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Short communication

Storage of *Yarrowia lipolytica* lipase after spray-drying in the presence of additives

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Abstract

Lipase from *Yarrowia lipolytica* is an enzyme that presents numerous potentialities for biotechnological applications. This work describes the development of powders obtained by atomization of supernatants lipase from *Y. lipolytica* LGx6481. Two formulations were studied: one formulation with skim milk powder and gum arabic, and the other with maltodextrin, calcium chloride and gum arabic. After drying, powders were stored at 4 and 20 °C in aluminium hermetic bags to evaluate their stability over a period of one year. The influence of water activity and glass transition temperature (T_g) on the powder's storage were also studied.

Keywords: *Yarrowia lipolytica* lipase; Formulation; Spray-drying; Storage

1. Introduction

Lipases (E.C.3.1.1.3) constitute a group of enzymes having the ability to hydrolyse triglycerides at lipid–water interfaces [1]. This reaction is reversible, so that these enzymes also catalyse the formation of acylglycerols from glycerol and free fatty acids. Over the last few years, there has been an increasing interest in the development of new applications for lipases in products and processes, particularly in the food, medical, and chemical industries. Extracellular microbial lipases can be produced relatively easily by fermentation and are available in large quantities for industrial use [2]. Spray-drying has been used for the dehydration of many enzymes, such as proteases or cellulases, and offers cost advantages compared to freeze-drying. However, dehydration by spray-drying causes a stress to proteins and their unfolding by thermal denaturation, leading to the loss of enzymatic activity. This unfolding is usually minimized by using additives, such as polysaccharide, some proteins and salts [3]. Spray-drying is a simple, fast, and economic technique to obtain a powder from a solution or a liquid suspension (e.g., an enzyme suspension). Powders which have dry matter content higher than 90% are easier to handle and preserve than liquid preparations. Unlike freeze-drying, spray-drying is generally considered as an attractive method for the preparation of large quantities owing to the low cost and complexity of the process [4]. Additives such as lactose contents in skim milk showed enzyme stabilizing abilities in several studies, mainly due to a readily attainable amorphous form [5–7] which reportedly enhances the preservation of activity of several enzymes upon spray-drying due to the increased initial skim milk total solids content. Maltodextrins are low converted starch products, with a DE-value range from 2 to 20. They are intermediate between starch and corn syrups; unlike starch, they are soluble in cold water, but unlike syrups, they are non-sweet. Consequently they have found wide application in the food industry as bodying agents, coatings, and carriers for flavours, fragrances and oils in cosmetics [8]. However, the use of the DE value has been shown to be inadequate to predict product performance in various applications [9]. Gum arabic is most often used as a flavour-encapsulating material. Its solubility, low viscosity, emulsification characteristics and good retention of volatile compounds make it very versatile for most encapsulation

Abbreviations: T_g, Glass transition; a_w, Water activity; DSC, Differential Scanning Calorimetry; GA, Gum arabic; MP, Milk powder; MD, Maltodextrin; DE, Dextrose Equivalent

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methods. In addition, its wall material is ideally suited for the encapsulation of lipid droplets as it fulfils the role of both surface-active agent and drying matrix, and thus preventing the loss of volatile compounds in contact with the atmosphere. However, its application within the food industry is limited because gum arabic is more expensive than maltodextrin [10,11] and its availability and cost are subject to fluctuations, hence there is a need to evaluate alternatives. Mixtures of gum arabic and maltodextrin have shown to be promising as high solid carriers, giving acceptable viscosity in studies of microencapsulation of oil by spray-drying [12]. Lipase from *Yarrowia lipolytica* presents many potential applications for the food industry, pharmacology, and for the environment. The enzyme is efficient for the hydrolysis of natural oils and fats, and keeps active while at low temperatures (4 °C) and low pH (3–4) [13]. Indeed, the activator effect of calcium ion (Ca^{2+}) on lipase was proven in this study. The lipase production process in a large bioreactor, as previously described by [14], used a mutant of *Y. lipolytica* obtained by mutagenesis with an *N*′methyl *N*′nitro *N* nitroso-guanidine from wild strain CBS 6303 [15]. The mutant strain called LGx 6481 produces 50 folds more activity than the wild strain. The preceding downstream process investigated included centrifugation to eliminate biomass, an ultrafiltration to concentrate the supernatant, and a spray-drying in the presence of milk powder and gum arabic. This study [14] indicated that after adding 12% (w/v) of milk powder alone, lipase recovery was highest, while adding 12% of milk powder and 3% (w/v) of gum arabic produced a fluent powder more compatible with commercial product standards. This paper also presents other formulations without lactose designed for people with lactase deficiencies and a characterization of different lipase powders related to their composition and their storage time (enzyme activities, dry matter, water activities, and glass transition temperature).

