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# Sustainable one-pot solvent-free enzymatic synthesis of capric acid-rich structured lipids to enhance the nutritional value of grape seed oil

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ABSTRACT

Grape seed oil (GSO) is a valuable resource for the pharmaceutical, cosmetic, and food industries due to its fatty acid composition, which provides anti-inflammatory, cardioprotective, antimicrobial, and anticancer properties. GSO is also recognized for its sensory attributes and serves as an essential component in our dietary intake, providing vital fatty acids for metabolic reactions. The enzymatic synthesis of structured lipids (SL) using GSO was investigated in this study. The SL was created by incorporating medium chain fatty acids (capric acid – C10) at the *sn*-1 and *sn*-3 positions and long chain fatty acids in the internal position of the triacylglycerols (TAG). GSO was selected based on its composition rich in unsaturated fatty acids, mainly linoleic acid. The acidolysis reactions were catalyzed by an immobilized commercial lipase Lipozyme TLIM®. The incorporation degree of C10 into GSO reached 36%. Health lipid indices were calculated to determine the C10 incorporation effect on the improvement of nutritional quality of oil. The SL showed lower oxidative stability index (OSI) values than the original oil and higher degree of fatty acid (DFA) values. The simpler, solvent-free method not only enhances the health benefits of oils but also gives valuable properties to GSO, making it an even more attractive option for food industries.

## 1. Introduction

Lipases, also known as triacylglycerol hydrolases, play a crucial role in the food industry due to their exceptional selectivity, efficient catalytic capabilities, and operational durability. These enzymes are highly proficient in hydrolyzing triacylglycerols into fatty acids and glycerol [1], as well as in targeting various ester bonds within triacylglycerols, enabling precise control over the integration and distribution of fatty acids in the glycerol framework. This intrinsic ability is a key mechanism for generating structured lipids (SL) [2,3], which can be further modified to enhance their nutritional, technological, and functional attributes. These improvements may lead to molecules possessing lower energy content, immune regulatory capabilities, enhanced fluidity, increased elasticity, improved melting point, and superior performance at high temperatures [4].

Microbial lipases, exemplified by Lipozyme® TLIM, exhibit *sn-1,3* regioselective triacylglycerol hydrolase activity, preventing acyl group exchange at the *sn-2* position due to steric hindrance. This feature enables precise control over the incorporation of fatty acids at the specific *sn-1* and *sn-3* positions within the glycerol chain of the triacylglycerol structure. This characteristic proves advantageous for the food industry, particularly in the formulation of SL. SL can be efficiently synthesized using sn-1,3-regioselective lipases through either acidolysis or interesterification processes [2]. This results in lipids with an energy content ranging from 5 to 7 kcal/g [5], in contrast to conventional fats and oils, which typically provide 9 kcal/g. Furthermore, SL remains a significant energy reserve with a lower caloric value, attributed to the reduction of long-chain fatty acids (LCFAs) [6]. Furthermore, SL is considered a healthy lipid due to its nutritional characteristics, such as rapid metabolization in the liver, preventing storage in adipose tissue [7]. Additionally, SLs enhance the availability of essential fatty acids in the *sn2* position.

Positioned as an alternative to traditional oils, SLs serve as functional foods with physiological properties aimed at protecting against the development of non-communicable diseases (NCDs) including diabetes, obesity, cancer, and coronary heart disease (CHD) [8]. In the last years,

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researchers have explored unconventional triacylglycerol sources with potential sustainable technological applications, particularly those rich in long-chain polyunsaturated fatty acids that can be obtained using green and biocompatible procedures[9,10]. A notable example is grape (*Vitis vinifera*) seed oil (GSO), distinguished by its high nutritional quality, primarily attributed to its elevated levels of unsaturated fatty acids (UFAs), including linoleic, oleic, and linolenic acids, which collectively constitute 76% of the oil [6,11]. This surpasses the reported content in palm oil (45%) [12]. The presence of essential fatty acids (EFAs) in GSO is nutritionally significant, as individuals actively seek foods rich in EFAs as part of their dietary requirements [13]. Among the EFAs, linoleic acid is predominant in GSO, constituting 48% [6,14], a higher proportion compared to oils such as avocado (10–14%) [15].

Despite its high nutritional quality, GSO can be further enhanced through SL, recognized as a type of oil or lipid characterized as low-calorie lipid, low-calorie triglycerides (TAGs), or dietary lipids [6,11, 16,17]. The synthesis of SL from GSO involves modifying the fatty acid composition within the GSO-TAGs. This process employs a 1,3 regio-selective lipase such as TLIM® and RO to remove LCFAs from the *sn1* and *sn3* GSO-TAGs positions through hydrolysis. Subsequently, S-MCFAs, such as capric acid (C10), can be incorporated into these positions through esterification without the need of solvents [18].

In fact, after the synthesis of SLs, the evaluation of lipid nutritional quality and health impact becomes paramount. This assessment is significantly influenced by Health Lipid Indices [19], providing crucial information for understanding the role of dietary SLs in preventing and treating chronic diseases [20]. Considering the previously mentioned information, the objective of this research is to produce MLM triacylglycerols (SLs) through the enzymatic acidolysis of GSO and C10 using Lipozyme TLIM® in a solvent-free environment within a stirred tank reactor. The proposed method is efficient and sustainable compared to conventional acidolysis methods. Enzymes are used as catalysts, and due to their specificity, it's possible to direct the reaction at specific TAG positions.

The primary objective is to assess whether the incorporation of C10 enhances the quality of GSO. To measure this improvement, several health lipid indices, including the PUFA/SFA ratio, OFA, DFA, NVI, AI, TI, and hHI, are meticulously evaluated.

## 2. Material and methods

### 2.1. Raw material and reagents

GSO (*Vitis vinifera* L.) was obtained through the cold pressing and filtration process by local producers in São Paulo, Brazil. Capric acid (C10) was procured from Merck (Darmstadt, Germany), and the standard for fatty acids methyl esters (FAMEs) (a 37-component FAME Mix) was sourced from Supelco. Lipozyme® TLIM (Novozymes A/S), a lipase derived from *Thermomyces lanuginosus* and immobilized on a non-compressible silica gel carrier, was used in the study. All other chemicals utilized for extraction and analysis were of high purity and analytical grade.

### 2.2. Characterization of raw material

GSO underwent fatty acid composition analysis through gas chromatography, following the AOCS Ce 2–66 method outlined by the American Oil Chemists' Society. Furthermore, acid, and peroxide values were assessed using the AOCS Ca 5a-40 and AOCS Cd 8b-90 methods, respectively [21].

### 2.3. Hydrolytic activity of biocatalyst

The hydrolytic performance of the biocatalyst was evaluated through the titration method using HCl (0.5 M). A single unit of activity (U) was defined as the amount of enzyme capable of liberating 1 μmol of fatty acid per minute under the specified assay conditions. To identify optimal conditions for their subsequent use in SL synthesis, the enzyme underwent characterization across a spectrum of temperatures (ranging from 25 to 90 ºC) and pH values (ranging from 2 to 10). This exploration aimed to pinpoint conditions that result in high relative activity.

