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Effects of Allyl Isothiocyanate on the Shelf-life of Gilthead Sea Bream (*Sparus aurata*) Fillets

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Abstract

Muscolino D., Giarratana F., Beninati C., Ziino G., Giuffrida A., Panebianco A. (2016): **Effects of allyl isothiocyanate on the shelf-life of gilthead sea bream** (***Sparus aurata***) **fillets**. Czech J. Food. Sci., 34: 160–165.

The effect of allyl isothiocyanate (AITC), a natural compound found in plants belonging to the family *Cruciferae*, on the shelf-life of fish fillets was evaluated. Preliminarily, the effectiveness of AITC was investigated *in vitro* against some strains of Specific Spoilage Organisms (SSOs). The inhibitory effect of AITC vapour was screened evaluating the bacterial growth in a jar. A strong inhibitory effect against the tested SSOs was observed. Successively, the shelf-life of gilthead sea bream fillets treated with AITC vapour and stored at 2°C was evaluated and compared with untreated samples. AITC inhibited the growth of SSOs effectively, and its use resulted in a shelf-life extension by ca. 8 days in treated fillets, compared to the control samples. The AITC addition in fillets yielded a distinct but pleasant flavour and contributed to a considerable reduction of fish spoilage.

Keywords: antibacterial activity; fish fillets; natural compound; Specific Spoilage Organisms

Allyl isothiocyanate (AITC) is a natural compound found in plants belonging to the family *Cruciferae*. It is found in the seeds, stem, leaves, and roots of cruciferous plants including horseradish, black and brown mustard, cabbage, Brussels sprouts, broccoli, cauliflower, kohlrabi, kale, turnip, rutabaga, watercress, wasabi, radish, and papaya (Zhang 2010). As known, AITC is bitter in taste with a strong, pungent, and mustard like smell and it is authorised as flavouring substances in foods by the European Regulation EC No 872/2012. Moreover, AITC possesses also antimicrobial, antifungal, antioxidant, and anticarcinogenic properties (Zhang 2010; Minarini *et al.* 2014). The antimicrobial activity of AITC was demonstrated on spoilage microorganisms, foodborne and postharvest pathogens, including gram-negative, gram-positive bacteria, and moulds (Lin *et al.* 2000; Maria *et al.* 2002; Rhee *et al.* 2003; Wilson *et al.* 2013; Zou *et al.* 2013; Dias *et al.* 2014). The antimicrobial activity of AITC is higher in the gas phase than in the liquid phase (Shin *et al.* 2010). In this regard, recently some authors have reported the effectiveness of AITC vapour as a preservative in several food, including fish products, as an alternative to common package techniques (Winther & Nielsen 2006; Mastromatteo *et al.* 2010; Pang *et al.* 2013; Giarratana *et al.* 2015). Microorganisms are the major cause of spoilage in seafood products. However, only Specific Spoilage Organisms (SSOs) are linked to the occurrence of off-flavours associated with seafood spoilage. The SSOs are typically present in low numbers and constitute only a very small fraction of the seafood microflora, including several bacterial species such as *Aeromonas* spp., *Enterobacteriaceae*, *Pseudomonas* spp., *Photobacterium phosphoreum*, *Shewanella putrefaciens*, and *Vibrio* spp. (Böhme *et al.* 2010). The major cause of seafood spoilage is their microbial growth and metabolism resulting in the formation of ammonia, biogenic amines, organic acids, sulphur compounds, and ketones, responsible for unpleasant and unacceptable off-flavours (Gram & Dalgaard 2002; Giuffrida *et al.* 2013). The improvement of preservation techniques to reduce the growth and activity of spoilage microorganisms in foods is crucial to increase their shelf-life and to reduce the losses due to spoilage.

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In this regard, nowadays, several natural compounds are paid increasing attention because of their potential functional and technological uses in the seafood industry, like for example to extend the shelf-life of fish products (Goulas & Kontominas 2007; Ojagh *et al.* 2010; Daniel *et al.* 2014). In this context, the use of AITC in seafood products could be interesting, due to the sensory attributes resulting from its use (pleasant smell and taste) and its relatively safe status.

