100 g, showed significant differences among samples (Table 2). Sample B exhibited the highest TPC value (33.2 mg GAE/100 g, $p < 0.05$), followed by sample A (27.8 mg GAE/100 g), which was significantly lower than sample B.

In contrast, the commercial samples C1, C2, and C3 had the lowest TPC values (2.1-4.3 mg GAE/100 g) and did not differ significantly from each other.

The elevated TPC in samples A and B can be attributed to the use of olive-derived ingredients, particularly olive oil and the olive pomace functional ingredient [5, 6, 9].

3.3. HT content

Regarding the HT content (Table 2), A and B formulations contained higher amounts of this phenolic compound (2.2-2.4 mg/100 g) in comparison to C2 (0.02 mg/100 g), due to the extra amount provided by the addition of both olive oil and the olive pomace functional ingredient. Conversely, the other two commercial spreads did not present HT, consistent with their lack of olive-based ingredients.

HT presents several health benefits, including antioxidant, anti-inflammatory, anticancer, antimicrobial, and heart-protective effects [15].

Table 2. Total phenolic content (TPC) and hydroxytyrosol (HT) content of the spreads.

Sample	TPC mg GAE/100 g	HT mg/100 g
A	27.8 ± 1.2^{b}	2.2 ± 0.1^{a}
B	33.2 ± 2.5^{a}	2.4 ± 0.1^{a}
C1	2.6 ± 0.2^{c}	$0.02 \pm <0.01^{b}$
C2	2.1 ± 0.2^{c}	0.0 ± 0.0
C3	4.3 ± 0.3^{c}	0.0 ± 0.0

Different letters in each column denote significant differences between the samples ($p < 0.05$). GAE, gallic acid equivalents.

3.4. FRAP

Concerning the antioxidant activity (Table 3), sample B presented the highest FRAP result (33 mg ferrous sulphate equivalents (FSE)/100 g), followed by sample A (26 mg FSE/100 g), then C3, C1, and C2 (22 > 16 > 12 mg FSE/100 g, respectively).

The higher antioxidant activity in samples A and B reflects their elevated TPC, confirming the effectiveness of the olive pomace-derived ingredient in enhancing the oxidative defense capacity of the spreads.

Table 3. Antioxidant activity of the spreads.

Sample	FRAP mg FSE/100 g	DPPH mg TE/100 g
A	26.3 ± 1.4^{b}	17.7 ± 0.8^{b}
B	32.7 ± 2.5^{a}	26.8 ± 2.6^{a}
C1	15.7 ± 0.8^{d}	8.5 ± 0.9^{d}
C2	11.9 ± 0.6^{e}	11.9 ± 0.7^{c}
C3	22.1 ± 1.3^{c}	25.2 ± 1.8^{a}

Different letters in each column denote significant differences between the samples ($p < 0.05$). FRAP, ferric reducing antioxidant power. DPPH$^{\bullet}$, 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity.

3.5. DPPH$^{\bullet}$ scavenging activity

In the DPPH$^{\bullet}$ scavenging activity assay (Table 3), the spreads B and C3 presented the highest values (25-27 mg Trolox equivalents (TE)/100 g), followed

by A, C2, and C1 (18 > 12 > 9 mg TE/100 g, respectively). The high scavenging capacity of B aligns with its superior TPC and HT values.
Interestingly, despite its lower TPC, C3 also exhibited strong DPPH activity, which could be attributed to the presence of other antioxidant compounds such as tocopherols or carotenoids typically found in seed oils like sunflower [14].

4. Conclusion

In conclusion, these results demonstrate that olive pomace extract is an effective natural functional ingredient for enhancing the nutritional and antioxidant profile of vegetable spreads, due to their richness in oleic acid, a fatty acid associated with cardiovascular health protection, and hydroxytyrosol, a potent natural antioxidant that protects against oxidative stress and inflammation.
Furthermore, the spreads developed with olive oil and olive pomace are distinct from those currently available on the market, as their main ingredients are obtained without the use of solvents, and their production contributes to reducing the environmental impact of olive oil production.
In future work, the sensory and textural characteristics of these spreads will be evaluated (e.g., spreadability tests and rheological analyses to determine flow behavior and viscoelastic properties), as such assessments would provide valuable insights into consumer acceptance and industrial applicability. Additionally, evaluating the shelf-life and oxidative stability of the developed formulations using the Rancimat method would further support their potential for large-scale production and commercialization.

Acknowledgments This work received financial support from the PT national funds (FCT/MECI, Fundação para a Ciência e Tecnologia and Ministério da Educação, Ciência e Inovação) through the project UID/50006 - Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos. Diana Melo Ferreira thanks FCT/MCTES for the PhD grant (doi.org/10.54499/2022.13375.BD).

Conflicts of interest / Competing interests: No conflicts of interest.

Ethics approval: No ethical situation.

Availability of data and material: The data is contained within the article.

