Food Production, Processing and Nutrition

RESEARCH Open Access

Mono- and dioleyl *p*-coumarate phenolipids and their antioxidant activity in a muscle food model system

Jiankang Wang[1*], Xiaojia Hu[1], Yan Huang[2], Ye Zou[2], Linxiao Han[1], Daoying Wang[2] and Fereidoon Shahidi[3*]

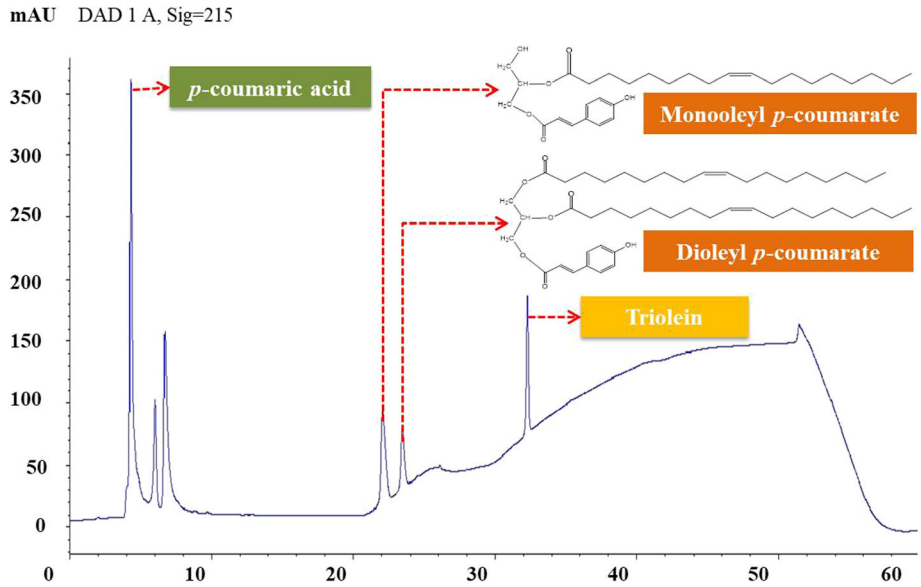
Abstract

Response surface methodology (RSM) was used to optimize the degree of esterification of *p*-coumaric acid to triolein via lipase-catalyzed acidolysis, and enzyme load, reaction time and mole ratio of substrates were selected as variables in the experimental design. The results showed that the model employed was highly sufficient for determining the effectiveness and interaction of three selected variables, enzyme load, reaction time and the mole ratio of substrates, on the dependent variable, the degree of esterification. Although the optimization point was not found in the selected range of the three variables, the steepest ascent analysis suggested that an increase of these three variables might lead to a stationary point. However, based on the limitations on increasing the range of tested variables, including possible oxidation of synthesized lipids and increased cost, the degree of esterification so yielded in the designed central composite design should be the one closest to the possible ideal optimized degree. The *p*-coumarates so produced exhibited varying antioxidant performance in the tested muscle food model, which could be explained by their different lipophilicity. Moreover, the potential health benefits of synthesized phenolic lipids have been discussed.

Keywords: Response surface methodology, Acidolysis, *P*-coumaric acid, Monooleyl *p*-coumarates, Dioleyl *p*-coumarates, Antioxidant activity

* Correspondence: rjiankangwang@outlook.com; fshahidi@mun.ca
[1]School of Food and Biological Engineering, Shaanxi University of Science and Technology, Xi'an 710021, People's Republic of China
[3]Department of Biochemistry, Memorial University of Newfoundland, St. John's, NL A1C 5S7, Canada
Full list of author information is available at the end of the article

Graphical Abstract



Introduction

It was well elucidated that dietary intake of phenolic compounds from plant sources leads to various health benefits (Kishida & Matsumoto, 2019). Investigations on the antioxidative efficacy of phenolic antioxidants, such phenolic acids, in foods have been widely reported, and most of them have proven to reduce the extent of lipid oxidation. Among phenolic acids, *p*-coumaric acid occurs in various food sources and beverages, including tomatoes, carrots, garlic as well as wines and vinegars (Ferguson et al. 2005). Its strong antioxidant property, anti-virus, anti-rheumatoid arthritis, anti-angiogenesis, and anti-cancer effects have been reported in a large body of literature (Wang & Shahidi 2014; Zhu et al. 2018; Tanida et al. 2015; Zhang et al. 2018). Acidolysis is a structural modification process, which incorporates desired fatty acids or other forms of organic acids, such as phenolic acids, into triacylglycerols by using enzyme catalysts (Ramadan 2021a; Ramadan 2021b; Senanayake & Shahidi 2002; Wang et al. 2020; Wang et al. 2021). For example, acidolysis of phenolic acids and triacylglycerols has been successfully conducted and reported in several studies (Sabally et al. 2006; Safari et al. 2006). Esterification of phenolic acids will change their chemical and biological properties due to steric hindrance exerted by esterified bulky moieties, decreased hydrogen atom donation tendency caused by interference of the formed ester bonds, decreased ionisation potential from added bulky moieties and an improved lipophilicity with fatty acid chains (Wang & Shahidi 2014).

In this work, a model study using *p*-coumaric acid and triolein was used to investigate the effects of various experimental conditions on the degree of acidolysis of triolein with *p*-coumaric acid via lipase-catalyzed reactions. To determine the optimum conditions for esterification of *p*-coumaric acid with triolein, response surface methodology (RSM) was used in this study. Based on the reported studies and the results from preliminary experiments, enzyme load, reaction time and mole ratio of substrates were selected as variables in the experimental design. The effects of these three experimental conditions on the degree of *p*-coumaric acid esterification as well as the interaction among them were examined in this study. In addition, the predicted conditions to optimize the degree of esterification were also considered and discussed. In order to obtain a better understanding of their antioxidant potential in food, antioxidant activity of the synthesized phenolipids was investigated in a muscle food model system. The potential of their biological function was also discussed regarding their future potential application in functional foods and pharmaceutical products for possible disease risk reduction and treatments.

Materials and methods

Materials

Triolein was purchased from Nu-Chek (Elysian, MN, USA). Novozyme 435 and *p*-coumaric acid were obtained from Sigma-Aldrich (Mississauga, ON, Canada). Fresh ground lean pork was purchased from a local supermarket. All solvents used were of analytical grade

and purchased from Fisher Scientific (Nepean, ON, Canada).

Experimental design for RSM

Response surface methodology (RSM) is a collection of mathematical and statistical techniques, which has been used for empirical model building to optimize a response influenced by several independent variables. The design of this experiment was generated with a three factor, three level central composite face-centred cube design using RSM. The design was composed of 17 experiments with 3 centre points 8 corner points and 6 facial points. Seventeen runs were performed in a totally random order for minimizing the effects of unexpected variability in the observed responses. The response is the degree of esterification of *p*-coumaric acid after acidolysis. Enzyme load (5–15 wt%), reaction time (144–288 h) and mole ratio of substrates (triolein to *p*-coumaric acid, 2:1–16:1) were chosen as the three tested variables. In the preliminary study, the increase of conversion rate continued to 288 h of reaction, which suggested that a longer reaction period was required to reach the peak conversion rate. However, as an unsaturated fatty acid, triolein is oxidizable and generates oxidation products during the reaction, and the level of oxidation increases as the reaction time is prolonged. Therefore, 288 h was selected for the longest reaction course in order to minimize the oxidation of this unsaturated oil. Details of the central composite face-centred (CCF) design are shown in Tables 1 and 2.

