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CHARACTERIZATION AND SPRAY DRYING OF LIPASE PRODUCED BY THE ENDOPHYTIC FUNGUS *Cercospora kikuchii*

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Abstract - A lipase from the endophytic fungus *Cercospora kikuchii* was purified, biochemically characterized and the effects of spray drying on stabilization of the purified enzyme were studied. The lipase was purified 9.31-fold with recovery of 26.6% and specific activity of 223.6 U/mg. The optimum pH and temperature were 4.6 and 35 °C, respectively, while the Vmax was 10.28 μmol/min.mg^{-1} protein and Km 0.0324 mM. All the metal ions tested enhanced the enzyme activity. The lipase retained almost 100% activity in the presence of strong oxidants and was also resistant to Triton X, Tween 80 and 20 and SDS, as well as to proteases. The purified lipase was spray dried and kept until 85.2% of enzymatic activity. At least 70% of the enzymatic activity was maintained for spray dried purified lipase during the storage period. The lipase produced by *Cercospora kikuchii* has properties useful for industrial application and showed adequate stabilization and retention of its enzymatic activity after spray drying.
Keywords: Lipase; *Cercospora kikuchii*; Enzyme characterization; Spray drying; Enzyme stabilization; Enzyme purification.

INTRODUCTION

Lipases (EC 3.1.1.3) are serine hydrolases produced by animals (Goswami *et al.,* 2012), plants (Barros *et al.,* 2010) and microorganisms (Hassan *et al.,* 2006); Carneiro *et al.,* 2014), although the majority of lipases used for many biotechnological purposes are extracted from bacteria, fungi and yeast cultures. Microbial enzymes are often more useful than enzymes derived from other sources because of the higher stability and more convenient and safer production (Hasan *et al*., 2006). Fungi are widely used to produce higher quantities of lipases. However, endophytic fungi are scarcely chosen as lipase producers even if they are able to live in association with several host plants such as oilseeds. We focused our attention on endophytic fungi as a potential new lipase producer (Costa-Silva *et al.*, 2010; Costa-Silva *et al*., 2011; Costa-Silva *et al.*, 2013).

Some commercial applications like biodiesel production and bioremediation do not require pure lipase preparations, but a certain purity degree enables a more efficient usage (Saxena *et al*., 2003a) However, purified lipase preparations are needed for the biocatalytic production of fine chemicals, pharmaceuticals and cosmetics. An overview of the literature presents different methods for purification and characterization of lipases. Most of the purification procedures reported involve gel filtration, ion-exchange chromatography and mainly hydrophobic interaction chromatography (Antonian, 1988; Taipa *et al*., 1992; Aires-Barros *et al*., 1994).

The commercialization of enzymes with potential industrial applications, including lipases, depends on

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their enzymatic activity stability. The removal of water reduces the movement freedom of the protein molecules and thus inhibits conformational changes leading to activity loss (Monsan and Combes, 1987). Consequently, dry solid formulations are often developed to provide an acceptable protein shelf life. The study of stable forms of protein structures has been important in providing valuable information regarding possible folding mechanisms and the interactions involved in the stabilization of protein structures (Bone, 1994). There are several drying techniques used in the production of dried plant extracts, fruit juices, blood products, microorganisms, and dehydrated foods, such as freeze drying, spouted bed drying and spray drying. Spray drying is used extensively for drying of heat sensitive materials, including enzymes (Nijdam and Langrish, 2005; Costa-Silva *et al*., 2010; Costa-Silva *et al*., 2011). Drying in general may also alter the chemical structure of the protein due to partial loss of protein hydration. The replacement of water by capable of forming hydrogen bonds with protein excipients, e.g., sucrose and lactose, that are can promote protein stabilization on drying (Namaldi *et al.,* 2006).

In the present paper, the endophytic fungus *Cercospora kikuchii* was used to produce inducible extracellular lipases in submerged culture. A single lipase was isolated, purified and its biochemical characterization was carried out. The purified lipase was submitted to spray drying in the presence of some carbohydrates as adjuvants (lactose, maltodextrin, and β-cyclodextrin). The stability of dried enzyme was determined during storage at 5, 25 and 40 °C for six months.

MATERIALS AND METHODS

Microorganism and Lipase Production

Cercospora kikuchii was isolated, identified and maintained according to Costa-Silva *et al*. (2010). The lipase production, as well as dialysis of culture broth, was carried out as described by Costa-Silva *et al*. (2011).

