# Enrichment of Hazelnut Oil with Long-Chain n-3 PUFA by Lipase-Catalyzed Acidolysis: Optimization by Response Surface Methodology

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**ABSTRACT:** Response surface methodology (RSM) was used to determine optimal conditions for the lipase-catalyzed enrichment of hazelnut oil by incorporating n-3 PUFA from menhaden oil. A four-factor, five-level central composite design was used, and hazelnut oil containing n-3 PUFA was successfully produced. The effects of incubation time, temperature, substrate molar ratio, and water content on the incorporation ratio were investigated. From the evaluation of response surface graphs, the optimal conditions for incorporation of long-chain n-3 PUFA into hazelnut oil were identified as 45–60°C for temperature, 30–40 h for reaction time, 1:1–2:1 (mol hazelnut oil/mol menhaden oil concentrate) for substrate molar ratio, and 3–5% (w/w) for water content. Experiments conducted at optimized conditions predicted by the model equation obtained from RSM yielded structured lipids with 19.6% n-3 PUFA. This value agreed well with that predicted by the model. This structured lipid containing PUFA may be nutritionally more beneficial than unmodified hazelnut oil.

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**KEY WORDS:** Enzymatic enrichment, hazelnut oil, lipase, n-3 PUFA, RSM.

Turkey produces about 580,000 t of hazelnuts annually, which is approximately 80% of the world's hazelnut production. Of this amount, it exports 75% of its hazelnuts, with a total annual revenue of US$1 billion (1). In addition to their economic value, hazelnuts provide food products with a desirable flavor and play an important role in human nutrition and health due to their specific bioactive components (2). In recent years, most hazelnut cultivars have been evaluated for hazelnut oil production, since the oil can be used in salads and as food ingredients. Hazelnut oil is a valuable product, being rich in monounsaturated FA and PUFA, as well as in vitamin E and sterols (2,3).

The long-chain n-3 FA EPA and DHA are essential for growth and development and may also play an important role in the prevention and treatment of cardiovascular diseases, inflammatory diseases, hypertension, diabetes, and rheumatoid arthritis (4,5). Vegetable and seed oils can be enriched with EFA to form new nutraceuticals or novel products having specific health benefits and functionality. In recent years, several studies have been carried out on the lipase-catalyzed enrichment of oils with EFA. Various types of fish oils; borage, evening primrose, sesame, melon seed, rapeseed, and linseed oils; and vegetable oils such as soybean and peanut oils have been the substrates in these studies. EPA, DHA, capric acid, GLA, and CLA are the EFA that have been used by many researchers for the production of enriched oils (6–13). In enzymatic reactions, lipases of *Pseudomonas fluorescens*, *Rhizomucor miehei*, *Candida antarctica*, and *C. rugosa* are generally used as biocatalysts. In these studies, the effects of enzyme type and amount, temperature, time, solvent type, substrate molar ratio, and water content on the incorporation of EFA into vegetable or seed oils have been investigated.

In the present study, response surface methodology (RSM) was used to model and optimize the reaction conditions for lipase-catalyzed incorporation of long-chain n-3 PUFA into hazelnut oil. In RSM, both mathematical and statistical techniques are used together in the modeling and analysis of situations in which a response is affected by several variables, alone or in combination (6,7,14). RSM also enables the behavior of different parameters to be predicted under a given set of conditions and provides sufficient information for statistically acceptable results with a reduced number of experiments; therefore, by choosing the appropriate experimental design, time, cost, wastage, and rework during production can be reduced (15,16).

The objectives of this study were to enrich hazelnut oil with long-chain n-3 PUFA by using lipase catalysis, to investigate the effects of different factors, such as incubation time, temperature, substrate molar ratio, and water content, on the incorporation ratio, and to optimize the reaction conditions by using RSM.

