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Direct measurement of rice bran lipase activity for inactivation kinetics and storage stability prediction

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ABSTRACT

Rice bran is a rich source of valuable nutrients and has potential for high-value applications. Endogenous lipases catalyze the hydrolysis of rice bran oil to free fatty acids, which initiates lipid oxidation. The evaluation of the success of rice bran stabilization processes in terms of the degree of lipid oxidation and shelf-life has so far relied on the measurement of free fatty acid content over a storage period of 3–6 months. In the present study, a photometric and a titrimetric pH-stat method for direct lipase activity measurement immediately after debranning were adapted to rice bran. The photometric method was further applied to determine rice bran lipase/esterase inactivation kinetics, which are useful to optimize stabilization treatments in order to prevent overprocessing and retain maximum level of nutrients. Rice bran was heat-treated in a specialized, hermetically sealable reactor at controlled holding times (5–40 min), temperatures (70–145 °C) and moisture contents (10–20%). Temperature dependency of the lipase/esterase inactivation rate could be described by the Arrhenius equation. Empirical findings on the importance of moisture content for effective rice bran stabilization could be quantified. Furthermore, the results demonstrate the great potential of the method to predict the shelf-life of stabilized rice bran without time-consuming storage tests.

1. Introduction

Rice bran is a side stream product of rice milling with an annual global production volume of about 70 million tons (FAOSTAT, 2012). It consists of the pulverized outer layers of the rice kernel and the germ and accounts for 10% of the processed brown rice. The vast majority of nutrients are concentrated in the bran fraction: oil including essential fatty acids, proteins, fibers, vitamins, antioxidants (e.g. γ-oryzanol), and other micronutrients. Presently, rice bran is mostly used as cost-efficient ingredient for animal feed or as raw material for oil extraction (Orthoefer, 2004). But there is an underestimated potential for high-value rice bran products for human nutrition, and several studies have been conducted to produce ingredients from rice bran that are rich in (soluble) dietary fibers or proteins. However, after the whitening step, rice bran oil (18–22% of rice bran) is exposed to the endogenous lipases, which catalyze the hydrolysis of rice bran oil. This leads to an increase in free fatty acids and to the subsequent generation of off-flavors (Orthoefer, 2004). A stabilization step to decrease lipase activity is therefore indispensable but should be as gentle as possible to retain a maximum level of nutrients. As free fatty acids produced through the activity of lipase are also better substrates for lipoxygenase enzyme and lipid autoxidation compared to the fatty acid moieties of glycerol esters (Gardner, 1995), inhibition of the initial formation of free fatty acids is a key to controlling rice lipid oxidation. Moreover, Orthoefer (2004) reports a lower inactivation temperature for lipoxygenase than for lipase and similarly, Vetrimani et al. (1992) found that lipase was more heat-stable than lipoxygenase when heat-treating rice bran.

So far, two types of lipases (EC 3.1.1.3) and two types of esterases (EC 3.1.1.1) have been purified from rice bran. Rice bran lipase I (relative molecular mass M_r 40'000) was purified by Funatsu et al. (1971) and later, Aizono et al. (1976) detected rice bran lipase II (M_r 33'300). In addition, lipases present in rice bran might also originate from microbes that proliferate upon grain storage especially under humid storage conditions (Huang, 1993). A rice bran esterase (M_r 27'000) was identified and characterized by Chuang et al. (2011) and another esterase (M_r 25'000) was isolated from rice bran by Hamada et al. (2012). Although it is not unambiguously proven whether esterases also contribute to the development of

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rancidity in unstabilized rice bran (Huang, 1993), it is rather likely as suggested by Goffman and Bergman (2003).

The current quantitative standard method for lipase activity determination in rice bran is an indirect measurement of the accumulation of the product (free fatty acids) over a storage period of 3–6 months (SLMB, 2012). Hence, a reliable conclusion on the shelf-life performance of stabilized rice bran can currently not be given before 3–6 months after debranning. A direct lipase activity method would therefore enable direct monitoring, evaluation and improvement of present hydrothermal stabilization processes. Many different direct lipase assays and fast test kits are well-established for high-moisture systems in medical and microbiological research, whereas the titrimetric pH-stat method is generally used as a specific reference lipase assay. Only a few of the existing lipase and/or esterase assays have been adapted to allow direct lipase activity measurement in low-moisture (cereal) systems. Among them is a titrimetric pH-stat method (Hoppe and Theimer, 1996), a titrimetric method (Prabhu et al., 1999), a copper soap method (O'Connor et al., 1992), an assay using radiolabelled substrate (O'Connor et al., 1992), a fluorimetric method (Saunders and Heltved, 1985), and a photometric method (Fretzdorff, 1992). However, only the titrimetric pH-stat method and the fluorimetric method additionally allow continuous-monitoring of initial lipase activity (measurement of maximum velocity). The other methods require a more time-consuming incubation step, in which aliquot samples are analyzed at different time points and maximum velocity is calculated from the reaction plot.