Protein Assay and Enzymatic Activity

The protein concentration was determined according to the method of Bradford (1976) using bovine serum albumin as standard. Lipase activity assay was performed using *p*-nitrophenyl palmitate (*p*NPP) as substrate according to Mayordomo *et al*. (2000). The optical density was measured at 410 nm. One unit (U) of lipase activity is the amount of enzyme that released 1 μmol of p-nitrophenol per minute.

Lipase Purification

The dialyzed culture broth was concentrated with polyethylene glycol 6000 (PEG-6000). For the enzyme concentration dialysis was used, which facilitates the removal of water from lipase in solution by selective and passive diffusion through a semi-permeable membrane. The polyethylene glycol was placed on opposite sides of the membrane. Lipases are larger than the membrane-pores and are retained inside of the membrane. The concentrated lipase was applied to a Butyl-Sepharose column (2.0 x 30.0 cm) previously equilibrated in 50 mM of phosphate buffer pH 6.5 according to Costa-Silva *et al*. (2011). The semi-purified enzyme obtained on the hydrophobic interaction column was concentrated using PEG-6000, prior to injection on a gel filtration column (1.0 x 58.0 cm) with a flow rate of 9.6 $mL.h^{-1}$. All purification steps were carried out at room temperature.

Electrophoresis and Molecular Weight Determination

Polyacrylamide gel electrophoresis (PAGE) under denaturing conditions was performed as described by Laemmli (1970), in 12 % running and 4% stacking gels. Protein was stained using the silver stain method reported by Blum (1987). Low molecular weight markers were used with the following reference proteins: phosphorylase B (94.0 kDa), bovine serum albumin (67.0 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20.1 kDa), and α-lactoalbumin (14.4 kDa) (Bio-Rad, Richmond, CA).

The molecular weight of the lipase was determined by SDS-PAGE (Laemmli, 1970) and by chromatography on Sephacryl S-200 HR (1.0 x 58.0 cm). Different protein molecular weight markers were used in the chromatography method: β-amylase (200.0 kDa), alcohol dehydrogenase (150.0 kDa), bovine serum albumin (66.0 kDa), α-lactalbumin (14.2 kDa), and cytochrome C (12.4 kDa). The column was pre-equilibrated with phosphate buffer (50 mM and pH 6.5) and the proteins were eluted with the same buffer containing 150 $mmol.L^{-1}$ NaCl at a flow rate of 6.0 $mL.h^{-1}$.

Determination of Kinetic Constants

Kinetic constants of *C. kikuchii* lipase were de-

termined by direct fitting of the Michaelis-Menten equation to experimental data (computational program Enzfitter version 1.05 published by Elsevier-Biosoft, 1987). The assays were carried out according to the standard procedure using a pure lipase sample (7.0 µg of protein/mL) and *p*NPP in a range of concentration from 0.015 to 0.4 mmol/L.

Effects of Temperature and pH on Enzyme Activity and Stability

The relative activity of *C. kikuchii* lipase at several temperatures (15-60 °C) was determined using the standard procedure described previously. Thermal stability experiments were conducted from 40 to 60 °C. The enzyme samples were incubated in 50 mM phosphate buffer (pH 6.5) at different temperatures for 120 min, and the residual activity was measured. To determine the optimum pH, 0.05 M solutions consisting of citrate-phosphate (pH 2.2-7.8) and Tris-HCl (pH 8.2-9.0) buffers were used in the standard assay. The effect of storage pH on lipase activity was measured by pre-incubating the enzyme for 1h at the desired pH values, after which the residual activity was assayed at the optimum pH (Belghith *et al.*, 2001).

Effect of Metal Ions, Surfactants and Inhibitors on the Enzymatic Activity

In these experiments, the enzyme was pre-incubated with metal ions, surfactants, or inhibitors for 60 min at 25 °C and then the residual activity was analyzed. The effects of 1 mM metal ions (Mg^{2+}, Cu^{2+}, Ca^{2+}, K^{+}, Hg^{2+}, Zn^{2+}, Mn^{2+}, Al^{3+} and Ba^{2+}) were investigated, as well as 1.0 % of surfactants (Tween 80, Tween 20, Triton X-100) and 1 mM SDS. Effects of 1% (v/v) oxidizing agents like hydrogen peroxide or sodium hypochlorite and proteases (bromelin and papain) were also analyzed.

