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Exploring the effect of inhibitors, cooking and freezing on melanosis in snow crab (*Chionoecetes opilio*) clusters

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ABSTRACT

Snow crab (*Chionoecetes opilio*) is a valuable crustacean either sold live or processed into two sections (i.e., clusters) and commercialized in a freshly-cooked or cooked-frozen form. The market value of snow crab clusters may be impaired by the development of melanosis, a blue-hued discoloration of enzymatic origin. This study explored the effectiveness of anti-melanosis treatments in solutions with commercially available melanosis inhibitors in conjunction with cooking and freezing. Digital image analysis, correlated to the response of a sensory panel, was used to determine melanosis progression during chilled storage. 4-Hexylresorcinol was the most effective melanosis inhibitor ($p < 0.001$). Phosphoric acid also showed a marginal, yet significant ($p < 0.05$), inhibitory effect. Ascorbic acid as well as cooking to a leg core temperature of 87 °C (±0.5) showed no effect on melanosis rate, which was instead accelerated by freezing or treatment with a mixture of acetic, ascorbic, citric and ethylenediaminetetraacetic acid. Overall, 4-hexylresorcinol has the potential to lower melanosis, which may otherwise occur very rapidly and markedly during chilled storage, especially in previously frozen clusters. Melanosis should be considered as a critical quality decay indicator in the shelf-life assessment of snow crab clusters.

1. Introduction

Snow crab (*Chionoecetes opilio*) is a decapod species widely distributed in the northern Pacific, northwestern Atlantic and along the marginal seas in the Arctic Ocean (Alvsvåg, Agnalt, & Jørstad, 2009). The adaptation of snow crab as a non-native species in the Barents Sea has prompted the rapid growth of the snow crab fishery in Norway with 3061 t landed in 2017 (Norwegian Fishermen's Sales Organization, 2018). Annual catches are expected to continue growing to a value of 1–5 billion NOK by 2020, thus providing the potential for a profitable industry (Tiller & Nyman, 2017).

Snow crabs are either sold live or processed into two separate sections, commercially referred to as clusters (Fig. 1). Snow crab clusters are usually marketed as freshly-cooked or cooked-frozen products (Lorentzen et al., 2018). Occasionally, the clusters may exhibit a blue-hued discoloration referred to as blueing or melanosis (Lorentzen et al., 2018). This is caused by the formation of dark pigments catalyzed by enzymes with phenoloxidase (PO) activity (Boon, 1975). Although melanosis does not pose a health concern, it negatively affects product acceptability (Ruddy, 2007) and may develop at a faster rate than microbial deterioration, hence representing a critical event determining product shelf-life (Nicoli, 2012).

Several biological aspects, including molting stage and catching season, may affect the susceptibility of crabs to melanosis. This is a multi-stage biochemical process triggered by physical damage, microbial invasions or *postmortem* physiological mechanisms (Opoku-Gyamfua, Simpson, & Squires, 1992). The cascade often starts with proteases, which, in turn, activate polyphenol oxidases (PPOs) (Amparyup, Charoensapsri, & Tassanakajon, 2013), a class of copper-containing enzymes abundant in crustaceans (Zamorano, Martínez-Álvarez, Montero, & Gómez-Guillén, 2009). Hemocyanin (Hc), which is a copper protein constituent of crab hemolymph and develops a blue color upon oxygenation, can also exhibit phenoloxidase activity (HcPO) (Fan et al., 2009). In the presence of oxygen, both PPO and HcPO can facilitate the conversion of monophenols into quinones which can polymerize or react with

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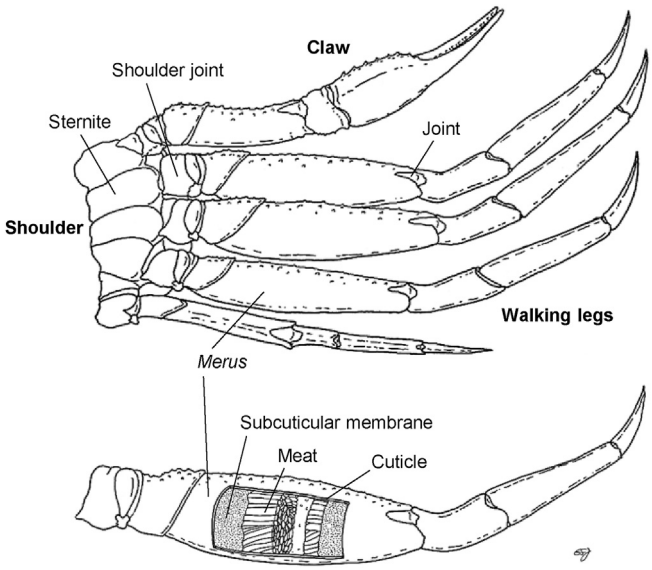

Fig. 1. Illustration of the ventral side of a cluster of snow crab and a section of the content of the *merus* of the walking legs.

proteins and amino acids, eventually leading to the formation of melanoid compounds (Kim, Marshall, & Wei, 2000).