The degree of esterification of *p*-coumaric acid to triolein was modeled from the experimental data using a second-order polynomial regression equation (Eq. 1).

$$Y = \beta_0 + \sum_{i=1}^{3}\beta_i X_i + \sum_{i=1}^{3}\beta_{ii}X_i^2 + \sum_{i<j=1}^{3} B_{ii}X_iX_j \quad (1)$$

Where Y is the predicted response variable (the degree of esterification), β_0, is the intercept, β_i is the first-order linear effect, β_{ii} is the interaction terms, β_{ij} is the squared effect, and X_i and X_i are the independent variables, including enzyme load, reaction time and mole ratio of substrates.

Table 1 Independent variables in the experimental design

Variables	Coded levels		
	−1	0	1
Enzyme load (wt%)	5	10	15
Reaction time (h)	144	216	288
Mole ratio of substrates (triolein to *p*-coumaric acid)	2	9	16

Table 2 The central composite design matrix employed for the three independent variables

Run	Variables		
	X_1	X_2	X_3
1[c]	0	0	0
2	1	1	1
3	−1	1	−1
4	0	0	−1
5[c]	0	0	0
6	1	−1	−1
7	−1	1	1
8	1	1	−1
9	0	0	1
10	−1	−1	1
11	1	0	0
12	0	1	0
13	1	−1	1
14	0	−1	0
15[c]	0	0	0
16	−1	−1	−1
17	−1	0	0

Note: X_1: enzyme load; X_2: reaction time; X_3: mole ratio of substrates
[c]: centre points; actual values of the independent variables seen in Table 1.

Equation 2 was used to calculate the degree of esterification after acidolysis.

$$\text{Degree of esterification}\ (\%) = 100{*}A_f/A_i \quad (2)$$

Where A_i is peak area of *p*-coumaric acid in the initial reaction mixture and A_f is total peak area of synthesized phenolic lipids including mono- and dioleyl *p*-coumarates in the final reaction mixture.

The statistical software *JMP* version 6.0.0 (SAS Institute Inc. Cary, NC, USA) was used to generate the experimental design and analyze the experimental data.

Acidolysis reaction

The lipase-catalyzed acidolysis of *p*-coumaric acid with triolein was carried out following the method reported by Safari et al. (2006) and Sabally et al. (2006). A stock solution of *p*-coumaric acid (10 mM) was freshly prepared in 2-butanone, and three stock solutions of triolein (6.67, 30, 53.36 mM) were prepared in hexane. Subsequently, 1 mL of *p*-coumaric acid stock solution and 3 mL of triolein stock solution were transferred to a 20-mL screwed-capped tube followed by the addition of different amounts of immobilized Novozyme 435 accordingly based on the generated experimental design.

The final reaction solutions contained 2.5 mM *p*-coumaric acid and a triolein solution of 5, 22.5 and 40 mM, according to the three different mole ratios of substrates. All tubes were flushed with nitrogen and sealed properly. The acidolysis reaction was performed in an orbital shaking water bath at 50 °C and 170 rpm. Reaction mixtures were removed from the water bath at different times according to the experimental design. The reaction mixture (0.1 mL) was transferred to a 4-mL vial, and the solvent was then removed under a stream of nitrogen. The sample was redissolved in 2 mL of solvent mixture consisting of methanol-acetonitrile (41:59, v/v) for high performance liquid chromatography-mass spectrometry (HPLC-MS) analysis. All reactions were run in duplicate.

HPLC-MS analysis

The composition of the reaction mixture was determined by reversed phase HPLC-MS according to a method described by Wang and Shahidi (2014). An Agilent 1100 HPLC unit (Agilent Technologies, Palo Alto, CA, USA) with a UV–diode array detector (UV-DAD) was used. Separation of different components in the sample was achieved on a C-18 column (4.6 mm × 250 mm coupled with a guard column, Agilent), using an isocratic elution with a mature of methanol-acetonitrile (41:59, v/v).

Purification and identification of mono- and dioleyl *p*-coumarates

Separation of components, especially mono- and dioleyl *p*-coumarates in the final acidolysis mixture was achieved using flash column chromatography according to a method described by Wang and Shahidi (2014). The mixture was eluted on a silica column (40 cm × 5 cm i.d.) packed with silica gel (Selecto Scientific, Suwannee, GA, USA) with a series of solvent mixtures.

Antioxidant activity in a muscle food model system

The inhibition effect of *p*-coumaric acid, monooleyl *p*-coumarate and dioleyl *p*-coumarate on the production of thiobarbituric acid reactive substances (TBARS) in cooked pork was determined according to Shahidi and Alexander (1998). Fresh ground pork (40 g) was mixed with 10 mL of distilled water in Mason jars. Test compounds dissolved in ethanol were added separately to the meat samples at a level of 80 μmol/kg (13 ppm of *p*-coumaric equivalent), and then the contents were thoroughly mixed with a glass rod. A control containing no test compounds was also prepared. Samples were cooked in a thermostated water bath at 80 °C for 40 min with intermittent stirring. The cooked meat was removed from the water bath and cooled to room temperature, and then samples were homogenized with a Polytron PT 3000 (Brinkmann Instruments, Rexdale, ON, Canada) homogenizer. The homogenized samples were transferred into plastic bags and stored at 4 °C for 14 days. The samples were taken on day 0, 3, 5, 7, and 14 for the measurement of secondary oxidation products in terms of TBARS formation according to the method described by Shahidi and Pegg (1994). Two grams of meat sample were weighed into a 50 mL centrifuge tube followed by addition of 5 mL TCA solution (10%, w/v) and 5 mL of 2-thiobarbituric acid (TBA) solution (0.02 M) in water, and then the contents were vortexed for 2 min. The samples were subsequently centrifuged at 3000×g for 10 min, and the supernatants were filtered through a Whatman No. 3 filter paper and collected in 20 mL screw-capped tubes. Samples were kept in a boiling water bath for 45 min, and then removed from the water bath and cooled to room temperature under running tap water. The absorbance of the resultant pink-coloured chromogen was read at 532 nm using a Hewlett-Packard model 845LA diode array spectrophotometer (Agilent, Palo Alto, CA, USA). A standard curve was prepared using 1,1,3,3-tetramethoxypropane as a precursor of malondialdehyde (MDA). The TBARS values of samples were calculated using the standard curve and expressed as μmol MDA equivalents per kg of sample.

Results and discussion

Identification of components from the acidolysis mixtures

The HLPC profiles were monitored by UV at both 215 and 300 nm (Figs. 1 and 2). Different components from the initial and final acidolysis mixtures are shown in Fig. 1. Each compound was identified by comparing its retention time with that of its corresponding standard and/or the characterization of molecular ion so generated from mass spectrometry. The compounds in peaks C and F were identified as *p*-coumaric acid derivatives as only those compounds with a *p*-coumaric acid moiety give an absorbance at 300 nm while other compounds from the final acidolysis mixture, including oleic acid, diolein, triolein are not detected at this wavelength. The major compounds in peaks C (t_R, 6.0 min) and peak F (t_R, 23.7 min) were identified as monooleyl *p*-coumarate and dioleyl *p*-coumarate, respectively, based on the fragmentation pattern and molecular ions yielded (Fig. 3). For monooleyl *p*-coumarate, the major molecular ion generated during fragmentation in peak C showed a m/z at 485.5, representing $[M + H - H_2O]^+$ of monooleyl *p*-coumarate, might have resulted from the loss of a H_2O and gain of a proton. For dioleyl *p*-coumarate, the abundant molecular ion yielded in peak F showed a m/z at 765.7, representing $[M - H]^-$, which resulted from the loss of a proton.

