Quality Evaluation of Plant Oil Blends Interesterified by Using Immobilized *Rhizomucor miehei* Lipase

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Abstract: The aim of this research was to evaluate the quality and oxidative stability of enzymatically interesterified plant oil blends. The model plant oil blends consisted of tomato seed oil and coconut oil, which were applied to enzymatic interesterification in the presence of a microbial lipase. To obtain quality characteristics of the enzymatically interesterified oil blends, the following analyses were performed: fatty acids composition and their distribution in internal position (sn-2) in triacylglycerols, oxidative induction time, melting profile, acid value (AV), and peroxide value (PV). The analyzed oil blends contain 6 to 25% monounsaturated fatty acids and 16 to 42% polyunsaturated fatty acids. Additionally, it was noticed that the major monounsaturated fatty acid was oleic acid, with its contribution ranging from 9 to 19%. In most cases, oleic and linoleic acids occupied the sn-2 position of the triacylglycerol molecules, with their contribution reaching 35 to 72% and 34 to 71%, respectively. The enzymatically interesterified oil mixtures were characterized by a relatively long oxidation induction time (41–87 min). Melting profiles of the tested samples revealed the presence of a diversified number of endothermic peaks. The AV and PV of the tested oil blends exceeded 10 mg KOH g\(^{-1}\) fat and 1 meq O\(_2\) kg\(^{-1}\) fat, respectively. In conclusion, the tested interesterified plant oil blends are characterized by acceptable thermal and oxidative stability and fatty acid profile.

Keywords: tomato seed oil; coconut oil; enzymatic interesterification; fatty acids composition; oxidation induction time

1. Introduction

Fats are recognized to be fundamental, highly energetic components of food. Fats of animal and vegetable origin, treated as a primary source of essential nutrients, have to be implemented in a balanced human diet. It is particularly noteworthy that vegetable oils contain various groups of fat-soluble compounds, such as tocopherols, tocotrienols, flavonoids, carotenoids, sterols, and also fatty acids [1–3]. Fatty acids are a significant structural component of the cell membrane and an energetic constituent. Furthermore, fatty acids play important biological, structural, and functional roles in living organisms [4]. Due to the aforementioned fatty acids properties, these compounds, especially unsaturated fatty acids, should be regularly supplied with food products. It has been reported that regular daily consumption of fatty acids might entail a positive effect on human health, including the stabilization of lipid metabolism, prevention of cardiovascular, metabolic, and inflammatory diseases and cancer, as well as an adequate evolution and functioning of the central nervous system [5–7]. Industrial and scientific surroundings and also consumers have placed tremendous attention on either the quality and proportion of edible vegetable oils to animal fats or processed lipids because of an abundance of harmful transfatty acids [8,9].
In recent years, it was observed that not only is consumer awareness becoming a crucial aspect driving the agroindustrial processing sector to develop new products with high nutritional value, but there is also a strong demand for the implementation of a circular economy (CE) concept. CE concentrates on renewable energy systems, recycling waste materials, and extending the product life cycle [10,11]. The food industry is progressively confronted with new challenges associated with the elaboration of efficient and environmentally friendly methods of managing plant waste materials [12]. Therefore, the food oil industry has to explore the use of less-known plant oil materials, especially byproducts from vegetable processing. A valuable alternative oil material could be tomato seeds, which are generated in huge amounts during tomato pomace processing. Tomato seed oil is characterized by a high content of antioxidants and fatty acids. Referring to the chemical composition of tomato seed oil, it could find application in food, cosmetics, and pharmaceutical areas [13,14].

