

Effect of selected starter cultures on physical, chemical and microbiological characteristics and biogenic amine content in Protected Geographical Indication *Ciauscolo* salami

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Abstract

The aim of the study was to evaluate the biogenic amine (BA) content of *Ciauscolo* salami made with and without the use of a selected starter culture. Two batches of salami were made following the guidelines of the Protected Geographical Indications: with and without adding a commercial starter culture made of *Lactobacillus plantarum* and *Staphylococcus xylosum*. Six samples of salami per batch were collected at different ripening times (0, 15, 30, 45 and 60 days) for physical, chemical and microbiological analyses and for the determination of BA content. No differences were recorded for physical, chemical and microbiological analyses except for *Staphylococcus* spp. count at the time of casing (T0) and total volatile basic nitrogen (TVBN) from 30 days (T2) to the end of the ripening time (60 days, T4). After 60 days of ripening, the use of selected starter culture significantly affected the amount of putrescine (195.15 vs 164.43 mg/100 g in salami without and with starters, respectively), cadaverine (96.95 vs 104.40 mg/100 g in salami without and with starters, respectively), histamine (81.94 vs 69.89 mg/100 g in salami without and with starters, respectively), and spermine (36.88 vs 33.57 mg/100 g in salami without and with starters, respectively). Despite significantly higher values of TVBN, the use of selected starter culture determined no significant effects on the BA content of the products.

Introduction

The cultural heritage and the connection to *local* production are two important intrinsic product attributes involved in consumer choice of high quality foods (Martinez *et al.*, 2010).

Among Italian *local* products, *Ciauscolo* salami is a fermented meat product with an ancient history, produced in a small geographic area in Central Italy and appreciated by consumers for its peculiar taste and traits (Rea *et al.*, 2003). The product is spreadable, slightly smoke flavoured and, due to its peculiar characteristics and geographical localization, in 2009 it was awarded with the Protected Geographical Indication (PGI) certification from the European Union (Reg. EC No 729/2009; European Commission, 2009). The product is traditionally marketed after about 4 weeks of ripening but according to the production guidelines (www.politicheagricole.it) not less than 20 days after casing (15 days of ripening). However, sometimes the product may undergo a more prolonged ripening (2-3 months) based on market requests (Rea *et al.*, 2005a). In reality, the real typical homemade *Ciauscolo* originally underwent a longer ripening because it was used as a break from farm fieldwork which restarted late in the spring after the winter rest (Rea *et al.*, 2003). Despite the generally artisanal production of PGI *Ciauscolo* salami, there is an industrial interest in the standardization of the product. The use of selected starter bacteria is probably the most common method for achieving a standard dry fermented product (Leroy *et al.*, 2006). The salami making technique, especially the starter culture used, could cause differences in the production of biogenic amines (BAs) (Martuscelli *et al.*, 2000; Rea *et al.*, 2005a). BAs are low molecular weight basic nitrogenous compounds occurring in many foods and are mainly due to amino acid decarboxylation performed by some microorganisms. Amino acid decarboxylation may play an important energy role for bacteria in nutritionally poor environments. It can generate a charge translocation through the cellular membrane influencing its potential (Martuscelli *et al.*, 2000; Suzzi and Gardini, 2003). In particular, the possibility that histidine decarboxylation and the following histamine (HIS) formation may represent an alternative pathway for lactic acid bacteria (LAB) to produce energy when insufficient amounts of fermentable sugars are present has been reported (Rea *et al.*, 2005a). The amino acid decarboxylation is not the only enzymatic system to form BAs. Some of them, such as putrescine (PUT) which is formed mainly from arginine decarboxylation, can be obtained through different pathways that produce intermediate compounds (ornithine and agmatine in particular) by both Gram negative and Gram positive bacteria following generally more complex reactions in the latter (Wunderlichova *et al.*, 2014). Moreover, some of the BAs present in food, especially PUT, cadaverine (CAD), spermine (SPM) and spermidine (SPD), are also produced by the animal organism itself for physiological purposes

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(Suzzi and Gardini, 2003). Polyamines are present in many types of body cells and their presence in food is often due to their endogenous origin, although in the human body the largest part of PUT comes from food intake (Wunderlichova *et al.*, 2014).

The presence of BAs can be considered from different points of view according to the meaning given to them (Silla Santos, 1996). Besides their real or potential action as toxic substances, BAs play several important biological roles. Most of these roles are still unclear: their contribution to the development of food aroma (Mascaro *et al.*, 2010); their role as potential precursors of carcinogenic compounds such as nitrosamines (Yamamoto *et al.*, 1982; Stratton *et al.*, 1991; Shalaby, 1996; Wunderlichova *et al.*, 2014); their role as possible indicators of freshness or spoilage levels of food (Rea *et al.*, 2008); their use in the evaluation of hygienic characteristics of raw matter used for food manufacturing, especially in fishery products (Veciana-Nogues *et al.*, 1997; Muscarella *et al.*, 2005; Visciano *et al.*, 2012; Wunderlichova *et al.*, 2014) but also in different meat products (Cantoni, 1995; Hernandez-Jover *et al.*, 1996; Rea *et al.*, 2008).