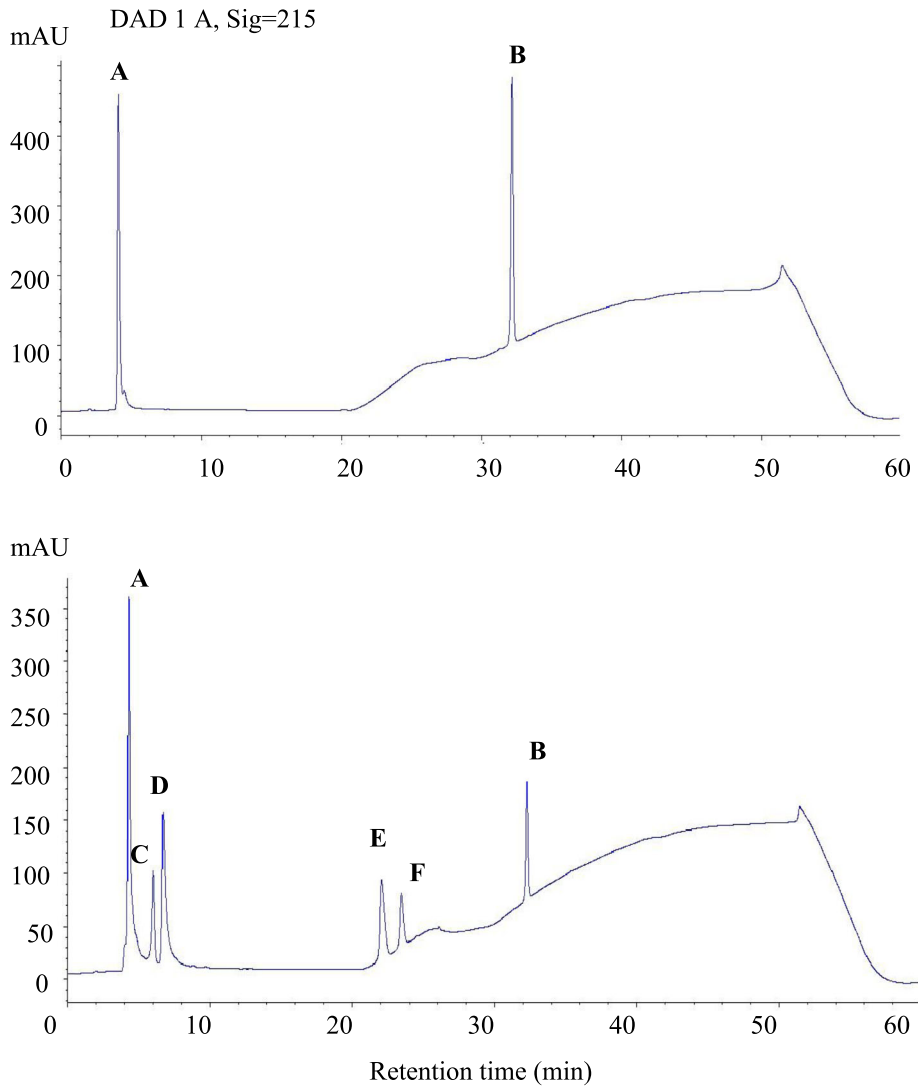


Fig. 1 High-performance liquid chromatography (HPLC) chromatograms of starting materials (top) and resultants (bottom) of acidolysis of *p*-coumaric acid and triolein at 215 nm. **A**: *p*-coumaric acid; **B**: triolein; **C**: monooleyl *p*-coumarate; **D**: oleic acid; **E**: diolein; and **F**: dioleyl *p*-coumarate

Estimated model

The model coefficients were generated with JMP software based on the measured responses given in Table 3. Based on the results (Table 3) of the experiments, the following second order polynomial equation (Eq. 3) giving the degree of *p*-coumaric acid esterification as a function of enzyme load, reaction time and mole ratio of substrates of the acidolysis reaction was obtained.

$$Y = 3.17 + 0.79X_1 + 1.21X_2 + 1.18X_3 + 0.39X_1X_2 + 0.48X_1X_3 + 0.34X_2X_3 - 0.35{X_1}^2 - 0.49{X_2}^2 - 0.26{X_3}^2 \quad (3)$$

The coefficient of determination, R^2 was 0.92, which indicates that the sample variation of 92.00% for the degree of esterification is attributed to three selected independent variables, including enzyme load, reaction time and mole ratio of the substrates. The closer the value of R^2 to 1, the better the model fits the experimental data, while the smaller value of R^2, the lesser will be the relevance of the dependent variable for explaining the behaviour of variations in the model (Cao et al., 2008; Tan & Shahidi 2012). Therefore, a R^2 of 0.92 indicates a strong fit between the model and the experimental data. The high significance of the model used was also supported by adjusted R^2, which was 0.95. Thus, both R^2 and adjusted R^2 provide a correspondence of the second order polynomial model to the obtained experimental data. Further, statistical testing of the regression model has been done by analysis of variance (ANOVA), which is also used to analyze the significance of the

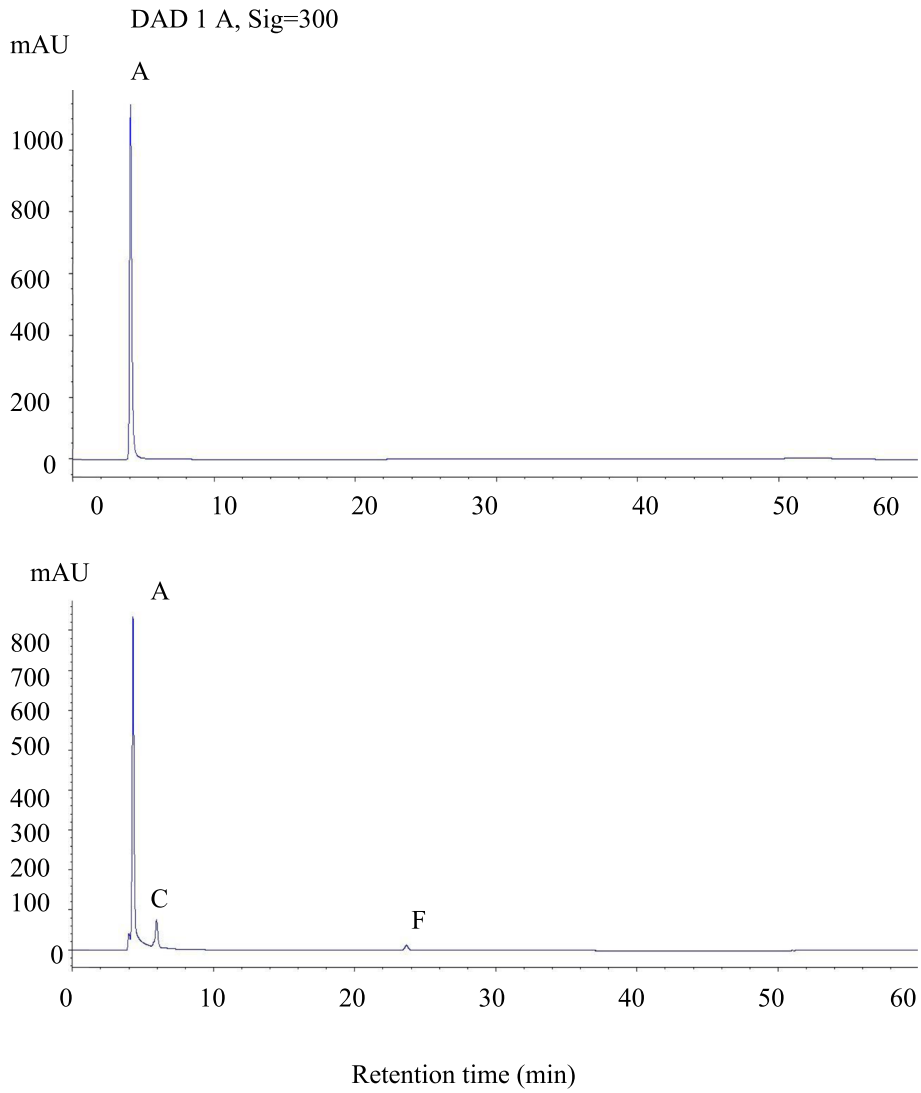


Fig. 2 High-performance liquid chromatography (HPLC) chromatograms of starting materials (top) and resultants (bottom) of acidolysis *p*-coumaric acid and triolein at 300 nm. **A**: *p*-coumaric acid; **C**: monooleyl *p*-coumarate; and **F**: dioleyl *p*-coumarate

model. In this analysis, the F value indicates the ratio of the mean square regression to the mean square due residuals. The higher the F value is, the greater the variation accounted for the model is compared to the unexplained variation. The ANOVA of the regression model revealed that the model is highly significant, with a high F value of 64.0479 and a small P value (< 0.0001). In addition, each of the observed actual response values were compared with the adequate predicted response values in the parity plot (Fig. 4), which shows an acceptable level of agreement (Peričin et al. 2009).

