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APPLICATION OF ENZYMATIC REACTIONS IN STRUCTURING AND OXIDATIVE STABILITY IMPROVEMENT OF OILS AND FATS

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1. INTRODUCTION AND AIM

In the first section of the thesis, the application of enzymatic reactions was used to solidify liquid vegetable oils containing mostly triacylglycerols (TAGs) into solid fats containing partial acylglycerols. This was done by enriching them with monoacylglycerols (MAGs) and diacylglycerols (DAGs) while keeping free fatty acids (FFAs) as low as possible and selectively removing saturated fatty acids (SFAs) from vegetable oils.

Solidifying liquid vegetable oils without compromising their nutritional properties has always posed a challenge for food scientists. Traditional methods such as partial hydrogenation, fractionation, physical blending with hard fats, and interesterification have been used for solidification. In this context, partial hydrogenation has been recently restricted due to the formation of trans fatty acids. However, innovative techniques have emerged in the last two decades, including oleogelation using lipidic or biopolymeric oleogelators, bi-phasic structured systems, and glycerolysis, offering successful alternatives for structuring liquid oils. These methods allow for the use of fats with lower SFAs while creating technologically functional fats, making them highly valuable in the field of food science. In this context, methods allowing the use of lower SFAs while developing technologically functional fat are of great importance.

With this in mind, our study aimed to structure vegetable oils by harnessing the acylglycerol formation ability and selectivity of lipases. Specifically, we sought to selectively remove SFAs from vegetable oils and enrich them with MAGs and DAGs. The outcomes of the hydrolysis reactions resulted in two target structured products: a crude hydrolyzed fat containing FFAs, MAGs, DAGs, and TAGs, and a deacidified hydrolysate with removed FFAs. Both of these products hold potential as specialty fats in the food industry.

Additionally, the application of enzymatic reactions was used to synthesis alkyl rosmarinates using ethyl rosmarinate as a model after investigating the impact of the oil-based food matrix on the antioxidant properties of rosmarinic acid derivatives.

The effectiveness of a particular antioxidant in oil-based food systems is significantly dictated by its chemical structure and concentration. Moreover, these properties are interconnected with the characteristics of the oil-based food matrix. In this context, the type of structuring agent or emulsifier utilized appears to have a significant impact, and there is a likelihood that particular interactions between the structuring agent or the emulsifier and antioxidants contribute to the additive or synergistic effect that influences their antioxidant activity. In this context, the hydrophobicity of the antioxidant can change its interaction in the oil-based food matrix. Therefore, understanding the intricate relationship between the structuring agent and its influence on antioxidant activity in relation to the hydrophobicity of antioxidants can lead to the formulation of enhanced food products with extended shelf life and improved oxidative stability.

Moreover, the presence of natural rosmarinate esters in plants are naturally trivial and the procedures for their isolation and refinement are normally difficult, calling for lipophilization techniques (Guan *et al.,* 2022; Wu *et al.,* 2019). Therefore, the synthesis of alkyl rosmarinates is necessary which can be done by structurally modifying rosmarinic acid.

Therefore, in this study, the antioxidant activity of alkyl rosmarinates (methyl and ethyl rosmarinates) were compared with rosmarinic acid with radical scavenging activity (*in vitro*), and in food systems, including bulk oil system, structured oil with MAGs, oleogel, O/W emulsion, and gelled O/W emulsion under accelerated oxidation condition at 35 °C during one month. Additionally, the lipophilization of rosmarinic acid with ethanol was done with Lipozyme 435 (Novozyme) as a model and optimized by considering reaction conditions,

including time, temperature, enzyme-to-substrate ratio, and the concentrations of rosmarinic acid and alcohols.

In view of the aforementioned points, this study has been designed with the following objectives:

First section (application of enzymatic reactions to solidify liquid vegetable oils)

1. Screening enzymes for the ability of enzymes to selectively enrich oils with MAGs (preferably) and DAGs and simultaneously remove SFAs.
2. Optimization of reactions for the selected enzyme for enriching them with MAGs and DAGs.
3. Expanding the application to vegetable oils with different degrees of saturation.
4. Deacidification of the obtained products.
5. Assessment of the properties of the obtained structured fats.

