Optimization of the degumming process for camellia oil by the use of phospholipase C in pilot-scale system

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Abstract In present study, phospholipase C (PLC) was applied in camellia oil degumming and the response surface method (RSM) was used to determine the optimum degumming conditions (reaction time, reaction temperature and enzyme dosage) for this enzyme. The optimum conditions for the minimum residual phosphorus content (15.14 mg/kg) and maximum yield of camellia oil (98.2 %) were obtained at reaction temperature 53 °C, reaction time 2.2 h, PLC dosage 400 mg/kg and pH 5.4. The application of phospholipase A (PLA) - assisted degumming process could further reduce the residual phosphorus content of camellia oil (6.84 mg/kg) to make the oil suitable for physical refining while maintaining the maximal oil yield (98.2 %). These results indicate that PLC degumming process in combination with PLA treatment can be a commercially viable alternative for traditional degumming process. Study on the quality changes of degummed oils showed that the oxidative stability of camellia oil was slightly deceased after the enzymatic treatment, thus more attention should be paid to the oxidative stability in the further application.

Keywords Enzymatic degumming · Phospholipase C · Camellia oil · Residual phosphorus content · Oil yield

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Abbreviations

PLC	Phospholipase
RSM	Response surface method
PLA	Phospholipase A
PUFAs	Unsaturated fatty acids
FFAs	Free fatty acids
DAG	Diacylglycerols
NHPs	Non-hydratable phospholipids
PC	Phosphatidylcholine
PE	Phosphatidylethanolamine
PI	Phosphatidylinositol
PA	Phosphatidic acid
NPPC	<i>p</i> -nitrophenylphosphorylcholine
PV	Peroxide value

Introduction

Camellia tea seeds, originated from China, have a long history of cultivation and utilization for over 2,300 years. In some countries where camellia tea seeds are abundantly available, camellia oil has been accepted as an edible oil (Sahari et al. 2004). China, India, Sri Lanka, Indonesia and Japan currently produce thousands of tons of camellia oil annually. Camellia oil is a main cooking oil in the southern provinces of China, especially Hunan, where more than 50 % of the vegetable cooking oil comes from camellia (Rajaei et al. 2005). It has been considered as a kind of high quality cooking oil with high contents of oleic acid and lipid accompanying compounds, and it can be stored well at room temperature. In addition, camellia oil is reputed to reduce blood pressure and cholesterol level, to have a high content of antioxidants, to be a rich source of emollients for skin care and to postpone the signs of aging (Fazel et al. 2008). Regardless of the way of extraction, camellia oil inevitably contains an amount of

impurities after rendering, crushing or solvent extraction, which may affect the quality, taste, smell and appearance of oil. Thus it is necessary to remove these impurities by refining process (Badan Ribeiro et al. 2008). Degumming is the first step in the refining process of crude vegetable oils, which removes phospholipids, protein and mucilaginous gums. The presence of substantial amounts of phospholipids can cause oil discoloration, serve as a precursor of off-flavors and bring problems to the downstream processing of the oil (Mei et al. 2013). In summary, inadequate degumming will directly affect the refining efficiency and the quality of final oils. Therefore, removal of nearly all of the phospholipids is essential for the production of high-quality final oils (Subramanian et al. 1999).

Traditional degumming processes including water degumming and acid degumming are not always optimally suited for camellia oil because of the high content of unsaturated fatty acids (PUFAs) existing in the oil. High degumming temperature could accelerate the oxidization of the oil and form pigments which will deepen the color of the oil (Zhi et al. 2010). Besides, some beneficial minor components such as tocopherols and sterols would be damaged during the alkali refining process followed by the traditional degumming processes. Compared to the traditional degumming processes, enzymatic degumming shows several advantages. Apart from the reduction in the amount of acid, alkali and wastewater used during the refining process, an enhancement in product yield and a reduction in operating costs have also been observed (Dijkstra 2010).