Oils derived from plants are widely utilized in food product preparation [15]. It is known that the chemical, physical, and nutritional properties of plant oils are related to the fatty acids profile and stereochemistry of triacylglycerol (TAG) [16]. The physicochemical attributes of TAGs are influenced by the fatty acid chain length, saturation degree, and position of the fatty acid on the glycerol backbone [17,18]. For that reason, most edible plant oils have limitations in technological applications due to the natural triacylglycerol structure, which is unable to provide desirable characteristics to the fat comestibles. In order to enhance the processing characteristics of crude plant oils, different techniques have been applied to modify the original composition of oils, such as blending, partial hydrogenation, interesterification, and fractionation [19,20]. Oil blending is the simplest, most physical, and most economical method widely employed in the food industry to produce specific oil products with desired sensory, oxidative, and nutritional characteristics. Oil blending could be performed by mixing diverse crude and modified plant oils with different physiochemical properties. The oils universally used in the oil industry sector (e.g., canola oil, palm oil, soybean oil, virgin olive oil, or coconut oil) are applied appropriately as a base for generating plant oil blends. Oil blending has been a common permissible technique in many countries, and there have been numerous reports that have presented a stepped-up interest in implementing this method in the edible oil industry [21]. The hydrogenation process of plant oils has been used for a long time to produce structured lipids. Nonetheless, hydrogenation is considered the cause of the formation of trans isomers of fatty acids, which indicate harmful effects on human health [22]. For this reason, food manufacturers tend to replace partially hydrogenated oil products with structured lipids obtained through an interesterification (IE) process. The IE reaction occurs in two stages: initial hydrolysis and a subsequent esterification reaction [23]. The IE process can be conducted by using two pathways such as chemical interesterification (CIE) and enzymatic interesterification (EIE). Despite the fact that CIE is commonly applied in the food industry, EIE has received increasing attention as an emerging technique to produce structured lipids with desired technological characteristics. The EIE technique has several advantages, contrary to the CIE process. EIE is investigated as a simple and efficient bioprocess that does not involve the use of hazardous chemicals or the formation of trans fatty acids at a high level. What is more, EIE can be conducted with milder reaction conditions in comparison to CIE. During the EIE process, the thermal degradation of plant oil bioactive compounds is inhibited; hence, the final products acquire a satisfactory nutritional quality and period of shelf life [24,25].

Taking the above into consideration, the main goal of the present study was to assess the quality and oxidative stability of structured lipids via the enzymatic interesterification of tomato seeds oil and coconut oil mixtures catalyzed by immobilized commercial sn-1,3-specific lipase from *Rhizomucor miehei*. 
2. Materials and Methods

2.1. Materials

Two commercially available oils on the market, tomato seed oil (A) and coconut oil (B), were selected to generate the model plant oil blends. Crude plant oils were mixed in different weight ratios (1:3, 1:1, 3:1) in order to obtain three formulations of plant oil blends (A25B75, A50B50, A75B25), respectively. Plant oil blends and crude oils were stored under refrigeration for further analysis.

2.2. Preparation of the Enzymatic Interesterification

The regioselective enzymatic interesterification with the use of a microbial lipase was based on the method of Bryš et al. [26]. The used enzyme, called Lipozyme (Merck KGaA, Darmstadt, Germany), is produced by immobilization of a lipase from *Rhizomucor miehei* fungi on a macroporous anion exchange resin and shows regiospecific activity against the ester bonds at the sn-1,3 position of triacylglycerol backbone. The model oil blends (20 g) were incubated for 10 min at 60 °C. To initiate the enzymatic interesterification (IE) reaction, lipase preparation (8% w/w) was added to all model oil mixtures. The IE reaction was conducted in a laboratory shaker Elpin Plus type 357 (Lubawa, Poland) for 2 h with simultaneous temperature control (60 °C) and continuous agitation. To terminate the IE reaction, the enzyme formulation was separated from the oil blends by filtration under vacuum in a Büchner funnel. Then, the obtained oil samples were transferred to plastic screw-cap containers and stored for further analysis.