## EXPERIMENTAL PROCEDURES

*Materials.* Hazelnut oil was purchased from a local market and menhaden oil was from Sigma-Aldrich (St. Louis, MO). Novozym 435 (immobilized *C. antarctica* lipase) was donated by Novozymes (Danbury, CT). The PUFA standard from menhaden oil was purchased from Supelco (Bellefonte, PA). All chemicals and organic solvents (analytical or chromatographic grade) were purchased from Merck (Darmstadt, Germany) or Riedel (Seelze, Germany).

*Preparation of FFA from menhaden oil.* FFA were prepared from menhaden oil according to the method of Wanasundara and Shahidi (16).

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*Preparation of n-3 FA concentrates from menhaden oil by urea complexation.* n-3 FA were separated from the hydrolyzed FA mixture of menhaden oil by urea–FA adduct formation according to the method of Wanasundara and Shahidi (16). The FA compositions of n-3 FA concentrates were determined using the GC procedure described in the following section.

*GC analysis of FA.* The FA compositions of substrates and reaction products were analyzed by GC. The samples were saponified using methanolic potassium hydroxide, and the FFA were methylated with boron trifluoride in methanol (17). The FAME were extracted with heptane, dried over anhydrous sodium sulfate, and analyzed by GC (Trace GC 2000 fitted with an FID; Thermo Quest, CE Instruments, Milan, Italy) on a DB-Wax capillary column with 30 m length, 0.32 mm i.d., and 0.25 μm film thickness (J&W Scientific Columns, Agilent Technologies, Folsom, CA). The injector and detector temperatures were held at 250 and 270°C, respectively. The initial temperature of the column was 180°C and was programmed to increase at a rate of 5°C/min to 225°C. Helium was the carrier gas, and the gas flow rate was adjusted to 1.5 mL/min. The individual FA esters were identified by comparing their retention times with standards obtained from Supelco.

*Acidolysis reaction.* The enzymatic acidolysis reaction mixture consisted of 30 mL of hexane, n-3 FA concentrate obtained from menhaden oil by urea complexation, and hazelnut oil at differing molar ratios (0.88 g hazelnut oil and 0.97 g menhaden oil concentrate at a 1:1 molar ratio). The amount of Novozym 435 was 10 wt% of reactants. Incubation was carried out at different water contents, at different temperatures, and for different time periods in an incubator with magnetic stirring at 400 rpm. The reaction was stopped by holding the samples at 90°C for 15 min.

*Analysis of product.* Reaction products were analyzed by TLC and column chromatography. Column chromatography was used to separate TAG fractions of the reaction products. A glass column (30 × 450 mm) and 0.5–1.0-mm particle size silica gel were used. Silica gel (2.5 g) was dried at 110°C for 3 h. After cooling to room temperature, it was mixed with hexane and poured into the column. Five milliliters of the reaction product was applied to the column. Hexane/diethyl ether (95:5, vol/vol) and hexane/diethyl ether (90:10, vol/vol) were used as the developing solvents. The flow rate of the eluting solvent was adjusted to 3 mL per minute. TAG fractions of the reaction products were collected from the column and confirmed by TLC analysis. In TLC, silica gel 60 plates were used and petroleum ether/ethyl ether/acetic acid (90:10:1, by vol) was used as the developing solvent. The bands were visualized after holding the plate in iodine vapor. Solvent from the TAG fractions collected from the column was removed in a rotary evaporator at 40°C, and the FA compositions of these fractions were analyzed by GC as described above.

*Experimental design.* A five-level central composite rotatable design was used for the RSM studies, and 35 experimental settings were generated with four factors (14). These factors were temperature, reaction time, substrate molar ratio, and water content. The ranges of settings for the variable factors were as follows: temperature, 40–55°C; reaction time, 18–54 h; substrate molar ratio, 2–4 mol/mol (hazelnut oil/menhaden oil concentrate); and water content, 1.5–4.5%. Including the extremes called for by the experimental design model, the ranges for those factors were 32.5–62.5°C, 0–72 h, 1–5 mol/mol, and 0–6%, respectively. The reaction conditions for each experimental setting are shown in Table 1. Experiments were run randomly. The regression analyses, statistical significance, and response surfaces were analyzed using STATISTICA 6.0 software (StatSoft® Inc., Tulsa, OK). Optimization of reaction conditions in terms of temperature, time, substrate/molar ratio, and water content was calculated by using the predictive equation from RSM.

