Food Chemistry 268 (2018) 498–503

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

The effects of endogenous proteases within abdominal muscle parts on the rheological properties of thermally induced gels from white croaker (*Pennahia argentata*)

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ARTICLE INFO

Keywords:
Abdominal muscle
Modori
Myosin heavy chain
Proteases
Proteolytic activity
Thermally induced gel
White croaker

ABSTRACT

Three types of material meats were prepared from a so-called normal muscle part of white croaker (*Pennahia argentata*) containing 0, 4.2 and 8.4% of an abdominal muscle part. Thermally induced gels were then prepared from these materials by pre-heating at 65 °C for 30 or 60 min and subsequent heating at 85 °C for 20 min. The breaking strength and breaking strain rate of thermally induced gels decreased with increasing contamination levels of the abdominal muscle part, in which degradation of myosin heavy chains was observed. The proteolytic activity in the abdominal muscle part homogenate was highest at 62.5 °C. These results suggest that the abdominal muscle part contains proteases that induce the modori phenomenon. Technical experts assume that a contaminated abdominal muscle part leads to quality deterioration in surimi production industries. Our findings will aid the production of high-quality surimi-based products.

1. Introduction

Surimi-based products such as "kamaboko" and "chikuwa" are traditional processed seafoods, made from fish and shellfish. In Japan, using this technique has led to imitation crab meat which is now distributed worldwide. Textural properties including elasticity, traditionally called "ashi" in Japanese, are closely related to the quality of these products along with their whiteness. This elasticity is developed when the meat is ground in the presence of neutral salt and then heated, resulting in the production of thermally induced gels (Iwata, Kanna, & Okada, 1977; Nishioka, Machida, & Shimizu, 1983; Akahane et al., 1984). These thermally induced gels are often produced by a two-step heating procedure, where the first step, known as "suwari" is carried out at a moderately high temperature of around 40 °C. The second step at a higher temperature exceeding 75 °C, is known as "honkanetsu".

Myosin, the major muscle protein, plays an important role in the formation of thermally induced gels. Myosin molecules are solubilised in the presence of neutral salt and form polymers through their heavy chains by the action of tissue-type transglutaminase at the suwari temperature, resulting in the formation of soft suwari gels. Subsequent heat treatment during honkanetsu produces elastic gels, where myosin polymers are further aggregated through hydrophobic interaction and disulphide bonding (Niwa, Nakayama, & Hamada, 1983; Numakura et al., 1985, 1987, 1989; Nishimoto et al., 1987; Kimura et al., 1991; Fukushima, Yoon, & Watabe, 2003; Fukushima et al., 2005). It is known that the rheological profiles of thermally induced gels differ depending on the thermal conditions used to prepare the gels, including temperature and heating time (Shimizu, Machida, & Takenami, 1981; Satoh, Nakaya, Ochiai, & Watabe, 2006; Fukushima, Okazaki, Fukuda, & Watabe, 2007; Matsuoka, Wan, Ushio, & Watabe, 2013). These profiles also differ among fish species (Fukushima et al., 2003, 2005). It has been shown that myosin exhibits different thermal properties, associated with the temperatures in the regions in which the fish inhabit (Nakaya, Watabe, & Ooi, 1995; Nakaya, Kakinuma, Watabe, & Ooi, 1997; Kakinuma et al., 1998). It has also been suggested that species-specific primary sequences of myosin heavy chains determine their rheological properties (Togashi et al., 2000; Togashi, Kakinuma, Nakaya, Ooi, & Watabe, 2002; Yoon, Kakinuma, Hirayama, Yamamoto, & Watabe, 2000; Hossain, Ikeda, Nomura, Fukushima, & Watabe, 2008).

The production of thermally induced gels having high elasticity is commercially important, as the quality of surimi-based products is regarded low when their elasticity is weak. As mentioned above, the suwari gels are formed at a moderately high temperature of about 40 °C and broken down in a temperature range of 50–70 °C. Such collapse of suwari gels in the latter temperature range is called "modori" and

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https://doi.org/10.1016/j.foodchem.2018.06.080
Received 24 February 2018; Received in revised form 17 May 2018; Accepted 18 June 2018
Available online 19 June 2018

thought to be induced by contaminating proteases. Alternatively, it has been reported that modori occurs due to the detachment of actin from myosin and the subsequent denaturation of actin (Sano, Noguchi, Matsumoto, & Tsuchiya, 1989; Ni, Nozawa, & Seki, 1999, 2001).

Various proteases have been identified from fish muscle and their properties have been determined (Busconi, Folco, Martone, Trucco, & Sanchez, 1984; Folco, Busconi, Martone, & Sanchez, 1988; Kinoshita, Toyohara, & Shimizu, 1990; Yanagihara, Nakaoka, Hara, & Ishihara, 1991). However, myosin heavy chains were not degraded when the homogenate prepared from the dorsal muscle was heated at 50–60 °C, while the elasticity of thermally induced gels remained high for both white croaker (*Pennahia argentata*) and walleye pollock (*Gadus chalcogrammus*) which are currently used as materials for high quality and conventional surimi-based products, respectively (Ueki, Wan, & Watabe, 2016a, 2016b). We further reported that proteolytic enzymes which induce modori phenomenon were contained in the intestinal extracts from white croaker and walleye pollock; these enzymes cannot be eliminated by washing procedures once fish muscle is contaminated with them (Ueki et al., 2016a, 2016b). Thus, we speculated that modori phenomenon observed during the processing of the surimi-based products is attributable to the action of exogenous proteases which would contaminate fish muscle rather than muscle endogenous proteases.