Drying of Purified Lipase

Purified lipase was dried using a laboratory spray dryer (model SD-05, Lab-Plant, Huddersfield, UK) with concurrent flow regime. The drying chamber has a diameter of 215 mm and a height of 500 mm. The main components of the system are presented in Costa-Silva *et al*. (2010). The purified lipase was fed to the spray dryer through a feed system consisting of a peristaltic pump, a two-fluid atomizer (with an inlet orifice diameter of 1.0 mm), and an air compressor. The spray drying conditions were determined in a previous study of optimization of spray drying of crude lipase extract (Costa-Silva *et al*., 2011). The feed flow rate of atomizing air was set at 17.0 L/min at a pressure 1.5 kgf/cm^2, the other drying conditions are shown in Table 3. The flow rate of the drying air was maintained constant at 60 m^3/h.

The drying operation started with the injection of the drying air into the SD-05 spray dryer. Lactose, maltodextrin (Dextrose equivalent of 10) and β-cyclodextrin at a proportion of 12.5% (wet basis) were used as adjuvants, with 0.1% Tween 80 (Labsynth), in order to evaluate the efficacy of the protection of the purified enzyme during drying and stabilization of enzyme activity in the storage period. Measurements of the outlet gas temperature, T_{go}, were taken at regular intervals in order to detect the moment when the dryer attained the steady state (15 min). Once the steady state was attained, samples of the dried product were collected and used to evaluate the dryer performance and product properties:

A) Enzymatic activity: The lipase activity assay was performed using *p*NPP as the substrate, with some modifications (Mayordomo *et al*., 2000). The difference was the dissolution of powder (adjuvants + purified lipase) in 50 mM of phosphate buffer, pH 6.5. The solution was used to evaluate the residual activity.

B) Efficiency of the powder production: The spray drying performance was evaluated by mass balance, through the determination of the product recovery (R_{EC}), defined as the ratio between the total mass of the product collected to the mass of enzymatic extract fed to the system (dry basis) (Souza and Oliveira, 2012).

C) Product moisture content (Xp): The moisture content of the spray dried product was determined by the oven drying method at 105 °C up to a constant weight and was calculated from triplicate analyses (WHO, 1998).

D) Water activity (Aw): The water activity of samples was measured at 25 °C using a water activity meter AQUALAB 4TEV-Decagon Devices. Standard salt solutions (Decagon) of known water activity were used for calibration of the sensor at the measuring temperature.

E) Morphological characterization: Analysis of the shape and surface characteristics of the powdered product were performed by scanning electron microscopy (SEM; model EVO-50, Zeiss, Cambridge, UK), with 5,000 X magnification. Powder samples were mounted on a double-coated conductive carbon tape adhered to a stub for observation. The samples were coated with gold under a vacuum of about 4 x 10^{-2} mbar (model SCD 050 Sputter Coater, Bal-Tec, Fürstentum, Liechtenstein). The coating time (spraying) was 130 s.

F) Enzyme stabilization: The enzyme stabiliza-

tion after spray drying was assessed by enzyme activity retention of the dried product during storage (6 months) at different temperatures: 5, 25 and 40 °C.

RESULTS AND DISCUSSION

Lipase Purification

Hydrophobic interaction chromatography has been used as method for most of the lipases (Saxena *et al*., 2003b). The retention of lipase on a hydrophobic support is dependent of several factors, including the salt concentration (Mhetras *et al*., 2008). The retention of the enzyme was observed on a phenyl sepharose column in the presence of ammonium sulphate. The main lipase activity (Pool IV) was eluted with distilled water with 80% recovery (Costa-Silva *et al*., 2011). The Pool IV from the Phenyl Sepharose column was concentrated and subsequently applied to a Sephacryl S-200HR column (Figure 1). The enzyme was purified approximately 9.31 fold, with an overall yield of 26.6% and specific activity of 375.0 U/mg (Table 1).

The purified lipase was a monomer with a molecular weight of 69.5 kDa, assessed by SDS–PAGE (Figure 2), and 73.5 kDa by gel filtration column. The molecular masses of most of the fungal lipases have been reported to be in the range of 11–70 kDa (Sugihara *et al*., 1988; Saxena *et al*., 2003a).

Effect of Temperature and pH on Lipase Activity

The optimum temperature for the purified lipase was 35 °C and the activity decreased dramatically at 50 and 60 °C (Figure 3A). The lipase was stable at 40 °C for 2 h and retained 74.3 and 68.5% of its activity at 45 and 50 °C for the same period (Figure 3B).