2.3. Analytical Methods

2.3.1. Determination of Fatty Acids Profile

The fatty acids profile was determined by using YL6100 gas chromatograph (Young Lin Bldg., Anyang, Hogye-dong, Korea) equipped with a flame ionization detector and capillary column BPX 70 (0.25 mm inner diameter × 60 m length × 0.25 µm film thickness) from SGE Analytical Science (Milton Keynes, UK). As a carrier gas, nitrogen was used with flow rate of 1 mL min⁻¹. Derivatization of fatty acids into methyl esters (FAME) was performed in accordance with standard PN-EN ISO 5509:2001 [27] and the procedure described in the paper by Bryš et al. [28]. Identification of fatty methyl esters was based on comparing the relative retention times of FAME peaks in the tested samples with FAME standard (Supelco 37 Component FAME Mix).

2.3.2. Determination of Fatty Acids Positional Distribution in Triacylglycerols

In accordance with the method described by [29,30], the hydrolysis of TAGs (triacylglycerols) was performed with some modification. Briefly, to investigate the hydrolysis of TAGs contained in the lipid fraction of the tested oil samples, lipase from porcine pancreas (Merck KGaA) was used, which shows regiospecific activity against ester bonds in the sn-1 and sn-3 positions. For this purpose, 1 mL TRIS-HCl, 0.1 mL CaCl₂ (2.2% w/w), and 0.25 mL bile salts (0.05% w/w) were added to 0.1 g of the analyzed oils samples. After stirring (30 s), 20 mg of lipase from porcine pancreas (Merck KGaA) was added and mixed again for 30 s. Then, the samples were incubated at 40 °C in water bath for 5 min, and 1 mL of 6N HCl and 4 mL of diethyl ether were then added and stirred for 1 min. Subsequently, the obtained mixtures were centrifuged for 5 min at 4000 rpm. The upper ether layer was transferred to a new tube. The samples were then applied to previously prepared chromatography plates (silica on TLC Alu foils, Fluka Analytical Sigma-Aldrich, St. Louis, MO, USA) and placed in a chromatography chamber with a solvent solution (hexane:ether:acetic acid in ratio 50:50:1 v/v/v).

The silica gel was scraped at the level of the monoacylglycerol (MAG) fraction, and then the samples were analyzed by gas chromatography as described in part related to the fatty acids profile determination.
2.3.3. Determination of Oxidative Stability

Pressure differential scanning calorimeter (DSC Q 20 TA Instruments, Newcastle, WA, USA) was applied to evaluate the oxidation induction time of the tested samples. The method was described in the paper by Brzezińska et al. [31]. Oil samples of 3–4 mg were placed in an aluminum pan. Measurements were carried out isothermally (120 °C) under an initial pressure of 1400 kPa in an oxygen atmosphere.

2.3.4. Determination of Melting Characteristics

Differential scanning calorimeter (DSC Q 200 Ta Instruments, Newcastle, WA, USA) was implemented to characterize melting profile of tested oil samples [32]. Then, samples were placed into nonhermetically sealed aluminum crucible with a lid, and 3–4 mg of the oil sample was weighed. The empty, identically sealed aluminum crucible was used as the reference sample. The measurement was performed under normal pressure and in a nitrogen atmosphere. The nitrogen was applied as cooling medium with a flow rate of 50 mL per min. The temperature program included a combination of heat–cool–reheat steps. Initially, the sample was heated to 80 °C and held at this temperature for 10 min. Then, the sample had to be cooled to −80 °C at rate of 10 °C/min and kept at this temperature for 30 min. Finally, the oil sample was reheated to 80 °C with a heating rate of 15 °C per min. Melting characteristics of the oil samples were obtained during second heating period.

2.3.5. Determination of Acid Value

According to the procedure described in PN-EN ISO 660:2010 [33], the acid value (AV) determination was performed. In beaker, the oil sample was weighed and dissolved in 50 mL of ethanol/diethyl ether mixture (1:1, v/v). Then, the sample was placed on a magnetic stirrer, and the titration method using ethanolic potassium hydroxide (KOH) solution (0.1 mol/L) as a titrant was started on a titrator TitraLab AT100 (HACH LANGE, Wrocław, Poland). AV was expressed as the mg KOH required to neutralize the acidic constituents present in 1 g of oil samples.

