

Shelf life of anchovy products (*Engraulis encrasicolus*): evaluation of sensory, microbiological and chemical properties

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Abstract

Fishery products have always been an important food in Italy. In the past, increased consumption was mainly due to the good quality of the products, easiness of use and their beneficial effects on health. Recently, owing to the national financial crisis, there has been a decline in the consumption of fish. In fact, in 2013, according to data from ISMEA, the consumption of fresh fish suffered a sharp contraction (-5%). This decline also concerns anchovy (*Engraulis encrasicolus*). This species, partly because of its low price, is a mainstay of traditional Italian food. The aim of this study was to evaluate sensorial, chemical and microbiological properties of anchovy-based (*Engraulis encrasicolus*) products during storage at 4 and -20°C. Fresh anchovies, obtained from the wholesale fish market of Pozzuoli (Southern Italy) were cut into small pieces and hand-prepared using bread, eggs, cheese and lemon juice. Samples were analysed after 0, 2, 4, 6 and 8 days of storage at 4°C. An aliquot was quickly frozen and analysed after 34 days at -20°C. Sensory assessment, microbiological (specific spoilage organisms, *Listeria* spp. and *Salmonella* spp.) and chemical (histamine, total volatile basic nitrogen, trimethylamine, thiobarbituric acid, pH and a_w) analyses were performed. Results showed that the shelf life of anchovy products was less than 5 days for the samples stored at 4°C. At -20°C, all anchovies preparations showed good sensory, microbiological and chemical properties for 34 days.

Introduction

Fishery products have always been an important food in Italy. In the past, increased consumption was mainly due to the good

quality of the products, ease of use and their beneficial effects on health.

Currently, there is a strongly decline in consumption of fish mainly related to the financial crisis so much so that in 2013 the consumption of fresh fish by Italian people suffered a sharp decline (-5%) (ISMEA, 2013). This negative trend also regards the anchovy (*Engraulis encrasicolus*) market. This fish species has always been a part of traditional, *poor* cuisine because, despite its status as *the most prized* of small fish, it actually costs little. Although the anchovy market is historically linked to the sale of fresh produce and produce preserved in salt and oil, it could be important to diversify this by developing new kind of products. The aim of this trial was to assess the shelf-life of anchovy (*Engraulis encrasicolus*) meatballs stored at 4°C for 8 days and at -20°C for 1 month by evaluating their microbiological, chemical and sensorial features.

Materials and Methods

For the study, a batch of fishballs was made at the workshop at Pozzuoli fish market using anchovies (*Engraulis encrasicolus*) fished no more than 5 h before in the nearby Gulf. The fishballs, which were bigger than a walnut, were obtained by manually mixing anchovies (cut with scissors), bread, eggs, cheese and lemon juice and coating the same with bread crumbs at the end. Then, 8 meatballs at a time were packed in aluminum semi airtight containers covered with foil and stocked at 4°C. One container was frozen and stored at -20°C for more than a month. All the refrigerated samples and raw materials were submitted to sensorial, chemical and microbiological analyses after 0, 2, 6, 8 storage days. The frozen sample, on the other hand, was analysed after 34 days of storage. All the analyses were performed at a food inspection laboratory.

Sensorial analyses

All samples were subjected to sensorial analyses by a test panel of 5 qualified people who evaluated the colour, flavour, texture and the presence of liquid in the containers. Afterwards, the meatballs were cooked in hot oil as preparation for a taste test.

Microbiological analyses

For the microbiological analysis, 25 gr of sample were homogenised in 225 mL of (w/v) sterilised 0.2% peptone water at 45°C in a Stomacher Lab-Blender 400 (PBI International, Milan, Italy) and consecutive ten-fold serial dilutions were made in sterilised 0.2% peptone water. Aerobic mesophilic counts

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Key words: *Engraulis encrasicolus*, Fish preparations, Shelf life, SSOs, Histamine.

Received for publication: 27 May 2013.
Revision received: 27 July 2013.
Accepted for publication: 29 August 2013.

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Licensee PAGEPress, Italy
Italian Journal of Food Safety 2014; 3:1678
doi:10.4081/ijfs.2014.1678

(AMC) were enumerated on plate count agar (PCA; Oxoid, Milan, Italy) after 48 h and 15 days of aerobic incubation at 32 and 4°C, respectively, enterobacteriaceae on violet red bile glucose agar (VRBG; OXOID), using the pour-plate and overlay technique, after 48 h of incubation at 37°C, *Pseudomonas* spp. on pseudomonas agar base+Pseudomonas C-N-C Supplement (OXOID) at 22°Cx48 h; *Aeromonas* spp. on Aeromonas Medium Base+Ampicilin Supplement (OXOID) at 30°Cx48 h, *Brochothrix thermosphacta* on SPAA Agar Base+STAA selective Supplement (OXOID) at 22°C x 48 h, *Listeria* spp. and *Salmonella* spp. according to ISO 6579:2008 (ISO, 2008) and ISO 11290-1:1996 (ISO, 1996), respectively. All analyses were performed in duplicate and the mean value of each count was expressed in Log cfu/g.