Second section (application of enzymatic reaction to synthesis alkyl rosmarinates after assessment of their antioxidant activity in oil-based food systems)

1. Studying the antioxidant activity of alkyl rosmarinates (methyl and ethyl rosmarinates) compared with rosmarinic acid through radical scavenging activity (*in vitro*), and in food systems, including bulk oil system, structured oil with MAGs, oleogel, O/W emulsion, and gelled O/W emulsion under accelerated oxidation condition at 35 °C during one month.
2. Examining the "Polar Paradox Hypothesis" and "cut-off Effect" by testing the antioxidant effects of the modified antioxidants in both oil systems and emulsions.
3. Enzymatic synthesis of alkyl rosmarinates using ethyl rosmarinate as a model.
4. Optimizing the reaction conditions to obtain the highest yield of the ethyl rosmarinate.

2. MATERIALS AND METHODS

2.1.Main materials

Novozymes, Denmark, generously provided three commercial enzymes: Lipozyme 435 (from *Candida antarctica*, Lipozyme TL IM, and Lipozyme RM IM. Amano, UK, provided Lipase AY "Amano" 30SD and Lipase DF "Amano" 15. All reagents, standards, and solvents used in the study were of analytical or HPLC grades and were purchased from either Sigma-Aldrich or Merck.

2.2. Methods - section 1 (application of enzymatic reactions to solidify vegetable oils)

2.2.1. Screening of lipases

Various lipases were assessed for their ability to synthesize MAGs through partial hydrolysis in palm olein. The reaction conditions were customized based on the supplier-declared optimum temperature and in the range of pH for each enzyme. A lipase dosage of 50 U/g of oil was used for Amano enzymes, while 5% per weight of oil was employed for Novozymes enzymes (500 PLU/g for Novozym 435, 12.5 IUN/g for Lipozyme RM IM, and 13.75 IUN/g for Lipozyme TL IM). The acylglycerol phase obtained from the hydrolysate was isolated through alkali deacidification, following the described procedure below. This phase was then utilized for measuring enzyme selectivity towards specific fatty acids or assessing the potential reduction of SFAs in the acylglycerol phase.

2.2.2. Hydrolysis reactions and optimization of partial hydrolysis

Lipase DF "Amano" 15 was chosen based on the results obtained in the previous section. The factors and their respective quantities included the water level (2-10 wt% of oil mass), enzyme load (10-90 units per gram of oil), temperature (22-54 °C), and time (1-5 hours). Design Expert 13 software from Minneapolis, Minnesota, USA, was utilized to create 30 experiments based on the central composite rotatable design. A desired pre-heated amount of water was added to

the oil, followed by stirring at 200 rpm using a magnetic stirrer and maintaining the desired temperature. The reaction was initiated by adding the required amount of lipase. After the specified duration of time, the water phase containing the lipase was separated from the reaction mixtures through centrifugation at 1610 g for 5 minutes. The resulting hydrolysate was then filtered and stored at -80°C for immediate analyses or for further processing steps.

2.2.3. Determination of acylglycerol composition

To analyze the lipid components, 5-10 mg of fat was dissolved in 1 mL of isooctane, and then thin-layer chromatography (TLC) was performed. In summary, 20 µL of the diluted sample was applied to a silica gel 60 TLC plate with glass support, measuring 20 cm × 20 cm. The plates were eluted twice using n-heptane: diethyl ether: formic acid (in a volume ratio of 55:45:2) and then dried at room temperature between runs. For staining, a solution of copper acetate-phosphoric acid was used. The densitometry evaluation of the stained lipids on the TLC plates was conducted using ImageJ software (version 1.54i).

