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Reduction in the ammonia content of salmon shark meat by a fermented rice bran suspension with the *Satoumi*-sourced yeast *Saccharomyces cerevisiae* Misaki-1 and lactic acid bacteria *Lactobacillus plantarum* Sanriku-SU8

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ABSTRACT

To reduce the ammonia content in the meat of the shark *Lamna ditropis*, the ammonia-reducing capacity of soaking solutions containing 10% sucrose and 5% NaCl, with or without selected strains of yeast *Saccharomyces cerevisiae* Misaki-1 and lactic acid bacteria (LAB) *Lactobacillus plantarum* Sanriku-SU8 was evaluated. After the 48 h soaking with the yeast and LAB, ammonia content in the meat was reduced to about from 150 to 50 mg/kg. *S. cerevisiae* Misaki-1 and *L. plantarum* Sanriku-SU8 grew well in 10% water extract suspension (AES) of rice bran (*nuka*). The fermented *nuka*-AES had a good flavour and demonstrated the ability to reduce the ammonia content of meat from about 290 to 100 mg/kg. These results suggest that soaking treatment with *S. cerevisiae* Misaki-1 and *L. plantarum* Sanriku-SU8 can reduce improve salmon shark meat flavour.

1. Introduction

According to fisheries data reported to the Food and Agriculture Organization of the United Nations (FAO), shark landings increased between 1950 and 1997 from 121,000 metric tons to 414,000 tons; the reported landings have decreased since 1997 (Worm et al., 2013; Pleizier, Gutowsky, Peddemors, Cooke, & Butcher, 2015). However, the trade volume of shark fins has continued to grow steadily, suggesting that there is a discrepancy between the catch and trade data. While it is well known that shark body meat is generally of low value, the fins (especially those containing ceratotrichia) are a valuable commodity considering the increase in the demand for shark-fin soup among the Chinese middle class (Cook, 1990). The removal of fins from sharks and subsequent discarding of the finless bodies is regarded as a very significant ecological problem (Worm et al., 2013; Carr et al., 2013; Friedrich, Jefferson, & Glegg, 2014; Eriksson & Clarke, 2015; Pleizier et al., 2015).

The blue shark *Prionace glauca* and the salmon shark *Lamna ditropis* frequently land in the North-east (Tohoku) coastal region of Japan, particularly in Kesennuma, a city in the Miyagi prefecture. Although the number of landings have decreased after the Tohoku earthquake and tsunami on 11 March 2011 (Fritz et al., 2012), the number of landings has increased moderately thereafter. In Japan, the meat from the body of the blue shark is an important ingredient used in *surimi* products such as *hampen* (Kananiawa, Kuboi, Chon, Taguchi, & Yokawa, 2011; Limpisophon et al., 2015). The heart of the salmon shark is consumed as part of the *sashimi* cuisine in Kesennuma, and its body muscle is traditionally consumed as part of both *sashimi* and cooked cuisines in other areas, particularly in inland regions.

In Japan, some of the population thinks that shark meat smells of ammonia. Generally, ammonia in meats is generated by amino acids via deamination. However, as recently as a decade ago, the scientific community maintained that shark meat is rich in urea, which is converted to ammonia during the storage process (Rose, 1996; Clarke, Francis, & Griggs, 2013). Generally, the smell of ammonia is absent in fresh and properly stored meat; however, in some instances, these ammonia compounds accumulate in the meat at the time of landing (Shimizu, Hibiki, & Fujita, 1953; Suyama & Tokuhiro, 1954). Several methods are available for the reduction of ammonia content in shark meat. For example, in Japan, it is well known that the water-leaching method elutes ammonia from the fish muscle meat that is prepared for *surimi* and other processed

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seafood (Ohta, 1980). However, the water-leaching process may also reduce the nutrient content and flavour. Volatile basic nitrogen compounds including ammonia, trimethylamine, and other amines are responsible for most of the pungent fishy odours emitted from seafood (Pacquit et al., 2007). These alkaline products are readily solvated in an acidified solution rather than in alkaline and neutral solutions. Therefore, if the meat is of pH 7 or higher, the pungent fishy odour is strong.

In this study, to reduce the ammonia content in shark meat, yeasts and lactic acid bacteria (LAB) were screened for ammonia-lowering capacity. The ammonia-lowering effects of the selected yeasts and LABs on soaking meat with sucrose and NaCl solution were examined. Furthermore, the potential of the water extract suspension of rice bran (*nuka*) for use as a growth medium, for the selected ammonia-lowering yeast and LAB was examined.

2. Materials and methods

2.1. Microbial cultures

Both the selected strains isolated in this study and strains previously reported and stored in our laboratory were used. Our stock cultures included *Saccharomyces cerevisiae* Misaki-1 (Accession No. LC093859), which was isolated from beach-cast algae in the Noto *Satoumi* area (Ishikawa, Japan; Kuda, Matsuda, Yasunaka, & Yano, 2011), and *Lactobacillus plantarum* Sanriku-SU1 (Accession No. AB968217), which was isolated from fermented squid prepared in the Sanriku *Satoumi* area (on the northeastern coast of the main island, Japan; Kuda, Kawahara, Nemoto, Takahashi, & Kimura, 2014).