2. Materials and methods

2.1. Lipase solution

Lipase was produced in 500 and 2000 l bioreactors (LSL Biolafitte, Poissy, France) in the same conditions as described previously [14]. Culture fluids were centrifuged on a BTPX205 continuous centrifuge (Alfa Laval, Sweden) at 12,000 × *g*, at a flow rate of 500 l h^{-1}. The supernatant was then clarified through a plate filter with 50 plates (SAPQ7, Filtreclair, France) of 2 μm porosity before being concentrated on a Niro UF/MF ultrafiltration apparatus equipped with 10 m^2 polysulfone membranes (cut off of 10 kDa, Kolding, Denmark). The concentrated supernatant was supplemented with loading materials for spray-drying.

2.2. Spray-drying of enzyme solution

Samples of concentrated supernatant produced in a 2000 l bioreactor (16,000 U ml^{-1}) in a ratio of concentration (950: 36 l) were supplemented with skim milk powder 120 g l^{-1}, and gum arabic 60 g l^{-1} (Roquette, France). They were spray-dried in a pilot spray dryer Niro Mobile Minor (Niro, Denmark) with inlet and outlet temperatures of 160 and 85 °C respectively, at a flow rate of 12 l h^{-1}. Samples of non-concentrated culture supernatant produced in the 500 l bioreactor (3600 U ml^{-1}) were supplemented with maltodextrin (DE 12) 120 g l^{-1} (Roquette, France), gum arabic 60 g l^{-1} and 0, 10, 20, or 30 g $l^{-1}$of calcium chloride (VWR Prolabo, Belgium). They were spray dried in the same spray dryer with inlet and outlet temperatures of 160 and 85 °C respectively, at a flow rate of 14 l h^{-1}.

2.3. Storage of dried powders

2.3.1. Effects of temperature

After separation from the cyclone and collection, dried powders were transferred into aluminium foil-lined plastic bags (Seal Pack Euro Bag, Belgium), sealed under vacuum to prevent rehydration [16], and stored at either 4 or 20 °C.

2.3.2. Effects of water activity

Samples of powders no sealed in aluminium bags were stored in different desiccators and measured at time intervals for water activity. Equilibrium RH (or a_w) that the powders achieved when exposed to the saturated salts solutions (VWR Prolabo, Belgium) was obtained. The salt solutions were as follows (a_w): lithium chloride (0.11), potassium acetate (0.23), magnesium chloride (0.33), potassium carbonate (0.43) sodium chloride (0.75), and potassium chloride (0.85). After three weeks equilibration, the enzymatic activity of powders was determined by titrimetric method.

2.4. Analytical procedure

Lipase activities were measured by the titrimetric method previously described by [13,14]. One unit of lipase is the amount of enzyme able to catalyse the release of 1 μmol of fatty acid per min at pH 7 and 37 °C. Dry matter of liquid and solid were measured after desiccation at 105 °C over a period of 48 h until a stable weight was obtained. Water activity (a_w) of the powders was determined after storage. A sample of powder was placed in a plastic cup and loaded into an osmosmeter (Aqualab CX3, Decagon Devices, USA), and a reading was taken after equilibration.

Glass transition temperatures (T_g) were determined for the powders produced with skim milk and calcium chloride using a Perkin Elmer DSC-7 differential scanning calorimeter/TAC-7 thermal analysis controller with an intracooler-2 cooling system (Perkin Elmer Instruments, USA). Perforated aluminum sealed 50 μl pans containing 2–5 mg powder samples were heated at a scanning rate of 10 °C min^{-1}, between 10 and 120 °C, using nitrogen as blanket gas. Empty sealed pans were used as reference cells (no thermal transition). Calibration was performed using cyclohexane and indium as standards. The glass transition temperature was determined using Pyris software ver. 3.81 (Perkin Elmer Instruments, USA) by considering the mid point temperature (T_{mid}) at the inflection point of the endothermic transition peak (average of three measurements).

3. Results and discussion

3.1. Effects of additives on lipase activity during spray-drying

The formulation carried out with skim milk powder was the first formulation most often used during the lipase downstream processing on pilot scale. In this study, non-concentrated culture supernatant formulated with maltodextrin gum arabic and $CaCl_2$ was compared to concentrated supernatant formulated with skim milk and gum arabic. This research was directed towards the combined effect of the two additives: milk powder 12% (w/v) and gum arabic 6% (w/v) in the first formulation. After addition of milk powder and gum arabic, the lipase concentrate formulated with an activity of 24,000 U ml^{-1} produced a powder with an activity of 55,800 U g^{-1}, which represented a yield of 64% from dehydration. Table 1 shows that the additives had a positive effect on the enzyme and led to an increase in lipase activity by 1.46-fold. The activation of lipase