### 2.4. Synthesis of structured lipids

The acidolysis process involving GSO and C10 was conducted within a stirred tank reactor of 60.61 mL. The jacketed reactor was connected to a thermostatic bath to maintain a constant system temperature of 45 °C. The reactions were performed at this temperature, with continuous agitation at 400 rpm, a molar ratio of fatty acid to oil set at 3:1, and a biocatalyst load of 5% (w/w) in relation to the total medium mass, sustained for 24 h. This molar ratio corresponds to the stoichiometric value required for the esterification of free fatty acids at *sn-1* and *sn-3* positions by *sn-1,3*-selective lipases. Subsequently, gas chromatography was employed to determine the fatty acid composition and incorporation degree.

### 2.5. Neutralization of triglycerides

After a 24-h reaction period, 3 g of the sample were measured. Next, a solution of 60 mL hexane and 20 mL of a 0.8 M hydroalcoholic KOH solution (30% ethanol) was added to remove the free fatty acids produced during the reaction. The mixture was left to stand for 30 min until biphasic system were visible. The organic phase was subsequently collected and processed using a rotary evaporator to eliminate hexane residues [22].

### 2.6. Methylation of triglycerides

In an Erlenmeyer flask connected to a capacitor, 0.1 g of GSO were precisely measured. To this, 4 mL of 0.5 M methanolic NaOH, preheated to 100 ºC for 5–10 min, were added. Subsequently, 5 mL of methanolic boron trifluoride was introduced for 2 min, followed by the addition of 4 mL of hexane for 1 min. The Erlenmeyer flask was then removed from the heat. After allowing it to stand for 5 min, saturated NaCl was added. The mixture was left until biphasic system were observed, at which point the organic phase was collected. The fatty acid esters obtained underwent fatty acid profile analysis.

### 2.7. Fatty acid profiles and incorporation degree (% ID) in SLs

Fatty acid methyl esters (FAMEs) were analyzed using an Agilent 6890 N gas chromatograph equipped with a flame ionization detector (FID), TR-CN100 capillary column (60 m x 250 μm x 0.20 μm ID), and a split/splitless injector with a Split ratio of 100:1. A 1.0 μL injection volume was used, with an injector temperature of 260 °C. The oven temperature program initiated at 90 °C for 7 min, increased at a rate of 5 °C/min up to 240 °C for 15 min, and the detector temperature was maintained at 300 °C. Helium served as the carrier gas at a flow rate of 1.1 mL/min. Fatty acid identification utilized a standard (37-component FAME Mix, Supelco). Results are expressed as the relative amount of each fatty acid (% of fatty acid/100 g of the sample). Concentrations of total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) were calculated as the sum of their respective families. The percentage of total fat was determined as the sum of SFA, MUFA, and PUFA. The percentage of fatty acid incorporation degree was calculated using Eq. 1 [23]:

$$\% \text{ ID} = (\text{MFA}/\text{MT}) * 100 \quad (1)$$

In which: *MFA* represents the moles of the C10 fatty acid, and *MT* denotes the total moles of fatty acids in the *TAG*.

### 2.8. Calculation of health lipid indices

The fatty acid profile of GSO was utilized to determine various health lipid indices for both unstructured and structured oils, with the aim of determining if the properties would be beneficial in the structured lipids. Short chain fatty acid (SCFA) were included as dietary FA having a desirable effect on hypercholesteremic These indices were calculated according to the following Eqs. 2 - 8:[24,25,26]

$$PUFA/SFA\ ratio = \sum PUFA/\sum SFA \quad (2)$$

$$NVI\ (Nutritive\ Value\ Index) = (C18{:}0 + C18{:}1)/C16{:}0 \quad (3)$$

$$AI\ (Atherogenic\ index) = (C12{:}0 + 4^{*}\ (C14{:}0) + C16{:}0)/\sum UFA \quad (4)$$

$$TI\ (Thrombogenicity\ index) = (C14{:}0 + C16{:}0 + C18{:}0)/\ [(0{,}5\ ^{*}\sum MUFA) + (0{,}5\ ^{*}\sum PUFA\ n\text{-}6) + (3^{*}\sum PUFA\ n\text{-}3) + (\sum PUFA\ n-3/\sum n\text{-}6)] \quad (5)$$

$$DFA\ (Dietary\ FA\ having\ a\ desirable\ effect\ on\ hypercholesteremic) = (\sum MUFA + \sum PUFA + (\sum C6{:}0 + C8{:}0 + C10{:}0) \quad (6)$$

$$OFA = (C14{:}0 + C16{:}0 \quad (7)$$

$$DFA\ (Dietary\ FA\ having\ a\ desirable\ effect\ on\ hypercholesteremic) = (\sum MUFA + \sum PUFA + (\sum C6{:}0 + C8{:}0 + C10{:}0)\ modified\ from\ Janiszewski\ et\ al.,\ 2016 \quad (8)$$

h/HI (Hypocholesterolaemic index): It is the cholesterol/saturated fat index (C18:0 + C20: 1 + C22: 5+ C22: 6+ C18+ C20: 4+ C18: 2+ C20: 5)/ (.C14: 0 + C16: 0) (9)

### 2.9. Statistical evaluation

The results were presented as the mean ± standard deviations. U de Mann-Whitney tests were applied for sample comparisons ($p < 0.05$). Pearson correlation analysis was performed using the corrplot test function from the stats package to investigate the relationship between factors. All analyses were conducted using Grad Pad 9.

## 3. Results and discussion

### 3.1. Evaluation of TLIM biocatalyst

In this study, an immobilized Lipozyme TLIM obtained commercially from *Thermomyces lanuginosus* (TLIM) was evaluated to establish ideal conditions for its subsequent use in SL synthesis. The enzyme underwent characterization across a spectrum of temperatures (ranging from 25 to 90 ºC) and pH values (ranging from 2 to 10). The goal was to pinpoint conditions that result in high relative activity and optimal incorporation of C10 (Fig. 1 and Figure S1 from Supplementary Material).

The temperature, a critical parameter influencing lipase activity, plays a pivotal role in SL biosynthesis. TLIM food-grade lipases were assessed under pre-established reaction conditions outlined in Fig. 1. TLIM® exhibited activity across the temperature range of 25–90 °C. The optimal temperature for enzyme activity was 55 °C (Fig. 1-A), with nearly approximately 100% relative activity.

Conversely, pH stands out as one of the pivotal factors impacting enzyme activity. TLIM® demonstrated moderate activity within the acidic pH range of 2.0–5.0, reaching its peak activity at 6.0 (Fig. 1-B). Following the screening of the lipase for C10 incorporation into GSO, the C10 incorporation degree into GSO was approximately 36% (Table S1 from Supplementary Material).