2.2.4. Alkali deacidification of hydrolysates

For the process, 10 grams of hydrolysates were neutralized using a 12 g/100 mL sodium hydroxide solution as part of the alkali deacidification. The reaction was carried out at 60°C for 10 minutes on a magnetic stirrer set at 50 rpm, continuing until no further soap formation occurred. Following neutralization, the mixture of soap stock and oil was mixed with 200 mL of hexane and stirred for 10 minutes. The resulting oil-hexane mixture was then mixed with hot water, allowing it to settle into two layers, after which the water layer was discarded. Finally, the deacidified oils underwent vacuum removal of hexane at 40°C (Buchi Rotavapor R-210).

2.2.5. Assessing changes in the properties of crude hydrolysates and deacidified hydrolysates

2.2.5.1.Fatty acid composition

The preparation of fatty acid methyl esters (FAMEs) followed the guidelines outlined in ISO 12966-2:2017. The analysis of the FAMEs was performed using an Agilent 6890 GC-FID system and a Phenomenex Zebron ZB-FAME column (60 m, 0.25 mm, 0.20 μm) with a cyanopropyl stationary phase. Hydrogen gas was used as the mobile phase at a flow rate of 1.2 mL/min, following a previously described method. The iodine value (IV) was measured based on the fatty acid composition according to the AOCS method Cd 1c-85.

2.2.5.2. Lipase selectivity

The enrichment number of each acyl moiety was calculated to investigate the enrichment or depletion of specific fatty acids in the acylglycerol section. The enrichment number of each fatty acid in the acylglycerol phase is determined using proportion of percentage of the fatty acid in the acylglycerol phase to percentage of the fatty acid in the initial oil.

2.2.5.3.Differential scanning calorimetry

Differential scanning calorimetry (DSC 3500 Sirius, Netzsch) was employed to study the crystallization and melting behavior of the samples. Between 8-12 mg of samples were put in aluminum crucibles, and then closed. Samples initially reached 100 °C (15 min), prior to studying their crystallization behaviors by reaching −70 °C (5 °C min^{-1}), and afterward melting behaviors by heating to 80 °C (5 °C min^{-1}) (Nicholson & Marangoni, 2021).

2.2.5.4. Rheological and textural properties

Different rheological measurements, including strain sweeps, frequency sweeps, and temperature sweeps, were conducted to study the viscoelastic behaviors of fats using a rheometer (Physica MCR 301, Anton-Paar, GmbH, Graz, Austria) equipped with parallel

measuring plates PP50 (50 mm in diameter and a 1000 μm gap) (Naeli *et al*., 2022). Strain sweep tests were performed at a strain amplitude ranging from 0.01% to 1000% and a constant frequency of 1 Hz at a temperature of 20 °C to determine the linear viscoelastic range. Frequency sweep tests were carried out with a frequency ramp of 0.1 to 50 Hz (at a constant strain of 0.02% in the linear viscoelastic range) at a temperature of 20°C. Temperature sweep tests were conducted with a constant frequency and strain of 1 Hz and 0.5%, respectively, over temperatures ranging from 10°C to 80°C, with an increase rate of 0.08°C per minute (Naeli *et al*., 2022). The textural analysis of the samples for the target product was performed at room temperature (20 °C) using a Stable Micro System TA-XT 2i universal device with the TTC Spreadability Rig (HDP/SR) attachment equipped with the male/female cone.

2.2.5.5. Oxidative stability of hydrolyzed samples

The oxidation induction period was assessed using a Rancimat machine (Model 743, Metrohm Herisau, Switzerland) with 3 grams of the sample at 120°C and an airflow rate of 20 L/h (Mardani *et al*., 2021).

2.3.Method - section 2 (oxidative properties and synthesis of alkyl rosmarinates)

2.3.1. *In vitro*-radical scavenging activity measurements

DPPH solution was created at the concentration of 0.2 mmol/L and then left in darkness at room temperature. Next, 100 μL samples were added to 96-well microplate and 100 μL ethanolic DPPH solution was added to each well. The absorbance was calculated at 515 nm. The FRAP solution was freshly made and kept at 37 °C for 1 h prior to the measurements. 10 μL sample solutions were included into a 96-well microplate, 100 μL FRAP solution and 100 μL distilled water were added to each well. The absorbance was determined at 593 nm. For ABTS scavenging activity, 7 mM ABTS stock solution mixing with 2.45 mM potassium persulfate was kept in a dark room at room temperature for 16 hours. To achieve an absorbance

of 0.7 ± 0.02 at 734 nm, the ABTS solution was diluted with ethanol. In a 96-well microplate, 16 µL of the sample solution and 184 µL of ethanolic ABTS solution were combined and incubated in darkness at room temperature for 30 minutes. The absorbance was subsequently measured at 734 nm.