The significance of each coefficient was assessed by Student's t test and P test (Table 4). The significance of corresponding coefficient was assessed with their correspondent t values and P values as they negatively correlated with P values and positively correlated with t values (Cao et al. 2008). The significance of the coefficient of the first order effects of three independent variables on the response, the degree of esterification of *p*-coumaric acid, can be explained by comparing either their corresponding P values or t values. Small P values (< 0.0001) indicate that significant coefficients of all three variables, enzyme load, reaction time and mole ratio of the substrates, which implies the significant influence of the variables on the investigated response. Furthermore, the magnitudes of the t values give the significance of their first order coefficient in a descending order, from reaction time, mole ratio of the substrates to enzyme load, which means the reaction time has the most influence, and enzyme load has the least effect. In addition, the results of the analysis on the quadratic effects of three variables showed that the coefficients of

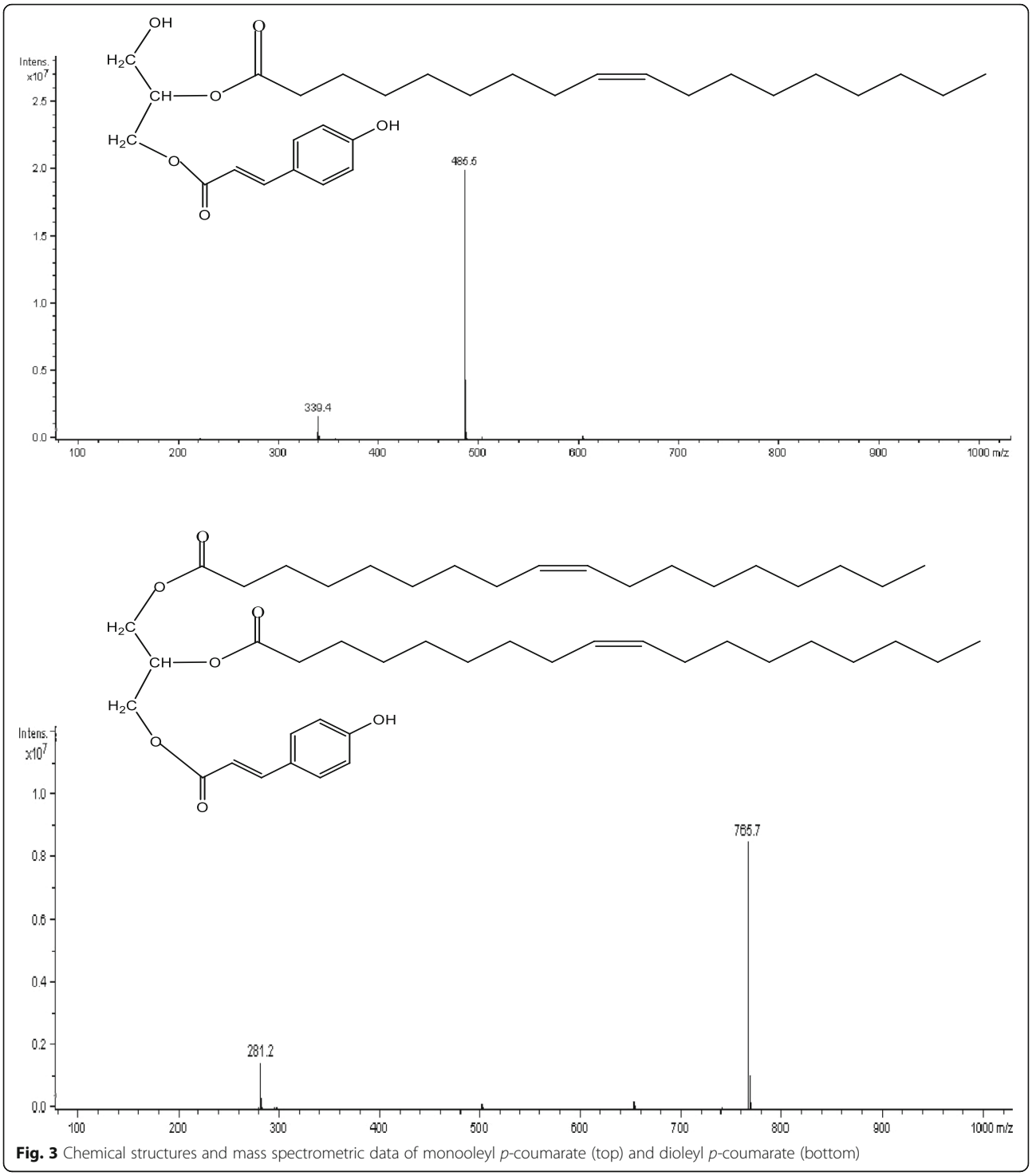


Fig. 3 Chemical structures and mass spectrometric data of monooleyl *p*-coumarate (top) and dioleyl *p*-coumarate (bottom)

enzyme load and reaction time are significant as small *P* values (< 0.05) occurred. The *P* value of 0.1267 from the third independent variable, i.e., the mole ratio of substrates, indicates an insignificant corresponding coefficient of quadratic effect of this variable.

Based on the analysis results (Table 4) of the cross-product, the significance of coefficient of the interactions between each pair of tested variables on the determined response were confirmed with smaller *P* values, all of which were lower than 0.002, and greater *t* value, which were greater than 3.5. For instance, the smallest *P* value, < 0.0001, and the largest *t* value, 4.96, from the cross-product of enzyme load and substrate ratio, indicate the most significant coefficient of the interaction between

Table 3 Central composite design and response for the enzymatic synthesis of mono- and dioleyl *p*-coumarates

Run	Pattern	Variables			Response (*Y*) degree of incorporation
		X_1 (wt%)	X_2 (h)	X_3 (ratio)	
1[c]	0 0 0	10	216	9	2.85 ± 0.11
2	+ + +	15	288	16	6.43 ± 0.40
3	− + −	5	288	2	1.25 ± 0.12
4	0 0 a	10	216	2	1.66 ± 0.29
5[c]	0 0 0	10	216	9	3.32 ± 0.08
6	+ − −	15	144	2	0.09 ± 0.04
7	− + +	5	288	16	2.93 ± 0.75
8	+ + −	15	288	2	2.23 ± 0.13
9	0 0 A	10	216	16	4.40 ± 0.67
10	− − +	5	144	16	0.98 ± 0.01
11	A 0 0	15	216	9	4.00 ± 0.34
12	0 A 0	10	288	9	4.13 ± 0.34
13	+ − +	15	144	16	2.31 ± 0.32
14	0 a 0	10	144	9	1.46 ± 0.28
15[c]	0 0 0	10	216	9	2.87 ± 0.07
16	−	5	144	2	0.04 ± 0.01
17	a 0 0	5	216	9	1.87 ± 0.45

Note: pattern identifies the coding of the factors. It shows all the codings with "+" for high, "-" for low factor, "a" and "A" for low and high axial values, and "0" for midrange; X_1: enzyme load; X_2: reaction time; X_3: mole ratio of substrates; and [c]: centre points

the enzyme load and mole ratio of the substrates. The less significant coefficient from the other two cross interactions are attested by their larger *P* values and smaller *t* values. Such effects are shown graphically in contour plots (Fig. 5).