Phospholipases (phospholipase A, B, C or D) are a class of hydrolytic enzymes that can hydrolyze the ester bonds of phospholipids. In the enzymatic oil-degumming process, phospholipases of type A and type C are commonly used (Jiang et al. 2011). Phospholipase A (PLA) can cause the production of lysophospholipids, which would be removed with water during the separation stage of the degumming process (Manjula et al. 2011). However, the enzymatic degumming processes using PLA have in common that they hydrolyze the fatty acid glycerol bond of phospholipids. Consequently, they catalyze the production of free fatty acids (FFAs). These FFAs will be concentrated in the oil being degummed from which they have to be removed as part of the refining process (De Maria et al. 2007). On the other hand, phospholipase C (PLC) does not cause the formation of FFAs because this enzyme hydrolyzes the bond between the acylglycerol and the phosphate group. Accordingly, it liberates oil-soluble diacylglycerols (DAG) and water-soluble phosphates. During the subsequent refining of oily phase, DAG will not be removed from the oil, thus it can be sold at full refined oil value (Casado et al. 2012). Above all, PLC has its own advantage in the industrial application because it can increase the oil yield during the degumming process.

So far, few researches have studied the degumming process of camellia oil by the use of PLC, though some researchers reported the PLA degumming process of camellia oil (Zhi et al. 2010; Li et al. 2008). Besides, the oil yield of camellia oil during the enzymatic degumming process has not been investigated. The primary objective of this study was to investigate the viability of PLC degumming process for camellia oil in the pilot-scale system, and the performance of PLC was tested with respect to reducing the residual phosphorus content and maximizing the yield of degummed camellia oil. Secondly, a combined enzyme technique was applied to make the oil suitable for physical refining (residual phosphorus < 10 mg/kg), while maintaining the maximal oil yield and not significantly increasing the content of FFAs. Thirdly, the qualities of degummed camellia oil after different kinds of treatments were studied to estimate whether the enzymatic degumming process affected the oil quality.

Materials and methods

Materials

Camellia oil was kindly supplied by Jinhao Camellia Oil Corp. Ltd. (Hunan, China) with a phosphorus content of 575.59 ± 3.40 mg/kg, and non-hydratable phospholipids (NHPs) content of 140.62 ± 1.28 mg/kg. PLC was prepared by our own laboratory using submerged fermentation of a genetically modified *Bacillus cereus*. Substrates of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) can be hydrolyzed by this kind of PLC to form DAG, while substrates of phosphatidylinositol (PI) or phosphatidic acid (PA) can not be hydrolyzed by this kind of PLC. PLA (Lecitase Ultra) was purchased from A/S Novozymes (Bagsvaerd, Denmark). The *p*-nitrophenylphosphorylcholine (NPPC) with purities over 98 % was purchased from Sigma Chemical Ltd. (St. Louis, MO, USA). A mixed standard of tocopherols (α , β , γ and δ) were obtained from Roche vitamins Inc Parsippany, New Jersey, 07054-USA. Isopropanol and n-Hexane purchased from J&K Scientific Ltd. were of HPLC purity. All other reagents of analytical grade were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China).

Determination of PLC and PLA activity

PLC activity was determined using NPPC as a substrate (Flieger et al. 2000). We defined 1 U/g of PLC as the amount of enzyme needed to produce 1 nM nitrophenol per minute by the hydrolysis of NPPC under appropriate condition: 100 μ L PLC was added to 2 mL of NPPC solution (10 mM NPPC, 250 mM Tris-HCl (pH 7.2), 60 % sorbitol and 1 mM ZnCl₂) in a test tube. The tube was incubated at 37°C for 30 min.

Substrate hydrolysis was then quantified by measuring absorbance at 410 nm. The activity of PLC was calculated by the following Eq. 1:

$$\text{PLC activity (U/g)} = 1.3636 \times 10^3 \times A/t. \quad (1)$$

Where A is the absorption value in 410 nm and t (min) is the time used in the substrate hydrolysis reaction. The activity of PLC used in this study was assayed to be 150 U/g.

PLA activity assay was performed with deoiled soy lecithin emulsion by using the method of Yang et al. (Yang et al. 2006). One U/g of PLA is the amount of enzyme solution which releases 1 μmol of titratable FFAs per minute under the described conditions. Substrate solution: 25 % deoiled soy lecithin and 4 % polyvinyl alcohol solution were emulsified at a volume ratio of 1: 4. Analysis conditions: 4 mL of deoiled soy lecithin emulsion, 5 mL of 0.01 M citric acid buffer (pH 5.0), and 1 mL of enzyme solution were mixed and incubated at 37°C for 10 min. The reaction was terminated with the addition of 95 % ethanol (15 mL) after incubation, and the liberated FFAs were titrated with 0.05 M NaOH. Blanks were measured with heat-inactivated PLA samples (95°C, 10 min). The activity of PLA used in this study was assayed to be 8670 U/g.

pH determination

pH determination was carried out according to Jahani et al. (Jahani et al. 2008).

