



Effects of high hydrostatic pressure processing on purine, taurine, cholesterol, antioxidant micronutrients and antioxidant activity of squid (*Todarodes pacificus*) muscles

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ABSTRACT

This study investigated the effect of high hydrostatic pressure processing (HHP, at 0.1 [control], 200, 400 or 600 MPa) on purine, taurine, cholesterol, antioxidant micronutrients (Mn, Se, Fe, Zn, Cu, vitamin B₂, A and E), DPPH, and reducing power of squids during 10-day storage at 4 °C. Compared with the control, pressurization did not change the contents of purine, vitamin A and E, Mn and Fe in the squid samples on day 0. After 10-days of storage, HHP at 600 MPa caused maximum decreases in cholesterol, hypoxanthine, adenine and Fe, and produced small reductions in guanine, vitamin B₂, DPPH, reducing power, and TBARS. No significant differences were found in cholesterol, reducing power, vitamin B₂ or A between 200 MPa and 400 MPa treated samples. Both pressurization and storage time did not affect the levels of taurine, DHA, EPA, Mn and Cu. This study provided a strategy to decrease the cholesterol and purine contents with minimal antioxidant activity loss in seafood using HHP.

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1. Introduction

Currently, nutrition, health, safety, convenience and diversity are the major factors driving the global food industry. Squid products have attracted considerable attention as a source of high amounts of good quality protein and healthy ω -3 series polyunsaturated fatty acids (Deng et al., 2011). Moreover, squids are also potential sources of antioxidant micronutrients (such as vitamins and minerals) (Li, 2003; Yang, Wang, Li, Huang, & Chi, 2013). These nutrients are essential for humans, and help to improve imbalanced dietary habits and prevent lifestyle-related diseases (Evans & Halliwell, 2001). For example, vitamin E is not only an essential nutrient for humans, but also acts as a chain-breaking antioxidant that specifically inhibits the oxidation of polyunsaturated fatty acids (PUFAs) (Evans & Halliwell, 2001; Moltó-Puigmartí, Permanyer, Castellote, & López-Sabater, 2011). High intake of vitamin E contributes to reduce the risk of disorders associated with free radicals, such as atherosclerosis, cancer, cataracts, and cell

damage connected to ischemia and reperfusion (Barba, Esteve, & Frigola, 2012a, b). Vitamin B₂ also acts as an antioxidant that can help rid the body of damaging free radicals and its intake has been associated with decreased plasma homocysteine levels. Sufficient vitamin B₂ could be obtained from natural food sources because of its water-solubility (Yang et al., 2013). The antioxidant activity of vitamin A (retinol) is conferred by the hydrophobic chain of polyene units that can quench singlet oxygen, neutralize thiyl radicals and combine with, and stabilize, peroxy radicals (Ahmed, Islam, Khan, Huque, & Ahsan, 2004). Some microelements (such as manganese, selenium, iron, zinc and copper) are commonly referred to as antioxidant minerals and are required for the activity of certain antioxidant enzymes (Ahmed et al., 2004; Evans & Halliwell, 2001).

Squids are currently regarded as a rich source of taurine, purine and cholesterol (Deng, Luo, Wang, & Zhao, 2015; Li, 2003). Taurine (2-aminoethanesulfonic acid) is a free amino acid found ubiquitously in the animal body that has important roles in several essential biological processes, such as calcium modulation, bile acid conjugation, antioxidation, membrane stabilization and immunity (Chen, 2006). Taurine synthetic activity in humans is weak, and supplemental taurine could be obtained from seafood (Chen, 2006;

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Li, 2003). Furthermore, human studies have revealed that the administration of taurine with n–3 PUFAs has hypolipidemic and antiatherogenic effects, compared with n–3 PUFAs supplementation alone (Chen, 2006; Yang et al., 2013). Like other seafood, squids are rich in purines (such as adenine, guanine, hypoxanthine and xanthine) and are thus classified as purine-rich foods. Purines play crucial roles in the storage of genetic information and in the processes of making and breaking nucleotides. Hypoxanthine and xanthine have been linked to off-flavors in food and are important markers for determining the freshness of seafood (Wang, Ling, Sun, Chu, & Zhou, 2008). Consumption of high-purine food raises uric acid and is associated with hyperuricemia and an increased risk of incident gout, which is a common and excruciatingly painful inflammatory arthritis (Lou, 1998). Therefore, low dietary purine intake is suggested for sufferers of gout to reduce the serum uric acid concentration (Wang et al., 2008). In addition, squids also have relatively high cholesterol, mostly the high-density lipoprotein cholesterol. Cholesterol is the major sterol (95% of total sterols), acting as a critical component of cell membranes, and is the precursor of all steroid hormones and vitamin D (Clariana & García-Regueiro, 2011; Medina-Meza, Barnaba, & Barbosa-Cánovas, 2014). On the other hand, consumption of cholesterol has been related to the incidence and severity of cardiovascular incidents (Chen, 2006). Squids have been designated as major contributors to dietary cholesterol; therefore, it is important to determine the level of cholesterol in food, including seafood.