The optimum pH of the lipase was pH 4.6 (Figure 3C). There are no reports on microbial lipases that are active at extremely acidic pH (<3.0). Such acidic lipases are useful in the food and flavor industries where aroma esters such as isoamyl acetate are formed in an acidic environment (Hassan *et al*., 2006). The purified lipase showed no significant loss of activity when stored in a pH range of 3.0 – 9.0 (data not shown).

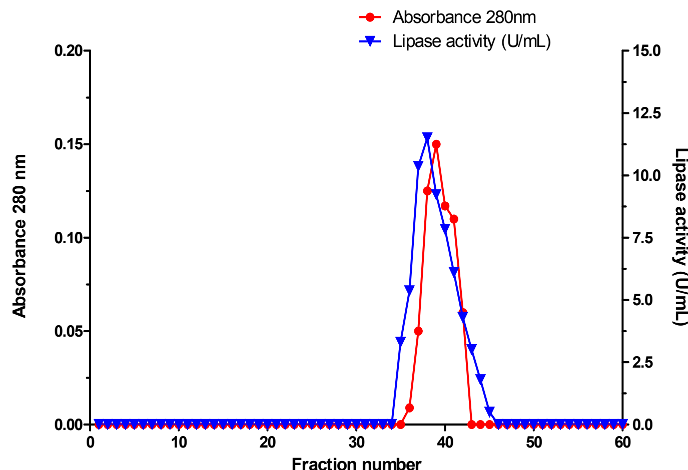

Figure 1: Gel filtration chromatographic profile of lipase from *Cercospora kikuchii*.

Table 1: Summary of purification of lipase from *Cercospora kikuchii*.

Purification step	U	Specific activity ($U.mg^{-1}$)	Total Protein (mg)	Fold purification	Yield (%)
Crude lipase	352.5	40.35	8.73	1.0	100.0
Hydrophobic Interaction	282.0	188.0	1.50	4.65	80.0
Gel filtration	31.3	223.6	0.14	9.31	26.6

(U); Total activity

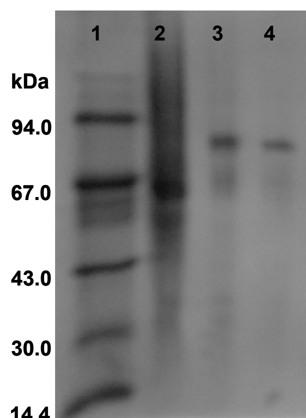

Figure 2: Polyacrylamide gel electrophoresis of purified lipase from *C. kikuchii*. Protein bands were stained with silver nitrate. Lane 1: molecular weight markers. Lane 2: Crude extract. Lane 3: Fractions containing lipase activity from the butyl sepharose column. Lane 4: Fractions containing lipase activity from the Sephacryl S-200 HR column.