## RESULTS AND DISCUSSION

*Concentration of n-3 FA from menhaden oil by urea complexation.* A concentrate of n-3 FA was obtained from menhaden oil FA by urea complexation, which is a technique frequently used for the elimination of saturated and monounsaturated FA (16). The FA compositions of the original menhaden oil and the n-3 FA concentrate are shown in Table 2. As a result of urea adduct formation, the saturated and monounsaturated FA in menhaden oil were eliminated, the EPA content was raised from 12.3 to 23.6%, and the DHA content was elevated from 13.5 to 42.1%. This n-3 FA concentrate was used to enrich hazelnut oil by enzymatic acidolysis.

*Statistical analysis of the effects of different reaction conditions on PUFA (EPA plus DHA) incorporation into hazelnut oil.* ANOVA was used to evaluate the significance of the relationship between linear and quadratic effects of dependent and independent variables, as well as their interactions (Table 3). A linear effect of temperature was found to be statistically significant ($P < 0.05$). Quadratic effects of time and linear effects of substrate molar ratio were also found to be statistically significant ($P < 0.01$).

The best-fitting quadratic model was determined for PUFA incorporation. The model equation can be written as follows:

$$\begin{aligned}\text{PUFA incorporation } \% = {} & 10.841 + 1.616 \cdot T - 1.217 \cdot T^2 + \\ & 0.108 \cdot t - 1.917 \cdot t^2 - 2.085 \cdot m + \\ & 0.744 \cdot m^2 + 0.548 \cdot s - 0.805 \cdot s^2 + \\ & 0.484 \cdot T \cdot t - 0.031 \cdot T \cdot m + 0.588 \cdot T \cdot s + \\ & 1.120 \cdot t \cdot m - 0.671 \cdot t \cdot s - 0.129 \cdot m \cdot s \end{aligned} \quad [1]$$

where $T$ is temperature, $t$ is reaction time, $m$ is substrate molar ratio, and $s$ is water content.

Predicted values according to the model and the observed responses from the experiments are shown in Figure 1. Predicted values were close to the observed responses, and a general linearity was obtained, which indicates that the model generated represents the actual relationships between reaction parameters.

*Impact of the interaction of parameters on PUFA incorporation.* According to the model equation, PUFA incorporation into hazelnut oil is affected by both first-order and second-order

**TABLE 1**
**Central Composite Rotatable Design Arrangement with Coded and Decoded Levels of Factors and Percentage of PUFA Incorporation into Hazelnut Oil**