Pilot-scale degumming system

The essential components of the pilot-scale design included a Nano Homogenize machine (ATS, Canada, model AH2010), a 10 L capacity reaction tank (Shenke, China, model RAT-10Dse) equipped with an agitation unit (Shenke, China, model S212-90; 100–1300 rpm), a heated oil bath with re-circulation (Shenke, China, model W-O-V; 20–200°C), a water vacuum pump with re-circulation (Shenke, China, model SHB-95) and a disc centrifuge (Haide, China, model SZSDH5). The agitation unit consisted of an impeller with a diameter of 200 mm. The schematic diagram was shown in Fig. 1.

Pilot-scale citric acid degumming process

The citric acid degumming process was modified according to Smiles et al. (Smiles et al. 1988), and 20 times were magnified in the pilot-scale experiments based on the laboratory-scale experiments. Crude camellia oil (8,000 g) was heated to 70°C and 7.12 mL of 45 % citric acid solution was added to make sure that 1 kg crude camellia oil contained 400 mg citric acid on a dry basis. The oil mixture was homogenized by using a

Nano Homogenize machine (60 Mpa, 8 L/h) and allowed to condition in the reaction tank at 70°C for 30 min under stirring at 500 rpm. Afterwards, a certain amount of distilled water (3 % relative to the weight of oil) was added, and the oil mixture was mixed by using a Nano Homogenize machine (60 Mpa, 8 L/h). Then the mixture was placed in the reaction tank with temperature maintained at 70°C under mechanical stirring at 500 rpm for another 30 min. After the citric acid degumming reaction, the temperature of the reaction tank was adjusted to 95°C for 10 min and then the oil mixture was centrifuged by using a disc centrifuge (6,500 rpm). The supernatant fluid was collected and dried by the rotary evaporator at 0.09 Mpa, 80°C for oil yield, residual phosphorus content and other analysis. All reactions were conducted in triplicate for each process condition.

Pilot-scale PLC degumming process

The first several steps of the pilot-scale PLC degumming process were carried out similar to the pilot-scale citric acid degumming process. After 30 min of citric acid treatment at 70°C under stirring at 500 rpm, the temperature of the oil mixture was decreased to a required temperature (40–60°C), and a certain amount of 16 % NaOH solution was added to make the mixture at predicted pH (4.8–6.0). After stirring for 5 min (500 rpm), a certain amount of distilled water (3 % relative to the weight of oil) and predicted quantity of PLC (0–500 mg/kg) were added, and the oil mixture was mixed by using a Nano Homogenize machine (60 Mpa, 8 L/h). Afterwards the oil mixture was placed in the reaction tank with temperature maintained at the required point (40–60°C), and stirred at 500 rpm for a given time (0.5–2.5 h). After the PLC degumming reaction, the temperature of the reaction tank was adjusted to 95°C for 10 min to inactivate the activity of PLC and then the oil mixture was centrifuged by using a disc centrifuge (6,500 rpm). The supernatant fluid was collected and dried by the rotary evaporator at 0.09 Mpa, 80°C for oil yield, residual phosphorus content and other analysis. All reactions were conducted in triplicate for each process condition.

Pilot-scale PLA-assisted degumming process

Degummed camellia oil treated with PLC under the optimum processing parameters was not suitable for physical refining (residual phosphorus > 10 mg/kg). Thus PLA was used in combination with PLC to make the degummed oil suitable for physical refining. The reaction condition for PLA was modified according to Yang et al. (Yang et al. 2006). The first several steps of the process were carried out the same as the pilot-scale PLC degumming process. After the optimum degumming treatment of PLC, a certain amount of PLA was added (10 mg/kg relative to the weight of oil), and the oil

Fig. 1 The schematic diagram of pilot-scale enzymatic degumming system (1- Nano Homogenize machine, 2- impeller, 3- reaction tank, 4- pump, 5- disk centrifuge, 6- degummed oil, 7- separated gums)

mixture was stirred at 500 rpm for another 30 min. Afterwards, the temperature of the reaction tank was adjusted to 95°C for 10 min to inactivate the activity of both PLC and PLA, and then the oil mixture was centrifuged by using a disk centrifuge (6,500 rpm). The supernatant fluid was collected and dried by the rotary evaporator at 0.09 Mpa, 80°C for oil yield, residual phosphorus content and other analysis. All reactions were conducted in triplicate for each process condition.