Chemical analyses

In all samples, pH (pH-meter FE20; Mettler, Toledo, Spain) were determined: a_w (activity water, con HigrLab rotonic PBI), total volatile basic nitrogen (TVB-N) (Antonacopoulos and Vincke, 1989), trimethylamine nitrogen (TMA-N) (AOAC, 1984), Thiobarbituric acid (TBA) (Pearson, 1973), Histamine (Embrog and Dalgaard, 2006).

Results

Sensorial analyses

After 4 days of storage, anchovy fishballs stored at 4°C showed a lively and bright colour, a firm texture, had a pleasing smell and there was no liquid in the package. From the 6th day of storage, they were grayish in colour, had a pungent odour, less firm texture and there was a turbid liquid in the package.

On the 8th storage day, a complete loss of freshness was observed. Regarding the taste test, the anchovy fishballs were acceptable up to the 4th day of storage. After thawing, the frozen samples showed sensorial features similar to those of the refrigerated fishballs on the first day of storage.

Microbiological analyses

In the refrigerated samples, TVC at 32 and 4°C, and enterobacteriaceae after 8 days of storage reached values of 8.63, 6.23 and 4.89 log CFU/g, respectively (Figure 1). *Aeromonas* spp. and *Pseudomonas* spp. increased gradually and constantly during storage (Figure 2).

In the frozen sample, TVC at 32°C and enterobacteriaceae showed lower values like those registered at the beginning of storage. On the contrary, TVC at 4°C increased by 4.59 log CFU/g compared to the values of raw material (Figure 1). *Aeromonas* spp. and *Pseudomonas* spp. were absent in the frozen sample (Figure 2). *Brochothrix thermosphacta*, *Listeria* spp. and *Salmonella* spp.

All bacteria results are shown in Figures 1 and 2.

Chemical analyses

TVB-N and TMA reached 12.14 and 34.42 mg/100gr on the 6th day, 22.14 and 35.42/100 gr after 8 days of storage at 4°C (Figure 3). In the chilled product, on 34th day values of 20.45 and 4.8 mg/100 gr for TVB-N and TMA respectively were revealed. From an initial value of 5 mg/100 gr, histamine increased during refrigerated storage up to 20 mg/100 g on 8th day (Figure 3). As regards TBA, concentrations of malonaldehyde of 0.98 mg/Kg in the refrigerated samples on 8th day and of 0.16 mg/Kg in the chilled sample were detected. In all samples, pH was 6.17 on first day and then decreased and showed the mean value of 5.64 until the end of storage. A_w showed the mean value of 0.95 during entire storage in all samples.

Chemical results are shown in Figure 3.

Discussion and Conclusions

Pseudomonas spp. and *Aeromonas* spp. are widely recognised as specific spoilage organisms (SSOs) of fish products (Gram and Huss, 1996; Gram *et al.*, 2002; Dalgaard *et al.*, 1995) deteriorating them when exceeding the value of 5×10^5 UFC/g (ICMSF, 1986) In this trial, these microorganisms showed a low charge in the raw material. From the 5th day, the gradual increase of TVC at 32 and 4°C, *Aeromonas* spp. and *Pseudomonas* spp. was accompanied by a gradual loss in freshness (Alasalvar *et al.*, 2001).

TVB-N and TMA was higher than the limits of 35 and 20 mg/100g in the refrigerated sam-

ples on the 8th day of storage. Histamine showed values lower than the limits of Reg. 2073/2005 (European Commission, 2005) until the end of storage. Regarding TBA, the concentration of malonaldehyde was always lower than the limit of 5 mg/Kg for products widely oxidised.

The frozen product had good sensorial, microbiological and chemical features after 34 days of storage at -20°C thanks to the rapid reduction of the temperature and to early processing times after the capture.

Our results allow us to define a shelf life of 4 days for refrigerated anchovy meatballs and of 34 days for the chilled product.

The anchovy is a fish species which is still under appreciated despite its high nutritional value. The development of new anchovy preparations could promote the consumption of this fish species especially by particular categories of consumers such as the elderly and children. Of course, in order to deliver a flawless product, it is necessary to start with fresh raw material and to respect the cold chain.