Strategies for melanosis control aim at inhibiting different stages of the reaction through the elimination of one or more of its crucial elements, namely copper, enzyme, oxygen, and the substrate (Gökoğlu & Yerlıkaya, 2008). Treatments based on the immersion in solutions with acetic, ascorbic, citric, phosphoric and ethylenediaminetetraacetic acid (EDTA) have been suggested (Nirmal, Benjakul, Ahmad, Arfat, & Panichayupakaranant, 2015). Also, 4-hexylresorcinol (4-HR), acting as an enzyme-competitive binder, has demonstrated to be an effective inhibitor at a low dosage with minimal influence on taste (Otwell, Iyengar, & McEvily, 1992). These melanosis inhibitors have been shown as valid alternatives to sulfites and can be used in combined formulations (Martínez-Álvarez, López-Caballero, Montero, & Gómez-Guillén, 2005), targeting different stages in the melanosis cascade. In this way, they have been suggested to work more efficiently by overcoming the limitations associated with their specific mode of action, adverse effects on physicochemical and sensory quality, and concentration limits set by the legislation (Regulation (EC) No. 1333/2008).

Crustaceans are often processed into cooked or frozen products to prolong the shelf-life. Cooking is an effective method of melanosis inhibition but severe heat treatments (i.e., 10–36 min at 90 °C) may be required to achieve substantial deactivation (99.9%) of crustacean PO (Huang et al., 2014; Williams, Mamo, & Davidson, 2007) with negative repercussions on product yield and sensory attributes (Niamnuy, Devahastin, & Soponronnarit, 2007). Quick freezing appeared to be a valid method to control melanosis in shrimps (Rotllant et al., 2002). However, melanosis may occur rapidly after thawing as potential PO activity is retained during freezing (Le Bris et al., 2016).

Several studies have investigated the effect of melanosis inhibitors in crustaceans (Gonçalves & de Oliveira, 2016). Nonetheless, to our knowledge, only one research work has focused on crab species (Encarnacion, Fagutao, Shozen, Hirono, & Ohshima, 2011), and no studies have yet addressed the problem in snow crab. To add to current research, the aim of this study was to explore the effect of inhibitors, cooking and freezing on melanosis in snow crab clusters. The melanosis was assessed by digital image analysis correlated to the response of a sensory panel.

2. Material and methods

2.1. Raw material

This study was carried out on mature male snow crabs (*Chionoecetes opilio*) of commercial size (608 g ± 103) caught using crab pots at a depth of 230–250 meters in the Barents Sea (between 75°34.100′ N–33°20.900′ E and 75°30.372′ N–33°14.957′ E) in April 2017.

Immediately after landing, the crabs ($N = 85$) were taken to the Aquaculture Research Station in Kårvik (Tromsø, Norway) and kept in 6 m^3 tanks with circulating seawater at a temperature of 1–3 °C for two weeks. Before the trials, the crabs were transported live for 45 min in polystyrene boxes with gel ice (Cold Inc., Oakland, CA, USA) to Nofima AS (Tromsø). The crabs were kept at 1 °C in dry storage in the boxes and processed within 16 h of arrival. On each day of the trials, the crabs were evaluated for vitality and inspected for injuries as previously described (Siikavuopio et al., 2017). Crabs with low vitality, damaged shell or mutilated legs were withdrawn from the study.

2.2. Research strategy and experimental design

The combined effects of inhibitors, cooking, and freezing on melanosis in snow crab clusters were studied by following a multistep research strategy (Fig. 2).

The experiment was arranged in a two-level fractional factorial design (2^{6-2}) of resolution IV. In addition to the 16 factor-level combinations, a treatment comprising of cooked clusters not treated with inhibitors was included in the design array. In total, 17 treatments were carried out over four consecutive days (Table 1).

The factors investigated included four melanosis inhibitors, cooking, and freezing. The melanosis inhibitors were selected as the most promising commercially available non-sulfite compounds on the basis of preliminary trials (unpublished) conducted on snow crab. The selected inhibitors were 4-HR (0.01% w/v; Sigma-Aldrich, Darmstadt, Germany), L-ascorbic acid (AA, 1.0% w/v; Sigma-Aldrich), disodium dihydrogen pyrophosphate (PPi, 1.0% w/v; Sigma-Aldrich), and a mixture of inhibitors (mACRA) as described by Montero, Martínez-Álvarez, and Gómez-Guillén (2004) with some modifications. This formulation consisted of acetic acid (0.3% w/v; Merck, Darmstadt), L-ascorbic acid (0.5% w/v; Sigma-Aldrich), citric acid (0.5% w/v; Merck), and EDTA (0.025% w/v; Merck). All the treatments were applied in freshwater solutions with 3.4% (w/v) sea salt (99.2% NaCl; KR Holst Engros AS, Harstad, Norway).

To account for the biological variability and the effect of crab size, each treatment included ten clusters obtained from five crabs which had been uniformly distributed across the treatments according to their wet body weight. The average crab weight in each treatment ranged from 583 g (±75) to 641 g (±111).

2.3. Sample preparation

2.3.1. Immersion in melanosis-inhibiting solutions

The trials were carried out in a temperature-controlled room (5 °C) and started with the slaughtering of the crabs, resulting in two clusters. Immediately after slaughter, the claws were withdrawn from the clusters obtaining experimental units consisting of four walking legs attached to the shoulder. Each cluster was individually weighed and coded with tags. Gills and entrails were removed from the cluster shoulder using a knife and by dipping the shoulder for 10 s into fresh water (5 °C) with sea salt (3.4% w/v). The clusters were then immersed for 1 h into 15 L melanosis-inhibiting solutions (5 °C), resulting in a cluster to solution ratio of approximately 1:2.5 (w/v). The solutions were gently stirred every 15 min.