| Experiment no. | Temperature | | Time | | Substrate molar ratio | | Water content | | Response |
|---|---|---|---|---|---|---|---|---|---|
| | Coded | Decoded | Coded | Decoded | Coded | Decoded | Coded | Decoded | % PUFA incorporation |
| 1 | −1 | 40 | −1 | 18 | −1 | 2 | −1 | 1.5 | ND[a] |
| 2 | +1 | 55 | −1 | 18 | −1 | 2 | −1 | 1.5 | 5.91 |
| 3 | −1 | 40 | +1 | 54 | −1 | 2 | −1 | 1.5 | 4.36 |
| 4 | +1 | 55 | +1 | 54 | −1 | 2 | −1 | 1.5 | ND |
| 5 | −1 | 40 | −1 | 18 | +1 | 4 | −1 | 1.5 | 1.31 |
| 6 | +1 | 55 | −1 | 18 | +1 | 4 | −1 | 1.5 | 1.82 |
| 7 | −1 | 40 | +1 | 54 | +1 | 4 | −1 | 1.5 | 1.38 |
| 8 | +1 | 55 | +1 | 54 | +1 | 4 | −1 | 1.5 | 4.16 |
| 9 | −1 | 40 | −1 | 18 | −1 | 2 | +1 | 4.5 | 8.17 |
| 10 | +1 | 55 | −1 | 18 | −1 | 2 | +1 | 4.5 | 7.56 |
| 11 | −1 | 40 | +1 | 54 | −1 | 2 | +1 | 4.5 | ND |
| 12 | +1 | 55 | +1 | 54 | −1 | 2 | +1 | 4.5 | 7.49 |
| 13 | −1 | 40 | −1 | 18 | +1 | 4 | +1 | 4.5 | 3.74 |
| 14 | +1 | 55 | −1 | 18 | +1 | 4 | +1 | 4.5 | 6.25 |
| 15 | −1 | 40 | +1 | 54 | +1 | 4 | +1 | 4.5 | 1.72 |
| 16 | +1 | 55 | +1 | 54 | +1 | 4 | +1 | 4.5 | 5.18 |
| 17 | −1 | 40 | 0 | 36 | 0 | 3 | 0 | 3 | 4.16 |
| 18 | +1 | 55 | 0 | 36 | 0 | 3 | 0 | 3 | 8.44 |
| 19 | 0 | 47.5 | −1 | 18 | 0 | 3 | 0 | 3 | 6.29 |
| 20 | 0 | 47.5 | +1 | 54 | 0 | 3 | 0 | 3 | 4.81 |
| 21 | 0 | 47.5 | 0 | 36 | −1 | 2 | 0 | 3 | 11.35 |
| 22 | 0 | 47.5 | 0 | 36 | +1 | 4 | 0 | 3 | 5.55 |
| 23 | 0 | 47.5 | 0 | 36 | 0 | 3 | −1 | 1.5 | 4.43 |
| 24 | 0 | 47.5 | 0 | 36 | 0 | 3 | +1 | 4.5 | 5.60 |
| 25 | +2 | 62.5 | 0 | 36 | 0 | 3 | 0 | 3 | 4.87 |
| 26 | −2 | 32.5 | 0 | 36 | 0 | 3 | 0 | 3 | 3.55 |
| 27 | 0 | 47.5 | +2 | 72 | 0 | 3 | 0 | 3 | 5.03 |
| 28 | 0 | 47.5 | −2 | 0 | 0 | 3 | 0 | 3 | ND |
| 29 | 0 | 47.5 | 0 | 36 | +2 | 5 | 0 | 3 | 4.85 |
| 30 | 0 | 47.5 | 0 | 36 | −2 | 1 | 0 | 3 | 11.65 |
| 31 | 0 | 47.5 | 0 | 36 | 0 | 3 | +2 | 6 | 3.98 |
| 32 | 0 | 47.5 | 0 | 36 | 0 | 3 | −2 | 0 | 5.36 |
| 33 | 0 | 47.5 | 0 | 36 | 0 | 3 | 0 | 3 | 6.70 |
| 34 | 0 | 47.5 | 0 | 36 | 0 | 3 | 0 | 3 | 7.19 |
| 35 | 0 | 47.5 | 0 | 36 | 0 | 3 | 0 | 3 | 4.38 |

[a]ND, not detected.

variables and parameter interactions. Because of the complexity of solving the model equation, and because too many solutions would be generated, the relationship between the responses and the parameters were examined by using contour plots. Six contour plots describing the interaction of four parameters on the incorporation of PUFA into hazelnut oil were obtained (Figs. 2A–F).

In Figure 2A, the interaction of temperature and reaction time on PUFA incorporation showed a maximal response surface. Both a decrease as well as an increase in temperature and time from the midpoint gradually decreased incorporation. The rate of decrease in the incorporation ratio was slower around the midpoint. As shown in Figures 2B and 2C, the interaction of water content with temperature and time showed the maximum response. PUFA incorporation decreased as the water content, temperature, and time increased or decreased from around the midpoints. The rate of decrease in PUFA incorporation was faster when moving around the midpoint.

