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Okara (soybean residue) biotransformation by yeast *Yarrowia lipolytica*

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ABSTRACT

Okara, or soybean residue, is a soy food processing by-product from the manufacture of soymilk and soybean curd (tofu). In this study, solid-state fermentation of okara was conducted over 5 days using yeast *Yarrowia lipolytica*, and the changes in proximate composition, antioxidant capacity, non-volatiles and volatiles were investigated. Yeast metabolism of okara significantly increased the amounts of lipid, succinate and free amino acids and enhanced the antioxidant capacity. In particular, there was a marked increase in important umami tastants after fermentation, with 3-fold increase in succinate and a 20-fold increase in glutamate. The final fermented okara contained 3.37 g succinate and 335 mg glutamate/100 g dry matter. Aldehydes and their derived acids in the fresh okara were catabolised by *Y. lipolytica* mainly to methyl ketones, leading to a reduced grassy off-odour and a slightly pungent, musty and cheese-like odour in the fermented okara. Amino acid-derived volatiles, such as 3-methylbutanal and 2-phenylethanol, were also produced. Overall, the okara fermented by *Y. lipolytica* had a greater amount of umami-tasting substances, a cheese-like odour, improved digestibility and enhanced antioxidant capacity. These changes highlight the potential of *Yarrowia*-fermented okara as a more nutritious, savoury food product or ingredient. *Y. lipolytica* was thus demonstrated to be suitable for the biovalorisation of this soy food processing by-product.

1. Introduction

Okara, or soybean residue, refers to the insoluble residue left after grinding soybeans and extracting the water-soluble components for the manufacture of soy-based food products, such as soymilk and bean curd (tofu). About 1.1 kg of fresh okara is produced from processing 1.0 kg of soybean to produce soymilk or tofu (Khare et al., 1995). Large quantities of okara are produced by the soy product manufacture industry, especially in Asian countries where soy foods are hugely popular. Okara is normally incorporated into animal feed or discarded by companies due to its high perishability and undesirable flavour and texture. However, okara is still highly nutritious; it contains about 40–60% carbohydrates (mostly as insoluble fibre), 20–30% protein and 10–20% lipid (all dry basis) (Li et al., 2012), and so okara is a suitable substrate for biotransformation.

The fermentation of okara by fungi and bacteria has been well-studied, as reviewed by Vong and Liu (2016a), but there are few studies on yeast fermentation of okara. Recently, Rashad et al. (2013) showed that yeast-fermented okara had changes in proximate composition and increased antioxidant capacity. In our previous work, we observed that yeast fermentation of autoclaved okara led to interesting changes in its volatile profiles (Vong and Liu, 2016b). Aldehydes in the okara, such as hexanal and *trans*-2-hexenal, were biotransformed into alcohols, ketones and/or esters, and the effect varied with the yeast employed. Okara fermented by dairy yeasts (yeasts typically associated with fermented dairy products) led to increase in cheese-like volatiles. Therefore, the present study further explored other biochemical changes in okara fermented by the dairy yeast, *Yarrowia lipolytica*.

Y. lipolytica is a strict aerobe and non-conventional dimorphic yeast that is generally recognised as safe (GRAS) (Groenewald et al., 2014). Naturally found on fermented dairy and meat products, such as cheese and sausage, *Y. lipolytica* plays an important role in the flavour development of these foods due to its high lipolytic and proteolytic activities. This yeast secretes microbial enzymes to break down milk lipids and proteins to generate amino acids, short-chain fatty acids and methyl ketones, all of which contribute to the flavour of cheese and sausage (Patrignani et al., 2007; Sørensen et al., 2011). The ability of *Y. lipolytica* to assimilate a wide range of hydrophobic substrates and carbon sources, including sugars, hydrocarbons and alcohols, has also driven the study of its potential biotechnological applications. Some of the

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products of *Y. lipolytica* in synthetic media include γ-decalactone (peach-like aroma) and organic acids from the tricarboxylic acid (TCA) cycle (Fickers et al., 2005a; Gomes et al., 2012). The application of *Y. lipolytica* in fermenting various agro-industrial wastes, such as olive-mill wastewater, waste cooking oil and industrial fats, has also been explored. Some of the value-added products include lipase, citrate and single-cell oil (Lanciotti et al., 2005a; Papanikolaou and Aggelis, 2003; Papanikolaou et al., 2008).

The aforementioned studies highlighted the robust characteristics of *Y. lipolytica* in producing a variety of substances from biotransforming protein- and lipid-rich substances. As okara also contains a significant amount of protein and lipid, we hypothesised that various compounds of interest, such as organic acids and flavour compounds, might also be produced after okara fermentation. Therefore, the present study was undertaken to investigate the biochemical changes during *Y. lipolytica* fermentation of okara in order to determine the feasibility of okara fermentation by this yeast, and to explore the possible applications of the fermented whole okara. By exploiting the biotechnological potential of *Y. lipolytica*, we aim to add value to okara such that it can be returned back to the food value chain as a nutritionally improved, wholesome food product or ingredient to achieve the goal of zero waste.

2. Materials and methods

2.1. Yeast strain and okara

Freeze-dried *Y. lipolytica* NCYC 2904 culture was obtained from the National Collection of Yeast Culture (Norwich, UK) and propagated in sterile yeast-malt (YM) broth (2% glucose, 0.25% yeast extract, 0.25% bacteriological peptone and 0.2% malt extract, all *w*/*v*, pH 5.0). The yeast culture was incubated at 30 °C, 150 rpm for 48 h to obtain a cell population of about 7 log CFU/mL. Glycerol was added to the pure culture at 15% v/v and it was stored at −80 °C before use.