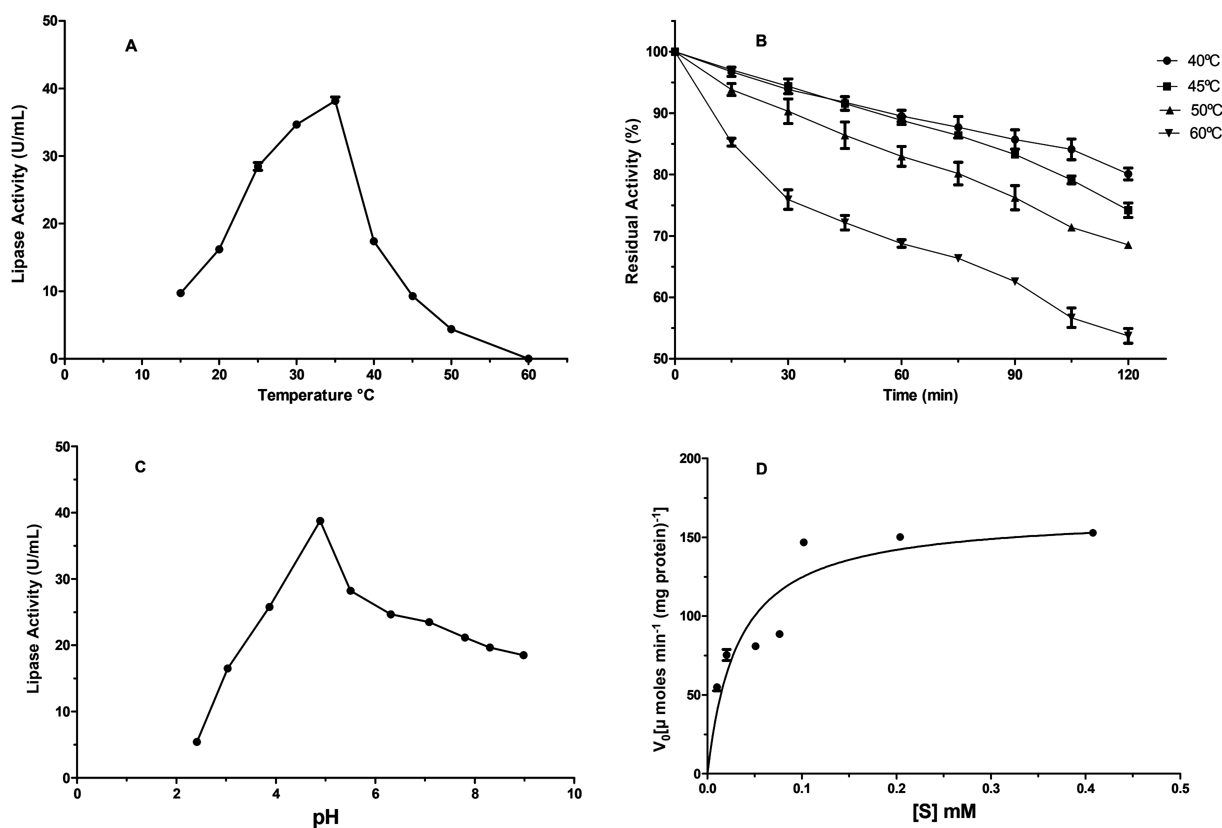

Figure 3: Characterization of purified lipase from *C. kikuchii*. The optimal temperature for activity was assessed by measuring the enzyme activity at different temperatures between 15 and 80 °C (A). For the thermostability test the native enzyme was incubated at different temperatures, 40 ºC (- ● -), 45 °C (- ■ -), 50 °C (- ▲ -) and 60 °C (- ▼ -) for a period of up to 120 minutes (B). The optimal pH of activity was assessed by measuring the enzyme activity at different pHs ranging from 2.2 to 9.0 (C). The Km value was determined by incubating the native enzyme at different concentrations of *p*NPP (D).

Effects of Substrate Concentration on Lipase Activity

In order to study the kinetic properties of purified lipase, the effect of increasing concentration of *p*-NPP on the initial rate of hydrolyse was studied. The V_{max} and K_m were 10.28 μmol/min/mg protein and 0.03240 mM, respectively, using *p*NPP as substrate (Figure 3D). Results showed that an increase in the substrate concentration (*p*-NPP) to 0.1 promoted a significant increment in the hydrolytic activity values for both free and immobilized lipases. The enzymatic reaction obeys the Michaelis–Menten equation and no product inhibition was observed. With a subsequent increase in the substrate concentration, the activity became essentially concentration independent and approached asymptotically to a constant rate (V_{max}). A slight reduction in the hydrolytic activity was verified for substrate concentrations higher than 0,1 μM, suggesting a substrate inhibition.

Effect of Metal Ions, Surfactants, Detergents, Oxidizing Agents and Proteases on Lipase Activity

The ions Ca^{2+}, Mg^{2+}, Al^{3+}, Zn^{2+}, Ba^{2+}, Hg^{2+} and Mn^{2+} enhanced the enzymatic activity of *C. kikuchii* lipase, while Cu^{2+}, K^{+} and Na^{+} had insignificant effects (Table 2). In the presence of Al^{3+} ions the lipase activity increased 129.3%. There is not much information available about promotion of lipolytic activity by this ion. The lipase produced by *C. kikuchii* was much more stable than that produced by *Fusarium solani* N4-2 and Lipolase®, whose activity was completely lost in the presence of Hg^{2+} ions (Liu et al., 2009). Activity of lipase from *Bacillus coagulans* BTS-3 was also enhanced in the presence of K^{+}, Mg^{2+}, Fe^{3+} and Hg^{2+} and from *Penicillium aurantiogriseum* by Mg^{2+}, Zn^{2+} and Mn^{2+} ions, but in this fungus it was completely inhibited by Hg^{2+} ions (Schmid and Verger, 1998).