### 3.2. Fatty acid profile of GSO and SL synthesized

Raw GSO was characterized by gas chromatography before and after

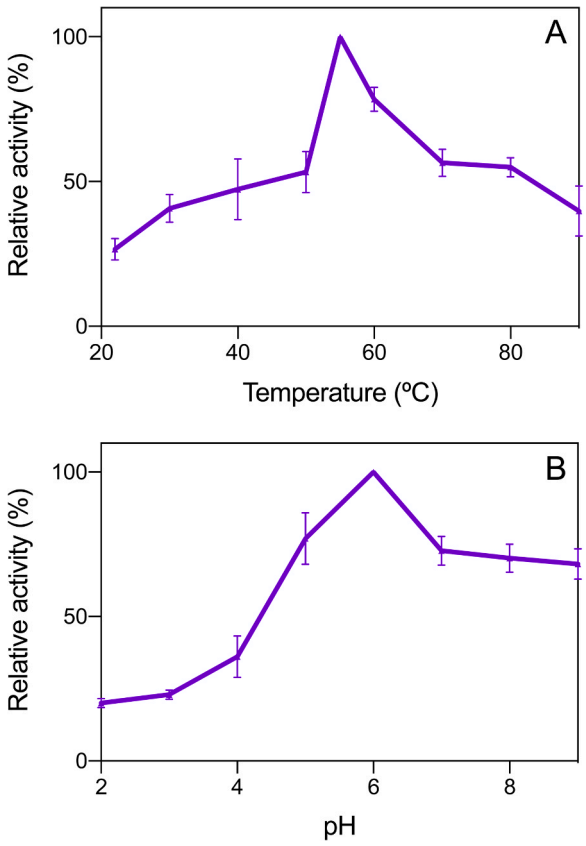


**Fig. 1.** A-B. Evaluation of food-grade lipase for potential SL synthesis: commercial immobilized Lipozyme® TLIM derived from *Thermomyces lanuginosus* (TLIM). A- Enzyme characterization conducted across a temperature range of 25–90 ºC and B- pH values spanning 2–10.

the synthesis of SL, namely GSO-E (grape seed oil enriched with C10) to determine the incorporation degree of C10. The chromatographic profile of GSO is depicted in Figure S1-A from Supplementary Material, while the SL (GSO-E) is shown in Figure S1-B from Supplementary Material. As observed, C10 peak number only appears on chromatogram of GSO-E (Fig. 1-B from Supplementary Material), representing the effective incorporation of C10 on GSO profile. Note that, both chromatograms (Fig. 1 A-B from Supplementary Material) display an ISTD peak corresponding to the internal standard added to the samples used for the quantification method. Fig. 2

As illustrated in Fig. 2, in addition to the effective incorporation of C10 in GSO-E, some significant differences in the fatty acid profiles

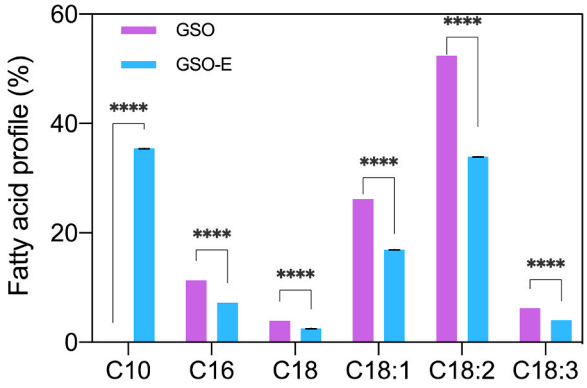


**Fig. 2.** Fatty acid composition profiles of GSO and GSO enriched with C10 (GSO-E) following 24 h of acidolysis catalyzed by lipase TLIM at pH 6.0 and 55 °C.

between raw GSO and GSO-E are observed. As an example, there is a nearly 20% decrease in the content of C18:1 and a 10% decrease in the content of C18:2. The decline in C18:1 and C18:2 levels in GSO-E, as opposed to raw GSO after the acidolysis process, can be attributed to the transformation of these fatty acids during the reaction. Acidolysis entails the interchange of fatty acids between triglycerides and free fatty acids, leading to the formation of new triglycerides. This process may lead to a decrease in specific fatty acids, such as C18:1 and C18:2, as they may participate in the formation of new triglycerides enriched with C10. A detailed fatty acid quantification was performed based on their representation in the samples. In GSO, linoleic fatty acid (C18:2) (47.6 ± 0.16) was the most abundant, followed by oleic acid (C18:1) (23.37 ± 0.07), palmitic acid (C16) (10.23 ± 0.02), linolenic fatty acid (C18:3) (5.68 ± 0.02), and stearic fatty acid (C18) (3.47 ± 0.01), in accordance with various studies [6,27]. In GSO-E, C10 was the most representative, as expected due to its incorporation degree in triacylglycerols (35.54 ± 0.02). The relative proportions of other fatty acids were maintained, although there were observed losses in the quantity of each fatty acid, approximately 30%, as anticipated with the increased value of C10. Similar findings have been reported in SL after capric acid incorporation in other studies [11,28]. The representativeness of fatty acids remained in the same order: C18:2 (30.82 ± 0.02), C18:1 (15.09 ± 0.01), C16 (6.54 ± 0.01), C18:3 (3.66 ± 0.01), and C18:0 (2.22 ± 0.01) (Table S1 from Supplementary Material).

To gain a comprehensive understanding of the fatty acid compositions and their distribution among SFA, MUFA, and PUFA, the samples were analyzed to identify the fatty acid profiles (Fig. 3).

As depicted in Fig. 3, in GSO, fatty acids are distributed approximately as follows: 19% SFA, 23% MUFA, and 55% PUFA, while in GSO-E, 45% SFA, 19% MUFA, and 37% PUFA. In GSO-E, three fatty acids are no longer present due to the process of C10 incorporation, estimated at 37% through the calculation of the incorporation degree described above. This value aligns with our previous work [4] and is slightly higher than those reported in some studies [29–32].

As previous mentioned, fatty acids are classified into SFA, MUFA, and PUFA. While SFA, particularly long-chain fatty acids (LCFAs), have been traditionally considered detrimental to health, recent studies highlight the harmful effects of long-chain saturated fatty acids (LCSFAs) [33]. To mitigate the negative impact, the incorporation of short-medium chain fatty acids (S-MFAs) in triacylglycerols (TAGs) is explored to enhance the beneficial effects of oil. In this work, SL were successfully synthesized with an estimated incorporation degree of 35.54%, a value consistent with the findings of Martínez-Galán et al. [11] and higher than those previous reported [30,31,34]. SLs facilitate the reduction of LCFAs, contributing to improved health indices.