2.3.6. Determination of Peroxide Value

The peroxide value (PV) was performed with regard to the method presented in PN_EN ISO 3960:2012 [34]. Oil sample was dissolved in 25 mL acetic acid/chloroform mixture (3:2, v/v), then 1 mL potassium iodide solution was added. The prepared mixture was stirred for 1 min. After magnetic stirring, the sample was stored in a dark place for 5 min. The peroxide value was measured by the use of a titrator TitraLab AT100 (HACH LANGE, Wrocław, Poland). During titration, a sodium thiosulphate solution (0.001 mol/L) was added as a titration agent. The result of PV was calculated in meq O₂ (milliequivalents of oxygen) per kg of oil sample.

2.4. Statistical Analysis

All experiments were in triplicate. Statgraphics Plus version 5.0 software (Statistical Graphics Corporation, Warrenton, VA, USA) was used for all calculations. Experimental data are presented as the mean ± standard deviation (SD). The collected data were analyzed using the one-way analysis of variance with Tukey’s (HSD) post hoc test. Tukey’s multiple range test was performed to compare significant differences among samples at a p-value of 0.05.

3. Results

3.1. Fatty Acids Profile

The composition of three fatty acid groups (saturated fatty acids, SFA; monounsaturated fatty acids, MUFA; polyunsaturated fatty acids, PUFA) in the tested samples is shown in Figure 1.
Figure 1. Composition of fatty acids in the enzymatically interesterified oil blends and raw oils. Data denoted with the same lowercase letter are not significantly different ($\alpha = 0.05$ with Tukey’s post hoc test) among the main fatty acids group.

The SFA group in the oil mixture samples was characterized by high lauric acid (C12:0) and myristic acid (C14:0), with contents ranging between 12.85% and 36.75% and 5.4% and 13.65%, respectively. Results indicate significant differences in the SFA percentage content between the analyzed samples.

GC analysis revealed that the main fatty acid in the MUFA group was oleic acid (C18:1, n-9c), with a content ranging between 10.1% and 19.35%. Moreover, in the case of sample A75B25, a trace of gadoleic acid (C20:1c) was observed, the presence of which was also found in crude tomato seed oil.

Considering the content of PUFA in the tested oil mixture samples, this group was dominated by linoleic acid (C18:2, n-6c), with 4.6–39.05% content, and $\alpha$-linolenic acid (C18:3, n-3), with 0.35–2.45% content. Additionally, in the case of sample A75B25, a trace amount of eicosatrienoic acid (C20:3 n-3) was observed.

3.2. Fatty Acids Distribution in Triacylglycerol Structure

The results concerning the composition of fatty acids at the sn-2 (internal) and sn-1,3 (external) TAG positions in the analyzed samples of oil mixtures are presented in Figure 2. It can be observed that the dominant acids present in the oil samples were lauric, myristic, palmitic, oleic, and linoleic acids.

In sample A25B75, with a tomato and coconut oil weight ratio of 1:3, the three fatty acids occupying the internal position were lauric, oleic, and linoleic acids, with a 34.1%, 53.5%, and 58.6% share, respectively. Palmitic acid occupied mainly the outer sn-1,3 positions. Sample A50B50 was similar to sample A25B75 with the exception of lauric acid, which occupied mostly the internal position in the latter.

In sample A75B25, fatty acids such as lauric (45.4% share), oleic (37.8% share), and linoleic acids (37.2% share) were present mostly in the sn-2 position.
3.3. Oxidative Stability

Results of the oxidation induction time (OIT) for the researched samples are presented in Figure 3.

Figure 2. Percentage share of fatty acids in sn-2 position in the enzymatically interesterified oil blends and raw oils. The black line indicates the constant share of fatty acid occupying sn-2 position assuming the equilibrium of all positions in the triacylglycerol molecule. Data denoted with the same lowercase letter are not significantly different (α = 0.05 with Tukey’s post hoc test) among the individual fatty acids.

