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Impact of fermentation conditions on the physicochemical properties, fatty acid and cholesterol contents in salted-fermented hoki roe

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ABSTRACT

Hoki (*Macruronus novaezelandiae*) roes were used to produce a salted fermented (*jeotgal*-like) roe product at 4 °C and 25 °C. The impact of the fermentation temperature on the microbiological status, proximate analysis, acidity, colour, fatty acid profile and cholesterol content were determined over a 4-week fermentation period. Total bacterial count and total LAB (expressed as log CFU) increased with fermentation time ($p < 0.001$). Fermentation temperature did not affect the proximate analysis, but fermentation time increased both the ash content and decreased the protein and moisture contents in the roe ($p < 0.001$, for all). Products produced at 25 °C had a lighter colour ($p < 0.001$) compared to products produced at 4 °C. Fermentation did not affect the roe fatty acid profile, but cholesterol content in the roe was reduced ($p < 0.001$) at both fermentation temperatures. Results suggest that fermentation can be a feasible approach to reduce cholesterol levels in fish roe.

1. Introduction

Fish roe refers to the eggs of fish when they are encased in skeins. In many parts of the world, fish roe products such as salted-dried, salted-fermented, or salted-cured roes are found under traditional names and are regarded as part of their cuisine. Fish roe is reported to be the source of many beneficial components (Bledsoe, Bledsoe, & Rasco, 2003), containing vitamins, minerals, well-balanced proteins and significant amounts of ω-3 PUFA, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) that can play an important role in the prevention of cardiovascular diseases (Nordøy, Marchioli, Arnesen, & Videbæk, 2001). However, a considerable amount of cholesterol can be found in fish roes (Moriya et al., 2007; Higuchi, Shirai, & Suzuki, 2008) and it can vary widely among various fish species (Bledsoe et al., 2003).

While several studies have documented the composition of fresh fish roe, very few studies have been performed on the changes that might occur in fish roe during the fermentation process. Fermentation may be a means to improve the nutritional benefits of fish roe and reduce the cholesterol content. It has been suggested that the bacterial degradation of cholesterol in cholesterol-containing food may be beneficial for human health (Watanabe et al., 1986).

In this study, hoki roes were used to produce a roe product similar to *jeotgal* (salted fermented seafood) from Korea. The changes in several physicochemical and microbial parameters (pH, titratable acidity, proximate, microbial, and fatty acids) and the potential of cholesterol degradation during the fermentation of hoki roe were followed over 24 days of fermentation.

2. Materials and methods

2.1. Samples

Frozen hoki (*Macruronus novaezelandiae*) roes (grade 1, packaged weight 20 kg) were obtained from a commercial fish processing company in New Zealand. The roes were thawed in a chiller (4°C) overnight, cleaned and washed thoroughly with ice-cold water. The weight, length and width of the individual roes were recorded. Individual roe skeins were found to have weights ranging from 80 to 700 g, lengths ranging from 11 to 28 cm and widths ranging from 4.5 to 15 cm. Skeins were selected to be as uniform as possible (568.4 ± 52.3 g) by eliminating extreme outliers and only whole (undamaged) roe skeins were used for processing.

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2.2. Processing of samples into salted and fermented roe (jeotgal-like product)

Thawed clean roes were individually mixed with 7% (of the raw weight) salt and 5% chilli powder. Roes were subsequently separated into 14 bags, each approximately 1 kg in weight and then vacuum packed. Half of the bags (n = 7) were placed in a chiller at 4 °C while the other 7 bags were placed in an incubator at 25 °C, to commence anaerobic fermentation. Over the next 24 days, 1 bag was removed at every 4 days interval from each temperature treatment (4°C and 25 °C). Sampling began on day 0 with fresh roes.

2.3. Chemical analysis

2.3.1. pH and titratable acidity measurements

2.3.1.1. pH measurement. The pH of the homogenised sample was measured with a calibrated pH meter (HANNA PH211 pH meter, HANNA Instruments, U.S.A). The pH measurements were obtained in triplicates from 3 separate roe skeins contained in each fermentation bag. The liquid released from the fermented samples was measured directly by inserting the pH probe into the solution, where 3 subsamples were pulled from the liquid contained within the fermentation bag.

2.3.1.2. Titratable acidity measurements. The titratable acidity, expressed as percent lactic acid, was determined using a 1 ml of the roe suspension (10 g sample and 90 ml deionized water) after being mixed with 9 ml of deionized water and the mixture was titrated with 0.1 N NaOH using phenolphthalein as indicator. Also, the titratable acidity of the fermentation liquid was carried out after a filtration step and 1 ml of the filtrate was mixed with 99 ml deionized water and the mixture was titrated with 0.1 N NaOH using phenolphthalein as indicator. The volume of NaOH used was recorded and 1 ml of NaOH was taken as equivalent to 9.0 mg lactic acid (Downes & Ito, 2001).