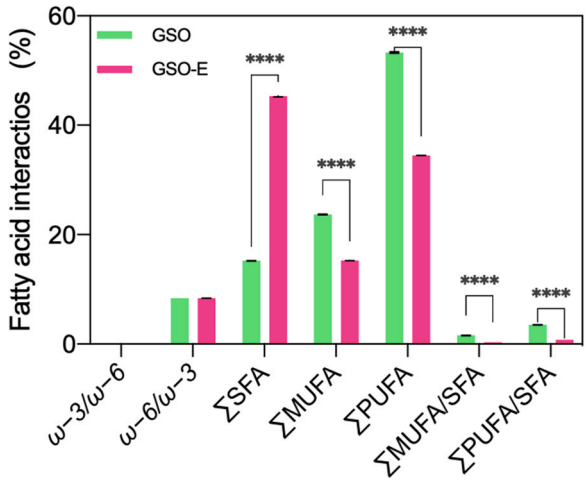


**Fig. 3.** Composition of fatty acids, including saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA), in both GSO and GSO-E.

S-MFAs, identified as low-calorie TAGs, mainly attributed to the fact that the enzymatic synthesis allows combining hydrolysis and esterification processes releasing LCFAs of interest and incorporating S-MFAs of interest in specific positions of the TAG making it more efficient compared to conventional acidolysis method, large-scale enzyme technologies contribute to sustainable development by enabling the cleaner and more sustainable synthesis of high-quality TAGs [35,36].

play a crucial role in mitigating metabolic disorders associated with fat absorption, obesity, and diabetes [11]. The SFA content was different in GSO and GSO-E. However, the composition of individual fatty acids differed, with reductions in C14:0, C16:0, and C18:0 in GSO-E (Table S1 from Supplementary Material), which are associated with the development of coronary heart diseases (CHD) [37]. In fact, fatty acids play a pivotal role as promoters or protectors in CHD development, with LCSFA linked to cholesterol elevation and arterial obstruction, while unsaturated fatty acids offer protective effects [20].

Both GSO and GSO-E are rich in C18:2 (omega-6), an essential fatty acid known for various benefits, including reducing liver fat and improving metabolism by decreasing inflammation associated with an SFA-rich diet. Additionally, C18:2 is associated with protective effects against cardiovascular diseases (CVD), diabetes type two, and immune system function [38]. C18:1 (omega-9), another representative MUFA in both samples, is known for its protective effects against CVD, reducing blood pressure, modulating inflammation, and aiding in lowering LDL cholesterol [39]. Furthermore, both samples contain C18:3 (omega-3), an essential fatty acid known for protective effects against CVD, reducing LDL cholesterol, obesity, diabetes type two, and supporting the immune system [38] and metabolic syndrome [40].

Ratios calculated from the fatty acid profile, as described in Fig. 3 and Table S2 from Supplementary Material, include the PUFA/SFA ratio, a significant indicator of protective or promotive effects on coronary diseases (CD). While a minimum recommended value for the PUFA/SFA ratio is 0.45 [41], both GSO and GSO-E surpassed this threshold. Notably, the GSO-E value was lower than that of GSO (0.82 ± 0.00 and 3.50 ± 0.01, respectively), in alignment with findings by Sánchez-Osorno et al. [42] and higher than those reported by Farajzadeh Alan et al. [43].

### 3.3. Health lipid indices

Health lipid indices play a crucial role in elucidating the desired or undesired effects of oils on health. While the n-6/n-3 ratio was initially a key parameter, various options are now available, with the atherogenicity index (AI) being one of the most widely used. In this work, the comprehensive health lipid indices were calculated for GSO and GSO-E (Fig. 4).

As depicted in Fig. 4-A, B, C, D, the NVI, AI, TI, and hHI of GSO and GSO-E values indicate no significant differences between both samples. This consistency is attributed to the maintenance of proportions of MUFAs and PUFAs after the incorporation of C10. TI is associated with a clotting tendency [44], with stearic acid considered to increase this tendency [45]. Values for GSO and GSO-E were very similar (0.26 ± 0.00; 0.26 ± 0.00, respectively), (Fig. 4-D) approaching zero and resembling values calculated in a study using camellia oil (TI: 0.1) [46]. These values are lower than those reported for sesame, olive, corn, rice, argan, palm, peanut, and pistachio oils (0.37, 0.39, 0.53, 0.47, 0.45, 1.88, 0.35 and 0.35, respectively) [47], indicating potential protective functions against coronary heart diseases (CHD) as previous described [48,49].

AI indicates the relationship between LCSFAs and UFAs in terms of inducing atherogenicity [44]. LCSFAs are deemed pro-atherogenic, promoting the adherence of lipids to immune and circulatory system cells [25]. AI values (Fig. 4-C) were close to zero and very similar (GSO: 0.13 ± 0.00; GSO-E: 0.14 ± 0.00), as reported in some oils such as soybean, sesame, olive, walnut, peanut, and pistachio (0.11, 0.11, 0.16, 0.10, 0.14 and 0.15, respectively), lower than corn, rice, argan, avocado,