Phosphorus content assay and determination of oil quality indices

The phosphorus content of the samples was carried out according to the colorimetric molybdenum blue method (GB/T 5537–2008, National Standard of the People's Republic China, 2008). The content of FFA was determined in accordance with GB/T 5530–2005. The peroxide value (PV) of the samples was determined in accordance with GB/T 5538–2005. Oxidative stability of the oils was analyzed by the Rancimat method using a Metrohm 743 Rancimat (Herisau, Switzerland) instrument. Samples of 3.0 g were analyzed under a heating block of 110°C at a constant air flow of 10 L/h. The tocopherols content of the samples was measured by HPLC-UV according to Suliman et al. (Suliman et al. 2013). All oil samples were performed in triplicate and the mean values and standard deviations were calculated. One-way ANOVA was carried out by using Tukey adjustment to determine the significant difference between treatments. Significant differences were declared at $P \leq 0.05$ (Origin8.0).

Experimental design

The experimental data was analyzed by the response surface method (RSM) to fit the following second-order polynomial model predicted for optimization of phosphorus content and degummed oil yield (Design Expert, State-Ease Inc., Statistics Made Easy, Minneapolis, MN).

$$Y = b_0 + \sum_{i=1}^3 b_i X_i + \sum_{i=1}^3 b_{ii} X_i^2 + \sum_{i=1}^2 \sum_{j=i+1}^3 b_{ij} X_i X_j \quad (2)$$

Y is one of the two responses (phosphorus content and degummed oil yield), X_i and X_j are the coded independent variables, and β_0 , β_i , β_{ii} and β_{ij} are the regression coefficients for the intercept, linear, quadratic and interactive terms, respectively.

Box-Behnken design for three independent variables was used to obtain the optimization, which allowed one to design a minimum number of experimental runs. For the present study, a total of 17 tests were necessary to estimate the coefficients. The significance of the second-order model was evaluated by analysis of variance (ANOVA). Additional experiments were also carried out to verify predicted optimal conditions.

Results and discussion

Pilot-scale citric acid degumming process

The treatment of citric acid could allow the dissociation of the salts of some NHPs to make them slightly more hydratable,

Fig. 2 Effects of reaction time, reaction temperature, PLC dosage (relative to the weight of oil) and pH on the residual phosphorus content and yield of degummed camellia oil: water amount=3 % (relative to the weight of oil) **a** reaction temperature=50°C, PLC dosage=300 mg/kg,

pH=5.0; **b** reaction time=2 h, PLC dosage=300 mg/kg, pH=5.0; **c** reaction time=2 h, reaction temperature=55°C, pH=5.0; **d** reaction time=2 h, reaction temperature=55°C, PLC dosage=300 mg/kg

facilitating the separation of NHPs from oils (Pan et al. 2000). In our study, the residual phosphorus content of camellia oil after citric acid treatment could be reduced from $575.59 \pm$

3.40 mg/kg to 28.76 ± 0.38 mg/kg, and the yield of degummed camellia oil after citric acid treatment was 97.3 ± 0.3 %.

Selection of independent variables and their levels by the single-factor experiments

Table 1 Independent variables and their levels used for the Box-Behnken design

Variables	Symbols	Levels		
		−1	0	+1
Reaction time (h)	X_1	1.5	2	2.5
Reaction temperature (°C)	X_2	50	55	60
PLC dosage ^a (mg/kg)	X_3	200	300	400

^a PLC dosage (relative to the weight of camellia oil)