2.3.2. Antioxidant activity measurements in oil-based food systems

Sunflower oil employed in this project was removed from its endogenous antioxidants in order to avoid affecting the oxidative strength of the samples. For production of structured fat with MAGs, MAGs were precisely measured and evenly distributed in stripped sunflower oil at 15% wt% using a magnetic stirrer based on the method previously explained with some. Then, rosmarinic acid and alkyl rosmarinates were added to 1 g of prepared oil at 1.31 mmol/L. For, production of ethyl cellulose oleogel, 7% ethyl cellulose was used in stripped sunflower oil and the oleogel was created by homogenizing using an Ultra-Turrax dispersing tool at 12000 rpm at 90 °C for 5 minutes. Then, rosmarinic acid and alkyl rosmarinates were added to 1 g of prepared oil at 1.31 mmol/L. For, preparation of sunflower oil O/W emulsion, a potassium phosphate buffer solution (40 mmol/L, pH 7) was slowly added to stripped oil containing Tween 80. The oil-to-water ratio stood at 1 to 10, while the ratio of oil to Tween 80 was 2 to 1. Initially, a magnetic stirrer set at 750 rpm was used to blend Tween 80 with stripped oil for 30 minutes. Following this step, the aqueous phase was introduced gradually into the oil phase while maintaining ongoing stirring of the system (Keramat et al., 2023). Rosmarinic acid and alkyl rosmarinates were added to 1 g of prepared emulsion while shaking for 5 minutes at 1.31 mmol/L. For, production of emulsion gel, potassium chloride was incorporated into the aqueous phase at a concentration of 1.25% (w/w). Initially, the emulsions were heated to 80°C for a duration of 5 minutes using a magnetic stirrer. Following that, kappa-carrageenan (2% w/w) was introduced and stirred for 10 minutes to evenly distribute it within the O/W emulsion. Rosmarinic acid and alkyl rosmarinates were added to 1 g of prepared emulsion gel while

shaking for 5 minutes at 1.31 mmol/L. Monitoring oxidation of food systems The vials containing bulk oil, structured fat with MAGs, oleogel, emulsion gel and non-gelled emulsion were stored in a forced air incubator at 35 °C. Samples were drawn on 0, 10, 20, and 30 days for assessments. In all cases, controls including samples with no added antioxidant and samples having BHT at the upper limit concentration of 200 ppm (0.9076 mM) were employed in the study. Conjugated dienes and p-anisidine values were utilized for observing the oxidative stability of samples based on the AOCS Method, Ti 1a-64 (Conjugated dienes, 1980), and Method Cd 18-90 (p-anisidine values, 1990) (AOCS, 2017, 2023). To extract the oil from emulsions, 1 g of either emulsion gel or non-gelled emulsion samples was combined with 1.5 mL of a mixture containing hexane and methanol in a 3:2 ratio (v/v). The samples underwent three rounds of 10-second vertexing. Afterward, they were subjected to centrifugation at 6000 rpm for 2 minutes. Following centrifugation, the upper phase was used for monitoring oxidative products after removal of solvent under vacuum at 60 °C.

2.3.3. Enzymatic synthesis and the optimization of reaction conditions

For a typical ethyl rosmarinate reaction, 1 mg of rosmarinic acid and 3 mL of n-hexane were mixed in a 10 ml tight screw-cap container. Additionally, 3 mg of molecular sieves (3 Å) was added to each reaction for absorption of water produced during esterification. Based on the model, the desired amount of ethanol (1-5 mmol/mmol rosmarinic acid) and enzyme (4-16 % relative to the total weight of substrates) was put into the reaction system which initiated the reaction at 150 rpm. The reactions were done in different conditions based on RSM to yield the highest conversion of rosmarinate esters as the response. A Randomized Box-Behnken design with 27 runs was used for the optimization of reaction conditions with RSM to assess the connection among a defined group of adjustable experimental parameters in the enzymatic production of ethyl rosmarinate.