The interactions of substrate molar ratio with temperature, time, and water content appeared as saddle surfaces (Figs. 2D–F). As shown in Figure 2D, PUFA incorporation decreased as the temperature increased or decreased from around the midpoint. Regarding the conditions valid within the experimental range, an increase in PUFA incorporation was positively correlated with the increase in temperature but was negatively correlated with the substrate molar ratio, until the midpoint of the response surface was reached. Around the midpoint, varying the substrate molar ratio had no effect on incorporation at any given temperature.

As shown in Figure 2E, longer or shorter reaction times gave lower PUFA incorporation ratios. Lower substrate molar ratios resulted in the incorporation of more PUFA into hazelnut oil. In Figure 2F, as the water content in the reaction medium decreased or increased from the midpoint levels in the surface, PUFA incorporation decreased at slow rates. Within the experimental ranges, at lower substrate molar ratios, PUFA incorporation decreased at high rates in the direction of increasing substrate molar ratio.

In the acidolysis reaction between hazelnut oil and the n-3 FA concentrate catalyzed by lipase, PUFA incorporation was

generally high at lower substrate molar ratios without any significant effects from temperature, reaction time, and water content. Moreover, in the interactions of temperature with time and water content, PUFA incorporation was greatest around the midpoint. Among all the experimental results, the highest incorporation ratio (21.5%) was obtained at 47.5°C, 36 h of reaction, 1:1 substrate molar ratio, and 3% water content (w/w). From the evaluation of response surface graphs showing the interactions of different factors such as temperature, reaction time, substrate molar ratio, and water content, the general optimal conditions were 45–60°C for temperature, 30–40 h for reaction time, 1:1–2:1 for substrate molar ratio (hazelnut oil/menhaden oil concentrate, mol/mol), and 3–5% (w/w) for water content for incorporation of long-chain n-3 PUFA into hazelnut oil.

In this study, increasing the temperature generally increased PUFA incorporation into the hazelnut oil, but above the optimal point incorporation decreased with an increase in temperature. It has been stated that enzyme activity and acyl migration are increased by an increase in temperature until a maximal rate is reached (15,18). According to Senanayake and Shahidi (8), this is because the temperature sensitivity of hydrogen bonds and other weak attractions hold the enzyme in its 3-D shape. In enzymatic modification reactions, increasing the temperature not only increases the enzyme activity but also improves the contact between the reaction substrate and enzyme as the viscosity of the substrate decreases (18).

**TABLE 2**
**FA Composition of Hazelnut Oil, Menhaden Oil, Its Concentrate Obtained by Urea Complexation and Hazelnut Oil Enriched with n-3 PUFA**

| FA | Hazelnut oil | Menhaden oil | Menhaden oil concentrate | Hazelnut oil enriched with n-3 PUFA[a] |
|---|---|---|---|---|
| 14:0 | ND[b] | 9.0 | 0.5 | ND |
| 16:0 | 5.2 | 16.7 | 0.3 | 4.4 |
| 16:1n-7 | 0.2 | 11.6 | 1.6 | 0.8 |
| 16:2n-4 | ND | 2.2 | 2.6 | ND |
| 16:3n-4 | ND | 1.5 | 3.8 | ND |
| 18:0 | 2.1 | 3.1 | 3.9 | 1.7 |
| 18:1n-9 | 82.3 | 6.8 | 0.2 | 63.3 |
| 18:1n-7 | ND | 3.2 | ND | ND |
| 18:2n-6 | 9.5 | 1.3 | 0.4 | 7.3 |
| 18:3n-4 | ND | 0.4 | 1.1 | ND |
| 18:3n-3 | 0.1 | 1.2 | 0.7 | 0.3 |
| 18:4n-3 | ND | 3.4 | 10.8 | 2.7 |
| 20:1n-9 | 0.1 | 1.1 | ND | ND |
| 20:4n-6 | ND | 0.8 | 0.9 | ND |
| 20:4n-3 | ND | 1.7 | 2.8 | 0.5 |
| 20:5n-3 | ND | 12.3 | 23.6 | 5.5 |
| 22:5n-3 | ND | 2.0 | 2.2 | ND |
| 22:6n-3 | ND | 13.5 | 42.1 | 11.4 |

