

## Levels of nitrates and nitrites in chili pepper and *ventricina* salami

Giampaolo Colavita, Michele Piccirilli,  
Luigi Iafigliola, Carmela Amadoro

Dipartimento di Medicina e Scienze della  
Salute, Università degli Studi del Molise,  
Campobasso, Italy

### Abstract

*Ventricina* is a traditional sausage made from pork meat produced in the Abruzzi and Molise regions. The aim of this study was to detect the content of nitrates and nitrites in local cultivars of chilli pepper, and their concentration in *ventricina* samples spiced with the same chilli pepper. Furthermore, it was examined whether, in the samples of *ventricina* with nitrate addition, the spicing with chilli pepper could exceed the maximum added dose. The concentration of nitrates and nitrites in the organic chilli pepper was 531.0±94.6 mg/kg and less than 5.0 mg, respectively, in the traditional chilli pepper it was 394.0±39.6 and less than 5.0 mg, while in the commercial it was 325.0±115.0 and less than 5.0 mg. The determination of nitrites and nitrates was carried out by high performance ion chromatography. In *ventricina* samples produced without added sodium nitrate, nitrates and nitrites were below 5.0 mg/kg at the case-filling time ( $t_0$ ) and after 50 days of aging ( $t_{50}$ ). In the samples of *ventricina* with added sodium nitrate, nitrate concentration values were 134.0±20.9 mg/kg at  $t_0$  and 129.0±15.4 mg/kg at  $t_{50}$ , while the nitrites were below 5.0 mg/kg at  $t_0$  and 28.8±15.8 mg/kg at  $t_{50}$ . Although in *ventricina* the amount of chilli pepper is quite relevant, it did not lead to a detectable concentration of nitrates. The maximum allowed amount was never exceeded.

### Introduction

*Ventricina* is a ripe sausage made from pork meat and abundantly spiced with sweet and hot chilli pepper. It is traditionally produced between Abruzzi and Molise regions. The origin of *ventricina* term derives from the custom of filling in pig stomach (ventricle). Currently, pig and bovine intestine bladders are used. The meat is cut into cubes of about 2-7 cm<sup>2</sup>. In the mixture salt (2.5% w/w), sweet and hot chilli pepper (1.5% w/w) (*Capsicum annum* L. var. *Longum* and *Capsicum annum* var. *Acuminatum*) are added, according to taste.

For its production technology *ventricina* is positioned between fermented sausages and cured meats made from entire anatomical parts (Amadoro *et al.*, 2011). According to tradition, the ripening process is carried out in household locals or in plug-in showcase cabinets for salami with temperature and humidity system control. In the cabinets the temperature values range between 18°C at the beginning and 12°C at the end of the process. Humidity ranges from an initial value of 85% to a final value of 75% (Piccirilli and Colavita, 2008; Amadoro *et al.*, 2011). Ripening and aging processes are slow (5-12 months). Moreover, the product ecosystem is characterised by microbial populations spontaneously selected and mainly constituted by lactic acid bacteria and coagulase negative cocci (CNC). As provided by law, in fermented meat products nitrates and/or nitrites are added to prevent the development of pathogenic microorganisms such as *Clostridium botulinum*. However, the addition of these additives can cause the formation of nitrosamines, *i.e.* substances with carcinogenic properties. According to the European Food Safety Authority (2003), it is the *maximum addition dose* and not the *maximum remaining dose* that contributes to the inhibitory activity against *C. botulinum*. The European Union provided a maximum allowed dose of 150 mg/kg both for nitrites and nitrates to limit the content of nitrosamines, restating the need to use them to manage the risk related to *C. botulinum* (Directive EC 2006/52; European Commission, 2006). Nitrites exert an intrinsic antimicrobial activity in synergy with the sodium chloride. On the contrary, nitrates do not possess a direct antibacterial activity, but are transformed into nitrites by pro-technological microbial populations, mainly represented by CNC (Duncan *et al.*, 1997; Cammack *et al.*, 1999; Colavita, 2005). Similarly to other vegetables, pepper plant (*Capsicum annum* L.) uses nitrates of the soil that accumulates in variable amounts depending on the cultivar of cultivation system. Furthermore, it contains high amounts of ascorbic acid, polyphenols, in particular flavonoids (quercetin and luteolin), oxygenated carotenoids (-carotene, -cryptoxanthin and xanthophylls) (Lee *et al.*, 1995; Hasler and Clare, 1998; Howard *et al.*, 2000). During the preparation of *ventricina*, chilli pepper is added in relatively high amount. For this reason, it is supposed that nitrates and nitrites in this spice can achieve an adequate concentration to carry out a sufficient antimicrobial activity to inhibit *C. botulinum*. Therefore, the aim of this study was to detect the levels of nitrates and nitrites supplied in *ventricina* sausages by a local cultivar of chilli pepper produced with two different agronomic regimes. At the same time, it was investigated whether the addition of chilli pepper could