Canonical analysis and steepest ascent analysis

The results from canonical analysis showed that the stationary point (a saddle point) predicted was located outside the range of the parameter space employed in the design of optimization, which indicates that the optimization conditions were not in the selected ranges of the three variables. In this situation, the directional search method, steepest ascent analysis was employed to determine the direction toward predictive higher responses with the use of the magnitude and sign of the linear effects in this study (Chen et al. 2002). In steepest ascent analysis, the path begins at the centre of the current design space and stretches well outside the design space. A sequence of equally spaced locations along the path is then normally selected and used to generate a new set of optimization experiments. Based on the results of steepest ascent analysis, the path of steepest ascent was to increase all of the three variables, enzyme load, reaction time and the mole ratio of substrates in order to improve the degree of esterification. However, there are limitations on options of ranges of selected

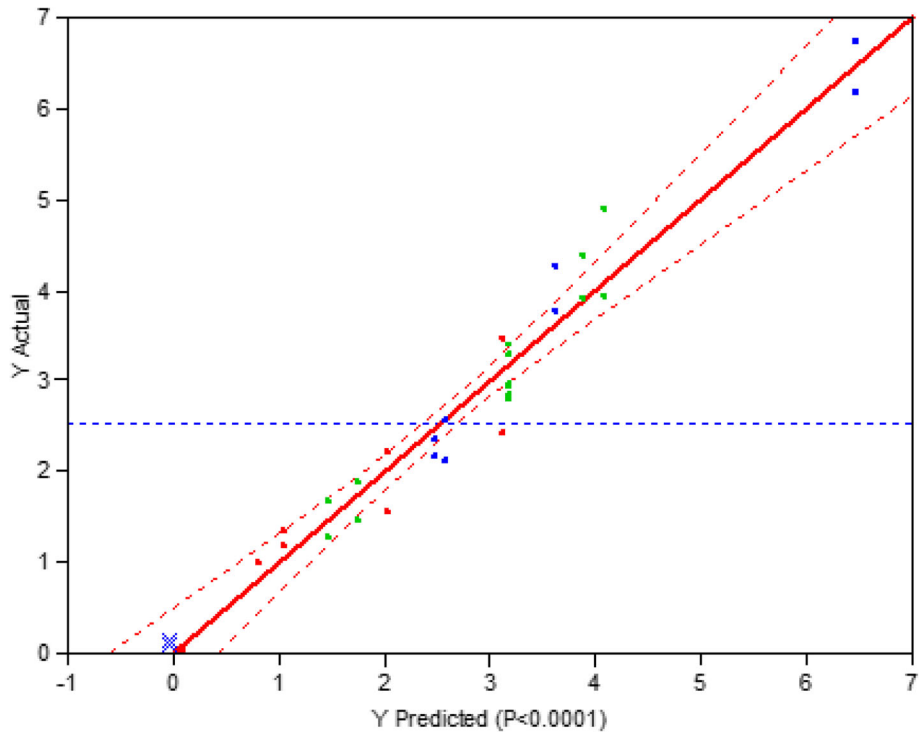


Fig. 4 Parity plot of actual response values and predicted response values; Y: response (the degree of esterification)

Table 4 Estimated coefficients and corresponding *t* and *P* values

Term	Coefficient	Std error	*t* Test	*P* value
Intercept	3.17	0.12	27.34	< 0.0001
Linear				
Enzyme load	0.80	0.09	9.35	< 0.0001
Reaction time	1.21	0.09	14.11	< 0.0001
Substrate ratio	1.18	0.09	13.76	< 0.0001
Cross-product				
Enzyme load * reaction time	0.39	0.10	4.06	0.0005
Enzyme load * Substrate ratio	0.48	0.10	4.96	< 0.0001
Reaction time * Substrate ratio	0.34	0.10	3.52	0.0017
Quadratic				
Enzyme load * Enzyme load	−0.35	0.17	−2.13	0.0440
Reaction time * Reaction time	−0.49	0.17	−2.99	0.0064
Substrate ratio * Substrate ratio	−0.26	0.17	−1.58	0.1267

variables due to the chemical quality and cost controls. For instance, the level of oxidation and concentration of oxidation products are positively correlated with the time course of storage under high temperature (Neff & Byrdwell 1998; Nevdakh et al. 1984). Therefore, the reaction time exceeding the used maximum reaction time course, 288 h, in the experiment should be the avoided. The maximum enzyme dose should not exceed 15% (wt) of the total substrate in order to control the cost in the industry, so the design of experiments regarding the use of enzyme should not be adjusted to the path shown in steepest ascent analysis to achieve a higher degree of esterification by compromising the quality of the lipids and increasing the cost. Therefore, the degree of esterification so yielded in the central composite design should be the closest value to the possible ideal optimized degree, and the maximum values of the three variables for the synthesis of *p*-coumaric acid with triolein is recommended.

Antioxidant activity in muscle food system

Phenolic compounds can be assessed in high surface-to-volume ratio systems such as the lipids in processed whole tissue foods (Porter 1993). Other than being one of the major components of oil-in-water emulsions such as salad dressing and spreads, lipids are also incorporated into processed whole tissue foods such as sausages and hams. Lipids, especially those containing high levels of unsaturated fatty acids, undergo oxidation when exposed to oxygen and generate oxidation products; therefore, lipid components are responsible for deterioration of processed whole tissue foods. Thus, lipid components require effective antioxidants to prevent their oxidation in lipid rich muscle foods as they are more vulnerable to oxidation during precooking and long storage time under high surface-to-volume conditions (Porter 1993). Thus, the inhibitory capacity of antioxidants in thermally processed whole or modified muscle foods, which are frequently associated with rapid lipid oxidation and development of "warmed-over flavour", is better evaluated (Jayathilakan et al. 2007; Lu et al. 2020; Shahidi et al. 1987). The strong inhibitory effects of epigallocatechin gallate (EGCG), the major phenolic compound from green tea, and its lipid derivatives synthesized from corresponding polyunsaturated fatty acids and phenolic extracts of millet on lipid oxidation in meat model systems have been demonstrated by Zhong and Shahidi (2012). In addition, resveratrol and its ester has also been proven to be highly effective antioxidants in the same model system (Oh & Shahidi 2018).

