Preliminary study on the inactivation of anisakid larvae in baccalà prepared according to traditional methods

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Abstract

The European Food Safety Authority stated that *many traditional marinating and cold smoking methods are not sufficient to kill* A. simplex and asked to *evaluate alternative treatments for killing viable parasites in fishery*. Baccalà is a well-liked traditional product. The aim of study was to evaluate the effectiveness of the salting process on the inactivation of nematodes of the genus *Anisakis* in naturally infected Baccalà fillets. N. 19 fillets, subjected to a dual salting process (brine and dry salting) were analyzed. Visual inspection and chloropeptic digestion were performed. Larvae viability was evaluated, and parameters such as NaCl (%), moisture (%), WPS and a_w were determined. In n. 17 samples parasites were found 123 parasites with a mean intensity of 7.23±4.78 and an mean abundance of 6.47±5.05. Visual examination has revealed 109 parasites. 61.8% of larvae were found in the ventral portions. The results show that salting process with a salt concentration of 18.6%, a_w values of 0.7514 and 24.15% WPS in all parts of baccalà fillets, devitalise *Anisakidae* larvae in a 15-day period.

Introduction

Salt-curing of fish have been used as preservation methods since ancient times; even if this method have lost importance for preservation purposes, due to the widespread use of new technologies in developed countries, salted cod (*Gadus spp*), is one of the main heavy-salted products consumed in Mediterranean countries. Salted cod, named *bacalhau* in Portugal or *baccalà* in Italy, is highly appreciated because of its characteristic taste and nutritional quality (Beraquet *et al*., 1975; Bjørkevoll *et al.,* 2003; Heredia *et al*., 2007; Lauritzsen *et al.,* 2004; Martìnez-Alvarez *et al*., 2005). *Baccalà* is considered a shelf stable product (low moisture and high salt content). Regarding parasites, Atlantic cod has an exceptionally rich and varied parasite fauna (*Pseudoterranova spp*, *Anisakis spp*, and others) compared with most other species of marine fish (Hemmingsen and MacKenzie, 2001). This may be due to its eating habits and to the fishing area rich of definitive hosts (Cipriani *et al*., 2015). Parasitic nematodes are a problem in cod processing industries (Margeirsson *et al*., 2007) because when are present in the cod flesh were considered the worst defects and contribute to an unpleasant appearance . On the other hand, *baccalà* is still consumed raw (marinated dish such as *carpaccio*) or semi-raw (partly cooked), which could represent a threat to human health. Thus, immediate removal of the viscera (gutting) after catching is fundamental to reduce the number of parasites in cod flesh (Rodrigues, 2006). Human anisakidosis, caused by parasites of the *Anisakidae* family, the most important fish parasites from a sanitary point of view, is associated with the consumption of raw or almost raw seafood products in which are present viable parasites. The European Food Safety Authority (EFSA, 2010) reported approximately 20000 anisakiasis cases worldwide prior to 2010, with >90% from Japan. Reg. EC 853/04 (Reg. EC 853/04; European Commission, 2004) states that fishery products to be consumed raw or almost raw and fishery products marinated and/or salted require freezing (-20° for 24 h or -35° for 15 h) if the ripening process is insufficient to kill nematode larvae.

Since freezing can affect the sensorial characteristics of salted fishery products (Stefànsson *et al*., 2000), several alternative methods were studied in order to obtain an equivalent effect (Adams *et al*., 1999; Anastasio *et al*., 2016; Giarratana *et al*., 2015). Aim of the study was to evaluate the effectiveness of the salting process on the inactivation of nematodes of the genus *Anisakis* in naturally infected Baccalà fillets.

Materials and Methods

Sampling

N. 19 samples of *baccalà* fillets (*Gadus morhua* - FAO area 27) belonging to the same lot with different weight categories (300-500 g; 500-800 g; 800-1200 g) were chosen for trial. Fillets were subjected to a double salting process: in Faroe Islands fresh cod fillets were pickle salted in a NaCl brine solution (13%) at 5°C for 24 h in a 1:1 fish-to-brine ratio; afterwards, in a food company located in Naples (Campania, Italy), fillets were dry salted and matured for 3 months at 5°C by stacking fish and salt (weight ratio 1:1) in alternating layers.