Fig. 2 showed the effects of four independent variables (reaction time, temperature, PLC dosage and pH) on the residual phosphorus content and yield of degummed camellia oil. With the addition of PLC, the residual phosphorus content of camellia oil could be reduced from 575.59 ± 3.40 mg/kg to less than 20 mg/kg within 2 h, and no obvious reduction was observed after 2 h. The yield of degummed camellia oil kept increasing slowly with an increase of reaction time (Fig. 2a). It might be explained that the PLC used in our study could only catalyze the split-off of the phosphate group from PC and PE,

Table 2 Experimental data for the three-factor, three-level surface analysis

Treatment No. ^a	Reaction time (h)	Reaction temperature (°C)	PLC dosage ^b (mg/kg)	Residual phosphorous (mg/kg)	Degummed oil yield (%)
	X ₁	X ₂	X ₃	Y ₁	Y ₂
1	0 (2)	−1 (50)	−1 (200)	20.87	97.8
2	1 (2.5)	−1 (50)	−1 (200)	18.61	98.1
3	0 (2)	1 (60)	0 (300)	25.52	97.6
4	0 (2)	0 (55)	0 (300)	17.02	98.1
5	0 (2)	0 (55)	0 (300)	16.85	98.1
6	−1 (1.5)	−1 (50)	0 (300)	23.02	97.7
7	1 (2.5)	0 (55)	1 (400)	16.20	98.2
8	−1 (1.5)	0 (55)	1 (400)	17.02	98.0
9	−1 (1.5)	1 (60)	0 (300)	26.45	97.6
10	0 (2)	1 (60)	1 (400)	23.13	97.7
11	1 (2.5)	−1 (50)	0 (300)	24.82	97.6
12	0 (2)	0 (55)	0 (300)	17.27	98.1
13	0 (2)	−1 (50)	1 (400)	17.35	98.1
14	1 (2.5)	0 (55)	−1 (200)	18.46	98.0
15	−1 (1.5)	0 (55)	−1 (200)	23.91	97.6
16	0 (2)	0 (55)	0 (300)	17.62	97.9
17	0 (2)	0 (55)	0 (300)	17.64	98.0

^a Treatments were run in random order. ^b PLC dosage (relative to the weight of camellia oil)

and had no activity with respect to PI or PA, which meant that oil treated with PLC required further degumming treatment for physical refining (Dijkstra 2009). On the other hand, the DAG generated by the hydrolysis of PC and PE could improve the oil yield after PLC degumming process.

The residual phosphorus content of camellia oil treated by PLC showed a decreasing-increasing pattern with an increase of reaction temperature, while the yield of degummed camellia oil followed an increasing-decreasing pattern (Fig. 2b).

High temperature can improve the reaction rate as it reduces the viscosity of the lipid mixture and certainly enhances the contact of enzyme and substrate on the oil–water surface (Han et al. 2011). However, temperature beyond optimal value will greatly reduce the stability and half-life of the enzyme (Xu et al. 2000).

With an increase of PLC dosage, the residual phosphorus content of camellia oil decreased first, and then the tendency of decrease became very slow. Besides, the yield of

Table 3 Regression analysis of variance for response surface quadratic model (ANOVA) pertaining to the predicted content of residual phosphorous

Source	Degree of Freedom	Sum of Squares	Mean Square	F-value	Prob>F ^a
Model	9	200.33	22.26	82.24	< 0.0001
X ₁	1	18.94	18.94	69.98	< 0.0001
X ₂	1	50.35	50.35	186.03	< 0.0001
X ₃	1	28.35	28.35	104.75	< 0.0001
X ₁ X ₂	1	1.93	1.93	7.14	0.0319
X ₁ X ₃	1	5.36	5.36	19.80	0.0030
X ₂ X ₃	1	0.32	0.32	1.18	0.3134
X ₁ ²	1	10.28	10.28	37.98	0.0005
X ₂ ²	1	80.87	80.87	298.78	< 0.0001
X ₃ ²	1	0.013	0.013	0.047	0.8345
Residual	7	1.89	1.89		
Lack of fit	3	1.4	1.4	3.74	0.1175 ^b
Pure error	4	0.5	0.5		
Cor Total	16	202.22			
CV=2.59 %	AdjR ² =0.9906				

^aP<0.05 indicates statistical significance; ^bP>0.05 indicates the Lack of Fit is not significant which is good

Table 4 Regression Analysis of Variance for Response Surface Quadratic Model (ANOVA) Pertaining to the Predicted Yield of Degummed Camellia oil

