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preliminary communication

Degumming of Vegetable Oil by a New Microbial Lipase

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Summary

Lecitase® Ultra lipase is an acidic lipase which exhibits maximal activity at pH=5.0. It takes part in the activity towards both phospholipid and triglyceride structures. When the temperature is over 40 °C, the phospholipase activity predominates, and the lipase activity is partly suppressed. It could be applied for the degumming of vegetable oil, and the phosphatides in the oil were easily converted by enzymatic catalyzed hydrolysis to less than 10 mg/kg within 5 h at 50 °C.

Key words: degumming, lipase, vegetable oil

Introduction

Oil degumming process plays a critical role in the physical refining of edible oil. Tracditional degumming processes, including water degumming (*1*), superdegumming (*2*), total degumming (*3*), ultrafiltration process (*4*), acid treatment (*5*), *etc.*, cannot guarantee the achievement of low phosphorus contents required for physical refining, and are not always optimally suited for all oil qualities. The yield loss, the apparatus requirement and the energy expenditure of these processes are also great (*6*). Enzymatic oil degumming is a suitable process for the physical refining, in which a phospholipase is used to convert the nonhydratable phosphatides into hydratable form. Apart from the reduction in the amount of acid, alkali and wastewater during the refining process, an enhancement in product yield and a reduction in operating costs can also be observed (*7*).

Enzymatic degumming was first introduced by the German Lurgi Company as the »EnzyMax process« (*8*). The EnzyMax process can be divided into four different steps: (*i*) the adjustment of the optimal conditions for the enzyme reaction, *i.e.* optimal pH with a citrate buffer and the optimal temperature; (*ii*) the addition of the enzyme solution; (*iii*) the enzyme reaction; (*iv*) the separation of lysophosphatide from the oil at about 75 °C. So far, only two kinds of enzymes, such as Lecitase® 10L (pancreatic phospholipase A_2) and Lecitase® Novo (microbial lipase), can be used for oil degumming in the industry. Lecitase® Ultra is a new microbial lipase developed by Novozymes, and it is a protein-engineered carboxylic ester hydrolase from *Thermomyces lanuginosus*/*Fusarium oxysporum* produced by submerged fermentation of a genetically modified *Aspergillus oryzae*. It has inherent activity towards both phospholipid and triglyceride structures. Some of the enzyme characterizations of Lecitase® 10L, Lecitase® Novo and Lecitase® Ultra are listed in Table 1.

The aim of this work was to study the characterization of Lecitase® Ultra, and to apply the enzyme in degumming of the rapeseed and soybean oil.

Materials and Methods

Determination of lipase and phospholipase activity

Lipase assay was performed with olive oil emulsion. One unit of lipase (U) is the amount of enzyme which

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Table 1. Summary of the characterizations of the three kinds of enzymes

Characteristics	Lecitase® 10L	Lecitase® Novo	Lecitase® Ultra
Source	Porcine pancreas	*Fusarium oxysporum*	*T. lanuginosus/F. oxysporum*
Specificity	Phospholipase A_2	Phospholipase A_1	Phospholipase A_1
Molecular mass/kDa	12–14	~28	~35
Ca^{2+} dependent	Yes	No	No
Td (DSC)	70–80 °C	50 °C	60 °C
Kosher/Halal	No	Yes	Yes

releases 1 μmol of titratable free fatty acids per minute under the described conditions. Substrate solution: olive oil and 4 % polyvinyl alcohol solution were emulsified at a volume ratio of 1:4 at 20 000 rpm for 10 min. Analysis conditions: 4 mL of olive oil emulsion, 5 mL of 0.05 M citric acid buffer and 1 mL of enzyme solution were mixed and incubated at 40 °C for 15 min. The reaction was terminated with the addition of 95 % ethanol (15 mL) after incubation, and the liberated fatty acids were titrated with 0.05 M NaOH. Blanks were measured with a heat-inactivated enzyme sample, for which an enzyme stock solution was kept at 100 °C for 15 min. After cooling to ambient temperature, the solution was used as described for the active enzyme sample.

Phospolipase activity was determined according to the lipase method, but using the phospholipid as a substrate. Substrate solution: 10 % phospholipid and 4 % polyvinyl alcohol solution were emulsified at a volume ratio of 1:4 at 20 000 rpm for 10 min.

All experiments were carried out in triplicate for the calculation of the mean value.

Enzyme degumming experiments

Crude rapeseed oil (150 g) was placed into a 250-mL conical flask fitted with stopple. The oil was heated to about 80 °C in a water bath, and then citric acid buffer (0.2 mL of 45 % citric acid and 1.1 mL of 4 % NaOH, pH=5.0) was added. After homogenization for 1 min with a homogenizer at 10 000 rpm, the mixture was incubated for 20 min at 80 °C in a water bath under mechanical stirring (500 rpm). The temperature of the oil then decreased to about 50 °C. Followed by the addition of deionized water (2 % of oil mass) and diluted Lecitase® Ultra solution (0.1 mL, 30 mg/kg of enzyme content), the mixture was mixed with high shear in a homogenizer at 10 000 rpm for 1 min to provide a large surface area through emulsification. Then the flask was placed in a prearranged temperature water bath to begin enzymatic degumming reaction. During the whole reaction, the oil was stirred with mechanical mixer (approximately 500 rpm). Samples were drawn for phosphorus and fatty acid analyses.