In this work, the inhibitory effect of *p*-coumaric acid and its lipid derivatives, mono- and dioleyl *p*-coumarates, on the production of thiobarbituric acid reactive substances (TBARS), the secondary products of lipid oxidation, in cooked ground pork was measured and expressed as μmol MDA equivalents per kg of sample. The results summarized in Fig. 6 show that TBARS values of all test samples increased throughout the experimental period. The TBARS values of the sample group with added *p*-coumaric acid were significantly lower ($P < 0.05$) than the control group at all test points, which indicated effective inhibitory effects of *p*-coumaric acid on lipid oxidation during the entire storage period. Monooleyl *p*-coumarate showed a slight prooxidative effect on its sample group as its TBARS value was significantly higher than that of the control group at day 3 test point while the TBARS values were similar to that of the control group at other test points. Dioleyl *p*-coumarate performed as a weak antioxidant against lipid oxidation as the TBARS values of its group were significantly

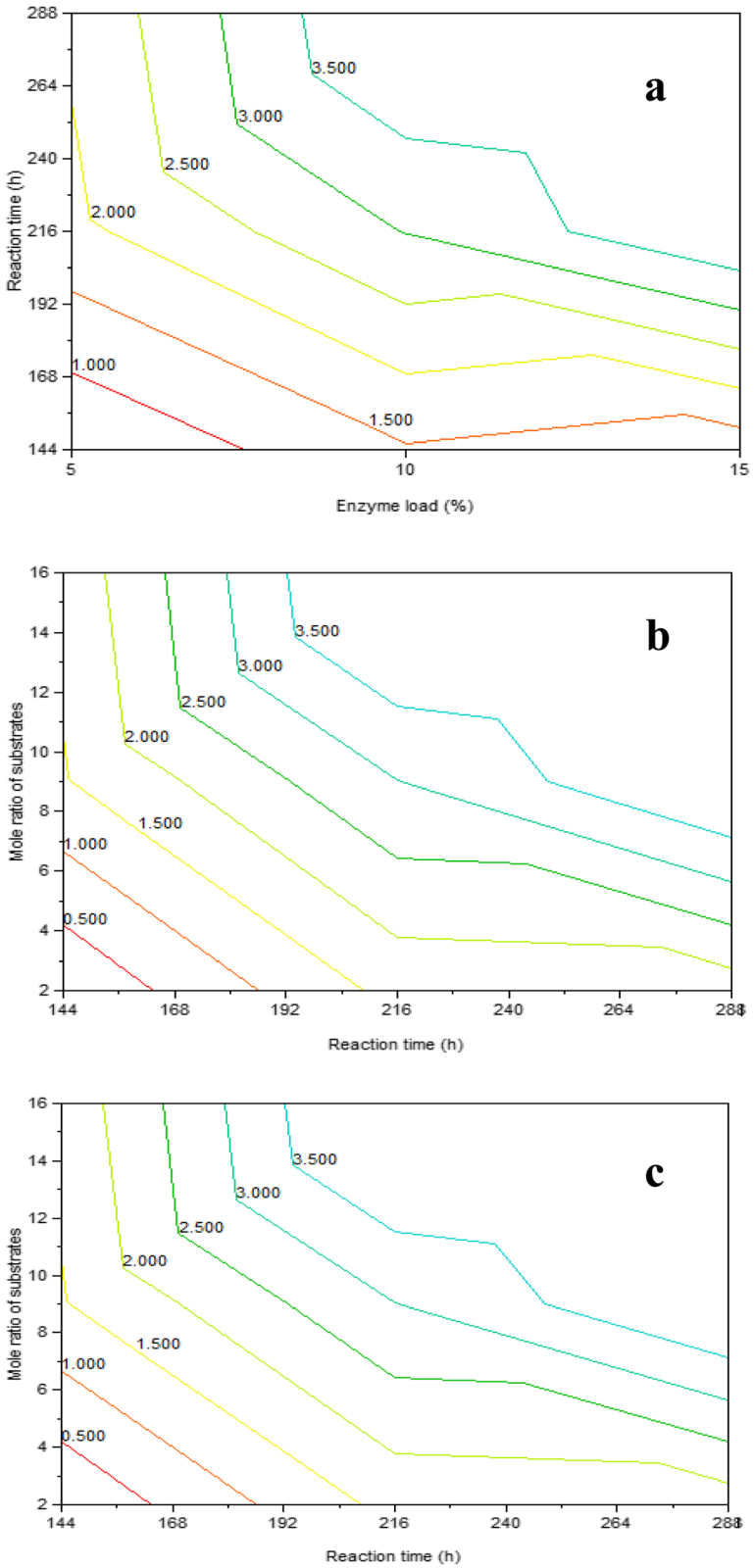


Fig. 5 Contour plots of two tested variables on the degree of esterification of p-coumaric acid; **a**: reaction time and enzyme load; **b**: mole ratio of substrates and enzyme load; and **c**: mole ratio of substrates and reaction time

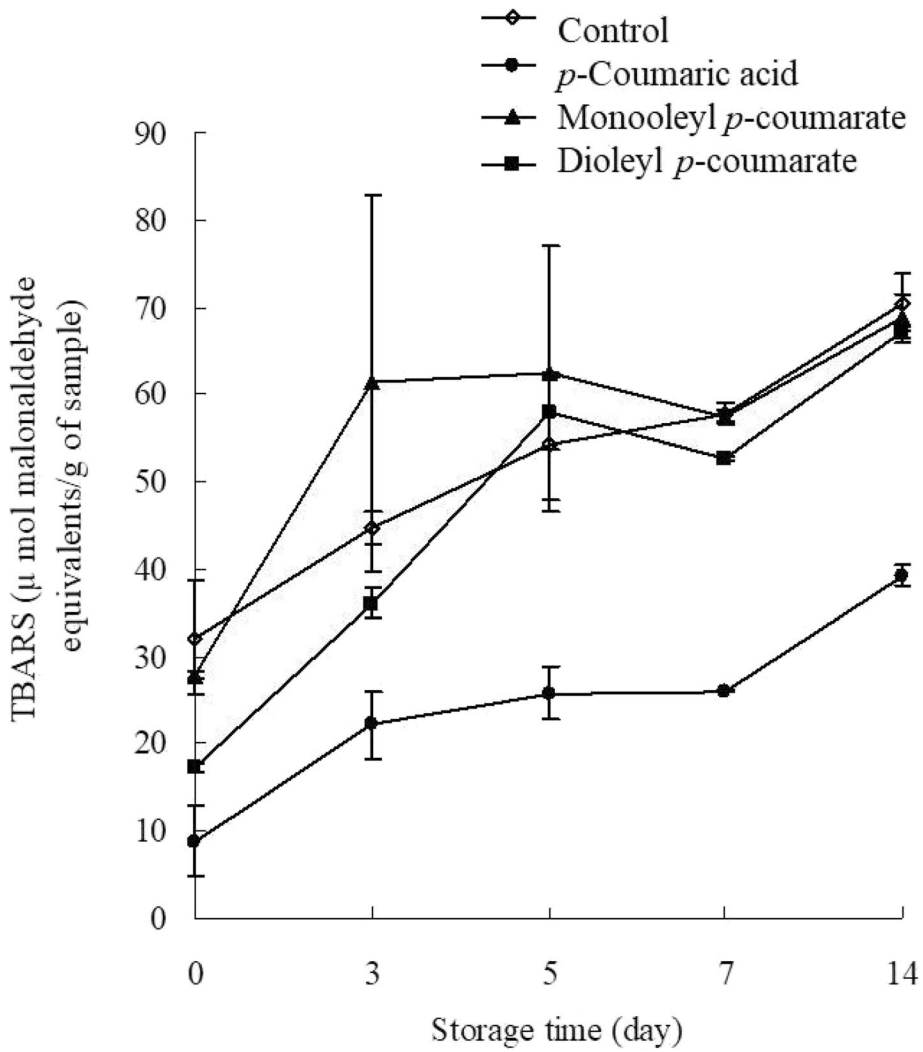


Fig. 6 TBARS values in cooked ground pork as affected by *p*-coumaric acid, mono- and dioleyl *p*-coumarates

lower than those of the control group only on day 0 and 7 test points and did not show significant difference at other test points. The test compounds added to fresh ground meat exerted effects at different levels on lipid oxidation during cooking of the meat prior to storage, which explains the presence of different TBARS values from test groups on day 0. Although oleic acid does not directly contribute to TBARS formation, its possible oxidation and free radical may indirectly compromise the effectiveness of monooleyl *p*-coumarate or dioleyl *p*-coumarate under high thermal conditions and longer storage period. In addition, higher lipophilicity of antioxidants may affect their better performance in cooked muscle food (Oh & Shahidi 2018; Zhong & Shahidi 2012). Thus, difference of antioxidant activity between monooleyl *p*-coumarate or dioleyl *p*-coumarate in this test may be caused by better lipophilicity of dioleyl *p*-coumarate compared to monooleyl *p*-coumarate. Hence, mono- and dioleyl *p*-coumarates with their weaker antioxidant activity may not be the best choice for use in cooked muscle foods for improving their oxidative stability and extending their shelf life.