The aim of this study was to evaluate the effect of AITC vapour on the growth of SSOs and on the shelf-life of packaged fresh fish fillets.

MATERIAL AND METHODS

Experimental plan. The present study was carried out in two different steps. The first one concerned *in vitro* tests where the effectiveness of different concentrations of AITC against SSOs was evaluated. The second experimental step was carried out on sea bream fillets with the addition of AITC in order to evaluate its effects on SSO growth and shelf-life.

In vitro test. A total of 60 fish isolates belonging to the group of SSOs were used for the assessment of AITC vapour inhibiting activity *in vitro*. They encompassed the following species: *Pseudomonas fluorescens* (n = 10), *P. putida* (n = 10), *P. syringae* (n = 10), *P. fragi* (n = 10), *Shewanella putrefaciens* (n = 10), and *S. baltica* (n = 10). These strains were selected from the Bacteria Collection of Food Hygiene Unit, Department of Veterinary Sciences, University of Messina, Italy. Bacteria strains tested were the predominant flora isolated from the skin and muscle of farmed gilthead sea bream (*Sparus aurata*) (Cattaneo *et al.* 2007). The isolates were identified by sequencing 16S rDNA region and by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF-MS) (Cattaneo *et al.* 2007). Bacterial inoculum suspensions for agar diffusion test were obtained from frozen stocks, stored at –80°C, and prepared according to the Clinical and Laboratory Standards Institute (2012). The inhibitory effect of AITC vapours was screened evaluating the bacterial growth in a jar where the known amount of AITC was evaporated. Pure AITC (purity ≥ 95%) was obtained from Sigma-Aldrich Corp. (St. Louis, USA). Mueller Hinton Agar plates (Oxoid Ltd., Basinkstoke, Hampshire, UK) were inoculated by dipping a sterile cotton swab into each strain culture and evenly streaking the swab in three directions over the entire surface of the plate. The plates were then placed into an airtight jar with sterile paper filters (1 × 3 cm) soaked with four different amounts of the compound (300, 100, 30, and 10 µl). The paper filters worked as an AITC carrier. The final concentrations of gaseous AITC in the jar, calculated according to Pang *et al.* (2013), were 131.11, 43.70, 13.11, and 4.37 mg/ l, respectively, assuming the AITC vapourised completely into the headspace. Control treatments consisted in a jar with filter papers added sterile distilled water. The jar was sealed immediately and incubated at 30°C for 24–48 hours. The inhibitory effect of AITC against the strains tested was determined by evaluation of bacterial growth on the surface of Mueller Hinton Agar plates after incubation. These studies were performed in three replicates.

Test on fish fillets

Preparation, packaging, and storage of fish samples. The present study was carried out on 84 skin-off fillets obtained from 42 gilthead sea breams (*Sparus aurata*) of a sea-based aquaculture facility located in Sicily (Italy). Immediately after harvest the fish were killed by immersion in ice-water slurry. Total size and weight of the fish were measured (average weight and length: 350 ± 20 g and 250 ± 4 mm). After death, the fish were skinned and threaded within 2 hours. Approx. 100 g of fillets were prepared and stored at 2 ± 0.5°C with and without addition of AITC. Each fish fillet was placed in transparent polypropylene trays. Each tray was then inserted into polypropylene bags operating with O_2, CO_2, and water vapour permeabilities of 3, 10, and 3 g/m^2/day, respectively, and hermetically sealed. The addition of AITC was done with a filter paper (1 × 3 cm) soaked with 10, 5, and 2 µl of the compound.

According to data obtained from *in vitro* tests**,** the concentrations used in this step were chosen in order to maintain antimicrobial effectiveness of AITC and preserve sensorial acceptability of fish fillets. The final concentrations in containers, calculated according to Pang *et al.* (2013), were 6.70, 3.35, and 1.34 mg/l, respectively. Gilthead sea bream fillets packaged without AITC were used as control. After packaging all samples were stored at 2 ± 0.5°C for 14 days. Microbiological and sensorial evaluations were carried out at 0, 2, 4, 7, 9, 11, and 14 days of storage by sampling three fillets for each time interval.