Correspondence: Giampaolo Colavita,  
Dipartimento di Medicina e Scienze della Salute,  
Università degli Studi del Molise, via F. de  
Sanctis, 86100 Campobasso, Italy.  
Tel. +39.087.4404877 - Fax: +39.087.4404778.  
E-mail: colavita@unimol.it

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lead to exceeded the maximum allowed amount of sodium nitrate when this was added.

### Materials and Methods

A cultivar of traditional chilli pepper of Abruzzi area has been cultivated (Paesanello di Altino) according to two agronomics regimes: traditional (A) and organic (B). After harvesting, the berries were dried and ground according a traditional process. Three samples of chilli pepper A and B were collected and analysed for the determination of nitrates and nitrites content. Furthermore, for knowledge and comparison purposes, nitrates and nitrites concentrations were determined also on 18 samples of commercial chilli powder and on 3 samples of sea salt used in dressing. The experimental protocol established the production of *ventricina* in an artisanal production plant, according to the traditional method (Piccirilli and Colavita, 2008). Organic chilli pepper powder of Altino (B) (Table 1) was used for spicing meat. The batch was divided in two portions: one with and the other without addition of sodium nitrate (150 mg/kg). *Ventricina* samples have been ripened in plug-in showcase cabinets. The ripening temperature was 18°C, the first week, and 12°C the remaining period. Humidity values ranged between 84.0 and 85.0% the first thirty days, and 74.0 to 75.0% the remaining ripening period. The determination of nitrates and nitrites was carried out after filling ( $t_0$ ) and after 50 days ( $t_{50}$ ). At  $t_0$  and  $t_{50}$ , 8 samples for each batch were collected and analysed. Five grams of sample were

weighed, then extracted with 100 mL of boiling ultrapure water and diluted 1:10 in ultrapure water. The diluted samples were filtered with PVDF disposable filters of 0.45 µm porosity and injected into high performance ion chromatography using an anion exchange column (AS9HC, 250x4 mm; Dionex Corporation, Waltham, MA, USA). Sodium carbonate 9 mM was used as mobile phase with 1 mL/min flow rate in an isocratic run. After passage through an anion suppressor (ULTRA-SRS Self-Regenerating Suppressor 4 mm; Dionex), nitrates were detected with a conductivity detector. Quantification was carried out using an external calibration curve. Statistical data processing was performed using Student's T test and values were considered significantly different for  $P < 0.05$ .

## Results

Table 1 shows that organic agronomic regime (B) caused a higher nitrate concentration ( $531.0 \pm 94.6$  mg/kg) if compared to the traditional system (A) ( $394.0 \pm 39.6$  mg/kg), although data were not statistically significant ( $P > 0.05$ ). Nitrites were below the analytical detection limit (5.0 mg/kg) in all samples of chilli pepper and of sea salt analysed. Moreover, in samples of commercial chilli, the nitrate concentration was very variable ( $325.0 \pm 115.8$  mg/kg) and significantly lower ( $P < 0.05$ ) compared to that observed in Paesanello di Altino (B).