Future perspectives on biological function of synthesized mono- and dioleyl *p*-coumarates

The synthesized mono- and dioleyl *p*-coumarates containing *p*-coumaric acid moiety and fatty acid chains exhibited varying antioxidant activity when different testing systems were employed, which may be due to steric hindrance exerted by esterified bulky moieties, decreased hydrogen atom donation tendency caused by interference of the formed ester bonds, decreased ionisation potential from added bulky moieties and an improved lipophilicity with fatty acid branch chains (Wang & Shahidi 2014). Conversion of phenolic compounds to their ester forms not only change their antioxidant

activity, but also may be due to the changes of their digestive stability, absorption, metabolism and biological function in human body (Oh et al. 2019; Yin et al. 2018; Yin et al. 2020). Yin et al. (2020) evaluated the pharmacokinetic characteristics in rats given tyrosol acyl esters, and the results revealed an increased duration of action in vivo in the plasma from rats orally administrated tyrosol esters when compared to those animals given tyrosol, which was supported by higher plasma half-life and mean residence time. Therefore, esterification of phenolic compounds may improve their oral bioavailability, and such effect will potentially enhance their biological function. The digestive stability of tyrosol esters with various alkyl chains was also evaluated in an in vitro simulated gastrointestinal tract model by Yin et al. (2018), and the results indicated a sustained-release of tyrosol in the digestive system once they were esterified.

Zhu et al. (2018) identified the active compounds in *Oldenlandia diffusa* in a rat model study that has commonly been used to treat rheumatoid arthritis. The results of the study revealed that *p*-coumaric acid was a key active compound from *Oldenlandia diffusa* that inhibited the collagen-induced arthritis in rats as administration of *p*-coumaric acid or the *Oldenlandia diffusa* extract leading to alleviated symptoms in tested rats when compared to that of the untreated animals. In addition, a decreased arthritis index and serum levels of TNF-α and IL-6 were observed when compared to that of the control group. The anti-virus potential of *p*-coumaric acid was reported in a study conducted by Tanida et al. (2015), in which the inhibitory effect of coffee extract and its major phenolic acid constituent, *p*-coumaric acid, on hepatitis C virus was proven with its inhibitory effect on the propagation of the virus in a Human hepatoma Huh-7.5.1 cells model system. Shirasago et al. (2019) also proved that *p*-coumaric acid can effectively decrease hepatitis C virus infectivity by acting on the virus particles. *p*-Coumaric acid methyl ester is one of the bioactive components of *Costus speciosus* (Koen) Sm. (Zingiberaceae) that has been used to treat ailments including catarrhal fevers and dyspepsia. Zhang et al. (2018) investigated the anti-angiogenic property of *p*-coumaric acid methyl ester and its associated mechanism using human umbilical vein endothelial cells and zebrafish. The results of this latter study revealed that *p*-coumaric acid methyl ester inhibited the proliferation of human umbilical vein endothelial cells, disrupted the formation of intersegmental vessels and the subintestinal vessels of zebrafish embryos, and inhibited tumor angiogenesis in the zebrafish cell-line derived xenograft model of SGC-7901. Therefore, *p*-coumaric acid methyl ester could be used as a multi-target anti-angiogenic drug.

Conversion of *p*-coumaric acid to its ester forms improves its lipophilic properties, and thus increases its cell membrane permeability, which helps to exhibit its biological function in human body. The effect of mono- and dioleyl *p*-coumarates on alleviating rheumatoid arthritis, inhibiting virus infection, preventing angiogenesis should be assessed in *in intro* and animal model studies prior to making any recommendations about their potential use in functional foods and pharmaceutical products for possible disease risk reduction and treatments.

Conclusion

Based on the analysis of experimental data, the model employed was highly sufficient for determining the effectiveness and interaction of three selected variables, enzyme load, reaction time and the mole ratio of substrates, on the dependent variable, the degree of esterification. Although the optimization point was not found in the selected range of the three variables, the steepest ascent analysis suggested that an increase of these three variables might lead to a stationary point. However, based the limitations on increasing the range of tested variables, including possible oxidation of synthesized lipids and increased cost, the degree of esterification so yielded in the designed central composite design should be the one closest to the possible ideal optimized degree. Thus, the maximum values of tested variables were recommended in the use of the acidolysis of *p*-coumaric acid with triolein. Mono- and dioleyl *p*-coumarates showed no or slight antioxidant activity in meat model system, and that may be explained by the indirect effect of oleic acid in mono- and dioleyl *p*-coumarates to oxidation under high thermal conditions and during longer storage period, and the difference of antioxidant activity between monooleyl *p*-coumarate or dioleyl *p*-coumarate in this test may be caused by better lipophilicity of dioleyl *p*-coumarate compared to monooleyl *p*-coumarate. The esterification of *p*-coumaric acid may also change its biological properties, which requires further studies.

Abbreviations

RSM: Response surface methodology; CCF: Central composite face-centred; HPLC-MS: High performance liquid chromatography-mass spectrometry; TBARS: Thiobarbituric acid reactive substances; TBA: Thiobarbituric acid

Acknowledgments

The authors acknowledge financial support from the Department of Science and Technology of Shaanxi Province, P. R. China (No. 2020NY-102). The author FS thanks the Natural Science and Engineering Research Council (NSERC) of Canada for support in the form of a Discovery Grant.

Authors' contributions

Authors contributed equally to the reported work. All authors read and approved the final manuscript.

Funding

The author JW is thankful for the support provided by Department of Science and Technology of Shaanxi Province, P. R. China (No. 2020NY-102).

The author FS is grateful to the Natural Science and Engineering Research Council (NSERC) of Canada for support in the form of a Discovery Grant.

Availability of data and materials
All data supporting this study are included in this manuscript. Further details are available from the corresponding author upon request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
Dr. Fereidoon Shahidi, Dr. Daoying Wang are members of Editorial Board, Yan Huang is editor in house of *Food Production, Processing and Nutrition*, and they are not involved in the journal's review of, or decisions related to this manuscript.

Author details
[1]School of Food and Biological Engineering, Shaanxi University of Science and Technology, Xi'an 710021, People's Republic of China. [2]Institute of Agricultural Products Processing, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, People's Republic of China. [3]Department of Biochemistry, Memorial University of Newfoundland, St. John's, NL A1C 5S7, Canada.

Received: 3 August 2021 Accepted: 6 March 2022
Published online: 26 March 2022

References
Cao, W., Zhang, C., Hong, P., & Ji, H. (2008). Response surface methodology for autolysis parameters optimization of shrimp head and amino acids released during autolysis. *Food Chemistry*, *109*(1), 176–183. https://doi.org/10.1016/j.foodchem.2007.11.080.

Chen, Q. H., He, G. Q., & Mokhtar, A. M. (2002). Optimization of medium composition for the production of elastase by bacillus sp. EL31410 with response surface methodology. *Enzyme and Microbial Technology*, *30*(5), 667–672. https://doi.org/10.1016/S0141-0229(02)00028-5.

Ferguson, L. R., Shuo-tun, Z., & Harris, P. J. (2005). Antioxidant and antigenotoxic effects of plant cell wall hydroxycinnamic acids in cultured HT-29. *Molecular Nutrition & Food Research*, *49*(6), 585–693. https://doi.org/10.1002/mnfr.200500014.

