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# Impact of high hydrostatic pressure on non-volatile and volatile compounds of squid muscles

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ABSTRACT

The effects of high hydrostatic pressure processing (HHP at 200, 400 or 600 MPa) on non-volatile and volatile compounds of squid muscles during 10-day storage at 4 °C were investigated. HHP increased the concentrations of $Cl^-$ and volatile compounds, reduced the level of $PO_4^{3-}$, but did not affect the contents of 5′-uridine monophosphate (UMP), 5′-guanosine monophosphate (GMP), 5′-inosine monophosphate (IMP), $Na^+$ and $Ca^{2+}$ in squids on Day 0. At 600 MPa, squids had the highest levels of 5′-adenosine monophosphate, $Cl^-$ and lactic acid, but the lowest contents of CMP and volatile compounds on Day 10. Essential free amino acids and succinic acids were lower on Day 0 than on Day 10. HHP at 200 MPa caused higher equivalent umami concentration (*EUC*) on Day 0, and the *EUC* decreased with increasing pressure on Day 10. Generally, HHP at 200 MPa was beneficial for improving *EUC* and volatile compounds of squids.



## 1. Introduction

Squid is one of the most popular seafood items worldwide, mostly due to its high nutritional value and organoleptic properties (Deng, Luo, Wang, & Zhao, 2015). Flavour plays an important role in the organoleptic characteristics of squids (Yang, Wang, Li, Huang, & Chi, 2013). Generally speaking, flavour compounds, including volatile and non-volatile components, contribute to the taste of squids. The classes of volatile compounds in seafood include aromatic hydrocarbons, aliphatic hydrocarbons, esters, aldehyde, alcohol, sulphur compounds, and ketones. The non-volatile compounds consist of nitrogenous (free amino acids, nucleotides, organic bases and related compounds) and non-nitrogenous compounds (organic acids and inorganic compounds) (Fratini, Lois, Pazos, Parisi, & Medina, 2012; Liu, Zhang, & Chen, 2013). In particular, umami is a specific taste of squid, mainly caused by two groups of non-volatile compounds: free amino acids and flavour nucleotides, such as 5′-inosine monophosphate (IMP), 5′-guanosine monophosphate (GMP), and 5′-adenosine monophosphate (AMP) (Johnson et al., 2013).

High hydrostatic pressure processing (HHP) has shown considerable potential as a technology to improve food safety and quality (Deng et al., 2013, 2014; Montiel, Martín-Cabrejas, Gaya, & Medina, 2014; Teixeira et al., 2014). For food subjected to HHP treatments, evaluation of processing parameters on the flavour components is vital to define proper treatment conditions, in order to prevent the loss of unique sensory properties of the seafood and to ensure consumer satisfaction. Recently, studies have investigated the effect of HHP on microorganism inactivation, protein denaturation, enzyme activation and inactivation, and modification of physicochemical properties (e.g., proximate composition, colour, texture and acidity) in squids and other seafood (Gou, Choi, He, & Ahn, 2010; Gou, Xu, Choi, Lee, & Ahn, 2010; Hu et al., 2013; Rastogi & Knorr, 2013; Zhang, Jiao, Lian, Deng, & Zhao, 2015). It is surprising that few studies have reported the changes of volatile compounds in seafood treated at various pressures (Cruz-Romero, Kerry, & Kelly, 2008; Hoover, Metrick, Papineau, Farkas, & Knorr, 1989).

Moreover, little attention has been given on the relationship between the non-volatile compounds in seafood and HHP treatments. Furthermore, no literature has reported squid flavour changes induced by HHP during storage. Therefore, the objectives of this work were (1) to investigate and compare the influences of different pressure levels on the non-volatile compounds (flavour 5′-nucleotides, free amino acids, inorganic ions, lactic acid, succinic acid and betaine) and volatile compounds in squid muscles, and (2) to assess the stability of flavour compounds of HHP-treated squids during storage at 4 °C. The findings of this comprehensive study could provide the theoretical basis for the HHP treatment of squid

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or other seafood, in order to enhance their nutritional and commercial value.

## 2. Materials and methods

### 2.1. Sampling, packaging and pressure treatment

Fresh squids (*Todarodes pacificus*) were purchased from a local fish market (Shanghai, China), and transported to the laboratory in iced water. Squids (310 ± 25 g, with a mantle length of 21.5 ± 2.8 cm) were cleaned and scrubbed thoroughly in iced water. After dissection, internal organs, arms and tentacles were removed in order to assess the mantle. The integrated mantle muscles, with an average length of 210 ± 25 mm (widest part of 165 ± 15 mm, and a thickness of 3.0 ± 0.5 mm), were retained as experimental samples. Subsequently, mantle muscles were gently blotted with tissue papers to remove excess water on the surface.

Squid samples were individually packaged in polyamide/chlorinated polypropylene (PA/CPP) complex film bags and heat-sealed with vacuum. Samples were divided into four groups: one batch was assigned as the control without HHP treatment, the other batches were processed at 200 MPa, 400 MPa, and 600 MPa, respectively. HHP treatments were performed at 20 °C for 10 min in an HHP-750 unit (Kefa High Pressure Food Processing Inc., Baotou, China) with a 2.5-L cylindrical pressure vessel and a pressure range of 0–700 MPa. The pressure increase rate was 8.3 MPa/min, and the depressurisation time was less than 4 s. After HHP treatments, all the samples were stored at 4 °C, and analysed after 0 and 10 days.