Increasing appreciation of the nutritional and functional properties of squid encourages studies on the effects of processing on product attributes to minimize quality degradation. High hydrostatic pressure processing (HHP) is considered a valuable non-thermal processing technology, allowing the extension of the shelf-life of food products (Kim & Ahn, 2013). It inactivates and inhibits microorganisms, and can activate or inactivate enzymes, as well as producing safe and minimally processed foods with satisfactory nutritional and organoleptic qualities (Andrés, Møller, Adamsen, & Skibsted, 2004; Chevalier, Le Bail, & Ghoul, 2001; Stollewerk, Jofré, Comaposada, Arnau, & Garriga, 2014; Vega-Gálvez et al., 2011; Zhang, Jiao, Lian, Deng, & Zhao, 2015). When HHP technology is employed in food processing, complete evaluation of the effects of process variables on essential nutrients and functional components is vital to define treatment conditions that avoid the loss of important food properties of foods and to obtain seafood with high health benefits for the consumer. 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, color, texture and acidity) in squids and other seafood (Gou, Choi, He, & Ahn, 2010; Gou, Xu, Choi, Lee, & Ahn, 2010; Hu et al., 2013; Zhang et al., 2015). However, little information is available on the effects of HHP treatment on the purine, cholesterol, taurine, antioxidant micronutrients and their antioxidant activities in squids.

Processing and storage may maintain, decrease or increase the levels of bioactive substances, and then modify bioavailability of these compounds in food. Therefore, to ensure that processed foods supply consumers with all nutrients in their most available form, comply with their requirements and maintain their organoleptic properties, it is fundamental to monitor and compare the influences of the main operative variable and storage conditions on nutritional components present in foods. The objectives of this work were 1) to investigate the influences of different pressure levels on the content and activity level of cholesterol, purines, taurine, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), thiobarbituric acid reactive substances (TBARS), and antioxidant micronutrients of squids; 2) to evaluate the relationships between

these determined compounds and their antioxidant activities; and 3) to assess the stability of these components and antioxidant activities in squids during refrigerated storage.

2. Materials and methods

2.1. Sampling, packaging and pressure treatment

Fresh squids (*Todarodes pacificus*) were purchased from a local fishery 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 to obtain the mantle. The integrated mantle muscle was retained as the experimental sample, with an average length of 210 ± 25 mm (widest part of 165 ± 15 mm, and a thickness of 3.0 ± 0.5 mm). Subsequently, mantle muscle was gently blotted with tissue paper to remove excess water on its surface.

Squid samples were individually packaged in polyamide/chlorinated polypropylene (PA/CPP) complex film bags (Yinuo Packaging Materials Co., Ltd., Shanghai, China), heat-sealed under a vacuum (Shengsong Machinery Manufacturing Co., Ltd., Shanghai, China). Sample pairs were divided into four groups: one batch was assigned as the control and was treated at 0.1 MPa; the second batch was allocated to pressurization at 200 MPa, the third batch was allocated to pressurization at 400 MPa, and the fourth batch was allocated to HHP at 600 MPa. HHP treatments were done at 20 °C for 10 min in a HHP-750 unit (Kefa High Pressure Food Processing Inc., Baotou, China) with a 2.5 L of cylindrical pressure vessel and a pressure range of 0–700 MPa. The pressure increase rate was 8.3 MPa/s, and the depressurization time was less than 4 s. After HHP treatments, all the samples were stored at 4 °C, and sampled at 0 and 10 d for analysis.

2.2. Determination of total cholesterol contents

Cholesterol analysis was conducted using the methodology described GB/T 22220-2008 (2008). About 10 mL of 60% KOH and 30 mL ethanol were added to squid samples (0.5 g) for saponification reaction at 100 °C for 1 h. The unsaponifiable portion was extracted with 40 mL petroleum ether/ether (1:1, v:v) three times. The organic layer was retained and further washed with deionized water until neutral pH, and dried over sodium sulfate. The sample was reduced to near dryness using a vacuum rotary evaporator, redissolved in 5 mL of ethanol and filtered through Millex 0.45 µm nylon membrane syringe filters for HPLC. Quantification by HPLC was carried out using an Alliance 2695 system (Waters Corp., Medford, MA, USA) liquid chromatograph system equipped with a UV–VIS detector at 206 nm. The chromatographic separation was carried out on a reversed-phase C18 column (5µ, 3.2×250 mm, Waters Corp., Medford, MA, USA.) and the mobile phase was methanol with a flow rate at 1.0 mL/min. The column temperature was set at 35 °C and the sample injection volume was 10 µL.

2.3. Determination of purine contents

The purine contents were determined by high performance liquid chromatography (Waters 2695, Medford, MA, USA) according to the method of Lou (1998), with some modifications. Briefly, the powdered samples (about 0.2 g) were digested with an 11 mL mixture of trichloroacetic acid/methanoic acid/H₂O (5/5/1: v/v) at 90 °C for 12 min. The resultant hydrolysates were transferred into a 250-mL flask and dried by a rotary vacuum evaporator at 55 °C. The solution was filtered via a 0.2 µm membrane filter. HPLC conditions were as follows: column, thermo scientific synchronis C18 (5 µ,

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