Samples A (tomato seeds oil) and B (coconut oil) differed from each other regarding the acids occupying the inner and outer positions of the TG molecules. In sample A, the oleic, linoleic, and α-linolenic acids occupied the sn-2 positions, while it was the capric, lauric, and oleic acids in sample B.

Figure 3. Oxidative stability of the enzymatically interesterified oil blends and raw oils. * OIT exceeded the analysis time on the PDSC apparatus (above 300 min). Data denoted with the same lowercase letter are not significantly different (α = 0.05 with Tukey’s post hoc test).
OIT of the tested oil samples ranged from 40.7 min to over 300 min. The enzymatically interesterified oil mixture with the highest OIT was sample A25B75. The difference between the shortest and longest oxidation induction time was about 45.8 min. OIT for crude coconut oil exceeded 300 min, as it was the highest time allocated to this experiment on the apparatus.

### 3.4. Melting Characteristics

Table 1 shows the experimental data obtained during DSC (differential scanning calorimetry) analysis concerning the melting characteristics of the enzymatically interesterified oil blends and crude plant oils. It was noticed that tested oil samples were characterized by various melting profiles with different numbers of endothermic peaks.

| Sample Type | Endothermic Peak 1 | Endothermic Peak 2 | Endothermic Peak 3 |
|-------------|--------------------|--------------------|--------------------|
|             | Minimum Temperature (°C) | Temperature Range (°C) | Minimum Temperature (°C) | Temperature Range (°C) | Minimum Temperature (°C) | Temperature Range (°C) |
| A50B50      | −77.0              | −80.1 to −60.0      | −35.0              | −44.0 to −26.0 | 10.4              | −16.5 to 22.5 |
| A75B25      | −76.7              | −80.0 to −60.2      | −37.0              | −45.5 to −21.3 | −6.0              | −21.3 to 15.0 |
| A           | −74.6              | −80.0 to −60.0      | −38.7              | −43.8 to −32.4 | −23.6              | −32.4 to 3.9  |
| B           | 24.0               | 6.7 to 30.0         | −                   | −                   | −                   | −                   |

Among the tested enzymatically interesterified oil blends, only sample A25B75 indicated different melting characteristics behavior. The DSC curve of this oil blend, combined with the highest proportion of coconut oil, had only one endothermic peak at a temperature range of −13.10 °C to 25.5 °C. According to the results of the melting profiles of the tested crude oils, tomato seed oil showed different thermal melting behavior in comparison to coconut oil.

### 3.5. Acid Value of Interesterification Products

The obtained results of acid values for the individual oil mixtures and oils from the raw materials are presented in Figure 4.

![Figure 4](image_url)

**Figure 4.** Acid value of enzymatically interesterified oil blends and raw oils. Data denoted with the same lowercase letter are not significantly different ($\alpha = 0.05$ with Tukey’s post hoc test).
According to the obtained acid value data, distinct differences between oils from the raw materials and prepared oil mixtures were determined. The acid values of tomato seed oil and coconut oil were below 0.1 mg KOH g\(^{-1}\) oil, while the interesterified oil blend samples showed acid values in the range of 9.8 to 13.3 mg KOH g\(^{-1}\) oil. The sample with the highest level of acid value was sample A25B75 (13.3 mg KOH g\(^{-1}\) oil).

### 3.6. Peroxide Value of Interesteerification Products

The obtained results of the peroxide values of samples are presented in Figure 5.

![Figure 5. Peroxide value of enzymatically interesterified oil blends and raw oils. Data denoted with the same lowercase letter are not significantly different (α = 0.05 with Tukey’s post hoc test).](image)

The highest level of peroxide value was observed for sample A (4.09 meq O\(_2\) kg\(^{-1}\) oil). The sample mixture difference in the oxidative stability between the lowest and the highest peroxide value was 0.75 meq O\(_2\) kg\(^{-1}\) oil.