Phosphorus and free fatty acids analyses

Preparation of samples

A 10-mL sample of oil emulsion was placed in a glass centrifuge tube. The emulsion was heated in a boiling water bath for 10 min and then centrifuged at 5000 × *g* for 10 min. The supernatant fluid was collected and mixed, and 8 g of oil was drawn for phosphorus and fatty acids analyses.

Phosphorus content analysis

Phosphorus analysis was carried out as follows: 100 mg of MgO were weighed in a porcelain dish and heated on a gas burner. A mass of 0.5–2 g of oil was added and ignited with a gas burner to become a black, hard mass, and then it was heated at 850 °C for 2 h until it turned into white ash. The phosphorus content of the ash was determined according to AOCS method Ca 12–55 (*9*). All experiments were carried out in triplicate for the calculation of the mean value.

Free fatty acid content analysis

Free fatty acid content of the oil samples was determined according to AOCS Ca 5a–40 (*10*). All experiments were carried out in triplicate, and the mean and standard deviation for each of the determinations were calculated and reported.

Results and Discussion

Effect of temperature and pH on lipase and phospholipase activity

As shown in Fig. 1, the enzyme displayed lipase and phospholipase activity. The enzyme was highly active in the acidic environment, and the optimal activity of the enzyme was observed at pH=5.0.

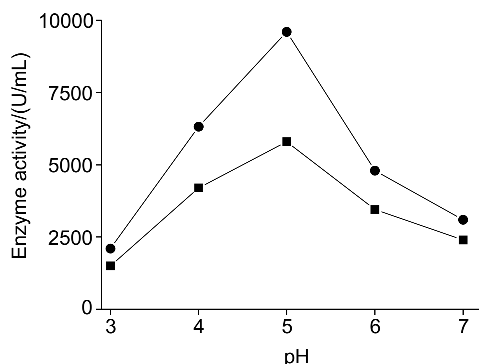

Fig. 1. pH optimum of the enzyme with lipase and phopholipase acitivity, measured in citrate buffer at 40 °C
■ phospholipase activity, ● lipase activity

The enzyme activity was dependent on temperature, as illustrated in Fig. 2. The lipase exhibited maximum activity at 40 °C, however, the maximal phospholipase

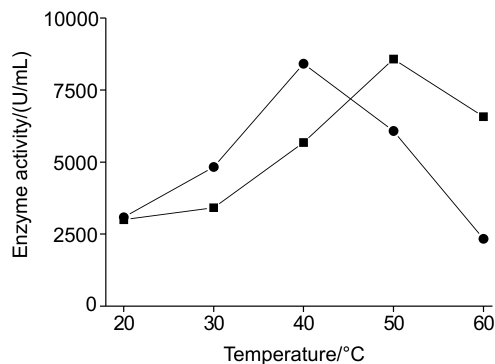

Fig. 2. Temperature optimum of the enzyme with lipase and phopholipase activity, measured at pH= 5.2
■ phospholipase activity, ● lipase activity

activity was at 50 °C. The temperature had different effect on the lipase and phospholipase activity. When the temperature was under 40 °C, the lipase activity was higher than phospholipase activity; when the temperature was above 40 °C, the phopholipase activity was predominant, and the lipase activity was suppressed.

Application in the degumming of oil

Reaction catalyzed by lipase occurred exclusively at the lipid-water interface, so it is critical to create the essential high surface area in the oil degumming reaction system. In the enzymatic oil degumming process, the high shear mixer was used to produce the stable emulsion of crude oil, citric acid, enzyme solution, and additional water. At the interface between the oil and water phase, the reaction of water-soluble enzyme and the oil--soluble phospholipid took place, and phospholipid was enzymatically hydrolyzed to lysophospholipid, which is soluble in the water phase.

Fig. 3 shows the results obtained for the degumming of crude oil. When the citric acid buffer was added to the oil and mixed at 80 °C for 20 min, the content of phosphorus in the rapeseed oil and soybean oil decreased from 123.1 and 150.4 mg/kg to 35.4 and 34.5 mg/kg, respectively. The addition of citric acid buffer had already caused coagulation and precipitation of part of phosphatides. Therefore, the phosphorus content in the oil was substantially lower than the phosphorus content of the crude oil after the acid treatment. When the reaction mixture was cooled to 50 °C, the enzyme solution (30 mg/kg of Lecitase® Ultra) was added. The oil was degummed to less than 10 mg/kg within 2 h, and then the phosphorus content was reduced to 8 mg/kg (rapeseed oil) and 6 mg/kg (soybean oil) by the enzymatic catalyzed hydrolysis within 5 h.