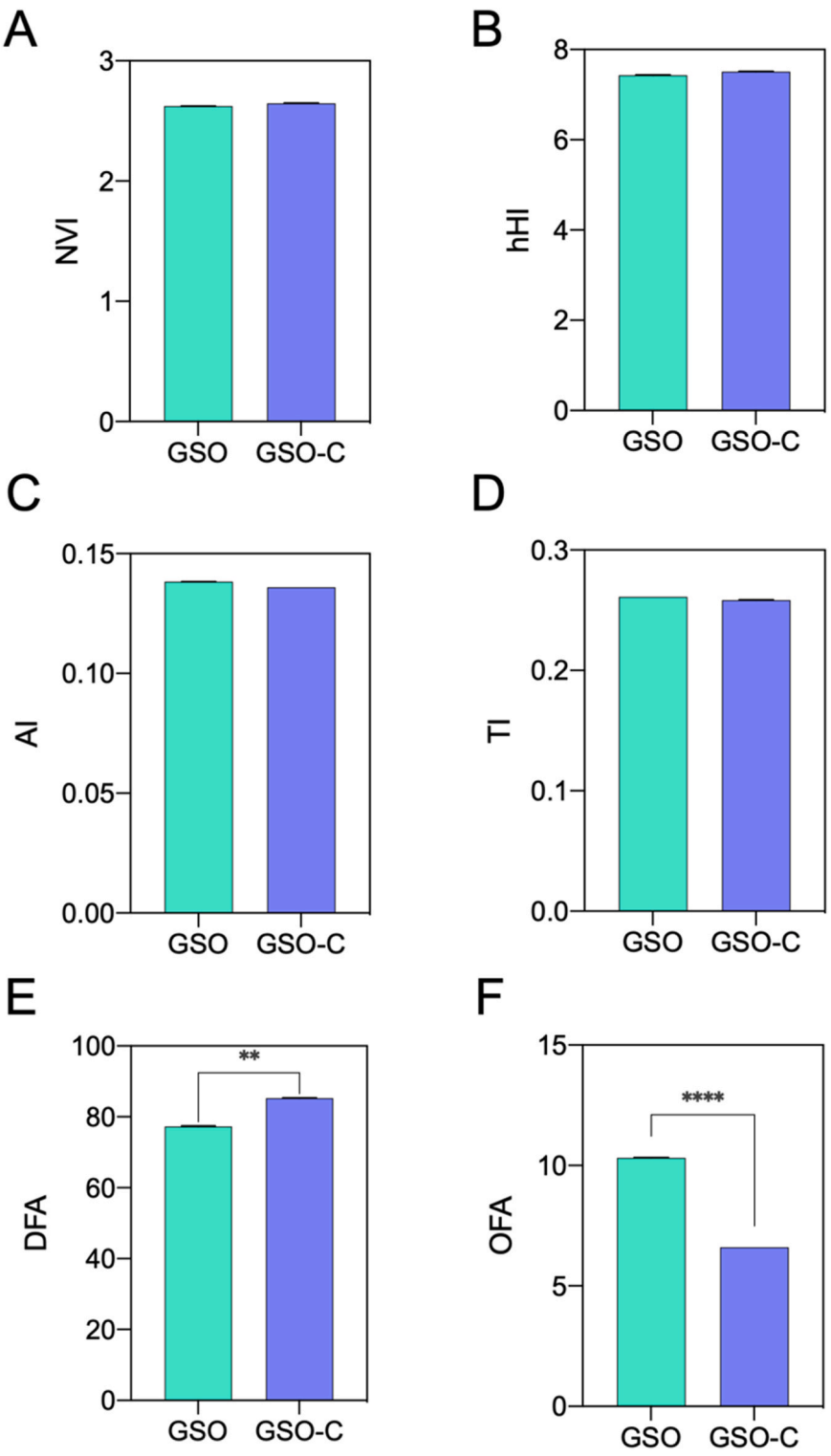


**Fig. 4.** Health lipids indices of GSO and GSO-E, * means significative differences ($p<0.05$).

palm oils (0.27, 0.27, 0.45, 0.41 and 1.88, respectively) [50], and reported in conventional oils [51], suggesting a protective effect against atherogenicity. Furthermore, myristic and palmitic fatty acids have been reported to promote AI [45,49]. Due to C10 incorporation, both fatty acids tend to decrease in percentage of incorporation degree, aiding in the reduction of AI index, confirming that, in all forms of oil, there is a potential function as a protector against CHD diseases.

The values of NVI (Fig. 4-A) were closely aligned (GSO: 2.62 ± 0.00; GSO-E: 2.65 ± 0.00), underscoring the significance of this observation. This consistency indicates that the nutritional value remains not only constant but potentially increases before and after C10 incorporation. The hHI index is focused on cholesterol metabolism [52]. It is supposed that a larger value indicates a more desirable effect. Values for GSO and GSO were similar (7.43 ± 0.01; 7.51 ± 0.01, respectively) (Fig. 4-B), both slightly lower than those reported in cold-preserved camellia oil [46], which may be attributed to the preservation method of the oil and

the different FA profiles. However, these values surpassed those documented in palm, soybean, and olive oils (1.06, 5.63, and 4.53, respectively [53], corroborating the positive effect of both oils on health.

OFA and DFA are indices that explain the types of fatty acids as having a desirable or undesirable effect on hypercholesterolemia. The OFA index, representing an undesired effect, is significantly lower in GSO-E than GSO (6.60± 0.00; 10.31 ± 0.03) (Fig. 4-F), suggesting that a 36% incorporation degree of S-MCFAs in the GSO can be positive for improving the nutritional value of lipids. DFA values (Fig. 4-E) were above 75% in both lipids, proving that nutritional quality is maintained after incorporation degree and even improved. The DFA value was higher in GSO-E than GSO (82.29 ± 0.10; 77.25 ± 0.26), as expected due to the incorporation of C10, indicating beneficial effects in cooperation with UFAs, which is good for the lipid quality.

Health lipid indices are equally important to describe desired or undesired effects on health. Correlation analyses between all evaluated indices indicate that C10 incorporation in GSO has some effect on all indices, more prominently on OFA and DFA with a very strong correlation, describing expected effects due to the remarkable reduction of C14:0 and C16:0 fatty acids and increase in C10 (Fig. 5).

Incorporation of C10 demonstrated a positive effect on improving the quality of GSO[54], particularly evident in the OFA and DFA indices. To explore the relationship between the degree of C10 incorporation and all indices (OFA, DFA, NVI, AI, TI, and hHI), a Pearson correlation test was conducted (Fig. 5). The results confirmed a very strong positive correlation with DFA and a very strong negative correlation with OFA (values 1.0 and −1.0, respectively). AI and hHI showed a positive medium correlation with C10 (both values of 0.36), while AI and TI exhibited a negative medium correlation with C10 (values −0.34 and −0.39, respectively). The overall behavior of all index values suggests that C10 positively impacts the oil quality by increasing desired effects and reducing undesired effects.

DFA and OFA were found to be inversely proportional (value of −1), with DFA displaying a medium positive correlation with NVI and hHI (values 0.35 and 0.34, respectively) and a medium negative correlation with AI and TI (values −0.33 and −0.38, respectively). OFA indicated a medium negative correlation with NVI and hHI (values −0.36 and −0.35, respectively) and a positive correlation with AI and TI (values 0.34 and 0.39, respectively). Furthermore, NVI demonstrated a very strong negative correlation with AI and TI (values −0.99 and −1, respectively) and a very strong positive correlation with hHI (values 1). Similarly, hHI exhibited a very strong negative correlation with AI and TI (values −0.99 and −1, respectively).

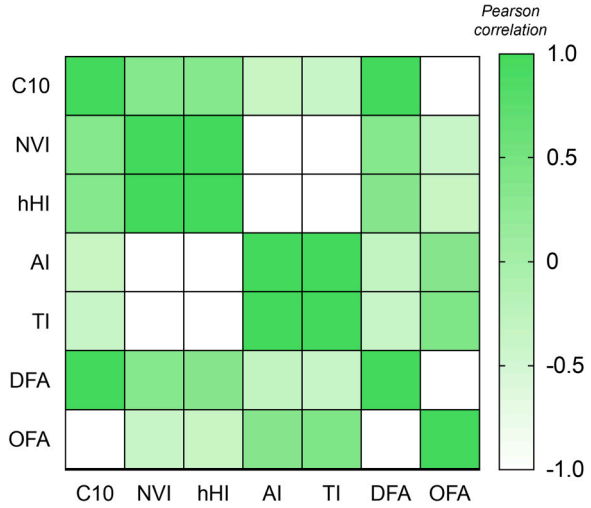


**Fig. 5.** Correlation coefficients between the incorporation of C10 and the values of various health lipid indices (NVI, hHI, AI, TI, DFA, and OFA,). Each cell represents the correlation between two variables. The color-coded scale reflects the values based on Pearson correlation statistics. Values between (0−1) indicate a positive correlation, while values between (0−1) indicate a negative.