In high-moisture food systems like in the dairy industry, inactivation kinetics of enzymes and microorganisms, which affect product quality, are an established tool for the planning and control of (thermal) processes in order to achieve an optimized quality of the respective food (Kessler, 1996). Concerning low-moisture rice bran stabilization, many different (mostly thermal) processes such as extrusion (Saunders and Heltved, 1985), ohmic heating (Loypimai et al., 2009) or microwave heating (Tao et al., 1993) have been reported. These stabilization processes were evaluated by free fatty acid measurement over storage time. In addition to this, Saunders and Heltved (1985) used a direct, fluorimetric lipase/esterase activity method. However, we know of no studies on lipase/esterase inactivation kinetics at different, well controlled moisture–time–temperature combinations in low-moisture (10–20%) cereal products. In particular, rice bran moisture content was not varied and could not be kept constant, and temperature could not be controlled exactly during the rice bran stabilization treatments mentioned above.

The aim of this study was to define inactivation kinetics of rice bran lipase with direct measurement of enzyme activity. To achieve this, we optimized and compared two direct methods for the continuous-monitoring of initial lipase and/or esterase activity in rice bran (i.e. low-moisture system). The optimal method was then used to study the thermal inactivation kinetics of rice bran lipase/esterase in a model stabilization process, allowing exact control of rice bran moisture content, temperature and holding time. Finally, the lipase/esterase activity measurements were compared to the development of free fatty acid content for storage stability prediction.

2. Materials and methods

2.1. Raw material

We analyzed the lipase activity in different rice grain varieties (*Oryza sativa* ssp. *indica*) of different origin: Puntal (center west Spain, Badajoz), Puntal (south Spain, Sevilla), Sirio (northern Italy), IRGA 409/417 (Brasil) and IR-64 (India), and used paddy rice as the sample material. Of these, the variety with the highest activity, namely the variety Sirio grown in northern Italy in 2011, was chosen for the subsequent studies. All the grains were stored in sealed containers at ambient conditions until dehulling.

2.2. Pilot plant installation for the simulation of industrial rice bran production

Dehulling of paddy rice was done on a Vario Roller Mill (Bühler MIAG GmbH, Germany) using rubber rollers. The fast roller was set to 400 rpm and the slow roller was operated at 150 rpm. The distance between the rollers was adjusted so that they were touching and consequently led to a power consumption of 0.1–0.2 kW. After two passages in the Vario Roller Mill, the hulls were removed by an air stream in a laboratory sifter (Röber, Germany). The resulting brown rice fraction was milled batchwise (500 g) in a vertical stone mill (Bühler MIAG GmbH, Germany) at a frequency of 50 Hz followed by a density based separation of the white rice from the bran until a defined rice whiteness between 38 and 42 (arbitrary units of the instrument) as measured in the whiteness tester C300-3 (Kett Electric Laboratory, Japan) was reached. Rice bran was finally sifted in a laboratory stack sifter (Perles AG, Switzerland) using a mesh size of 710 μm. The rice bran was used immediately for the experiments.

2.3. Specialized reactor for heat-treatment experiments at constant rice bran moisture

The closely controlled heat-treatment of rice bran was conducted in a hermetically sealable specialized reactor allowing fast and repeatable heating without loss of moisture by evaporation (devise described and illustrated by Amrein et al., 2006). Analysis of rice bran moisture content was done using a Halogen Moisture Analyzer HR 73 (Mettler Toledo, Switzerland) before and after each heat-treatment. After the adjustment of the rice bran moisture content, 10 g of rice bran were distributed uniformly between the two thin stainless steel plates of the reactor and it was then closed by a silicone gasket. The compressed rice bran sample in the reactor had a diameter of 100 mm and a thickness of 1.6 mm. The rice bran sample in the reactor was heated using a liquid bath (Sinth 200, Haake GmbH, Germany) on a heating plate coupled with a temperature regulator (Heidolph EKT 3001, Germany), allowing temperature adjustment of ±1 °C. After a defined holding time in the preheated bath, the reactor was immediately brought to room temperature in a cooling bath.

2.4. Sample preparation: extraction and concentration of rice bran lipase/esterase

Extraction of rice bran lipase/esterase was performed as described by Prabhu et al. (1999) with some modifications. Rice bran first was defatted using an accelerated solvent extractor (Dionex ASE 200, USA). Each cell (cell volume 22 mL) was loaded with 2 to maximal 6 g of rice bran. The temperature was set to 40 °C and the pressure to 500 psi. Petroleum ether was used as solvent and after 5 cycles, the extraction cell was purged with nitrogen for 300 s. Defatted rice bran was transferred quantitatively into a 50 mL Falcon tube. Then, 5 mL of extraction buffer per gram of non-defatted rice bran was added and the samples were shaken vigorously for 1 min. Extraction buffer was 50 mM potassium phosphate buffer containing 0.1% Triton X-100 at pH 8.0 for the photometric method and 5 mM MOPS (3-(N-morpholino)propanesulfonic acid) buffer containing 0.2 M $CaCl_2$ at pH 7.0 for the titrimetric pH-stat method. After centrifugation for 15 min at 4750 rpm and 4 °C,

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