Every 15 days, until the 90th day, n. 3 fillets belonging to the 3 different weight category were sampled and delivered to the Food Chemistry laboratory of the Department of Veterinary Medicine and Animal Production, University of Naples Federico II for analytic determination.

Physical-chemical analyses

The following determinations were performed, except pH, on pooled samples: pH was measured with a digital pH-meter (CrisonMicroTT 2022, Crison Instruments, Barcelona); a_w was measured by Aqualab 4 TE (Decagon Devices Inc., USA); Moisture (%) was determined by oven drying for 24 h at 105°C (AOAC, 1990). Water phase salt (WPS) was determined according to Kenneth and Hilderbrand (1991). The salt content (% NaCl) was measured with the Volhard method (AOAC, 2000). All tests were done in duplicate.

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Parasitological survey

Each sample was first skinned, desalted in water for 2 h and separated into the hypaxial (ventral) and epaxial (dorsal) regions, following the horizontal septum. According to Reg. EC 2074/05 (Reg EC 2074/05; European Commission, 2005) each portion was subjected to a visual inspection; afterwards, each part was digested separately in a chloropeptic solution, according to Llarena-Reino et al. (2013). All anisakid larvae were identified at the genus level by microscopic examination of diagnostic characters (Mattiucci *et al*., 2011). Infection indexes were established according to Bush *et al*. (1997).

Larvae viability

At each sampling time, to test viability, all larvae collected from fillets were put into a pepsin digestion solution (0.5% w/v Pepsin in 0.063 M HCl) and inspected under a stereomicroscope at 37°C for one hour (CODEX, 2004). Viability scores were assigned according to Hirasa and Takemasa (1998): viable, score 3; motility reduction, score 2; motility only after stimulation by a needle, score 1; and death, score 0, when no motility was observed.

Results

Physical-chemical analyses

PH, a_w, NaCl (%), moisture (%) and WPS (%) contents are shown in Table 1. PH, a_w and moisture values remained constant until the 90th day of curing. Salt content ranged from 18.6 and 21.4%; WPS ranged from 24.15 to 26.55. Moisture content and WPS were typical of a ripened and shelf stable product (FDA, 2011).

Infection indexes

In n. 17 fillets were found parasites: a total of 123 Anisakidae larvae (prevalence 89.47%) were detected; n. 109 larvae were detected by visual inspection (88.61%). Chloropeptic digestion has revealed n. 14 parasites. The mean±SD larva abundance and the mean±SD larva intensity were 6.47±5.05 and 7.23±4.78, respectively. All larvae were identified as belonging to the genus *Anisakis* (80.49%) and *Pseudoterranova* (19.51%) by their morphological characters. A positive correlation between the size and the number of parasites was noted: infact fillets belonging to weight category 300-500g had a mean parasite abundance±SD of 4.4±4.47; fillets belonging to weight category 500-800g had a mean parasite abundance±SD of 7±2.94; fillets belonging to weight category 800-1200g had a mean parasite abundance±SD of 10.2±5.76; smaller weight samples showed a deeper larvae encystation. 61.8% of larvae were found in the ventral portions (Figure 1).

Larvae viability

Salt killing effect was evaluated every 15 days: at the first sampling time all tested larvae probed with a dissection needle or with a nipper did not show any movement, and were considered dead (score 0). Furthermore at every sampling time until the 90th day of the ripening process, all tested larvae were dead.

Discussion

According to Codex Alimentarius, potential hazards in fish and fishery products during filleting, skinning, trimming and candling are viable parasites, while parasites are *only* potential defects. Codex Alimentarius also states that brining or pickling may reduce the parasite hazard if the products are kept in an adequate combination of salt content and curing time (CAC/RCP 52-2003).