Microbiological analysis. Approx. 20 g of fillets were sampled under sterile conditions for microbiological assays. Each sample was transferred into a stomacher bag and 0.1% peptone water was added at a 1 : 9 ratio of (w/v); samples were homogenised for 60 s at 230 rpm

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with a stomacher (Stomacher® 400 Circulator; International PBI s.p.a., Milan, Italy) and tenfold dilutions in 0.1% peptone water were prepared. One ml aliquots were plated, in duplicate, on Lyngby Iron Agar plates (Oxoid Ltd., Basinkstoke, Hampshire, UK) (Gram *et al.* 1987). Hydrogen sulphide-producing and hydrogen sulphide non-producing bacteria were enumerated after 3–5 days of incubation at 20°C. Black colonies were recorded as sulphide-producers, whereas white colonies were counted as sulphide non-producers.

Sensory analysis. For sensory analysis, the quality index method scheme developed by some authors (Cardenas Bonilla *et al.* 2007; Ozogul *et al.* 2014) was used with modification (Table 1). The scheme consisted of four quality parameters (flesh texture, flesh odour and colour, brightness). The scheme had several descriptors, scoring demerit points from 0 to a maximum of 3, where 0 represented best quality and higher score (3), indicated poorer quality. The total sum of demerit points was 8. Sensory evaluation was performed according to ISO guidelines (ISO 6658: 1985 – Sensory analysis – Methodology – General guidance; ISO 8589:1988 – Sensory analysis – General guidance for the design of test rooms; ISO 5886-1:1993 – General guidance for the selection, training and monitoring of assessors: Part 1. Selected assessors 1993). The panel consisted of six assessors regularly trained in fish quality assessment and selected among the staff of the Inspection of Food of Animal Origin (Department of Veterinary Sciences, Messina, Italy). Triplicate samples were taken at regular intervals for sensory analysis.

Sensory evaluation for cooked fish fillets was carried out according to a scheme reported in Table 1. Fish fillets were cooked in a microwave for 1 min (300 W) and then served to the panellists to assess. Panellists scored for texture, colour, odour, and taste. The scheme had several descriptors, scoring demerit points from 0 to a maximum of 3, where 0 represented best quality and higher score (3) indicated poorer quality. The total sum of demerit points was 10.

RESULTS AND DISCUSSION

In vitro test: Agar disc-diffusion test. The results revealed a strong activity of AITC vapour against all strains tested. No bacterial growth was observed at all concentration used.

Microbiological analysis. The growth of sulphite non-producing and sulphite-producing bacteria for all groups is shown in Figure 1. The initial count of sulphite non-producing bacteria of sea bream fillets was 3.04 ± 0.89 log CFU/g (Figure 1A). Although these bacteria increased gradually in untreated and treated samples, the samples treated with AITC maintained microbial populations at a significantly lower level during storage compared with the control samples. The maximum reductions were about 4 log CFU/g in all treated groups within 7 days of storage, as compared with untreated fillets. The fillets treated with 3.35 and 6.70 mg/l of AITC had lower counts of sulphite non-producing bacteria (6.55 ± 0.12 and 6.04 ± 0.05 log CFU/g, respectively) after

Table 1. Quality index method for fresh and cooked fillets

Quality parameter	Description	Score	Quality parameter	Description	Score
Texture	firm	0	texture	firm	0
	soft	1		soft	1
	very soft	2		very soft	2
Odour	fresh, sea-weedy	0	odour	typical	0
	neutral	1		neutral	1
	slight off odour	2		slight off odour	2
	strong off odour	3		strong off odour	3
Colour	white	0	colour	white	0
	greyish	1		greyish	1
	yellowish	2		yellowish	2
Brightness	lucid	0	taste	pleasant	0
	opaque	1		neutral	1
				bitter	2
				unpleasant	3