Source	Degree of Freedom	Sum of Squares	Mean Square	F-value	Prob>F ^a
Model	9	0.71	0.079	17.29	0.0005
X ₁	1	0.11	0.11	23.76	0.0018
X ₂	1	0.16	0.16	35.70	0.0006
X ₃	1	0.11	0.11	23.76	0.0018
X ₁ X ₂	1	0.032	0.032	7.12	0.0321
X ₁ X ₃	1	0.011	0.011	2.42	0.1635
X ₂ X ₃	1	8.100×10 ⁻³	8.100×10 ⁻³	1.78	0.2239
X ₁ ²	1	0.032	0.032	7.04	0.0328
X ₂ ²	1	0.23	0.23	50.99	0.0002
X ₃ ²	1	6.318×10 ⁻⁴	6.318×10 ⁻⁴	0.14	0.7205
Residual	7	0.032	4.551×10 ⁻³		
Lack of fit	3	5.975×10 ⁻³	1.992×10 ⁻³	0.31	0.8198 ^b
Pure error	4	0.026	6.470×10 ⁻³		
Cor Total	16	0.74			
CV=0.069 %	AdjR ² =0.9569				

^a $P < 0.05$ indicates statistical significance; ^b $P > 0.05$ indicates the Lack of Fit is not significant which is good

degummed camellia oil showed a continuous increase with the increment of PLC dosage, and the tendency of increase became slight when the PLC dosage was beyond 300 mg/kg (Fig. 2c). Phospholipase-catalyzed reaction takes place at the interface between the aqueous phase containing the enzyme and the oil phase containing phospholipids (Dijkstra 2010). When the interfacial area provided by mechanical-stirring is fully accommodated by the substrates (phospholipids) and enzyme, there is no need to add more enzyme. Besides, enzyme dosage at higher concentrations is more liable to agglomeration, which will reduce the actual effective reaction area on the phases (Jiang et al. 2014).

With other variables fixed, the residual phosphorus content of camellia oil reached lowest at pH 5.4. Meanwhile, the highest yield of degummed camellia oil was obtained at pH 5.4 (Fig. 2d). In fact, each kind of enzyme has its optimal range of working pH, and values without its optimal condition can lead to a partial or complete inactivation of the enzyme. Working pH can influence the ionization state of enzyme and then leads to the change of its active sites, affecting the activity and affinity of the enzyme and substrates. In addition, pH can also change the charged state of the substrates, influencing the interactions between the enzyme and substrates (Liu et al. 2008). Thus, the optimal pH of 5.4 was chosen for further study.

Overall, reaction time, reaction temperature, PLC dosage and pH had their respective effects on the reduction of phosphorus content and yield of degummed camellia oil. RSM was used to study the mutual effects of reaction time, reaction temperature and enzyme dosage on the PLC degumming process. With a fixed reaction pH of 5.4, the lower, middle,

and upper levels of the three independent variables (time, temperature and PLC dosage) were chosen in Table 1.

Model fitting

Table 2 showed the independent variables, their levels, the experimental design and the observed responses. Under experimental conditions, the residual phosphorus content ranged from 16.20 to 26.45 mg/kg, and the degummed oil yield ranged from 97.6 to 98.2 %. Further analysis on the results showed that there existed second-order polynomial equations that could relate residual phosphorus content and degummed oil yield to the parameters studied. The quadratic models are given in Eq. 3 and Eq. 4 as below.

$$Y_1(\text{mg/kg}) = 17.28 - 1.54X_1 + 2.51X_2 - 1.88X_3 + 0.69X_1X_2 + 1.16X_1X_3 + 0.28X_2X_3 + 1.56X_1^2 + 4.38X_2^2 + 0.055X_3^2 \quad (3)$$

$$Y_2(\%) = 98.04 + 0.12X_1 - 0.14X_2 + 0.12X_3 - 0.09X_1X_2 - 0.052X_1X_3 - 0.045X_2X_3 - 0.087X_1^2 - 0.023X_2^2 - 0.012X_3^2 \quad (4)$$