2.3.2. Proximate analysis

Proximate analysis (moisture, lipid, protein, ash) of the roe samples was carried out in duplicate for 3 samples (separate skeins) per sampling time for the 2 fermentation temperatures (4°C and 25 °C) as described by Bekhit, Morton, Dawson, and Sedcole (2009) and Bekhit, Morton, Dawson, Zhao, and Lee (2009). Carbohydrates were calculated by subtracting the sum of moisture, protein, lipid, and ash from 100%. Energy value was calculated using the following formula as described by Falch, Overrien, Solberg, and Slizyte (2010):

$$[\text{Energy (kcal/100·g)} = (\text{lipid} * 9) + (\text{protein} * 4) + (\text{carbohydrate} * 4)]$$

2.3.3. Colour measurements

The colour of the roe skeins was measured as the L^*, a^* and b^* values of CIE using a calibrated colourimeter (Hunterlab MiniScan XE Plus, Hunter Associates Laboratory Inc., Reston, Va, USA.) as described by Bekhit, Morton, Dawson, and Sedcole (2009). The measurements were carried out for the 2 different temperature treatments on 3 separate roe skeins with 2 readings taken on each roe skein.

2.3.4. Microbiological analysis

Roe samples (25 g each) from skeins (n = 3) at each fermentation temperature treatment were macerated for 2 min in 225 g of sterile 0.1% peptone using a stomacher (Model No. BA6021, Seward Limited, West Sussex, UK.). The homogenate was serially diluted in 0.1% peptone and used for the enumeration of microorganisms by the surface spread plate method (Swanson, Petran, & Hanlin, 2001). Counts of total bacteria and lactic acid bacteria were determined on plate count agar and lactobacilli MRS agar, respectively (Difco™, Becton, Dickinson and Company, Sparks, USA). All agar plates were incubated anaerobically at 25 °C for 72 h.

2.3.5. Fatty acid and cholesterol analysis

2.3.5.1. Lipid extraction and fatty acid methyl esters preparation. The extraction of the lipids and the simultaneous preparation of methylated fatty acids and cholesterol were carried out as described by Meier, Mjøsb, Joensen and Grahl-Nielsen (2006). Lipids were extracted from roe samples (60–100 mg) with a mixture of chloroform and methanol (1:2 v/v) following the method of Bligh and Dyer (1959). The solution was vortexed for 1 min and a 1.25 ml of chloroform and a 1.25 ml NaCl solution (8% w/v) were successively added with 2 min of vortexing after each addition. The solution was then centrifuged at 2500 rpm for 5 min. This produced a biphasic solution where the lower chloroform phase containing the lipids was transferred to 15 ml KIMAX glass tubes with Pasture pipettes and evaporated to dryness under a stream of nitrogen gas. Fatty acids were methylated by acid-catalysed transesterification at 80 °C for 12 h (Christie, 1993). A 3 ml volume of 6% sulphuric acid (v/v) in methanol solution was added to the isolated lipid fraction as a methylating agent. After cooling to room temperature, 2 ml of hexane followed by 1 ml of water were added to the sample with 2 min of vortexing between each addition. The upper hexane layer containing the fatty acid methyl esters (FAME) was removed and stored at -20 °C until gas chromatography analysis. The fatty acid and cholesterol content in the roe samples were initially identified using GC–MS and quantified using GC-FID following the same analysis conditions. A series of cholesterol standard (5-α-cholestan-3-β-ol) was used to construct a standard curve as described in Section 2.3.5.4. The samples were methylated using 6% sulphuric acid in methanol and extracted using hexane as described above.

2.3.5.2. GC–MS operating conditions. The fatty acid methyl esters and cholesterol content in standards were identified using a GC–MS system. The GC–MS system consisted of a 6890N Network GC system (Agilent Technologies U.S.A) and a 5973 Network Mass Selective Detector (Agilent Technologies USA). Hydrogen was used as a carrier gas at a flow rate of 15.5 ml/min. The injection port temperature was set at 200 °C. The GC oven temperature was increased by 1 °C/min to 210 °C from an initial temperature of 190 °C. The MS detector was turned on after 6 min of running time. The split ratio was set at 10:1.

2.3.5.3. GC-FID operating conditions. The fatty acid methyl esters (FAME) and cholesterol content in the roe samples were separated using a Hewlett Packard HP-5890 GC. The compounds in the roe samples were identified by matching their retention times with the FAME and cholesterol standard solutions that had also been put through the GC-FID system and compared to their respective GC–MS standard chromatograms. The gas chromatography system consisted of a 5890 GC equipped with an auto-sampler (HP7673) and ChemStation Integrator (Hewlett Packard, Avondale, PA). FAMEs and cholesterol were separated using a DB225 capillary column (30 m × 0.53 mm inner diameter), 0.25 μm film (Agilent Technologies USA). Hydrogen was used as the carrier gas at a flow rate of 15.5 ml/ min. The injection port temperature was set at 200 °C. The GC oven temperature was increased by 1 °C/min to temperature 210 °C from an initial temperature 190 °C. The FID detector was turned on after 2 min of running time. The split ratio was set at 12.5:1.

2.3.5.4. Identification of cholesterol. Standard cholesterol (5-α-cholestan-3-β-ol, chromatography grade, Sigma Chemical Inc, U.S.A.) solution was prepared by dilution from a stock solution (4 mg/ml). The stock solution was used to prepare working solutions containing 0.25, 0.50, 1.00 and 2.00 mg/ml cholesterol. The cholesterol standard solutions were methylated prior to injection. By reference to the NIST Mass Spectral Search Program (Version 2.0a, build July 2002), using GC-FID, cholesterol was detected at the retention time of 23.69 min.

2.3.5.5. Identification of fatty acids. Identification of peaks corresponding to fatty acid methyl esters was accomplished by means