Jayathilakan, K., Sharma, G. K., & Radhakrishna, K. (2007). Antioxidant potential of synthetic and natural antioxidants and its effect on warmed-overflavour in different species of meat. *Food Chemistry*, *105*(3), 908–916. https://doi.org/10.1016/j.foodchem.2007.04.068.

Kishida, K., & Matsumoto, H. (2019). Urinary excretion rate and bioavailability of chlorogenic acid, caffeic acid, pcoumaric acid, and ferulic acid in non-fasted rats maintained under physiological conditions. Heliyon, 5(10), e02708.

Lu, T., Shen, Y., Wang, J. H., Xie, H. K., Wang, Y. F., Zhao, Q., … Shahidi, F. (2020). Improving oxidative stability of flaxseed oil with a mixture of antioxidants. *Journal of Food Processing & Preservation*, *44*(3), e14355. https://doi.org/10.1111/jfpp.14355.

Neff, W. E., & Byrdwell, W. C. (1998). Characterization of model triacylglycerol (triolein, trilinolein and trilinolenin) autoxidation products via high-performance liquid chromatography coupled with atmospheric pressure chemical ionization mass spectrometry. *Journal of Chromatography A*, *818*(2), 169–186. https://doi.org/10.1016/S0021-9673(98)00553-6.

Nevdakh, O. P., Agabekov, V. E., & Mitskevich, N. I. (1984). Kinetics of the formation and decomposition of hydroperoxide in the oxidation of 8-pentadecanone. *Reaction Kineties and Catalysis Letters*, *27*, 225–230.

Oh, W. Y., Chiou, Y., Lee, P., Pan, M., & Shahidi, F. (2019). Lipophilized resveratrol affects the generation of reactive nitrogen species in murine macrophages and cell viability of human cancer cell lines. *Journal of Food Bioactives*, *7*, 73–77. https://doi.org/10.31665/JFB.2019.7201.

Oh, W. Y., & Shahidi, F. (2018). Antioxidant activity of resveratrol ester derivatives in food and biological model systems. *Food Chemistry*, *261*, 267–273. https://doi.org/10.1016/j.foodchem.2018.03.085.

Peričin, D., Radulović-Popović, L., Vaštag, Ž., Mađarev-Popović, S., & Trivić, S. (2009). Enzymatic hydrolysis of protein isolate from hull-less pumpkin oil cake: Application of response surface methodology. *Food Chemistry*, *115*(2), 753–757. https://doi.org/10.1016/j.foodchem.2008.12.040.

Porter, W. L. (1993). Paradoxical behavior of antioxidants in food and biological systems. *Toxicology and Industrial Health*, *9*(1-2), 93–122. https://doi.org/10.1177/0748233793009001-209.

Ramadan, M. F. (2021a). Chemistry and functionality of Lipo-phenolics. In *Phenolipids and Lipo-phenolics*. Cham: Springer. https://doi.org/10.1007/978-3-030-67399-4_3.

Ramadan, M. F. (2021b). Chemistry and functionality of Phenolipids. In *Phenolipids and Lipo-phenolics*. Cham: Springer. https://doi.org/10.1007/978-3-030-67399-4_1.

Sabally, K., Karboune, S., St-Louis, R., & Kermasha, S. (2006). Lipase-catalyzed transesterification of dihydrocaffeic acid with flaxseed oil for the synthesis of phenolic lipids. *Journal of Biotechnology*, *127*(1), 167–176. https://doi.org/10.1016/j.jbiotec.2006.06.010.

Safari, M., Karboune, S., St-Louis, R., & Kermasha, S. (2006). Enzymatic synthesis of structured phenolic lipids by incorporation selected phenolic acids into triolein. *Biocatalysis and Biotransformation*, *24*, 272–279.

Senanayake, N. S. P. J., & Shahidi, F. (2002). Enzyme-catalyzed synthesis of structured lipids via acidolysis of seal (*Phocagroenlandica*) blubber oil with capric acid. *Food Research International*, *35*, 745–752.

Shahidi, F., Yun, J., Rubin, L. J., & Wood, D. F. (1987). The hexanal content as an indicator of oxidative stability and flavour acceptability in cooked ground pork. *Canadian Institute of Food Science and Technology Journal*, *20*(2), 104–106. https://doi.org/10.1016/S0315-5463(87)71099-2.

Shahidi, F., & Pegg, R. (1994). Hexanal as an indicator of meat flavor deterioration. Journal of Food Lipids, 1(3), 177-186.

Shahidi, F., & Alexander, D. (1998). Green tea catechins as inhibitors of oxidation of meat lipids. Journal of Food Lipids, 5(2), 125-133.

Shirasago, Y., Inamori, Y., Suzuki, T., Tanida, I., Suzuki, T., Sugiyama, K., Wakita, T., Hanada, K., & Fukasawa, M. (2019). Inhibition mechanisms of hepatitis C virus infection by caffeic acid and tannic acid. Biological and Pharmaceutical Bulletin, 42(5), 770-777.

Tan, Z., & Shahidi, F. (2012). Optimization of enzymatic synthesis of phytosteryl caprylates using response surface methodology. *Journal of the American Oil Chemists Society*, *89*(4), 657–666. https://doi.org/10.1007/s11746-011-1949-y.

Tanida, I., Suzuki, R., Abe, R., Wakita, T., Hanada, K., & Fukasawa, M. (2015). Inhibitory effects of caffeic acid, a coffee-related organic acid, on the propagation of hepatitis C virus. Japanese Journal of Infectious Diseases, 68(4), 268-275.

Wang, J., Han, L., Wang, D., Li, P., & Shahidi, F. (2020). Conjugated fatty acids in muscle food products and their potential health benefits: A review. *Journal of Agricultural and Food Chemistry*, *68*(47), 13530–13540. https://doi.org/10.1021/acs.jafc.0c05759.

Wang, J., Han, L., Wang, D., Sun, Y., Huang, J., & Shahidi, F. (2021). Stability and stabilization of omega-3 oils: A review. *Trends in Food Science & Technology*, *118*, 17–35. https://doi.org/10.1016/j.tifs.2021.09.018.

Wang, J., & Shahidi, F. (2014). Antioxidant activity of monooleyl and dioleyl *p-coumarates* in *in vitro* and biological model systems. *European Journal of Lipid Science and Technology*, *116*(4), 370–379. https://doi.org/10.1002/ejlt.201300348.

Yin, F., Wang, X., Hu, Y., Xie, H., Liu, X., Qin, L., … Shahidi, F. (2020). Evaluation of absorption and plasma pharmacokinetics of tyrosol acyl esters in rats. *Journal of Agricultural and Food Chemistry*, *68*(5), 1248–1256. https://doi.org/10.1021/acs.jafc.9b05112.

Yin, F. W., Hu, X. P., Zhou, D. Y., Ma, X. C., Tian, X. G., Huo, X. K., … Shahidi, F. (2018). Evaluation of the stability of tyrosol esters during *in vitro* gastrointestinal digestion. *Food & Function*, *9*(7), 3610–3616. https://doi.org/10.1039/C8FO00788H.

Zhang, H. Z., Li, C. Y., Wu, J. Q., Wang, R. X., Wei, P., Liu, M. H., & He, M. F. (2018). Anti-angiogenic activity of Para-coumaric acid methyl ester on HUVECs *in vitro* and zebrafish *in vivo*. *Phytomedicine*, *48*, 10–20. https://doi.org/10.1016/j.phymed.2018.04.056.

Zhu, H., Liang, Q. H., Xiong, X. G., Wang, Y., Zhang, Z. H., Sun, M. J., … Wu, D. (2018). Anti-inflammatory effects of *p-Coumaric* acid, a natural compound of oldenlandia diffusa, on arthritis model rats. *Evidence-based Complementary and Alternative Medicine 2018*, 1–9. https://doi.org/10.1155/2018/5198594.

Publisher's Note