[a]Reaction conditions for incorporation: 47.5°C temperature, 36 h of reaction time, 2:1 substrate molar ratio, and 3% water content (w/w).
[b]ND, not detected.

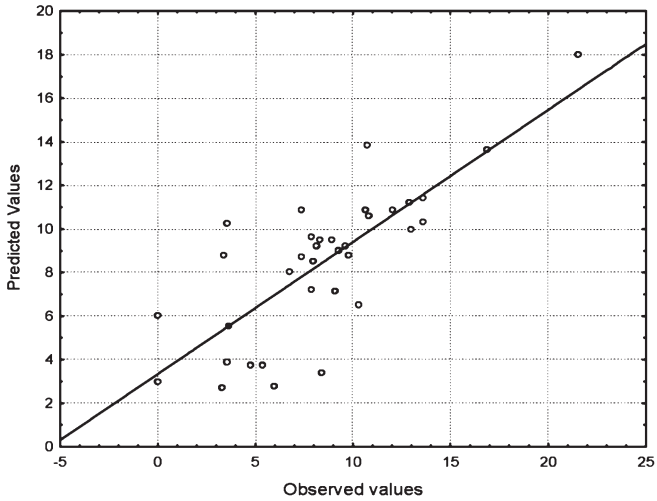


**FIG. 1.** Plot indicating the relationship between the PUFA incorporation ratios predicted by the model and the observed values obtained by the experimental results.

**TABLE 3**
**ANOVA Results**

| Factor | Sum of squares | Degrees of freedom | Mean squares | *F* | *P* |
|---|---|---|---|---|---|
| Temperature (L) | 67.9108 | 1 | 67.9108 | 5.1225 | 0.0349[a] |
| Temperature (Q) | 43.3874 | 1 | 43.3874 | 3.2727 | 0.0855 |
| Time (L) | 0.3015 | 1 | 0.3015 | 0.0227 | 0.8816 |
| Time (Q) | 107.7483 | 1 | 107.7483 | 8.1274 | 0.0099[b] |
| Substrate molar ratio (L) | 113.0279 | 1 | 113.0279 | 8.5256 | 0.0085[b] |
| Substrate molar ratio (Q) | 16.2163 | 1 | 16.2163 | 1.2232 | 0.2819 |
| Water content (L) | 7.8101 | 1 | 7.8101 | 0.5891 | 0.4517 |
| Water content (Q) | 18.9734 | 1 | 18.9734 | 1.4312 | 0.2456 |
| Temperature × time | 3.7442 | 1 | 3.7442 | 0.2824 | 0.6010 |
| Temperature × substrate molar ratio | 0.0156 | 1 | 0.0156 | 0.0012 | 0.9730 |
| Temperature × water content | 5.5225 | 1 | 5.5225 | 0.4166 | 0.5260 |
| Time × substrate molar ratio | 20.0704 | 1 | 20.0704 | 1.5139 | 0.2328 |
| Time × water content | 7.2092 | 1 | 7.2092 | 0.5438 | 0.4694 |
| Substrate molar ratio × water content | 0.2652 | 1 | 0.2652 | 0.0200 | 0.8889 |
| Error | 265.1481 | 20 | 13.2574 | | |
| Total sum of squares | 673.3015 | 34 | | | |

[a]Statistically significant ($P < 0.05$).
[n]Statistically significant ($P < 0.01$). L, linear; Q, quadratic.