After completion of the reaction, samples were diluted 10 times with methanol to stop the reaction. The solutions were then diluted 10 times with the HPLC mobile phase (solvent A was 0.1% acetic acid in water and solvent B was acetonitrile and the solvent ratio was 50:50) and analyzed by HPLC after filtration through a 0.22 μm PTFE filter into an HPLC vial. A reverse phase XBridge C18 column (3.5 μm, 2.1 × 50 mm, Waters Corp., Milford, MA, USA) was used for the optimization of ethyl rosmarinate reactions with a UV detector at 290 nm. Isocratic elution at room temperature was used for the separation of the rosmarinic acid and ethyl rosmarinate. The flow was set to 0.6 mL/min. Commercially available rosmarinic acid and ethyl rosmarinate were used for the determination of retention times and method developments. Additionally, HPLC system with a diode array detector (DAD) was coupled to an Agilent (Santa Clara, CA USA) 6530 quadrupole – time-of-flight (q-TOF) hybrid mass spectrometer, equipped with a dual spray ESI source based on the method described by Abrankó *et al*. (2015).

2.4.Statistical analysis

Significant levels were based on the confidence level of 95% ($P < 0.05$). Results were analyzed by applying a two-way analysis of variance (ANOVA) using IBM SPSS-25 software. Tukey's post hoc test was used when homogeneity of variances was assumed to be valid. In cases where homogeneity of variances was not met, the Games-Howell post hoc test was utilized.

3. RESULTS AND DISCUSSIONS

3.1.Section 1 (application of enzymatic reactions to solidify liquid vegetable oils)

3.1.1. Screening of lipase for maximizing MAGs formation

Lipase DF demonstrated remarkable efficacy in MAGs preparation through hydrolysis, with 5.61% MAGs (21.61% FFAs) after only 0.5 h of reaction. Subsequently, the MAGs level slightly increased, reaching a maximum of 8.76% after 5 h of reaction (34.76% FFAs). Furthermore, the selectivity of different enzymes towards various fatty acids was investigated after the separation of FFAs. Lipase DF reduced the overall SFAs after 2 hours, reaching an IV of 63.71 (39.19% SFAs). In conclusion, considering the MAGs content and partly reducing the SFAs in the acylglycerol phase, which is known to be nutritionally desirable for structured fats, Lipase DF "Amano" 15 was selected for the optimization studies and extending the experiments to other vegetable oils.

3.1.2. Modeling and optimization of selective partial hydrolysis

RSM was utilized to model and optimize the reaction conditions with four factors. The investigation focused on the main effects and interactions between contributing factors, including water level (2-10 wt% of oil mass), enzyme load (10-90 units per gr of oil), temperature (22-54 °C), and time (1-5 h), to assess their influence on the MAGs in hydrolyzed oil. The resulting acylglycerol compositions, including FFAs, MAGs, DAGs, and TAGs, were monitored. The data were fitted to the model to explain the dependent variables as a function of independent variables. Additionally, the proportion of MAGs/FFAs and DAGs/FFAs is also presented and modeled. The R-squared (R^2) values and adequate precisions were found to be high for all models, indicating a good fit of the data. In conclusion, the reaction conditions for the next stage were chosen based on the optimized tested model as follows: a water level of 5.11%, 70 U/g enzyme, and 45 °C, based on a maximum desirability of 0.605 and the acylglycerol profile were monitored until 10 hours.