### 4. Discussion

#### 4.1. Fatty Acids Profile

The distinctive biological properties and the impact of fats on the human body depend on the profile of fatty acids. These compounds are divided according to the type and number of bonds: saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), as well as polyunsaturated fatty acids (PUFA). The specific composition of fatty acids in triacylglycerol molecules is directly related to the type and origin of fats.

The literature shows that the typical content of unsaturated fatty acids in tomato seed oil is around 80% [35,36] which is similar to the results obtained in this study (81.55%). In addition, according to Zuorro et al. [35], the main unsaturated fatty acids in tomato seed oil are linoleic, oleic, and linolenic acids, with contents of 54.52%, 22.36%, and 2.06%, respectively. These results are also comparable to the ones obtained in this study. According to the data reported by Marikkar et al. [37], coconut oil is comprised of around 92% saturated fatty acids and 8% unsaturated fatty acids. These values are in agreement with those reported in this study, in which the saturated fatty acid content was 92.4%, and the unsaturated fatty acid content was 7.6%. According to the authors, the main fatty acids there were lauric (46.03%) and myristic (19.03%) acids, which also largely coincides with the data obtained in this experiment in which the content of lauric acid was 44.5% and myristic acid was 19.95%. In the research by Avezado et al. [38] on the fatty acid profile of cocoa oil, coconut oil, and cocoa butter, the lauric acid content in the samples was shown to be 38.4% and 20.2% for myristic acid. The value for lauric acid is lower...
than that obtained in this work, while the content of myristic acid is very similar to the 19.95% content obtained in this research.

4.2. Fatty Acids Distribution in Triacylglycerol Structure

Triglycerides (TGs) are the main constituents of oils and can contain various fatty acids esterified with glycerol leading to several positional isomers. Triglyceride biochemistry and physical properties are determined by the stereospecific structure of TG and, thus, have an influence on lipid metabolism. Acidic radicals can occupy specific positions in the triacylglycerol molecule. In order to correctly determine the structure of triglycerides, methods with enzymes from the lipase group are used. These enzymes are characterized by high regio- and stereospecificity. Because of that, these substances can be successfully used to selectively cleave acidic radicals from triglyceride molecules [39].

TGs can also be considered the characteristic indicators of oil quality control [40]. In each of the mixture samples, palmitic acid (C16:0) was present only in the external position. Fabritius et al. [41], in their work on the regiospecificity of triglycerides in human milk, found that in the case of vegetable oils, palmitic acid is usually in the sn-1,3 position, while unsaturated fatty acids such as oleic and linoleic acids are located at the sn-2 position. The results obtained in this study are comparable with the literature. Oleic and lauric acids were always present in the internal positions of TGs, whereas palmitic acid was present in the external positions of TGs.

The composition of fatty acids in the internal position of TG does not change during interesterification in the presence of regiospecific lipases because the process undergoes in the external positions. Therefore, when this type of modification is carried out, there are mainly unsaturated fatty acids in the internal position of triacylglycerols in the mixtures, as in the initial raw oils. Minor changes in TG distribution may be associated with acyl migration during interesterification, which leads to the transfer of unsaturated fatty acids from the sn-1 and 3 positions to the sn-2 position [42].

4.3. Oxidative Stability

The oxidative stability of oils and fats is one of the most important parameters for assessing their quality. Many methods have been developed for this evaluation, some of which are based on the measurement of the induction time of oil oxidation (e.g., differential scanning calorimetry or the Rancimat method). Vegetable oils, in addition to being used in the food, cosmetics, pharmaceutical, and chemical industries, have found a huge market in the biofuel industry. Oils and fats are also extremely important in human nutrition. However, oils and fats are very susceptible to oxidation processes in which essential fatty acids are degraded, resulting in an unpleasant smell and taste as well as the generation of compounds that are potentially toxic to humans [43,44].