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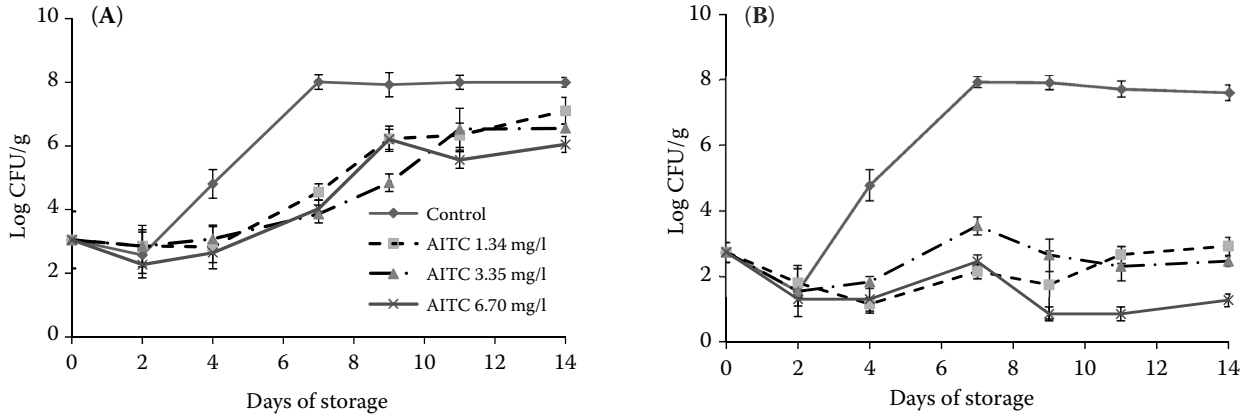

Figure 1. Trend of (**A**) hydrogen sulphite non-producing and (**B**) hydrogen sulphite-producing bacteria in gilthead sea bream fillets at 0, 2, 4, 7, 9, 11, and 14 days of storage at 2 ± 0.5°C

14 days of storage than the control sample (8.00 ± 0.05 log CFU/g). Similar final counts (7.10 ± 0.11 log CFU/g) were reached for fillets treated with a lower concentration of the compound 1.34 mg/l.

The initial count of sulphite-producing bacteria of sea bream fillets was 2.72 ± 0.30 log CFU/g (Figure 1B). In control samples the bacterial population tended to increase in about the same manner as sulphite non-producing bacteria, reaching the concentration of 7.92 ± 0.07 log CFU/g already within 7 days of storage. On the contrary, in fillets with the addition of AITC, the bacteria load never exceeded 3.5 log CFU/g until the end of storage (Figure 1B). Untreated fillets exceeded the value of 7 log CFU/g for SSOs, considered as the upper acceptability limit for fresh fish fillets on day 7 (Giuffrida *et al*. 2013).

As shown in Figure 1B, the major effectiveness of AITC was observed on the growth of sulphite-producing bacteria (black colonies) that represent the predominant microbial spoilage fraction in fish stored under refrigerated conditions (Giuffrida *et al*. 2013). Their reduction during the storage causes the absence of off-flavour related to the production of H_2S. Thus, guaranteed microbiological shelf-life extensions of 5 and 8 days were achieved for 1.34 and 6.70 mg/l, respectively, as determined by SSOs data (Figure 1A).

Sensory analysis. Figure 2A shows the sensory score of raw fillets stored at 2°C. Demerit points of raw fillets increased with the storage time in all groups. During the first days of storage every group presented the same sensorial characteristics, then the demerit points of treated groups were lower than those of the control group. The addition of AITC at a lower concentration (1.34 and 3.35 mg/l) does not affect the natural odour and taste of fish. Moreover, the fillets treated with a higher AITC concentration (6.70 mg/l) showed a marked AITC odour after opening the package that disappeared after exposure to the air in a few seconds. Although the initial sensory scores for control and treated groups were similar,

