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Effects of addition of anka rice on the qualities of low-nitrite Chinese sausages

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ABSTRACT

Anka rice (AR), previously inoculated with *Monascus purpureus*, was added during manufacturing of low-nitrite Chinese sausages. Chemical compositions and water activities of sausages were not affected. "*L*", "*a*", and "*b*" values of sausages with less nitrite (25 ppm) and 0.5% AR added were not significantly different from those with more nitrite (100 ppm) added. Colours of the sausages without AR were light red whereas those with AR added were darker red. Addition of AR did not inhibit lipid oxidation. Higher VBN (volatile basic nitrogen) values of the samples with AR added were observed. With addition of AR, the nitrite degrading rate was retarded. Microbial counts of the sausages with AR added were significantly higher than those of the controls (100 ppm nitrite). The low-nitrite Chinese sausage with addition up to 1.5% AR was acceptable when stored at 4 °C for 28 days.

1. Introduction

Monascus spp. which is a food fungus, has been widely applied for making wines and other fermented food products, especially in Taiwan, China, and many Asian areas for many years (Tseng, 1999). Many metabolic derivatives, such as ethanol, monascus pigments (red in colour), γ-aminobutyric, monacolins K, can be produced by *Monascus* spp. (Ma et al., 2000). In addition, it has also been reported in many ancient Chinese scripts to have some beneficial effects, such as improvement of food digestion and blood circulation (Ma et al., 2000). In Taiwan, as a natural food additive source, *Monascus* spp. has been granted edible natural colourant status and allowed in foods (DOH, 2008). Anka rice, also known as *Monascus*-fermented rice, monascal rice, and red koji, is a fermented food made by cultivating *Monascus* spp. on cooked rice, and has been widely used as a colouring and flavouring agent to make many Chinese roasted products (Tseng, Chen, & Lin, 2000).

Nitrite salts, mainly sodium nitrite and potassium nitrite, have been used in the preparation of cured meats for years and for many purposes. First, nitrite can be used as a potent anti-bacterial agent to provide protection against food microorganisms (Gill & Holley, 2003), as well as a potent antioxidant. In addition, nitrite is reduced to nitric oxide, which then interacts with myoglobin to produce nitric oxide myoglobin, which contributes to the characteristic pink cured meat colour (Honikel, 2008). Nitrite can also be applied to preserve desirable meaty flavour (Hedrick, Aberle, Forrest, Judge, & Merkel, 1994). Even though nitrite is known to participate in numerous reactions in cured meats, with many desirable functions, as mentioned previously, concern regarding the levels of nitrite used in meat curing has arisen because of the possibility of nitrosamine, which is a known carcinogen, being formed in cured meat (Osterlie & Lerfall, 2005). Therefore, many studies have been conducted to attempt to reduce the nitrite contents in cured meat products. Walsh et al. (1998) indicated that a combination of dietary vitamin E supplementation at a level of 500 mg α-tocopheryl kg feed^{-1} with nitrite at the level of 50 mg nitrite kg meat^{-1} could be used to produce cured pork products similar to those obtained from meat containing high nitrite levels (100 mg nitrite kg meat^{-1}). Osterlie and Lerfall (2005) recommended mixing minced meat with a lycopene-containing product that could be used to reduce nitrite levels. Jiménez-Colmenero, Carballo, and Cofrades (2001) pointed out that combining several compounds, which together have a cumulative effect on colour, flavour, antioxidant, and antimicrobial activity, could be considered when attempting to reduce the addition of nitrite in meat products. Functionality, such as colour enhancement and antioxidative properties, makes anka rice a potent candidate to be applied in low-nitrite products. Fink-Gremmels, Dresel, and Leistner (1992) indicated that *Monascus* extracts might be used as an alternative to nitrite in some meat products. Therefore, the purposes of this study were to compare the effects of anka rice on the qualities of low-nitrite Chinese sausages during refrigerated storage and to identify the volatile compounds from the anka rice and the sausages with anka rice added.

2. Materials and methods

2.1. Anka rice preparation

Monascus purpureus (CCRC No. 31499) was obtained from the Culture Collection and Research Center, Food Industry Research

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and Development Institute, Hsinchu, Taiwan. The methods described by Tseng (1999) were utilised. *M. purpureus* was inoculated into a yeast glucose broth medium which contained 10% glucose and 0.8% yeast extract; the pH was adjusted to 5.5 and it was incubated at 35 °C with 120 rpm shaking (Yih Der LM-570R, shaker incubator, Taiwan) for 7 days. Then, the inocula were homogenised with a sterile blender (Waring Commercial, New Hartfor, Connecticut, USA) at high speed for 2 min. The microbial suspension was prepared accordingly. After being rinsed and soaked in water for 12 h, indica rice was shaken to remove excess water, autoclaved for 30 min, and then removed for cooling. The microbial suspension prepared previously [5% (w/w), with the yeast glucose broth as 100%], yeast glucose broth and pre-autoclaved rice (same weight as the yeast glucose broth) were mixed thoroughly, and then cultivated at 30 °C for 20 days. After drying in an oven (Model RHD, S-Tai Co., Kaohsiung, Taiwan) at 45 °C for 24 h, and cooling at room temperature, the mixture was ground with a grinder (Type 780A, Krups, Ireland), and then stored at −20 °C.