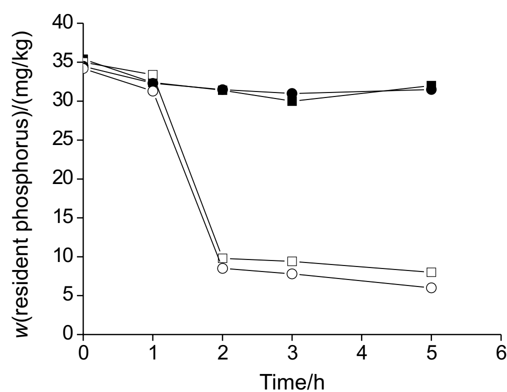

Fig. 3. Residual phosphorous content in the oil phase as function of time in oil degumming experiments
■ rapeseed oil, no enzyme was added, □ rapeseed oil, 30 mg/kg of Ultra enzyme was added, ● soybean oil, no enzyme was added, ○ soybean oil, 30 mg/kg of Ultra enzyme was added

Treatment of crude oil with the enzyme could result in an increase of free fatty acids in the oil. As shown in Table 2, it was observed that the free fatty acids in the rapeseed and soybean oil increased less.

Table 2. Free fatty acids in the oil from the degumming experiments of rapeseed oil and soybean oil at different time

Reaction time/h	*w*(free fatty acids in the oil)/%	
	Rapeseed oil	Soybean oil
0	2.26±0.03	1.04±0.07
1	2.28±0.04	1.08±0.11
3	2.38±0.07	1.19±0.08
5	2.41±0.05	1.20±0.06

Results are the mean of triplicates ± S.D.

It is well known that one fatty acid will be removed from one phospholipid molecule in the enzymatic degumming process. As a result, there will be about 0.1 % increase of the free fatty acids (FFA) if the phosphorus content is 100 mg/kg. In this study, it was found that the amount of FFA increase fitted well to that of the decrease of phosphorus. Therefore, it was concluded that the increase of free fatty acids was the consequence of the hydrolysis of phospholipids rather than triglycerides. It was an interesting phenomenon that the Lecitase® Ultra enzyme was able to identify only the phospholipids as substrate, and did not hydrolyze the triglyceride in the enzymatic oil degumming system, although the lipase activity of the Lecitase® Ultra enzyme was remarkable in the other system. The mechanism of this enzymatic reaction needs to be explored in the future research.

So far, some microbial enzymes with both lipase and phospholipase activity are known. The microbial lipase from *Aspergillus niger* could be more efficient in the hydrolysis of soybean phospholipids, and it exhibits a much higher phospholipase than lipase activity (*11*). Winter *et al.* (*12*) used the microbial lipase from *Aspergillus* sp. RH 3046 to hydrolyze the phospholipids in the soybean, rapeseed and sunflower oil, and it was found that both the phospholipase and lipase activity were exhibited, but phospholipase was predominant. The microbial enzyme is significantly different from pancreatic phospholipase. Pancreatic phospholipase is regarded as a true phospholipase activity without lipase activity. It was found that Lecitase® 10L (pancreatic phospholipase) preferentially hydrolyzed phospholipids in aqueous phase, while Lecitase® Novo (microbial lipase from *Fusarium oxysporum*) preferentially hydrolyzed phospholipids in oil phase (*13*). For different microbial lipase the ratio of

lipolytic to phospholipase activity varies widely, it is not only related to the structure of lipase (*14*), but also to the reaction system.

Conclusions

The Lecitase® Ultra enzyme is a new microbial lipase (E.C. 3.1.1.3) from *Thermomyces lanuginosus/Fusarium oxysporum* produced by submerged fermentation of a genetically modified *Aspergillus oryzae*. It is an acidic lipase, which exhibits maximal activity at pH=5.0, and it has inherent activity towards both phospholipid and triglyceride structures. When the temperature is over 40 °C, the phospholipase activity predominates, and the lipase activity is partly suppressed. It is intriguing to observe that the enzyme is able to identify only the phospholipids as substrate to hydrolyze them in the oil degumming process, and the yield loss due to triglyceride hydrolysis is only marginal. Phosphatides in the oil were easily converted by enzymatic catalyzed hydrolysis to less than 10 mg/kg within 5 h at 50 °C. It was indicated that Lecitase® Ultra enzyme could be used in the degumming of the vegetable oil in the plant.

Acknowledgement

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Degumiranje biljnog ulja s novom bakterijskom lipazom

Sažetak

Lecitase® Ultra lipaza je kiselinska lipaza s maksimalnom aktivnosti pri pH=5,0. Enzim omogućava hidrolizu fosfolipida i triglicerida. Pri temperaturi višoj od 40 °C prevladava fosfolipazna aktivnost, a lipazna je aktivnost djelomično potisnuta. Enzim se može koristiti za degumiranje biljnog ulja, a fosfatidi u ulju smanjeni su enzimskom kataliziranom hidrolizom na manje od 10 mg/kg tijekom 5 sati pri 50 °C.