Surfactants are usually present in detergents. They can potentially denature the lipases by disrupting their tertiary structures. On the other hand, they can inhibit the aggregation of the lipases, so their addition may increase the lipolytic activity (Schmid and Verger, 1998). The lipase from *C. kikuchii* was stable in the presence of four surfactants, keeping 98 -100 % of enzymatic activity (Table 2). It was also surprisingly stable after 1 h in the presence of oxidizing agents, mainly sodium hypochlorite, where 98.2% of the activity was kept. Under the same condition the residual activity of the commercial enzyme Lipolase® was 46.3% (Liu *et al*., 2009). Proteases are often present in detergents together with lipases, so for this industrial application the lipase needs to exhibit protease toleranc. In this regard, the lipase produced by *C. kikuchii* showed appreciable stability towards papaina and bromelin (Table 2).

Table 2: *Cercospora kikuchii* lipase stability in the presence of ions, surfactants, detergents, oxidizing agents and proteases.

Additives Tested	Residual Activity (%)
Metal ions	
$CuSO_4$	103.1 ± 1.83
$CaCl_2$	**133.0 ± 1.21**
$MgCl_2$	**121.6 ± 0.75**
KCl	102.5 ± 1.29
$BaCl_2$	111.3 ± 1.86
$AlCl_3$	**229.3 ± 1.72**
NaCl	100.5 ± 1.53
$MnCl_2$	**128.2 ± 1.24**
$HgCl_2$	**137.1 ± 1.89**
Surfactants	
Triton X-100	101.5 ± 1.55
Tween 80	100.2 ± 1.25
Tween 20	99.8 ± 0.79
SDS	102.4 ± 1.20
Oxidizing agents	
Hydrogen peroxide	100.4 ± 1.59
Sodium hypochlorite	99.2 ± 0.74
Proteases	
Papaina	83.9 ± 0.95
Bromelin	92.4 ± 0.89

Metal ions/surfactants/detergents/oxidizing agents (1%, w/v or v/v)/proteases (0.05 $mgml^{-1}$)

Spray Drying Stabilization of Purified Lipase

Table 3 shows the effect of spray drying in the presence of different adjuvants on residual lipase activity (R_{AE}), process yield (R_{EC}), outlet drying gas temperature (T_{go}), moisture content (Xp), and water activity of the dried product (A_w). The residual enzymatic activities with all adjuvants used were above 85.2% after spray drying. However, the activity was completely lost in the absence of adjuvants. Among all preparations evaluated, those containing β-cyclodextrin with 0.1% Tween 80 (Labsynth) showed the best result because they retained almost 100% of activity after drying, followed by lactose (87.7%) and maltodextrin DE10 (85.2%).

Several researchers have examined the effects of the operating conditions on enzyme activity retention after spray-drying process. Alloue *et al*. (2007) showed the powders produced by spray drying supplemented with maltodextrin or gum arabic, both with and without calcium chloride, were also stored for 12 months at 4 or 20 °C without loss of activities. In all cases, additives used to stabilize lipase allowed for

Table 3: Drying conditions and effect of adjuvants on lipase activity recovered after spray drying.

ADJ*	W_s (g/min)	T_{gi} (°C)	T_{go} (°C)	R_{AE} (%)	R_{EC} (%)	X_P (%)	A_w (-)
Lactose	4.0	100.0	50.5	87.7±1.12	53.0±1.15	7.0±1.56	0.3±0.08
Maltodextrin	9.36	153.6	61.5	85.2±0.89	45.5±1.43	3.2±1.04	0.3±0.05
β-Cyclodextrin	9.36	153.6	63.3	99.6±1.03	42.5±1.23	8.4±1.62	0.3±0.07
Without adjuvants	4.0/9.36	100.0/153.6	70.1/119.3	-	-	-	-

ADJ= drying adjuvant; Ws= feed flow rate; T_{GI}= inlet gas temperature; T_{go}= outlet gas temperature; R_{AE}= retention of the enzymatic activity; R_{EC}= product recovery; X_P= moisture content; A_w=water activity; *with 0.1% Tween 80.

longer periods of storage at these two temperatures. Others enzymes have been stabilized by spray drying too. In work of Belghith *et al*. (2001) found that, although 1% maltodextrin had a negative effect on enzyme recovery, it completely stabilized cellulases even after a long period (8 months) of storage at 30 °C.