Where Y_1 and Y_2 were the predicted values for the residual phosphorus content (mg/kg) and the yield of degummed camellia oil (%), respectively. X_1 , X_2 and X_3 were the coded variables as described in Table 1. An analysis of variance was conducted to determine the significant effects of process variables on each response, and the results are shown in Table 3 and Table 4. All P values of the coefficient (β) except X_2X_3 , X_3^2 in Y_1 and X_1X_3 , X_2X_3 , X_3^2 in Y_2 for the two models were

Fig. 3 Contour plots of residual phosphorus content (a-c) and yield of degummed camellia oil (d-f), pH=5.4: **a** PLC dosage (relative to the weight of oil)=300 mg/kg; **b** reaction temperature=55°C; **c** reaction time=2 h; **d** PLC dosage=300 mg/kg; **e** reaction temperature=55°C; **f** reaction time=2 h

below 0.05, which implied that the models were statistically significant and adequate to explain most of the variability. X_2X_3 , X_3^2 in Y_1 and X_1X_3 , X_2X_3 , X_3^2 in Y_2 , despite insignificance, were not eliminated from the models in order to support the hierarchy of the models.

The coefficients determination (R^2) of the models for residual phosphorus content and yield of degummed camellia oil were 0.9906 and 0.9569, respectively, indicating that the models adequately represented the real relationships among the selected parameters. According to analysis of variance, P values of lack of fit for the two models were both >0.05 (residual phosphorus content, 0.1175; yield of degummed camellia oil, 0.8198), which meant the models fit well.

The mutual interaction of reaction time, reaction temperature and enzyme dosage was shown in Fig. 3. The relationship between reaction factors and their corresponding responses would be better illustrated by the three-dimensional response surface graphs (not given). As seen in Fig. 3a–f, generally, an increment in reaction time and enzyme amount could reduce the residual phosphorus content and improve the yield of degummed camellia oil. The reaction temperature should be limited within the range from 50.25 to 56.25°C, in which the minimal phosphorus content and the maximal oil yield could be gained.

Optimization of reaction and model verification

The optimal conditions were determined by RSM with interactive calculations in the range selected. With pH of 5.4 as a fixed treatment condition, the optimal temperature, reaction time and PLC dosage for the minimal residual phosphorus content and maximum yield of degummed camellia oil were 53°C, 2.2 h, and 400 mg/kg (relative to the weight of oil), respectively. Under the optimal conditions, the predicted values of residual phosphorus content and yield of degummed camellia oil were 15.03 mg/kg and 98.2 %, respectively. In our study, the measured value of residual phosphorus content of camellia oil under the optimal conditions was 15.14 mg/kg, which was lower than the

conditions before optimization, and the yield of degummed camellia oil was 98.2 %. Both of the values were very close to the predicted values above, which further proved the model could fit very well. Compared to the pilot-scale citric acid degumming process without the addition of PLC, a rise from 97.3 ± 0.3 to 98.2 ± 0.2 % of the oil yield was observed under the optimal conditions in the pilot-scale system of PLC. The result was highly consistent with other researchers who reported that there was an increase of about 1 % in oil yield for every 500 mg/kg phosphorus presented in the crude oil (Barton 2008). The main reasons for this oil yield increase are: firstly, the DAG formed by the hydrolysis of PC and PE is counted as oil; secondly, the absolute amount of oil retained by the gums is decreased in combination with the declination of phospholipids hydrolyzed by PLC (Dayton and Galhardo 2008). The application of PLC to improve the oil yield during degumming process is quite a promising research area and more efforts may be expected in this area.

Pilot-scale PLA-assisted degumming process

Based on the optimum reaction parameters mentioned above (reaction temperature 53°C, reaction time 2.2 h, PLC dosage 400 mg/kg and pH 5.4), the measured residual phosphorus content of camellia oil was 15.14 mg/kg, which was unsuitable for physical refining. After the PLA-assisted degumming process (reaction temperature 53°C, reaction time 0.5 h, PLA dosage 10 mg/kg and pH 5.4), the residual phosphorus content of degummed camellia oil could be further reduced to 6.84 ± 0.67 mg/kg and the oil yield was 98.2 ± 0.3 %. It means that PLA-assisted degumming process combined with the treatment of PLC is an efficient way to reduce the phosphorus content to less than 10 mg/kg, while maintains the maximal oil yield treated by PLC.