Correlation analyses among all indices (Fig. 5) reveal that C10 incorporation has a notable impact on various indices, with a stronger association observed in OFA and DFA, as indicated by a very strong correlation. This effect aligns with expectations, given the significant reduction in C14:0 and C16:0 fatty acids and increase in C10 observed in SLs synthesis studies [6,11]. Desirable effects, represented by NVI, DFA, and hHI indices, exhibit a medium positive correlation among them, reflecting their protective nature. Conversely, these indices show a medium positive correlation with undesirable effects indices (OFA, AI, and TI), as reported by Szabo et al. [53]. This association suggests a positive link with cardiovascular disease, emphasizing that oils with lower values of these indices could be an alternative for mitigating CVD. NVI demonstrates a particularly strong positive correlation with hHI, emphasizing specific desirable effects on cardiovascular health, and a strong negative correlation with AI and TI, indicating a reduction in blood cholesterol. Oils with lower IA and IT values boast better nutritional quality, potentially reducing the risk of coronary heart disease (CHD). While recommended values for IA and IT are not provided, aiming for lower values is preferable, considering the influence of FA composition on oils.

AI and TI display a very strong negative correlation with hHI. Szabo et al. [53] evaluated different types of oils before and after heating, noting that AI and TI values increased while hHI values decreased. This underscores the impact of external factors, such as temperature, on diminishing oil quality and is directly linked to the observed negative strong correlation between AI and TI indices in the present study, as well as the strong positive correlation between AI and TI.

Remarkably, to our knowledge, previous research has not detailed the effects of SL synthesis on indicators like OFA, DFA, IA, TI, and hHI ratios. These indices, crucial in the context of cardiovascular health (CHC) diseases, are commonly investigated in fried foods and certain traditional oils. In contrast to earlier studies concentrating on one or two indices, our investigation comprehensively assessed seven nutritional indices, providing a more holistic insight into their impact on CHC diseases.

## 4. Conclusion

The incorporation of C10 into GSO through GSO-E synthesis has yielded positive effects on various health lipid indices, particularly those associated with cardiovascular health. The GSO-E was successfully synthesized with an estimated 36% incorporation degree of C10 into GSO using a solvent-free bioprocess. The fatty acid composition profiles highlighted significant changes in the GSO-E compared to the original GSO, including a decrease in the content of certain fatty acids (e.g., C18:1 and C18:2), attributed to the acidolysis process. Health lipid indices, including NVI, AI, TI, hHI, DFA, and OFA, were calculated and compared between GSO and SL. The GSO-E exhibited positive effects, maintaining, or even enhancing the nutritional value, as indicated by consistent NVI values. Moreover, GSO-E showed improvements in desired effects (DFA) and reductions in undesired effects (OFA), emphasizing potential health benefits. Correlation analyses further supported the positive impact of C10 incorporation, revealing strong associations between C10 incorporation degree and indices such as DFA and hHI. The GSO-E demonstrated a favorable influence on cardiovascular health, as evidenced by the medium to strong correlations between indices.

## CRediT authorship contribution statement

**Cassamo Ussemane Mussagy:** Writing – review & editing, Validation, Supervision, Formal analysis, Data curation, Conceptualization. **Rodrigo Valenzuela:** Writing – review & editing, Supervision, Data

curation, Conceptualization. **Julian Paul Martinez:** Resources, Project administration, Data curation, Conceptualization. **Marta Mediavilla:** Writing – review & editing, Supervision, Investigation, Data curation. **Angie Vanessa Caicedo:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Camila Farías:** Writing – original draft, Investigation, Data curation, Conceptualization.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.procbio.2024.05.020.