This enzyme stabilization is the result of the occurrence of different interactions between the enzyme and the adjuvants used. However, the mechanisms of stabilization in the solid state are still not completely understood (Chang and Pikal, 2009). From the enzymatic activity results for spray dried lipase, it was concluded that the enzyme activity of dried lipase using β-cyclodextrin was higher than with the other adjuvants. This is not a surprising result owing to the interaction between the enzyme and this carbohydrate. It is well known that lipases are enzymes which have a high affinity for hydrophobic surfaces, and this interaction could cause a hyper-activation in the lipase activity. This is a consequence of the interfacial activation that involves the movement of a polypeptide chain called the lid that can isolate the active site from the medium (Verger, 1997). The surroundings of the active center of the lipase and the internal face of the lid are hydrophobic and, after the lid movement, the lipase is adsorbed on drops of oils (natural substrate). Thus, the hydrophobic nature of the β-cyclodextrin cavity, shown in Figure 4, could permit the adsorption and hyper-activation of lipases via this mechanism. Other researchers have been shown the usefulness of β-cyclodextrin in the immobilization of different lipases (Ozmen and Yilmaz, 2009; Yilmaz and Sezgin, 2012).

The moisture content could have an impact on the properties of the solid formulations, mainly on enzyme preparations (Hageman, 1988). In general, low values of moisture content (and water activity as well) are excellent for product stability. It can be seen in Table 3 that the product moisture content was in the range of 4.2–7.0% (dry basis). These values are within the range of the recommended content moisture for biological products. The water activity is another important parameter linked to the quality of dry products. The values of A_w could indicate the possibility of microorganism growth and toxin production. According to Beauchat (1981) water activity values above 0.6 could promote microorganism growth and above 0.8 facilitate toxin production. Table 3 shows that the water activity values (0.3) for the three adjuvants used are below the maximum value recommended. The temperature of the exhaust gas, T_{go}, is an important drying parameter affecting both the water activity and product moisture content.

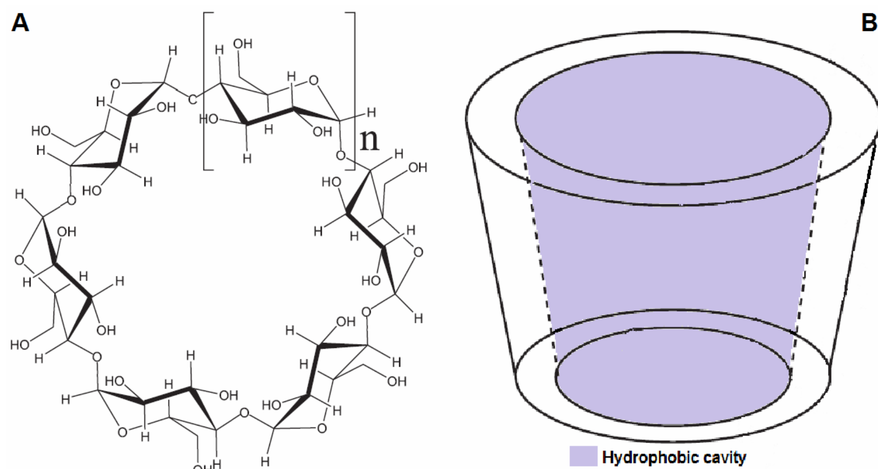

Figure 4: Chemical structure of β-cyclodextrin (A) and its hydrophobic central cavity (B).