3.1.3. Selective partial hydrolysis of different vegetable oils

The optimized conditions achieved for palm olein were extended to other vegetable oils with varying degrees of SFAs. As predicted by the model, the reaction reached equilibrium after only two hours during lipase-catalyzed hydrolysis. In general, a sharp change was observed in the first half-hour of the reaction for all acylglycerol classes. As time passed, the level of TAGs slightly and continuously decreased, while the level of FFAs slightly and continuously increased. Additionally, DAGs and MAGs gradually increased and started to decrease after two hours (except in the case of sunflower oil, where DAGs showed a slight increase until 5 hours and then decrease). In conclusion, the high proportion of MAGs/FFAs and DAGs/FFAs was observed when the reaction reached equilibrium, representing the peak of hydrolysis where MAGs and DAGs reached high levels. Beyond this point, MAGs and DAGs started to undergo hydrolysis as well.

3.1.4. Properties of structured fats obtained from selective partial hydrolysis

Properties of treated oils were assessed and it was shown that one step hydrolysis reaction can produce a high level of MAG and DAG (sum of above 40%), with the lowest proportion of FFAs to MAG at 2 hours. FFAs were removed from the treated oils and it was found that deacidification methods can weaken the thermal, textural, and rheological properties of the obtained fats which were proved to be due to the refining loss of neutral lipids and partial removal of SFAs. In all samples, with the exception of sunflower oil which has the lowest SFAs content, lipase DF exhibited higher rates of cleavage for SFAs (both palmitic and stearic acids), indicating the highest lipase selectivity. The oxidative stability of the structured hydrolyzed fats was evaluated before and after deacidification and compared to that of the initial oils. However, the oxidative stability of all samples improved after the removal of FFAs, despite the decrease in SFAs, compared to both the initial oils and the hydrolyzed oils/fats. The thermograms revealed significant changes in crystallization and melting behaviors for each of

the samples. In all cases, noticeable shifts or broadening of the bulk crystallization or melting peaks were observed, and at least one new peak emerged at higher temperatures. After deacidification, peaks were shifted to lower temperatures, resembling the initial oils, with some small peaks remaining at higher temperatures. This shift can be attributed to the loss of partial lipids, particularly DAGs and MAGs, during deacidification. Finally, the hardness of the treated fats was examined and correlated with their rheological behaviors. The hardness of fats is influenced by various factors, including their levels of SFAs, acylglycerol composition, storage conditions, and structural characteristics.

3.2.Section 2 (oxidative properties and synthesis of alkyl rosmarinates)

The purpose of this section was to study the antioxidant activity of alkyl rosmarinates (methyl and ethyl rosmarinates) compared to rosmarinic acid through radical scavenging activity (*in vitro*) and in various food systems, including bulk oil, structured oil with MAGs, oleogel, O/W emulsion, and gelled O/W emulsion under accelerated oxidation conditions at 35°C in one month. The research also includes the enzymatic synthesis of alkyl rosmarinates using ethyl rosmarinate as a model and aims to optimize the reaction conditions to achieve the highest yield of ethyl rosmarinate.

3.2.1. Antioxidant activity measurements

The impact of the oil-based food matrix on the antioxidant properties of rosmarinic acid derivatives with different chain length was studied. This was done in common oil-based food systems under accelerated oxidation conditions at 35 °C, measuring conjugated diene formation and p-Anisidine values. All *in vitro* antioxidant activity measurement results were reasonably comparable with each other. Rosmarinic acid illustrated the highest radical scavenging activities, followed by methyl rosmarinates, and ethyl rosmarinate. In bulk oil, both conjugated dienes and p-AnV values reached a peak in the following order after 30 days: ethyl

rosmarinate > methyl rosmarinate > rosmarinic acid = BHT > control. In structured fat with MAGs, methyl rosmarinate was shown to be more effective than both methyl and ethyl rosmarinates. Finally, for ethyl cellulose oleogel, emulsion, and gelled emulsion systems, rosmarinic acid was shown to be the most effective.

3.2.2. Synthesis of ethyl rosmarinate and optimization of the reaction conditions

After confirming the importance of the food matrix on the antioxidant activity of rosmarinic acid derivatives and as the presence of natural rosmarinate esters in plants is limited and isolating them is challenging, synthesizing alkyl rosmarinates through structural modification of rosmarinic acid was done. The lipophilization of rosmarinic acid with ethanol was optimized as a model with Lipozyme 435 (Novozymes) in hexane with conversion yield as high as 85.59% as quantified by HPLC-UV and confirmed by HPLC-DAD-ESI-qTOFMS. The coefficient of determination of the model was 0.96, indicating the model is fitting for representing the connection between the parameters of the reaction condition. Additionally, the predicted R^2 of 0.89 was acceptable similarity to the adjusted R^2 of 0.93.