## References

[1] A.L. Reyes-Reyes, F. Valero Barranco, G. Sandoval, Recent advances in lipases and their applications in the food and nutraceutical industry, Page 960, Catalysts 2022 Vol. 12 (12) (2022) 960, https://doi.org/10.3390/CATAL12090960.
[2] S. Ferreira-Dias, N. Osório, C. Tecelão, Bioprocess technologies for production of structured lipids as nutraceuticals, BV (2022), https://doi.org/10.1016/b978-0-12-823506-5.00007-2.
[3] T. Simões, J. Ferreira, M.F.L. Lemos, A. Augusto, R. Félix, S.F.J. Silva, S. Ferreira-Dias, C. Tecelão, Argan oil as a rich source of linoleic fatty acid for dietetic structured lipids production, Life 11 (2021), https://doi.org/10.3390/LIFE11111114.
[4] A.V. Caicedo-Paz, C. Ussemane Mussagy, V. Mesa, R.H. Miotti Junior, R. Valenzuela, A. Veloso de Paula, J.P. Martinez-Galan, Enzymatic synthesis of structured lipids from sacha inchi (Plukenetia volubilis) oil with capric acid via acidolysis reaction in stirred tank and packed bed mini reactors, Food Biosci. 58 (2024) 103769, https://doi.org/10.1016/J.FBIO.2024.103769.
[5] N. Bassan, R. Hatanaka, R. Monti, C. Tecelão, S. Ferreira-dias, A.V. Paula, LWT - Food Science and Technology Enzymatic modi fi cation of grapeseed ( Vitis vinifera L.) oil aiming to obtain dietary triacylglycerols in a batch reactor, LWT Food Sci. Technol. (2018) 1–7, https://doi.org/10.1016/j.lwt.2018.05.013.
[6] N. Bassan, R.H. Rodrigues, R. Monti, C. Tecelão, S. Ferreira-Dias, A.V. Paula, Enzymatic modification of grapeseed (Vitis vinifera L.) oil aiming to obtain dietary triacylglycerols in a batch reactor, Lwt 99 (2019) 600–606, https://doi.org/10.1016/j.lwt.2018.05.013.
[7] A. Valenzuela B, J. Sanhueza C, Structured lipids and fat substitutes, the future lipids? Rev. Chil. De. Nutr. 35 (2008) 394–405, https://doi.org/10.4067/S0717-75182008000500001.
[8] N. Di Daniele, The role of preventive nutrition in chronic non-communicable diseases, Page 1074, Nutrients 2019 Vol. 11 (11) (2019) 1074, https://doi.org/10.3390/NU11051074.
[9] J.A.;Lee, K.T.E. ac. kr Shin, Lipase-Catalyzed Synthesis of Structured Lipids with Capric and Conjugated Linoleic Acid in a Stirred-Batch Type Reactor, Journal of The Korean Society of Food Science and Nutrition 33 (n.d.).
[10] N.S.;Lee, K.T.E. ac. kr Kim, Enzymatic Synthesis of Structured Lipids Containing Conjugated Linoleic Acid from Extracted Corn and Peanut Oil, Journal of The Korean Society of Food Science and Nutrition 33 (n.d.).
[11] J.P. Martínez-Galán, C.M. Ontibón-Echeverri, M. Campos Costa, A. Batista-Duharte, V. Guerso Batista, V. Mesa, R. Monti, A. Veloso de Paula, A. Martins Baviera, Enzymatic synthesis of capric acid-rich structured lipids and their effects on mice with high-fat diet-induced obesity, Food Res. Int. 148 (2021), https://doi.org/10.1016/j.foodres.2021.110602.
[12] M.P. St-Onge, A. Travers, Fatty acids in corn oil: role in heart disease prevention, Handb. Lipids Hum. Funct. Fat. Acids (2016) 131–140, https://doi.org/10.1016/B978-1-63067-036-8.00006-8.
[13] I. Djuricic, P.C. Calder, Beneficial outcomes of omega-6 and omega-3 polyunsaturated fatty acids on human health: an update for 2021, Page 2421, Nutrients 2021 Vol. 13 (13) (2021) 2421, https://doi.org/10.3390/NU13072421.
[14] J.C. Bassan, T.M. de, S. Bezerra, G. Peixoto, C.Z.P. Da Cruz, J.P.M. Galán, A.B. dos, S. Vaz, S.S. Garrido, M. Filice, R. Monti, Addendum: immobilization of trypsin in lignocellulosic waste material to produce peptides with bioactive potential from whey protein, Materials 9 (2016), [Materials 9 (357) (2016) 5, https://doi.org/10.3390/ma9080705.
[15] M. Flores, C. Saravia, C.E. Vergara, F. Avila, H. Valdés, J. Ortiz-Viedma, Avocado oil: characteristics, properties, and applications, Page 2172 24, Molecules 2019 Vol. 24 (2019) 2172, https://doi.org/10.3390/MOLECULES24112172.
[16] S. Sivakanthan, A.P. Jayasooriya, T. Madhujith, Optimization of the production of structured lipid by enzymatic interesterification from coconut (Cocos nucifera) oil and sesame (Sesamum indicum) oil using Response Surface Methodology, LWT 101 (2019) 723–730, https://doi.org/10.1016/J.LWT.2018.11.085.
[17] D. Moreria, P. Santos, A. Gambero, G. Macedo, Evaluation of structured lipids with behenic acid in the prevention of obesity, Food Res Int (2017).
[18] Q.D. Utama, A.B. Sitanggang, D.R. Adawiyah, P. Hariyadi, Lipase-catalyzed synthesis of medium-long-medium-type of structured lipids from refined bleached deodorized olein, Appl. Food Biotechnol. 7 (2020) 85–94, https://doi.org/10.22037/AFB.V7I2.26807.
[19] J. Wołoszyn, G. Haraf, A. Okruszek, M. Wereńska, Z. Goluch, M. Teleszko, Fatty acid profiles and health lipid indices in the breast muscles of local Polish goose varieties, Poult. Sci. 99 (2020) 1216–1224, https://doi.org/10.1016/j.psj.2019.10.026.
[20] J. Chen, H. Liu, Molecular Sciences Review Nutritional Indices for Assessing Fatty Acids: A Mini-Review, (n.d.). https://doi.org/10.3390/ijms21165695.
[21] AOCS, Official methods and recommended practices, in: American Oil Chemists' Society, 6th ed, 2011.
[22] X. Wang, R. Xu, R. Wang, A. Liu, Transcriptome analysis of Sacha Inchi (Plukenetia volubilis L.) seeds at two developmental stages, BMC Genom. 13 (1) (2012), https://doi.org/10.1186/1471-2164-13-716.
[23] L. Casas-godoy, A. Marty, G. Sandoval, S. Ferreira-dias, Optimization of medium chain length fatty acid incorporation into olive oil catalyzed by immobilized Lip2 from Yarrowia lipolytica, Biochem Eng. J. 77 (2013) 20–27, https://doi.org/10.1016/j.bej.2013.05.001.
[24] HMSO, Nutritional aspects of cardiovascular disease, Rep. Health Soc. subjets no 46 (1994).
[25] T.L.V. Ulbricht, D.A.T. Southgate, Review article - coronary heart disease: seven dietary factors, Lancet 338 (1991) 985–992.
[26] P. Janiszewski, E. Grześkowiak, D. Lisiak, B. Borys, K. Borzuta, E. Pospiech, E. Poławska, The influence of thermal processing on the fatty acid profile of pork and lamb meat fed diet with increased levels of unsaturated fatty acids, Meat Sci. 111 (2016) 161–167, https://doi.org/10.1016/j.meatsci.2015.09.006.
[27] G. Arslan, A.Y. Sönmez, T. Yank, Effects of grape Vitis vinifera seed oil supplementation on growth, survival, fatty acid profiles, antioxidant contents and blood parameters in rainbow trout Oncorhynchus mykiss, Aquac. Res 49 (2018) 2256–2266, https://doi.org/10.1111/ARE.13686.
[28] D.G. de Oliveira, V.T. Mazziero, B.W. Picão, R.M. de, Magalhães Gomes Pontes Ribeiro, M.N. Esperança, A.V. de Paula, M.O. Cerri, Effect of geometrical modifications on the mixing and enzymatic conversion of structured lipids in internal-loop airlift bioreactors, Biochem Eng. J. (2024) 109316, https://doi.org/10.1016/J.BEJ.2024.109316.
[29] S.M. Abed, X. Zou, A.H. Ali, Q. Jin, X. Wang, Synthesis of 1,3-dioleoyl-2-arachidonoylglycerol-rich structured lipids by lipase-catalyzed acidolysis of microbial oil from Mortierella alpina, Bioresour. Technol. 243 (2017) 448–456, https://doi.org/10.1016/j.biortech.2017.06.090.
[30] M.M. Zheng, T. Sun, Z. Dong, J. Wang, F.H. Huang, Ultrasound-assisted interfacial immobilization of lipase on hollow mesoporous silica spheres in a pickering emulsion system: a hyperactive and sustainable biocatalyst, ACS Sustain Chem. Eng. 8 (2020) 17280–17290, https://doi.org/10.1021/ACSSUSCHEMENG.0C06271.
[31] S.A. Willett, C.C. Akoh, Application of taguchi method in the enzymatic modification of menhaden oil to incorporate capric acid, J. Am. Oil Chem. Soc. 95 (2018) 299–311, https://doi.org/10.1002/AOCS.12043.
[32] S. Silroy, A. Sengupta, D.K. Bhattacharyya, M. Ghosh, Optimization of reaction parameters of acidolysis reaction between mustard oil and capric acid by using Thermomyces lanuginosus lipase, J. Food Sci. Technol. 51 (2014) 715–721, https://doi.org/10.1007/S13197-011-0543-5/TABLES/4.
[33] A.L. Unger, M. Torres-Gonzalez, J. Kraft, Dairy fat consumption and the risk of metabolic syndrome: an examination of the saturated fatty acids in dairy, Page 2200, Nutrients 2019 Vol. 11 (11) (2019) 2200, https://doi.org/10.3390/NU11092200.
[34] S.M. Abed, W. Wei, A.H. Ali, S.A. Korma, A.H. Mousa, H.M. Hassan, Q. Jin, X. Wang, Synthesis of structured lipids enriched with medium-chain fatty acids via solvent-free acidolysis of microbial oil catalyzed by Rhizomucor miehei lipase, LWT 93 (2018) 306–315, https://doi.org/10.1016/J.LWT.2018.03.057.
[35] A. Mustafa, S. Fathy, O. Kutlu, F. Niikura, A. Inayat, M. Mustafa, T.M. M. Abdellatief, A. Bokhari, O.D. Samuel, C. Pastore, L. di Bitonto, M.A. Tawfik, M. Munir, R. Mohsen, Cleaner and sustainable synthesis of high-quality monoglycerides by use of enzyme technologies: techno-economic and environmental study for monolaurin, Clean. Technol. Environ. Policy 25 (2023) 3263–3283, https://doi.org/10.1007/S10098-023-02577-1/TABLES/11.
[36] A. Mustafa, S. Faisal, I.A. Ahmed, M. Munir, E.P. Cipolatti, E.A. Manoel, C. Pastore, L. di Bitonto, D. Hanelt, F.O. Nitbani, Z.M. El-Bahy, A. Inayat, T.M.M. Abdellatief, K. Tonova, A. Bokhari, A. Abomohra, Has the time finally come for green