The results of this study show that salting process of cod with a salt concentration of 18.6% for at least 15 days, which is considered the minimum time required in order to obtain commercial salted cod (Andrés *et al*., 2005), devitalise all *anisakidae* larvae present in flash. The salting process causes osmotic damages to cuticle and digestive tract modifying permeability of the membrane with a leakage of cell content such as ions (Bakkali *et al*., 2008). Previous studies reported the ability of salt to inactivate

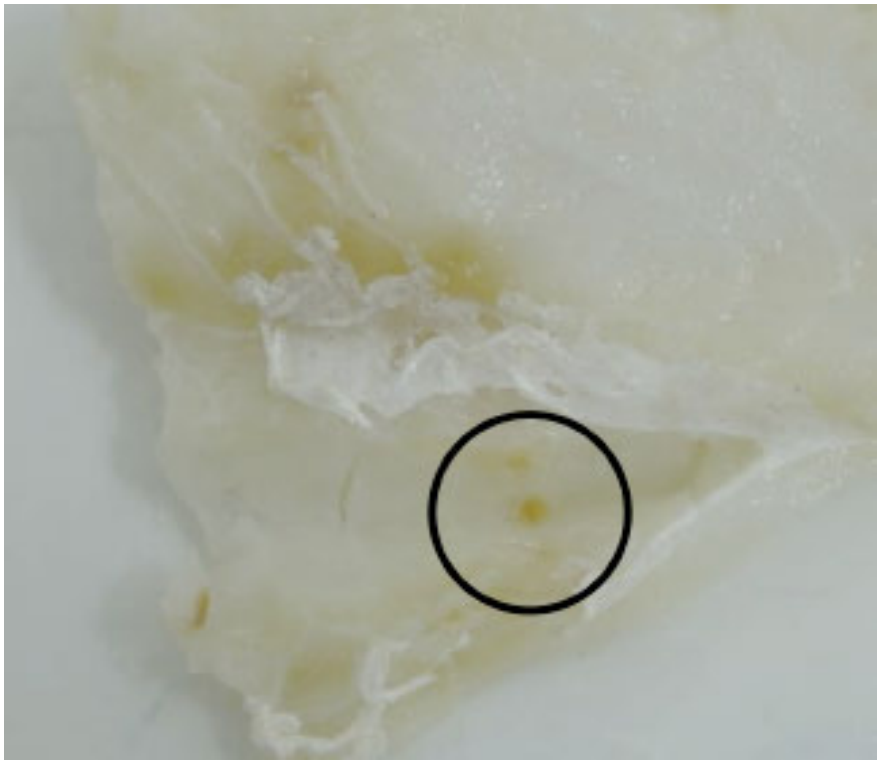

Figure 1. Baccalà belly flap with encysted anisakidae larvae.

Table 1. Trend of pH, aw, NaCl (%), moisture (%) and water phase salt (%) contents in baccalà during the ripening process.

Variables	Ripening days					
	15	30	45	60	75	90
aw	0.7514	0.7403	0.7401	0.7395	0.7388	0.7381
pH	6.21	6.22	6.18	6.2	6.15	6.15
NaCl	18.6	20.7	20.9	20.9	21.3	21.4
Moisture	58.4	58.7	58.7	59	59.1	59.1
WPS	24.15	26.07	26.25	26.15	26.49	26.55

WPS, water phase salt.

nematodes. Wootten and Cann (2001) showed that brining with 21% salt for 10 days killed all *Anisakis* larvae. Regarding salted cod, Rodrigues (2006) proved that number of parasites in salted cod was generally lower than in fresh cod because of gutting, visual inspection during filleting, manipulation and removal of the parasites that are located superficially on visceral peritoneum; moreover he stated that a curing period by salting of 13 days is enough to devitalise parasites.

Anastasio (2016) showed that the dry salting process of anchovies with a salt concentration of 21% in all the edible parts, for at least 15 days, kill *Anisakis pegreffii* larvae present in ripened anchovies.

In Spain the *Agencia Española de Seguridad Alimentaria y Nutrición* (AESAN, 2007) have specified the technical salting parameters and curing period, excluding freezing of products, able to inactivate anisakidae larvae: when salt concentration in fish is above 9% for at least six weeks, between 10% and 20% for at least four weeks, or more than 20% for at least three weeks freezing of fish products can be avoided.

Conclusions

EFSA asked to *evaluate alternative treatments for killing viable parasites in fishery* which could preserve traditional production methods ensuring food safety and product quality. The results of our study show that for *baccalà* a salting period of 15 days with a 18.6% NaCl concentration, combined with a_w and WPS values of 0.7514 and 24.15%, is effective to inactivate anisakidae larvae present in the fillets. This result might have practical outcomes because freezing treatment, at this time mandatory for EC, may adversely affect organoleptic features of salted fish products (Stefânsson *et al*., 2000).

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