In the case of the oxidative stability analysis, the primary principle is that the longer OIT, the greater the oxidative stability of the oil. The rate of oxidation processes, on the other hand, may depend on the presence of anti- or prooxidants.

Kowalski et al. [45], in their research into the oxidative stability of vegetable oils, stated that oils and fats that contain PUFA are the most exposed to oxidative changes because they undergo faster oxidation processes. Results obtained in this study showed that the lowest oxidative stability was in the sample containing the most tomato seed oil (A75B25), which is known to be rich in polyunsaturated fatty acids.

OIT for crude coconut oil showed very high oxidative stability (OIT over 300 min). This result is in agreement with the work of Ramezan et al. [46], who obtained OIT above 550 min.

The study performed by Zhang et al. [47] indicated that the oxidation induction time of interesterified palm-based oil blends decreases in comparison with unmodified blends, which supports our observations. Blending and interesterification reduce the oxidative stability of coconut oil, as blends composed of 75% coconut oil are characterized by a much shorter oxidation induction time than raw coconut oil.
4.4. Melting Characteristics

Differential scanning calorimetry (DSC) is a thermoanalytical technique widely used to characterize phase transitions in vegetable oils. It is an established method that has many advantages, as it does not require any chemical or complicated pretreatment or time-consuming steps before each measurement. Various vegetable oils exhibit complex thermal properties that are mainly due to the great variety of triglycerides as their main constituents, as well as their natural polymorphism [42,48].

Based on the results obtained in this study, it can be assumed that tomato seed oil is a rich source of unsaturated fatty acids because the endothermic peaks occurred at low temperatures. In contrast, crude coconut oil is rich in monounsaturated and saturated fatty acids due to the fact that the resulting endothermic peaks occurred at higher temperatures.

Benitez et al. [49], in their work on the valorization of tomato processing byproducts using the DSC method, obtained results that dry tomato pomace showed two slight endothermic peaks at temperatures $-22 \degree C$ and $26 \degree C$, which were related to the seed fraction. The lower temperature peak could be associated with the unsaturated fatty acid esters present in tomato seed oil, such as linoleic and oleic acid; however, the peak at $26 \degree C$ was attributed to saturated homologs (palmitic and stearic acids). This agrees with the results obtained in this study (last endothermic peak at $-23.55 \degree C$ for tomato oil sample).

As for crude coconut oil (sample B), the obtained data are in agreement with the experimental data reported by Marikkar et al. [37], who acquired an endothermic peak at a minimum temperature of $23.4 \degree C$ in comparison with our study in which the endothermic peak was achieved at $24 \degree C$.

Srivastava et al. [50] observed in their research that along with an increase in the percentage of coconut oil in the mixture, the melting peaks shifted from a lower temperature to a higher temperature. An almost identical temperature range of endothermic peaks was observed in this study. The mixture containing a greater percentage of coconut oil (sample A25B75) than tomato seed oil showed the farthest shift in endothermic peaks toward higher temperatures. As a consequence, mixture A25B75 showed the highest melting point, while oil blend A75B25 indicated the lowest melting point.

4.5. Acid Value of Interesterification Products

According to the Codex Alimentarius recommendations, the levels of acid value in vegetable oils should not exceed 4 mg KOH/g of oil [51]. Based on the acquired data, none of the tested oil blends met the level of the acid value mentioned in this recommendation. Sample A25B75 was characterized by the highest level of acid value among all the samples, so this enzymatically interesterified oil mixture was supposed to be the least hydrolytically stable.

The esterification process caused a significant increase in the acid value of all tested samples. This is directly related to the hydrolysis of triglycerides to glycerol and free fatty acids, which takes place during interesterification [52]. Taking into account an interesterification reaction with the presence of a regiospecific enzyme, the high levels of acid value are associated with the release of a greater amount of free fatty acids formed by the partial hydrolysis of triacylglycerol and the esterification of acylglycerols. This causes an increase in the amount of free fatty acids, monoacylglycerols, and diacylglycerols in the final product, resulting in its low stability [53].