oleochemicals and biodiesel production using large-scale enzyme technologies? Current status and new developments, Biotechnol. Adv. 69 (2023) 108275, https://doi.org/10.1016/J.BIOTECHADV.2023.108275.
[37] C. Papandreou, A. Sala-Vila, S. Galié, J. Muralidharan, R. Estruch, M. Fitó, C. Razquin, D. Corella, E. Ros, J. Timiraos, J. Lapetra, L. Serra-Majem, S. Carlos, O. Castañer, E.M. Asensio, J. Salas-Salvadó, M. Bulló, Association between fatty acids of blood cell membranes and incidence of coronary heart disease, Arterioscler. Thromb. Vasc. Biol. 39 (2019) 819–825, https://doi.org/10.1161/ATVBAHA.118.312073.
[38] S. Gutiérrez, S.L. Svahn, M.E. Johansson, Effects of omega-3 fatty acids on immune cells, Page 5028, Int. J. Mol. Sci. 2019 Vol. 20 (20) (2019) 5028, https://doi.org/10.3390/IJMS20205028.
[39] M.A. Farag, M.Z. Gad, Omega-9 fatty acids: potential roles in inflammation and cancer management, J. Genet. Eng. Biotechnol. 20 (2022) 1–11, https://doi.org/10.1186/S43141-022-00329-0/FIGURES/5.
[40] T. Yary, S. Voutilainen, T.P. Tuomainen, A. Ruusunen, T. Nurmi, J.K. Virtanen, Omega-6 polyunsaturated fatty acids, serum zinc, delta-5- and delta-6-desaturase activities and incident metabolic syndrome, J. Hum. Nutr. Diet. 30 (2017) 506–514, https://doi.org/10.1111/JHN.12437.
[41] J. Wołoszyn, G. Haraf, A. Okruszek, M. Wereńska, Z. Goluch, M. Teleszko, Fatty acid profiles and health lipid indices in the breast muscles of local Polish goose varieties, Poult. Sci. 99 (2020) 1216, https://doi.org/10.1016/J.PSJ.2019.10.026.
[42] D.M. Sánchez-Osorno, A.V. Caicedo Paz, M.C. López-Jaramillo, A.L. Villa, J. P. Martínez-Galán, Protection of mono and polyunsaturated fatty acids from grapeseed oil by spray drying using green biopolymers as wall material, Foods 11 (2022), https://doi.org/10.3390/foods11243954.
[43] D. Farajzadeh Alan, M.H. Naeli, M. Naderi, S.M. Jafari, H.R. Tavakoli, Production of Trans-free fats by chemical interesterified blends of palm stearin and sunflower oil, Food Sci. Nutr. 7 (2019) 3722–3730, https://doi.org/10.1002/FSN3.1231.
[44] L. Senso, M.D. Suárez, T. Ruiz-Cara, M. García-Gallego, On the possible effects of harvesting season and chilled storage on the fatty acid profile of the fillet of farmed gilthead sea bream (Sparus aurata), Food Chem. 101 (2007) 298–307, https://doi.org/10.1016/J.FOODCHEM.2006.01.036.
[45] M. Wereńska, G. Haraf, J. Wołoszyn, Z. Goluch, A. Okruszek, M. Teleszko, Fatty acid profile and health lipid indicies of goose meat in relation to various types of heat treatment, Poult. Sci. 100 (2021), https://doi.org/10.1016/j.psj.2021.101237.
[46] K. Ratusz, E. Symoniuk, M. Wroniak, M. Rudzińska, Bioactive compounds, nutritional quality and oxidative stability of cold-pressed camelina (Camelina sativa L.) oils, Appl. Sci. 8 (2018), https://doi.org/10.3390/app8122606.
[47] S.K. Tilami, L. Kouřimská, Assessment of the nutritional quality of plant lipids using atherogenicity and thrombogenicity indices, Nutrients (2022) 3795, https://doi.org/10.3390/NU14183795.
[48] L. Hooper, C.D. Summerbell, R. Thompson, D. Sills, F.G. Roberts, H.J. Moore, G. D. Smith, Reduced or modified dietary fat for preventing cardiovascular disease, Sao Paulo Med. J. 134 (2016) 182, https://doi.org/10.1590/1516-3180.20161342T1.
[49] G. Manuela A, V.-A. Robert, N. Yoannis, L. Elvira, R. Rossana, M. Roberta, D. Giacomo, G. Daniele, Fatty Acids Profile, Atherogenic (IA) and Thrombogenic (IT) Health Lipid Indices, of Raw Roe of Blue Fin Tuna (*Thunnus thynnus* L.) and Their Salted Product "Bottarga,", Food Nutr. Sci. 2011 (2011) 736–743, https://doi.org/10.4236/FNS.2011.27101.
[50] S.K. Tilami, L. Kouřimská, Assessment of the nutritional quality of plant lipids using atherogenicity and thrombogenicity indices, Nutrients (2022) 3795, https://doi.org/10.3390/NU14183795.
[51] S. Filip, J. Hribar, R. Vidrih, Influence of natural antioxidants on the formation of trans-fatty-acid isomers during heat treatment of sunflower oil 113 (2011) 224–230, https://doi.org/10.1002/ejlt.200900231.
[52] J. Santos-Silva, R.J.B. Bessa, F. Santos-Silva, Effect of genotype, feeding system and slaughter weight on the quality of light lambs: II. Fatty acid composition of meat, Livest. Prod. Sci. 77 (2002) 187–194, https://doi.org/10.1016/S0301-6226(02)00059-3.
[53] Z. Szabo, T. Marosvölgyi, E. Szabo, V. Koczka, Z. Verzar, M. Figler, T. Decsi, Effects of repeated heating on fatty acid composition of plant-based cooking oils, Foods 11 (2022) 192, https://doi.org/10.3390/FOODS11020192/S1.
[54] R.H. Miotti, S.R. do Amaral, A.N. Freitas, H.B.S. Bento, A.K.F. de Carvalho, F. L. Primo, A.V. de Paula, Enzymatic production process of capric acid-rich structured lipids: development of formulation as a new therapeutic approach, Int J. Biol. Macromol. 257 (2024) 128641, https://doi.org/10.1016/J.IJBIOMAC.2023.128641.