2.2. Sausage preparation

Frozen pork hams, frozen pork backfat, and salted natural pork casing were purchased from a local market in Nantou, Taiwan. Lean tissue and pork backfat were ground through a 10 mm plate. Ground pork was mixed thoroughly with non-meat ingredients, including 10% sugar, 2% rice wine, 1.6% salt, 0.5% monosodium glutamate, 0.2% polyphosphate, 0.08% onion powder, 0.05% white pepper powder, 0.05% five-spice powder, 0.02% clove powder, 0.02% sodium erythorbate, and sodium nitrite (25 ppm for treatments A, R1, R2, and R3 samples, and 100 ppm for treatment C samples), and then cured under refrigeration at 7 °C for 3 days. In addition, 0.5%, 1%, and 1.5% anka rice which were previously inoculated with *M. purpureus* were also added to the formula for the sample treatments R1, R2, and R3, respectively. Then, ground pork backfat (25%) was mixed with the meat mixture, and stuffed (Stuffer, Dick D-73779, Germany) into natural casings, which were previously soaked in water. Raw sausages were dried in a preheated oven (Model RHD, S-Tai Co., Kaohsiung, Taiwan) at 50 °C for 3 h. Following drying, sausages were cooled, vacuum-packaged (HAS02G, Europack, Holland) and stored at 4 °C.

2.3. Proximate composition, water activity and pH

Samples were first ground with a grinder (Type 780A, Krups, Ireland). Proximate compositions of samples, including moisture, crude fat, crude protein, and ash contents, were measured according to AOAC (1990) methods. Crude fat was measured using a fat extractor (Sotec System HT 1043 Extraction Unit, Tecator Co., Sweden) with ethyl ether as a solvent and extracted for 16 h. Crude protein was measured by the Kjeldahl method, using a digester (Model 2006, Foss tecator, Sweden) and a distillation unit (Model 2100, Foss tecator, Sweden). Approximately 2 g of ground samples were put into a holding cup, and measured with a water activity analyser (Aqualab-CX2, Decagon Devices Inc., USA). Eleven gramme samples were blended with 99 ml distilled water in a polyethylene bag for 1 min using a stomacher (Stomacher 400, Seward Ltd., England) at high speed, and then the pH of the mixture was measured using a pH meter (Micro-computer pH meter, Model 6210, Taiwan).

2.4. Instrumental colour measurement

Ground samples were placed in a measuring container, and then the "*L*" (lightness), "*a*" (redness), and "*b*" (yellowness) values of samples were measured with a colour meter (Spectrophotometer, Model TC1, Tokyo Co., Ltd. Japan). A standard plate with "*Y*" = 86.53, "*X*" = 82.45, and "*Z*" = 91.28 was used as a reference.

2.5. Thiobarbituric acid (TBARS) values and volatile basic nitrogen (VBN)

TBARS values of the samples were determined according the methods described by Faustman, Specht, Malkus, and Kinsman (1992). TBARS value was expressed as mg malonaldehyde/kg meat. Volatile basic nitrogen was determined according to CNS (1982) by the Conway micropipette diffusion method.

2.6. Nitrite residue

Nitrite residue was measured according to the methods of Lin (1984), briefly as follows: five grammes of ground sausage samples in a 250 ml flask were homogenised with 100 ml of 80 °C reverse osmosis water, using a homogenizer (Type PT 10/35, Brinkmann Instruments Inc., Westbury, NY, USA) for 60 s at 10,000 rpm. The homogenate was washed with reverse osmosis water to 150 ml totally, sealed with an aluminium foil cap, and heated for 30 min in an 80 °C shaking water bath (50 rpm). After adding 2 ml of saturated $HgCl_2$, cooling with ice water to room temperature, and filtering (Toyo No. 1) immediately, 10 ml of filtrate were transferred into a tube; 2 ml of Griess solution (a combination of sulfanilic acid solution and α-naphthlamine solution in a 1:1 ratio; 0.5 g sulfanilic acid was added to 150 ml of 15% acetic acid solution and 0.1 g α-naphthlamine was dissolved in 20 ml of boiled reverse osmosis water and 150 ml of 15% acetic acid solution) were placed in the tube, covered with aluminium foil, and kept for 30 min. OD values were measured, using a Spectrophotometer (U3210, Hitachi, Japan) at 540 nm wave-length. A nitrite standard curve was made by adding 0.2 g of $NaNO_2$ dissolved in 1000 ml DW and 10 ml of solution were withdrawn and diluted to 1000 ml. The concentration values were then determined according to the equation: $Y = 5.3527 \times X - 0.089$, with Y representing the concentration (ppm), and X representing OD values at 540 nm wave-length.

2.7. Microbial evaluation

Microbial qualities of samples were evaluated according to Bacteriological Analytical Manual for Foods (FDA, 1996), briefly follows: at specified sample times, sausages were aseptically removed from the bags. Eleven gramme samples were placed in a sterile bag containing 99 ml of sterile distilled water and homogenised with a stomacher (Stomacher blender, Model 400, Seward) for 1 min. Serial dilutions were then made. Plate count agar (PCA, Merck) and potato dextrose agar (PDA, Merck) were used for enumeration of total plate count, and mould count, respectively, using the pour plate method to enumerate bacteria. Total microflora and mould were incubated at 37 °C for 48 h and 37 °C for 96 h, respectively. For anaerobic plate counts, dilutions were poured into anaerobic agar (AA, Merck), placed in an anaerobic jar (BBL GasPak System, USA) and incubated at 37 °C for 48 h. Microbial counts in this study were expressed as $\log_{10}$ colony forming units (CFU) per gramme of sample.

2.8. Sensory evaluation

At days 0, 28 and 56, during storage, sausages were first cooked on a grill, until the internal temperature of the sausages reached and was held at 75 °C for 8 min, cooled to room temperature (approximately 25 °C), sliced (approximately 0.3 cm thickness), and then served to a sensory panel which consisted of 10 meat science-majored faculty and students. Sensory attributes, including colour, flavour, odour, and overall acceptance, was conducted using