According to Karim and Yangomodou [54], who researched the chemical properties of the tomato seed oil acid value, the tomato seed oil reached approximately $3.3 \text{ mg KOH/g}$ of oil. This acid value differs from that obtained in this study. On the other hand, Giuffrè et al. [55] obtained values in the range of $0.48$ to $2.5 \text{ mg KOH/g}$ of oil. Arlee et al. [56], in their research into differences in the content of chemical components and substances with antioxidant properties in coconut oil, obtained an acid number ranging from $0.06$ to $0.63 \text{ mg KOH/g}$ of fat, which is in accordance with the results obtained in this study ($0.06 \text{ mg KOH/g}$).
4.6. Peroxide Value of Interesterification Products

Peroxide value depends mostly on the content of polyunsaturated fatty acids (PUFAs) in the tested sample. The higher the PUFA content in the product, the more susceptible it is to an accelerated oxidation reaction. The peroxide value is mainly dependent on the molecular structure of triglycerides as well as on storage conditions and the amount of the nontriacylglycerol fraction [57].

Sample A75B25 was characterized by the highest peroxide value among all mixture variants. The high peroxide value in this mixture is directly related to the large number of polyunsaturated acids (PUFAs) present in tomato seed oil, which is additionally confirmed by the high peroxide value obtained for sample A (crude tomato seed oil).

The peroxide value of tomato seed oil described in the literature ranges between 2.47 meq O$_2$ kg$^{-1}$ oil [58] and 4.52 meq O$_2$ kg$^{-1}$ oil [59], which is in agreement with the results obtained in this research.

Subermanian et al. [60], in their work on lowering the level of malondialdehyde in the heart tissue of rats by consuming crude coconut oil, obtained a peroxide value at a level below 1.5 meq O$_2$ kg$^{-1}$ oil. In the case of research conducted by Marina et al. [61] on the chemical properties of fresh coconut oil, the peroxide value obtained was in the range of 0.21–0.63 meq O$_2$ kg$^{-1}$ oil, which is consistent with the results in this study.

5. Conclusions

As demonstrated in this research, the application of a blending technique, as well as a regioselective enzymatic interesterification process, might significantly influence the quality and thermal stability of produced plant oil mixtures. The tested crude plant oils were characterized by a varied composition of fatty acids. The dominant group of fatty acids in coconut oil was saturated fatty acids, while in tomato seed oil, it was polyunsaturated fatty acids. Additionally, all tested enzymatically interesterified oil blends were characterized by a low contribution of saturated fatty acids and also a relatively high level of essential fatty acids. Nevertheless, all analyzed variants of the interesterified oil mixtures indicated acceptable thermal and oxidative stability, but their hydrolytic stability was at an insufficient level. Palmitic acid did not occupy the internal position of triacylglycerols in any of the tested interesterified oil blends. As aforementioned, enzymatic interesterification could be a promising type of oil modification to assess the satisfactory digestibility level of the obtained oil blends.

Author Contributions: Conceptualization, R.B., J.B. and O.G.; methodology, R.B., J.B. and E.O.-L.; formal analysis, R.B., J.B. and O.G.; investigation, O.G.; data curation, O.G.; writing—original draft preparation, R.B., J.B. and A.B.; writing—review and editing, A.G. and M.W.-W.; visualization, A.B.; supervision, A.G., E.O.-L. and M.W-W.; funding acquisition, A.G. All authors have read and agreed to the published version of the manuscript.

Funding: Research equipment was purchased as part of the “Food and Nutrition Centre—modernisation of the WULS campus to create a Food and Nutrition Research and Development Centre (CZiZ)”, cofinanced by the European Union from the European Regional Development Fund under the Regional Operational Programme of the Mazowieckie Voivodeship for 2014–2020 (Project No. RPMA.01.01.00-14-8276/17).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.
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