Table 2 shows that in *ventricina* sausages without nitrates, their values did not exceed the threshold of analytical detectability (5.0 mg/kg). However, in *ventricina* samples with sodium nitrate added, an average quantity of nitrates at  $t_0$  of  $134 \pm 20.9$  mg/kg was detected, whereas nitrites have never been found (5.0 mg/kg) at  $t_0$ , while after 50 days of ripening values of nitrates and nitrites were  $129.0 \pm 15.4$  and  $28.8 \pm 15.8$  mg/kg, respectively.

## Discussion

Biological farming has led to a greater concentration of nitrates in the pepper studied, but the difference with traditional method of cultivation was not significant. Chilli samples used to spice *ventricina* sausages in this study showed high values of sodium nitrate if compared with the commercial one and with that studied by Yordanov *et al.* (2001). Nevertheless, nitrates concentration was irrelevant in *ventricina* sausages without sodium nitrate added. Moreover, the maximum dose added (150 mg/kg) in batch of *ventricina* sausages with sodium nitrate was never

**Table 1. Average concentration of nitrates and nitrites in chilli powder and salt used.**

	Paesanello di Altino (mg/kg)		Sea salt (mg/kg)	Commercial chilli pepper (mg/kg)
	A	B		
Nitrates	$394.0 \pm 39.6$	$531.0 \pm 94.6^*$	<5.0	$325.0 \pm 115.8^*$
Nitrites	<5.0	<5.0	<5.0	<5.0

A, traditional agronomic system; B, organic agronomic system. \* $P > 0.05$ .

**Table 2. Average concentration of nitrates and nitrites in ventricina samples at 0 ( $t_0$ ) and after 50 days ( $t_{50}$ ) of ripening.**

	<i>Ventricina</i> samples without nitrates added (mg/kg)		<i>Ventricina</i> with nitrates added (mg/kg)	
	$t_0$	$t_{50}$	$t_0$	$t_{50}$
Nitrates	<5.0	<5.0	$134.0 \pm 20.9$	$129.3 \pm 15.4$
Nitrites	<5.0	<5.0	<5.0	$28.8 \pm 15.8$

exceeded. After 50 days of ripening ( $t_{50}$ ), a low concentration of sodium nitrite was also detected. This is due, presumably, to a low nitrate-reductase activity of CNC, which, as it is known, characterises ripened non-minced sausages, with slow maturation and low acidity, such as *ventricina* (Amadoro *et al.*, 2013).

## Conclusions

In this study the content of nitrates in the organic chilli pepper used to spice *ventricina* salami was lower than that observed in other vegetables like lettuce, spinach, potatoes, *etc.* (Cantoni, 2004; Ionescu *et al.*, 2010). Nevertheless, it was higher than that reported by Yordanov *et al.* (2001), who, however, observed differences in the content of nitrates between home-grown chilli (10.1 mg/kg) and commercial paprika (38.1 mg/kg). The content of nitrates in the organic chilli pepper was higher than that observed in samples obtained by the traditional cultivation method. Nonetheless, the content of nitrates and nitrites was irrelevant in *ventricina* where it was added. Therefore, the results of this study showed that the addition of high amounts of organic chilli pepper has no influence on the concentration of nitrates and nitrites in sausages. It can also be inferred that the preserving activity of chilli pepper can be due to i) the high content of sodium ascorbate (isoascorbic sodium/sodium erythorbate), which has a strong activity in preventing the oxidative processes and setting the colour (European Journal of Food Safety Authority, 2003), and ii) compounds with antimicrobial activity like capsaicinoids (Paleari *et al.*, 1989; Dorantes *et al.*, 2000; Careaga *et al.*, 2003) rather than to nitrates and nitrites.

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