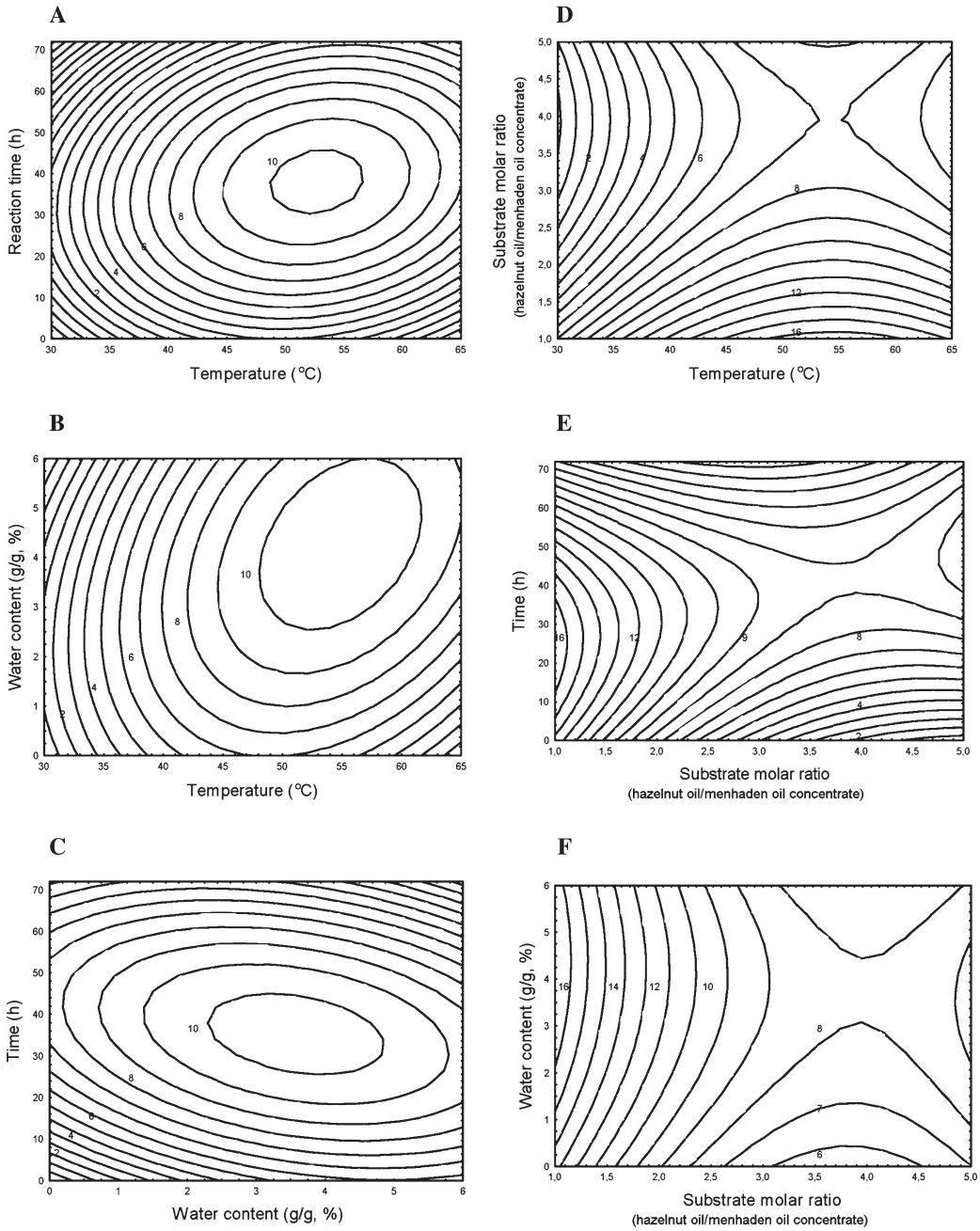


**FIG. 2.** Contour plots for the incorporation of PUFA into hazelnut oil. (A) Reaction time and temperature; (B) water content and temperature; (C) time and water content; (D) substrate molar ratio and temperature; (E) time and substrate molar ratio; (F) water content and substrate molar ratio.