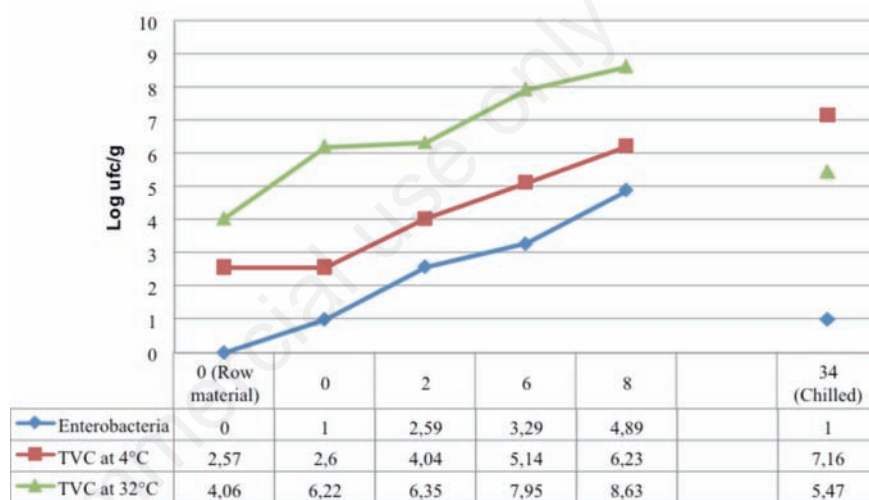


Figure 1. Average values of total viable count at 32 and 4°C, and Enterobacteria in anchovy fishballs.

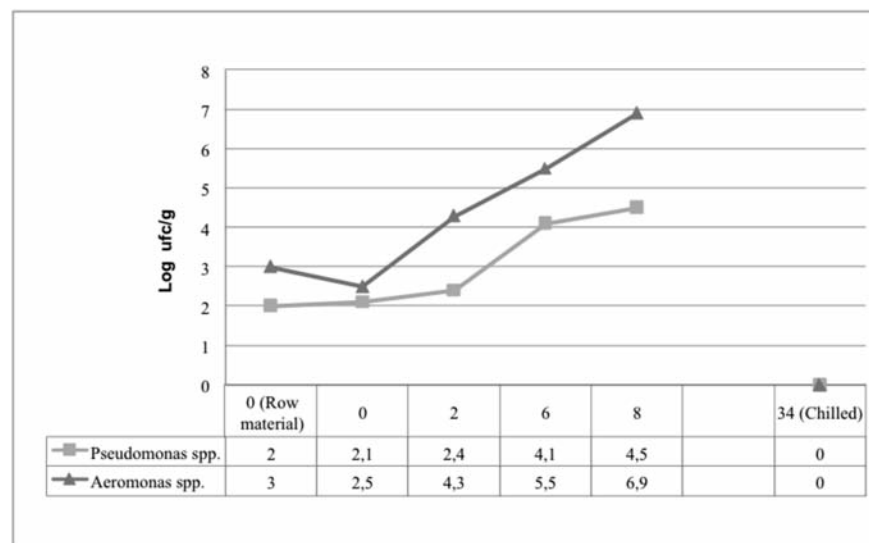


Figure 2. Average values of *Aeromonas* spp. and *Pseudomonas* spp. in anchovy fishballs.

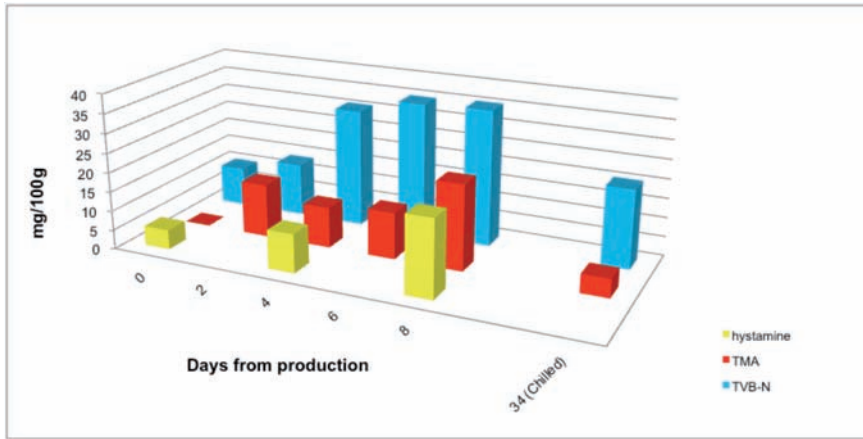


Figure 3. Average values of total volatile basic nitrogen, trimethylamine and histamine in anchovy fishballs.

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