### 2.2. 5′-Nucleotide analysis

The 5′-nucleotide contents in squid samples were measured according to the method described by Chiang, Yen, and Mau (2007), with some modifications. Briefly, one gram of sample was homogenised in 30 mL of 8% cold perchloric acid for 15 min, and extracted with ultrasound for 10 min, then centrifuged at 10,000 rpm for 15 min at 4 °C. The extraction process was repeated once with 20 mL of cold perchloric acid. The combined filtrate was immediately neutralised to pH 6.5 with 1 M potassium hydroxide solution. After standing at 1–2 °C for 30 min, the precipitated potassium perchlorate was removed by filtration through 0.45-μm filters before the samples were analysed by high-performance liquid chromatography (HPLC) (Waters 2695, Milford, MA). HPLC conditions were as follows: column, Thermo Scientific Syncronis C18 (5 μm, 4.6 × 250 mm); mobile phase, potassium dihydrogen phosphate (0.05 M)-methanol (95:5, *v/v*, pH = 4.1); flow rate, 0.5 mL/min; column temperature, 25 °C; and detector wavelength, 254 nm. The identity and quantity of the nucleotides were assessed by comparing the retention times and peak areas of each nucleotide to a standard (Sigma–Aldrich, St Louis, MO).

### 2.3. Free amino acid analysis

Free amino acid contents in squid samples were determined using an amino acid analyser (L-8900, Hitachi, Japan) according to the method described in Deng et al. (2014).

### 2.4. Determination of succinic acid, lactic acid and betaine contents

Lactic acid and succinic acid were extracted and analysed as described by Liu et al. (2013), with some modifications. Briefly, the samples (about 2.5 g) were homogenised in 30 mL of 2% ammonium dihydrogen phosphate (pH = 2.5) for 15 min, and extracted with ultrasound for 10 min, then centrifuged at 10,000 rpm for 10 min at 4 °C. The extraction process was repeated once with 20 mL of ammonium dihydrogen phosphate and samples were then filtered through 0.45-μm filters prior to HPLC (Waters 2695) analysis. HPLC conditions were as follows: column, Thermo Scientific Syncronis C18 (5 μm, 4.6 × 250 mm); mobile phase, potassium dihydrogen phosphate (2%)-methanol (97: 3, *v/v*, pH = 2.5); flow rate, 0.8 mL/min; column temperature, 25 °C; and detector wavelength, 210 nm. The identity and quantity of lactic acid and succinic acid were assessed by comparing the retention times and peak areas to standards (Sigma).

Betaine analysis was carried out according to the reineckate method described by the Chinese Standard NY/T 1746-2009 (2009) and Chi et al. (2012). The absorbance was measured at 525 nm by spectrophotometer (Thermo Spectronic Genesys 10 UV, Thermo Fisher Scientific Inc., Waltham, MA).

### 2.5. Determination of inorganic ions

The concentrations of Ca, K, Mg and Na in squid samples were measured using an inductively coupled plasma optical emission spectrometer (OPTIMA-7000DV, Perkin-Elmer, Waltham, MA) according to the method described by Qian et al. (2012). The phosphate and chloride concentrations were determined using an 882 ion chromatograph system (Metrohm, Riverview, FL) equipped with a high capacity anion exchange analytical column (Metrosep A Supp 5, 250 mm × 4.0 mm). Sample (1.5 g) was carbonised on anelectric stove in porcelain crucibles, and ashed in a muffle furnace at 500 °C for 24 h. After dissolving in deionised water, it was transferred to a 100-mL flask, and deionized water was added to bring total volume to 100 mL. The electrical conductivity of the sample solution was adjusted to 350 μS/cm with ultrapure water. Then the sample solution was filtered through a 0.22-μm membrane and injected into the ion chromatography system. All experiments were performed at room temperature at flow rate of 0.7 mL $min^{-1}$. The isocratic elution was carried out using a mixture of 3.2 mM $Na_2CO_3$ and 1.0 mM $NaHCO_3$.

### 2.6. Equivalent umami concentration (EUC)

*EUC* is defined as the concentration of monosodium glutamate (MSG, g/100 g) as equivalent to the umami intensity given by umami amino acids (aspartic acid (Asp) or glutamic acid (Glu) and 5′-ribonucleotides (AMP, GMP and IMP) (Chen & Zhang, 2007). *EUC* was calculated using the following equation (Chiang et al., 2007; Liu et al., 2013):

$$\mathrm{EUC} = \sum \alpha_i \beta_i + 1218\left(\sum \alpha_i \beta_i\right)\left(\sum \alpha_j \beta_j\right)$$

where *EUC* value was expressed as g/kg (g MSG/100 g); $\alpha_i$ was the concentration (g/100 g) of Asp or Glu, $\alpha_j$ was the concentration (g/100 g) of IMP, GMP or AMP; $\beta_i$ was the relative umami concentration (*RUC*) of Asp (1) and Asp (0.077), $\beta_j$ was the *RUC* of IMP (1), GMP (2.3) and AMP (0.18); and 1218 was the synergistic constant based on the concentration of g/100 g used.

### 2.7. Volatiles analysis

Squid volatiles were collected using simultaneous distillation and extraction (SDE) performed according to the method described by Deng et al. (2015). GC–MS analysis was conducted using a 7890A gas chromatograph coupled with a 5975C mass spectrometer (Agilent Technologies, Santa Clara, CA). A DB-5MS capillary column (30 m × 0.25 mm i.d., 0.25 μm $d_f$) from Agilent was used to separate the compounds in the extract. The injector port was heated to 250 °C and injections (1 μL) were performed in splitless