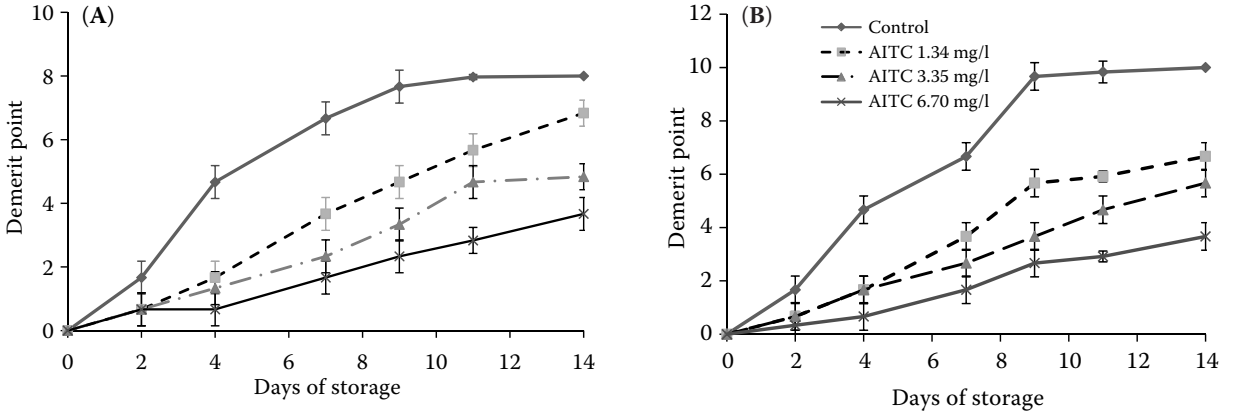

Figure 2. Quality index method scores of (**A**) raw and (**B**) cooked gilthead sea bream fillets at 0, 2, 4, 7, 9, 11, and 14 days of storage at 2 ± 0.5°C

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after 7 days of storage these scores for the control were higher than those of treated groups. Untreated fillets presented a strong off-odour with the texture tending to be very soft. On the contrary, fillets treated with AITC maintained constant and fully acceptable features until the last day. The limit for acceptability of untreated fillets was 7 and 14 days for treated groups. When fillets were rejected by panellists, demerit scores were found higher than 6.

Sensory scores of cooked fillets are shown in Figure 2B. The sensory scores in control and treated groups increase significantly during storage. After cooking, a slight garlic odour was noticed for fillets treated with 6.40 mg/l of AITC, stronger when the fillets were still hot, perceptible even to chew, but not nasty. Fillets treated with 1.34 and 3.35 mg/l of AITC were preferred by panellists because of their desirable flavour. As spoilage progressed, the off-flavour increased in intensity until the control fish were no longer edible within 7 days. The treated fillets maintained constant and fully acceptable features until the last day of storage. QIM results show that the AITC addition does not affect sensorial characteristics with the exception of garlic odour of the cooked product that, however, did not appear unpleasant to panellists.

CONCLUSION

Results show the high effectiveness of AITC against SSOs *in vitro* and in raw sea bream fillets stored at refrigerated conditions. The use of AITC resulted in a shelf-life extension of ca. 8 days, attributed to the antimicrobial effects of the compound known to exert antimicrobial activity also against some fish isolates (Pang *et al.* 2013). QIM results show that the AITC addition does not affect sensorial characteristics with the exception of garlic odour of the cocked product that, however, did not appear unpleasant to panellists. It is noteworthy that the presence of AITC in cooked gilthead sea bream fillets produced a distinct but organoleptically acceptable pleasant odour, well received by the panellists. The use of AITC at higher concentrations (> 10 µl) would probably result in a further increase of the shelf-life of fish fillets, but it would probably impart unpleasant sensorial effects (strong garlic taste and odour) on the quality of swordfish fillets. The effects of AITC on the shelf-life of fish fillets are similar to those of essential oils (EOs), although EOs seem to be more effective in combination with other EOs or with packaging technique (vacuum, modified atmosphere packaging) (Goulas & Kontominas 2007; Kykkidou *et al.* 2009; Ojagh *et al.* 2010). However, further investigation needs to be conducted in order to evaluate the antimicrobial effectiveness of AITC vapours at lower concentrations, and to optimise the range of AITC concentrations acceptable to consumers.

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