References

1. Ferreira, D. M.; de Oliveira, N. M.; Chéu, M. H.; Meireles, D.; Lopes, L.; Oliveira, M. B.; Machado, J. Updated organic composition and potential therapeutic properties of different varieties of Olive leaves from *Olea europaea*. *Plants* **2023**, 12 (3), 688; doi.org/10.3390/plants12030688.
2. Olive Oil Market Report. Price Adjustments Continue, Demand Grows Steadily. www.certifiedorigins.com/olive-oil-market-report-june-2025 (accessed 2025-10-23).
3. Olive oil world congress. Almost 90% of olive oil 2024/25 has already been produced in the EU. www.oliveoilworldcongress.com/almost-90-of-olive-oil-2024/25-has-already-been-produced-in-the-eu (accessed 2025-10-23).
4. Di Giovacchino, L.; Preziuso, S. M.; Di Serio, M. G.; Mucciarella, M. R.; Di Loreto, G.; Lanza, B. Double Extraction of Olive Oil in Large Oil Mills of Southern Italy: Effects on Extraction Efficiency, Oil Quality, and Economy of the Process. European *Journal of Lipid Science and Technology* **2017**, 119 (1). doi.org/10.1002/ejlt.201600161.
5. Nunes, M. A.; Palmeira, J. D.; Melo, D.; Machado, S.; Lobo, J. C.; Costa, A. S. G.; Alves, R. C.; Ferreira, H.; Oliveira, M. B. P. P. Chemical composition and antimicrobial activity of a new olive pomace functional ingredient. Pharmaceuticals **2021**, 14 (9), 913; doi.org/10.3390/ph14090913.
6. Ferreira, D. M.; Barreto-Peixoto, J.; Andrade, N.; Machado, S.; Silva, C.; Lobo, J. C.; Nunes, M. A.; Álvarez-Rivera, G.; Ibáñez, E.; Cifuentes, A.; Martel, F.; Oliveira, M. B. P. P.; Alves, R. C. Comprehensive analysis of the phytochemical composition and antitumoral activity of an olive pomace extract obtained by mechanical pressing. *Food Bioscience* **2024,** 61, 104759; doi.org/10.1016/j.fbio.2024.104759.
7. ISO 12966-2:2017 Animal and vegetable fats and oils - Gas chromatography of fatty acid methyl esters Part 2: Preparation of methyl esters of fatty acids (2017).
8. Capannesi, C.; Palchetti, I.; Mascini, M.; Parenti, A. Electrochemical sensor and biosensor for polyphenols detection in olive oils. *Food Chemistry* **2000,** 71 (4), 553–562; doi.org/10.1016/S0308-8146(00)00211-9.
9. Lu, Y.; Zhao, J.; Xin, Q.; Yuan, R.; Miao, Y.; Yang, M.; Cong, W. Protective effects of oleic acid and polyphenols in extra virgin olive oil on cardiovascular diseases. *Food Science and Human Wellness* **2024,** 13(2), 529–540; doi.org/10.26599/FSHW.2022.9250047.
10. Serra, J. J.; Fagoaga, C.; Mura, J.; Sempere-Ferre, F.; Castellano, G. Effectiveness of natural antioxidants on oxidative stability of margarines. LWT **2024,** 214, 116997; doi.org/10.1016/j.lwt.2024.116997.

11. Prasanna, N. S.; Selvakumar, M.; Choudhary, N.; Raghavarao, K. S. M. S. Virgin coconut oil: wet production methods and food applications–a review. *Sustainable Food Technology* **2024,** 2(5), 1391–1408; doi.org/10.1039/D4FB00183J.
12. Lin, Y.; Knol, D.; Valk, I.; van Andel, V.; Friedrichs, S.; Lütjohann, D.; Trautwein, E. A. Thermal stability of plant sterols and formation of their oxidation products in vegetable oils and margarines upon controlled heating. *Chemistry and Physics of Lipids* **2017,** 207, 99–107; doi.org/10.1016/j.chemphyslip.2017.06.002.
13. Ferreira, D. M.; Machado, S.; Espírito Santo, L.; Nunes, M. A.; Costa, A. S.; Álvarez-Ortí, M.; Oliveira, M. B. P. Defatted flaxseed flour as a new ingredient for foodstuffs:
14. Comparative analysis with whole flaxseeds and updated composition of cold-pressed oil. *Nutrients* **2024,** 16(20), 3482; doi.org/10.3390/nu16203482.
15. Pointner, T.; Rauh, K.; Auñon-Lopez, A.; Veličkovska, S. K.; Mitrev, S.; Arsov, E.; Pignitter, M. Comprehensive analysis of oxidative stability and nutritional values of germinated linseed and sunflower seed oil. *Food Chemistry* **2024,** 454, 139790; doi.org/10.1016/j.foodchem.2024.139790.
16. Wang, Z.; Lei, Z.; Zhang, H.; Liu, Z.; Chen, W.; Jia, Y.; Shi, R.; Wang, C. From Biosynthesis to Legislation: A Review of Hydroxytyrosol's Biological Functions and Safety. International *Journal of Molecular Sciences* **2025**, 26(10), 4470; doi.org/10.3390/ijms26104470.