Fresh okara was provided by Super Bean International Pte Ltd (Singapore) from a single batch. Raw, soaked and non-genetically modified soybeans were ground into fine particles at room temperature and then filtered to obtain the okara. The okara was stored at −20 °C before use.

2.2. Solid-state fermentation of okara

A thawed pure yeast culture was added to sterile YM broth at 1% v/v and sub-cultured twice under the aforementioned conditions to obtain a cell count of about 6 log CFU/mL. It was then centrifuged (8000 *g*, 15 min, 4 °C) and the supernatant discarded. The pellet was washed twice with 10% v/v phosphate saline buffer (pH 7.4), and the washed cells were resuspended in the same buffer to obtain the yeast pre-culture.

Frozen okara was thawed, and 600 g of okara were placed in a glass container and autoclaved at 121 °C for 15 min. Yeast pre-culture was added to the sterilised okara at 2% *v*/*w* to and mixed evenly with a sterilised metal spoon, obtaining an initial cell count of about 4 log CFU/g okara. The airtight containers were incubated at 30 °C for 5 days. Aerobic condition within the container was maintained by ensuring sufficient headspace (bed height: 3–3.5 cm; headspace: 3.5–4 cm) and by mixing the substrate bed daily to introduce oxygen during sampling under aseptic conditions. Uninoculated, autoclaved okara incubated under the same conditions served as the control, while fresh, unheated okara served as the blank. All treatments were prepared in triplicate. Sampling was conducted daily over 5 days for the determination of viable yeast cell count, sugars, organic acids, amino acids and volatiles. Samples collected on days 0 and 5 were also subjected to proximate composition and antioxidant capacity analyses. Samples collected were stored at −20 °C before the analyses.

2.3. Yeast growth determination

To 90 mL of 0.1% *w*/*v* sterilised peptone water, 10 g of okara was added and the mixture was homogenised with a stomacher for 90 s. The homogenised mixture was appropriately diluted and spread plated on potato dextrose agar plates, which were then incubated at 25 °C for 48 h before yeast enumeration. At least two samples were taken from each container to obtain an average count.

2.4. Proximate composition

Moisture content was measured with a moisture analyser (MOC-120H Shimadzu, Kyoto, Japan). Total nitrogen content was determined following AOAC 920.87 semi-micro Kjeldahl method (AOAC, 1995). The conversion factor 5.71 was used to transform nitrogen into protein. Ash content was determined by the direct ashing method (AOAC, 2000). Fat content was measured with a Soxtec apparatus (Soxtec™ 2050 FOSS, Hillerød, Denmark). Fat was extracted from 3 g of freeze-dried okara with 90 mL of petroleum ether at 80 °C for 120 min. The defatted okara was collected and dried under nitrogen gas, and used for subsequent non-volatile and antioxidant extractions. It was stored at −20 °C before use. The combined carbohydrate and fibre content was calculated by mass differences.

2.5. Analysis of sugars, organic acids and amino acids

Sugars and amino acids were extracted from okara following the procedures of Giannoccaro et al. (2006) with slight modifications. Five grams of freeze-dried, defatted okara were extracted with 80 mL of 80% v/v ethanol in a water bath (50 °C, 150 rpm, 30 min). Lactose was added as an internal standard. Extraction was conducted thrice, and the combined extracts were concentrated to 10 mL using a rotary evaporator. One part of concentrated extract was added to 2 parts of acetonitrile, and the mixture was stored at 4 °C for at least 24 h to allow complete precipitation of proteins. The precipitate was removed by centrifugation and the supernatant was stored at −20 °C before use.

Organic acids were extracted from 3 g of defatted freeze-dried okara with 50 mL of 0.1% v/v sulphuric acid in a water bath (50 °C, 150 rpm, 45 min). The extract was then centrifuged (10,000 *g*, 20 min) to remove the okara, and the supernatant was stored at 4 °C for at least 24 h to allow complete precipitation of proteins. The precipitate was removed by centrifugation and the supernatant was stored at −20 °C before use.

HPLC analysis of sugars, organic acids and amino acids followed the methods of Chen et al. (2014) with slight modifications. For HPLC analysis of sugars, it was conducted at 30 °C with a flow rate of 1 mL/min instead of 40 °C at 1.4 mL/min.

2.6. Antioxidant capacity

Extraction of antioxidants followed the method optimised by Singh et al. (2011). One gram of freeze-dried okara was extracted with 50 mL of 25% v/v acetone in a water bath (40 °C, 150 rpm, 15 min). The mixture was then centrifuged (5000 *g*, 10 min) to remove the okara, and the supernatant was used in subsequent antioxidant assays, both measured using a microplate reader (BioTek, Winooski, Vermont, USA). Total phenolic content (TPC), expressed as gallic acid equivalent, was determined with Folin–Ciocalteu reagent as described by Isabelle et al. (2008). Oxygen radical absorbance capacity (ORAC), expressed as Trolox equivalent, was measured following the method of Huang et al. (2002).

2.7. Analysis of volatiles

In a 20-mL glass vial, 3 g of sample and 3 mL of saturated sodium chloride solution (Goodrich Chemical Enterprise, Singapore) were added and capped with a polytetrafluoroethylene septum. Volatiles