In total, 71 samples of leaves, flowers, seeds, algae, stones, sands, and fermented foods (Table 1) were obtained from Sanriku *Satoumi* areas between 2014 and 2015, kept in cold storage, and delivered within 2 days of collection. To isolate and screen the yeasts, approximately 2–5 g of sample was soaked in 25 mL of glucose-yeast extract-peptone (GYP) broth (glucose 20 g/L, yeast extract 10 g/L, and polypeptone 10 g/L) containing 5 mL/L lactic acid and 100 mg/L chloramphenicol. After incubation at 30 °C for 3–7 days, a loopful of the culture was streaked onto potato dextrose agar (PDA, Oxoid, Basingstoke, UK) and incubated at 30 °C for 3 days. Gas formation and cell morphology of the culture in 20% (w/v) glucose-containing GYP broth incubated at 30 °C for 3 days were observed. Finally, the ammonia-lowering capacity of the gas-producing isolates having *Saccharomyces*-like morphology was evaluated.

To isolate LAB strains, 2–5 g of sample was soaked in 30 mL of de Man, Rogosa, and Sharpe (MRS) broth (Oxoid) containing 3 g/L bile (Wako Pure Chemical, Osaka, Japan). After incubation at 30 °C for 3–7 days, a loopful of the culture was streaked onto MRS agar and incubated anaerobically at 30 °C for 3 days by using an AnaeroPack System (Mitsubishi Gas Chemical, Tokyo, Japan). In total, 540 colonies were isolated and inoculated into MRS broth with 5 g/L bile at pH 4.

2.2. Ammonia-lowering capacity of the test culture broths

The selected yeast and LAB strains were inoculated into 3 mL of the ammonia test GYP and the ammonia MRS broths, respectively; these broths contained 1 g/L ammonium sulphate, 60 g/L sucrose, and 60 g/L NaCl. After incubation at 30 °C for 2 days, the ammonia concentration was determined using a commercial water analysis kit with the indophenol-blue method (No. 7, Kyoritsu, Tokyo, Japan; An, Kuda, Yazaki, Takahashi, & Kimura, 2014). The effects of a co-culture of selected yeast and LAB strains were also determined after 4 days of incubation using the ammonia test in Tryptone Soy Broth (TSB, Oxoid) containing 1 g/L ammonium sulphate, 60 g/L sucrose, and 60 g/L NaCl. One strain of yeast and one strain of LAB were selected and identified by sequencing the 26S(D1/D2) rRNA and 16S rRNA genes, respectively, followed by a BLAST analysis, as previously reported (Kuda et al., 2011; Kuda, Kawahara, et al., 2014; Kuda, Noguchi, et al., 2014).

2.3. Lowering of ammonia content of salmon shark meat by soaking with or without the selected yeast and LAB strains

Two experiments testing the ammonia-lowering capacity of the strains in soaking solution were carried out under different conditions. In the first experiment, two yeast strains (*S. cerevisiae* Misaki-1 and the selected isolate) and two LAB strains (*L. plantarum* Sanriku-SU1 and the selected isolate) were incubated in 40 mL of GYP and MRS broths, respectively, at 30 °C for 2 days. Then, the yeast and LAB culture cells were suspended and adjusted to $OD_{600} = 4.5$ (1.5 of yeast and 3.0 of LAB) in 6% sucrose and 6% NaCl solution. Five grams of fresh salmon shark meat (an approximately 1.5–2.0 cm cube) was soaked in 10 mL of the cell suspension in a 50 mL tube (Polypropylene; TPP, Trasadingen, Switzerland) and incubated at 10 °C for 2 days. From the results of these experiments, one strain each of yeast and LAB was selected. In the second experiment, 50 g of salmon meat was soaked in 100 mL of 100 g/L

Table 1
Screening of yeasts and lactic acid bacteria from Sanriku satoumi region.

		Screening for yeasts				Screening for lactic acid bacteria			
Kinds of samples	Number of samples	Growth in GYP[*1]	Number of isolates	Round Gas[*2]	Ammonia lowering	Growth in MRS[*3]	Number of isolates	pH 4.5 0.5% bile[*4]	Ammonia (−) fast growth[*5]
Leaves	4	2	20	10	–	1	10	–	
Flowers	36	19	270	90	–	19	290	35	–
Seeds	2	–				1	20	–	–
Algae	17	6	50	10	–	13	140	25	3[*7]
Stones	3	–				3	30	–	
Sands	2	2	30	–		–			
Fermented foods	7	5	60	10	10[*6]	5	50	3	1[*8]

[*1] Number of samples that showed turbidity in GYP contained 0.5% (w/v) lactic acid and 0.01% (w/v) chloramphenicol.
[*2] Number of selected isolates that round shape and well CO2 production in broth containing 20%(w/v) glucose.
[*3] Number of samples that showed turbidity in MRS broth containing 0.3%(w/v) bille.
[*4] Number of isolates that grew in MRS adjusted pH 4.5 and containing 0.5% (w/v) bile.
[*5] Isolates did not produce ammonia and well growth ammonia containing broth.
[*6] Isolates from a Chinese cabbage kimuchi.
[*7] Isolates from a algal cast on Hikado fishery port.
[*8] Isolate from a fermented sandfish.