4. CONCLUSION AND RECOMMENDATIONS

In this thesis, different vegetable oils with varying levels of saturation were subjected to enzymatic reactions after screening various commercial lipases. The aim was to enrich the acylglycerol content, primarily MAGs, and partially DAGs. Four reaction parameters, including water content, enzyme load, temperature, and time, were adjusted to optimize the MAGs and DAGs contents while minimizing FFAs production. The results led to the identification of conditions for maximizing MAGs through a one-step enzymatic hydrolysis reaction. This method presents a significant advantage over glycerolysis reactions, as it allows for the creation of fats with lower saturation levels, particularly useful for cost-effective vegetable oils such as palm oil. However, it was observed that the deacidification reactions led to a high loss of neutral lipids, indicating that alkali refining through saponification may not be a suitable method due to the considerable loss of partial acylglycerols.

Additionally, in this study, the antioxidant activity of alkyl rosmarinates (methyl and ethyl rosmarinates) were compared with rosmarinic acid through radical scavenging activity (*in vitro*) and in different oil-based food systems (under accelerated oxidation condition at 35 °C). The antioxidant activities resulting from *in vitro* analysis were in line with the antioxidant activity of alkyl rosmarinate and rosmarinic acid in ethyl cellulose oleogel, emulsion, and gelled emulsion systems (based on the most effective followed an order of rosmarinic acid > methyl rosmarinate > ethyl rosmarinate). In structured fat with MAGs, methyl rosmarinate was shown to be the most effective antioxidant (methyl rosmarinate > ethyl rosmarinate > rosmarinic acid). In both oil-in-water emulsion and bulk oil systems, we noticed a discrepancy with the polar paradox. Following the recognition of the significant role played by the food matrix in influencing the antioxidant activity of rosmarinic acid derivatives, the process of lipophilizing rosmarinic acid with ethanol was fine-tuned using Lipozyme 435 (Novozymes) in hexane as a model system. This optimization effort resulted in an impressive conversion

yield reaching as high as 85.59%. In conclusion, further examinations are essential to assess the potential application of each bioactive compound by the relevant industries. In addition to the challenges discussed, microbiological and toxicological analyses are imperative to guarantee the future safe utilization of these modified phenolics.

Based on the conclusions of this thesis, it is recommended to expand the scope of future research by exploring additional enzymatic and chemical modifications of vegetable oils to further optimize the production of MAGs and DAGs. Future studies should investigate alternative deacidification methods that minimize the loss of neutral lipids while maintaining high MAG and DAG yields. Additionally, the antioxidant properties of alkyl rosmarinates and their interactions with different food matrices should be studied in greater depth, focusing on their behavior in real-world food systems under various storage and processing conditions. Exploring the synergistic effects of combining different antioxidants with modified vegetable oils could also provide valuable insights. Furthermore, comprehensive microbiological and toxicological evaluations of these modified phenolics are crucial to ensure their safety and efficacy for potential industrial applications.

5. NEW SCIENTIFIC RESULTS

1) Five types of the enzyme were examined for their MAG-producing ability while keeping FFAs as low as possible in palm olein through partial hydrolysis and their ability to produce MAGs, DAGs, and TAGs was compared. Lipase DF Amano 15 was selected for the optimization studies and extending the experiments to other vegetable oils due to production of 9.75 % MAGs in comparison to 0.64-2.35% produced by other enzymes and partial removal of SFAs in the acylglycerol phase.

2) RSM was employed to model and optimize the reaction conditions for Lipase DF. The main effects and interactions between contributing factors that influence the MAG level in hydrolyzed oil, such as enzyme load, temperature, and water content were investigated. The model was validated by experimenting two sets of tests according to the model-predicted conditions.