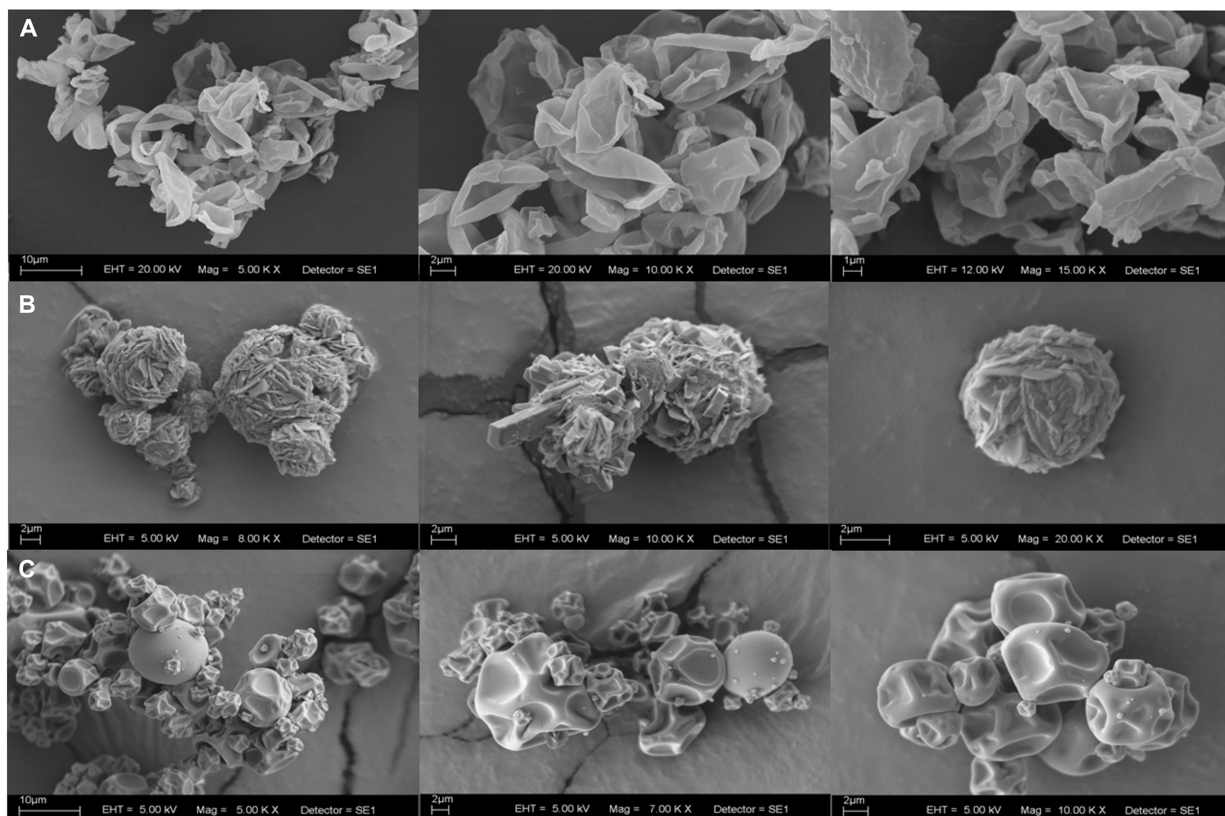

Figure 5: Scanning electron photomicrographs of the spray-dried lipases with different adjuvants: β-cyclodextrin (A), lactose (B), and maltodextrin (C).

The process yield during spray drying was evaluated through the determination of product recovery, R_{EC}. Table 3 shows R_{EC} in the range of 53.0–42.5%, which is considered adequate for lab-scale spray dryers. To solve the problem of the low yield, it is necessary to use more efficient powder collection systems or change the spray drying conditions.

The external morphology of the purified lipase powder, using lactose, maltodextrin, and β-cyclodextrin as adjuvants, is shown in Figure 5. The appearance of the product morphology varied according to the adjuvants used. The particle surface appeared to be grooved when maltodextrin was used as the adjuvant and, in the case of β-cyclodextrin, the particles of dry powder did not show a defined format. With lactose as the adjuvant, the particle surface showed a rounded and a rough surface. The particle size ranged from 2 to 10 μm in all cases.

Stability tests were performed for the spray dried purified lipase powders. The samples were stored at three temperatures, namely 5, 25, and 40 °C, for up to 6 months. After this period the powders were used to evaluate the residual enzymatic activity. The experimental results are presented in Table 4.

Table 4: Stability testing of product spray dried in the presence of various additives after 6 months' storage at different temperatures.

Adjuvants	Temperature °C	Residual activity %
Lactose	5	74.8±0.78
	25	49.1±1.19
	40	32.9±1.02
Maltodextrin	5	70.9±0.76
	25	45.5±0.55
	40	28.7±1.12
β-Cyclodextrin	5	77.6±1.23
	25	53.2±0.88
	40	39.4±1.43
Lipase in aqueous solution	5	9.3±1.44

The enzyme residual activity decreased with increasing temperature. Positive effects of the additives over the storage period become clear since almost 100% of lipase activity was lost without additive. Higher values of retention of enzymatic activity were obtained for samples stored at 5 °C (69.0–77.6% of

the original value). Significant losses of enzymatic activity were observed for samples stored at 25 and 40 °C. Therefore, 5 °C is the best storage temperature for the spray-dried product. Among all adjuvants tested, β-cyclodextrin at 5 °C provided the best result.

CONCLUSIONS

The purified lipase from *C. kikuchii* presented desirable properties for industrial application, for example in aroma production. High relative activity and stability of dried purified lipase can be achieved by using carbohydrates as drying adjuvants. Given the typically better economy of spray-drying compared to freeze-drying, this suggests that the former technique has potential for drying of protein-containing systems.

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