Oil quality analysis

An overview of the quality changes among crude, pilot-scale PLC degummed and pilot-scale PLA-assisted degummed

Table 5 Overview of the FFA, PV, oxidative stability and tocopherols content of crude, PLC, degummed and PLA-assisted degummed camellia oil

Parameters	Crude	Pilot-scale PLC ^a	Pilot-scale PLA-assisted ^b
FFA (mg KOH/g)	0.38 ± 0.04 ^A	0.36 ± 0.02 ^A	0.39 ± 0.02 ^A
PV (mmol/kg)	0.87 ± 0.03 ^A	1.46 ± 0.02 ^B	1.52 ± 0.04 ^B
Oxidative stability (h)	10.68 ± 0.36 ^A	8.59 ± 0.25 ^B	8.47 ± 0.37 ^B
Tocopherols content (mg/kg)	325.71 ± 16.32 ^A	256.92 ± 10.28 ^B	243.56 ± 13.56 ^B

The letters of A and B represent the differences among different treatments: the same letter indicates no significant difference ($P > 0.05$), different letters indicate a significant difference ($P \leq 0.05$) Optimal conditions in pilot-scale PLC system: reaction temperature=53°C, pH=5.4, reaction time=2.2 h, PLC dosage=400 mg/kg. ^b Optimal conditions in pilot-scale PLA-assisted system: reaction temperature=53°C, pH=5.4, PLC reaction time=2.2 h, PLC dosage=400 mg/kg, PLA reaction time=0.5 h, PLA dosage=10 mg/kg

camellia oils under optimal conditions was given in Table 5. In our study, the FFA content of crude, PLC degummed and PLA-assisted degummed camellia oils were 0.38 ± 0.04 , 0.36 ± 0.02 and 0.39 ± 0.02 mg KOH/g, respectively. There was no significant difference between crude and pilot-scale PLC degummed oils ($P > 0.05$), and the result was consistent with the fact that PLC would not generate additional FFA during the degumming process (Dayton et al. 2013). Besides, as most of the phospholipids were firstly hydrolyzed by PLC, the additional FFA generated by the following treatment of PLA was also not significant ($P > 0.05$). The peroxide value (PV) gives a measure of the extent to which an oil sample has undergone primary oxidation. Enzymatic degummed camellia oils did not undergo noticeable changes in PV between the pilot-scale PLC degummed (1.46 ± 0.03 mmol/kg) and the pilot-scale PLA-assisted degummed systems (1.52 ± 0.04 mmol/kg), but showed a significantly higher value compared to the crude camellia oil (0.87 ± 0.03 mmol/kg) ($P \leq 0.05$). It meant that the crude camellia oil was partially oxidized during the enzymatic degumming process. Besides, the oxidative stability of crude camellia oils (10.68 ± 0.36 h) was significantly better than that of degummed oils ($P \leq 0.05$), which might be explained that phospholipids were a kind of natural antioxidants and could postpone the rate of oil oxidation (Kashima et al. 1991). In addition, the tocopherols content of crude camellia oil (325.71 ± 16.32 mg/kg) was significantly higher than that of enzymatic degummed camellia oils (Seen in Table 5). The decrease in the total tocopherols level during the degumming process is in agreement with the results obtained by other researchers (Suliman et al. 2013), and the declination of tocopherols may be due to the fact that tocopherols are unstable in the presence of air and light for longer contact time. These results indicated that more attention should be paid on the oxidative stability of degummed oils in further application.

Conclusion

A pilot-scale PLC degumming process for camellia oil was developed in this study. The residual phosphorus content of degummed camellia oil could be reduced to 15.14 mg/kg by the treatment of PLC under optimal conditions. With the following treatment of PLA, the residual phosphorus content could be further reduced to less than 10 mg/kg, while maintaining the maximal oil yield. Quality analysis of the enzymatic degummed oils indicated that PLC degumming process in combination with PLA treatment would not significantly increase the FFA content of camellia oil. However, the crude camellia oil was partially oxidized during the enzymatic degumming process and its oxidative stability was decreased with the removal of certain amounts of natural antioxidants

such as phospholipids and tocopherols. These results indicate that PLC degumming process in combination with PLA treatment can be a commercially viable alternative for traditional camellia oil degumming process. However, more attention should be paid on the oxidative stability of degummed oils in further application.

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