3) Using a 2-hour partial hydrolysis, palm olein, rice bran and pumpkin seed oils were converted to plastic fats, which was evidenced by the formation of MAGs, DAGs, and FFAs. All vegetable oils, including palm olein, were liquid at room temperature before partial hydrolysis. After initial hydrolysis, except sunflower oil, all vegetable oils showed a solid like behavior which is attributed to the presence of approximately 10% MAGs and around 30% DAGs. The properties of treated oils were evaluated, revealing that a one-step hydrolysis reaction can yield a high level of MAGs and DAGs (totaling over 40%), with the lowest ratio of FFAs to MAGs achieved at 2 hours. This process enhances the thermal, textural, and rheological properties of the resulting fats, irrespective of whether FFAs are present or removed.

4) The impact of the oil-based food matrix including bulk oil, structured fat with MAGs, ethyl cellulose oleogel, emulsion, and gelled emulsion systems on the antioxidant properties of

rosmarinic acid derivatives was investigated for the first time in this study by considering both hydrophobicity of antioxidant and effect of food matrix. In bulk oil, both conjugated dienes and p-AnV values reached a peak in the following order after 30 days: ethyl rosmarinate > methyl rosmarinate > rosmarinic acid = BHT > control. In structured fat with MAGs, methyl rosmarinate was shown to be more effective than both ethyl rosmarinate and rosmarinic acid. For ethyl cellulose oleogel, emulsion, and gelled emulsion systems, rosmarinic acid was shown to be the more effective.

5) Additionally, after confirming the importance of the food matrix on the antioxidant activity of rosmarinic acid derivatives, the lipophilization of rosmarinic acid with ethanol was optimized for the first time as a model with Lipozyme 435 in hexane. A conversion yield of as high as 85.59 % for ethyl rosmarinate was achieved, as quantified by HPLC-UV and confirmed by HPLC-DAD-ESI-qTOFMS. The coefficient of determination (R^2) of the model was 0.96, indicating the model is fitting for representing the connection between the parameters of the reaction condition.

6. LIST OF PUBLICATIONS IN THE FIELD OF STUDIES

Mardani, M., Badakné, K., Szedljak, I., Sörös, C. and Farmani, J., 2024. Lipophilized rosmarinic acid: Impact of alkyl type and food matrix on antioxidant activity and optimized enzymatic production. ***Food Chemistry***, p.139518. **Q1 - IF 8.8**

Mardani, M., Badak-Kerti, K., Tormási, J. *et al*. Selective Partial Hydrolysis as a Novel Strategy to Produce Specialty Structured Fats from Vegetable Oils: Optimization of Monoacylglycerol Formation and Assessment of the Final Product 2024. ***Food and Bioprocess Technology.*** https://doi.org/10.1007/s11947-024-03460-7 **Q1 - IF 5.6**

Mardani, M., Badakné, K., Farmani, J. and Shahidi, F., 2022. Enzymatic lipophilization of bioactive compounds with high antioxidant activity: a review. ***Critical Reviews in Food Science and Nutrition*****,** pp.1-18. **Q1 - IF 11.2**

Tirgarian, B., Yadegari, H., Bagheri, A., Neshagaran, E., **Mardani, M.** and Farmani, J., 2023. Reduced-fat chocolate spreads developed by water-in-oleogel emulsions. ***Journal of Food Engineering*****,** *337*, p.111233. **Q1 - IF 5.5**

Mardani, M., Badakné, K., Farmani, J. and Aluko, R.E., 2023. Antioxidant peptides: Overview of production, properties, and applications in food systems. ***Comprehensive Reviews in Food Science and Food Safety*****,** *22*(1), pp.46-106. **Q1 - IF 15.78**

Mardani, M., Somogyi, L., Szedljak, I., Prauda, I., Farmani, J. and Badakné Kerti, K., 2021. Efficiency of sea buckthorn extract in oxidative stability improvement of high oleic sunflower oil. ***Acta Alimentaria*****,** *50*(4), pp.527-536. **Q3 - IF 0.650**