Observing the progress of enzymatic reactions is useful in determining the shortest time necessary to obtain the highest incorporation of EFA and to reduce production costs (8,9). In this study, the optimal incorporation occurred at around 30–40 h. On the other hand, in similar studies the optimal reaction time changed depending on the enzyme and substrate used (8,9). In incorporating DHA into borage oil using *C. antarctica* lipase as the biocatalyst, Senanayake and Shahidi (8) reported that DHA incorporation increased as the incubation time increased up to 24 h, whereas Akoh and Moussata (9) reported 40 h as the optimal reaction time for the incorporation of EPA and capric acid into borage oil.

In the enzymatic synthesis of structured lipids, substrate molar ratios can be adjusted according to the desired amount of EFA in the product. In this study, the incorporation of n-3 FA into hazelnut oil increased by increasing the molar ratio of n-3 FA, without any significant effect from other factors. Other authors have also stated that the incorporation ratio increased with an increase in substrate molar ratio when incorporating n-3 FA into seed oils (8–10). The incorporation rate can be improved by choosing higher substrate molar ratios and the reaction time can be shortened, but Jennings and Akoh (11) pointed out that using excess amounts of EFA may result in lipase inhibition and a decrease in product yield

while removing excess FA when the modification is carried out on a large scale.

In lipase-catalyzed reactions, both hydrolysis and esterification occur. The equilibrium is affected by the amount of water in the reaction mixture, as water is one of the products of the hydrolysis reaction (19). An excess amount of water would lead to hydrolysis instead of synthesis and would decrease the product yield. Thus, the amount of water should be adjusted to maximize the reaction rate. Additionally, water content is very critical in maintaining enzyme structure and stability (15). In incorporating n-3 FA into vegetable and seed oils using *C. antarctica* lipase as the biocatalyst, excess water has been found to decrease the incorporation ratio (6,9).

*Validation of the model and optimization*. Based on the RSM studies, a new set of experiments was carried out at the optimized conditions predicted by the model. We observed that, at optimal conditions, the product contained 19.6% n-3 PUFA as compared with the predicted maximum of 18.0%. The verification results indicated that the predicted values from these models were reasonably close to the observed values. Therefore, when experiments were conducted at a temperature condition of 47.5°C, a reaction time of 36 h, a substrate molar ratio of 1:1, and a water content of 3%, a structured lipid containing 19.6% PUFA could be obtained.

In a similar study, Rao *et al.* (7) used RSM to optimize reaction conditions in the modification of coconut oil TAG using lipase-catalyzed acidolysis in hexane to incorporate n-3 or n-6 PUFA. They stated that the optimal conditions generated from their models indicated that maximal incorporation of n-3 PUFA occurred at a 1:4 molar ratio of TAG/FFA when incubation was carried out for 34 h at 54°C. In another study, Cerdán *et al.* (19) aimed to model the synthesis of PUFA-enriched TAG by lipase-catalyzed esterification. They pointed out that the optimal reaction conditions were 60°C, 0.5% (vol/vol) water, 24 h of reaction, and a glycerol/FA molar ratio of 1:3.

In this study, we showed for the first time that hazelnut oil can be modified to incorporate PUFA enzymatically; we efficiently increased the n-3 PUFA content of hazelnut oil. This structured lipid containing PUFA may have many health benefits and can be used for the prevention of some cardiovascular diseases, inflammations, and rheumatoid arthritis, since it contains desirable functional FA. However, health and nutritional benefits of the modified oil should be investigated by clinical studies. Future research should concentrate on increasing the amount of enzyme to decrease the reaction times.

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