Besides the detrimental effects of contaminating visceral organs on gel elasticity, technical experts in the kamaboko factories believe that modori is also induced by the presence of contaminated abdominal muscle in surimi materials. In fact, even for white croaker, the elasticity of thermally induced gels prepared from frozen surimi, manufactured with the aim of obtaining an extremely high yield for large scale production, declined when treated at around 60 °C in the suwari step (Matsuoka et al., 2013). Such decline in the elasticity of thermally induced gels was not observed when only the dorsal muscle was used as a material while avoiding any contamination with the abdominal muscle, as mentioned above (Ueki et al., 2016a). However, the differences in the level of modori phenomenon between the dorsal and abdominal muscles of fish has not previously been compared. Of which, the abdominal muscle is not contaminated with visceral organs.

Under these circumstances, we investigated the rheological properties of thermally induced gels prepared from the abdominal muscle of white croaker and further examined proteolytic activities when the abdominal muscle was added at various ratios to a so-called normal muscle part (normal material part) currently used for the production of high-quality surimi-based products (excluding the abdominal muscle).

2. Materials and methods

2.1. Materials

Normal and abdominal muscles parts (Fig. 1) were prepared from white croaker, which were captured in the East China Sea and subsequently stored on ice for two days during transportation. The normal muscle part defined in the present study indicates one which is used for thermally induced gels at the industrial level, excluding the abdominal muscle part. The reagents were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), unless otherwise specified.

2.2. Preparation of washed meat from normal and abdominal muscle parts

After heading and gutting, normal and abdominal muscle parts were separated from white croaker (Fig. 1). The wet weight ratios of these two muscle parts recovered were 91.6 and 8.4%, respectively, from 24 individuals (Table 1). The two muscle parts were separately washed with tap water to avoid any contamination with proteases from internal organs and subsequently dissected with a kitchen knife. Then, two types of material meats were prepared for the production of thermally induced gels. One type consisted of 458 g normal muscle part and 42 g abdominal muscle part, accounting for 8.4% abdominal muscle part in the total weight, thus mimicking the original ratio of normal to abdominal muscle part. The other type consisted of 479 g normal muscle part and 21 g abdominal muscle part, accounting for 4.2% abdominal muscle part (around half the original ratio). We defined samples containing 8.4% and 4.2% abdominal muscle part as high and moderate contamination levels (HCL and MCL), respectively. The two types of material meats, along with the normal muscle part only (control) were washed with four volumes of tap water at 15 °C for five minutes and subjected to centrifugation at 15,000 × *g* and 4 °C for 15 min.

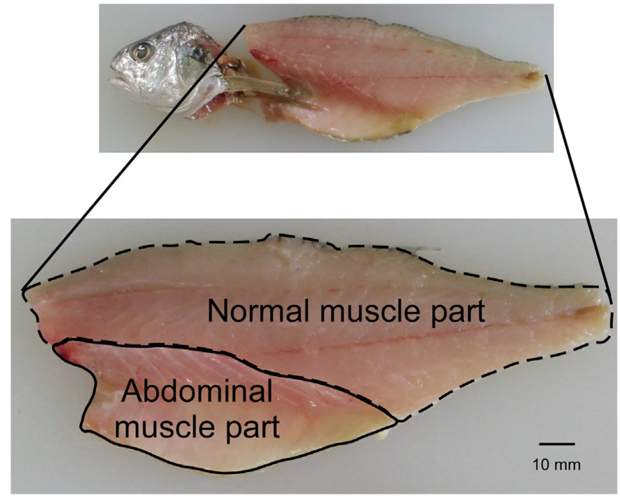

Fig. 1. Photographs indicating normal and abdominal muscle parts.

Table 1
Weights of normal and abdominal muscle parts collected from 24 individuals of white croaker.

Part	Weight (g)	Ratio (%)	
		Per total weight	Per total muscle weight
Normal muscle	1517	35.0	91.6
Abdominal muscle	140	3.2	8.4
Other*	2673	61.8	
Total	4330	100	

* Including a head, internal organs, skins, and bones.

2.3. Preparation of thermally induced gels

After the three types of washed meats (mentioned above) were adjusted to a water content of 89.0% with MilliQ water (v/w), NaCl was added to a final concentration of 2.9% (w/w). These mixtures were ground using a food processor (MK-K61, Panasonic Corporation, Osaka, Japan), while the temperature maintained was no higher than 8 °C. Subsequently salt-ground surimi was stuffed into cylindrical plastic cases (total volume 20 mL, inner diameter 36 mm, height 20 mm) (Yamayu Plastic Medical Products, Osaka, Japan). The cases were preheated at 65 °C in a water bath (TBA103DA, Adventec, Tokyo, Japan) for 30 or 60 min, then heated at 85 °C for 20 min. Obtained gels were immediately cooled in ice-cold water for 30 min and stored at 4 °C until use in the following experiments.

2.4. Rheological properties measurement

The rheological properties of thermally induced gels were measured