Many studies have been carried out regarding BAs as toxic compounds and the aspects related to their toxicity have been largely reviewed (Stratton *et al.*, 1991; Shalaby, 1996; Silla Santos, 1996; Wunderlichova *et al.*, 2014), even specifically in dry fermented sausages (Suzzi and Gardini, 2003). Their biological roles have also been reported by several authors: polyamines act as modulators of cellular function, as reviewed in detail by (Igarashi and Kashiwagi (2010); are indispensable com-

ponents of living cells, especially in rapidly growing tissues and the intestine in particular; and are essential to maintain the metabolic activity of the immunological system of the gut (Silla Santos, 1996). Some BAs, such as histamine (HIS) and catecholamines, fulfill important metabolic functions in the nervous system, control blood pressure, and mediation of allergic responses (HIS), as reported by Silla Santos (1996). Furthermore, polyamines may act as radical scavengers and inhibit the oxidation of polyunsaturated fatty acids. Putrescine is also a precursor in the synthesis of other polyamines such as SPM and SPD (Wunderlichova *et al.*, 2014). Many of the functions described are related to the polycationic nature of polyamines which allows them to interact with many important negatively charged molecules (DNA, RNA, proteins, phospholipids) (Igarashi and Kashiwagi, 2010; Wunderlichova *et al.*, 2014).

Different papers have recently considered *Ciauscolo* salami from different perspectives (Aquilanti *et al.*, 2007; Trani *et al.*, 2011; Ranucci *et al.*, 2013; Federici *et al.*, 2014), and a preliminary evaluation of BA content in this product has been reported by Rea *et al.* (2005b). No data is available on the relationships between the addition of a mixed starter culture of *Lactobacillus plantarum* and *Staphylococcus xylosus* and BA content in *Ciauscolo* salami and the aim of this work is to evaluate the effect of the use of selected starter culture on different characteristics and BA content in such a product.

Materials and Methods

Two batches of *Ciauscolo* salami were made from the same raw materials and ingredients (pork meat/fat, salt, pepper, garlic) in a local processing plant of central Italy with and without the use of starter culture. Products made without starters were considered as control. Commercially available starter cultures (Lyocarni SHA-24; Sacco srl., Cadorago, Italy) containing *Lactobacillus plantarum* and *Staphylococcus xylosus* were added to the minced meat, according to manufacturer's guidelines, followed by the other ingredients and additives (ascorbic acid – E300, and potassium nitrate – E252), as dictated by regulation. Formulation was in accordance with the PGI guidelines for *Ciauscolo* salami.

Six samples from each batch were collected from each group after casing (T0) and every 15 days until 60 days of ripening (T1=15 days; T2=30 days, T3=45 days, T4=60 days) were reached. Samples were sent under refrigeration to the laboratory for physical, chemical and microbiological analyses and for BA determination.

Some physical and chemical analyses were performed on each sample and at each sampling time. In particular: pH was determined according to Bendall (1977) using a pHmeter (MP 230; MettlerToledo, Schwerzenbach, Switzerland) equipped with a routine electrode; water activity (a_w) was determined by HygroLab 3 hygrometer (Rotronic, Huntington, NY, USA); and total volatile basic nitrogen (TVBN) was determined according to Pearson (1991). Only after 60 days of ripening the proximate chemical composition and salt (NaCl) content were determined according to AOAC methods (1990).

For microbiological analyses, 25 g of each sample were collected after aseptic removal of the casing and were placed in a sterile bag with buffered peptone water (Oxoid, Basingstoke, UK). Samples were homogenized (Stomaker 400 circulator; PBI International, Milan, Italy) for 2 min at room temperature and serial decimal dilutions in buffered peptone water were then prepared. *Lactobacillus* spp. (on MRS agar, Oxoid, incubated at 37°C for 72 h under anaerobic conditions), *Enterobacteriaceae* (according to ISO 21528-2:2004; ISO, 2004), *Enterococcus* spp. (according to ISO 7899-2:2003; ISO, 2003) and *Staphylococcus* spp. [on Baird Parker agar with Egg Yolk Tellurite Emulsion (Oxoid) incubated at 37°C for 48 h] counts were determined. BA contents were determined according to Rea *et al.* (2005b).

An unpaired T test (Statview, SAS Institute Inc., Cary, NC, USA) was used to compare the differences in proximate chemical composition between samples with and without starter cultures. For the other parameters, an ANOVA

model (Statview) with time (T0, T1, T2, T3 and T4) and groups (with and without starters) as fixed factors, followed by Tukey's test, was used. Significance level was set at $P < 0.05$.

Results

The proximate chemical composition and salt content of *Ciauscolo* salami, at the end of a 60-day ripening period with starter culture added or not added, is reported in Figure 1. No differences were recorded in all the parameters considered in the two groups. The evolution of pH and a_w of the samples throughout the ripening is reported in Figure 2. The pH dropped to a value below 5.6 after 15 days of ripening (T1) with a significantly ($P < 0.05$) lower value in the products made with the addition of starter culture than in samples without starters (5.39 and 5.54, respectively). No differences were recorded in the pH values registered at T2, T3 and T4. The results of physical and chemical analyses showed the total compliance with the production guidelines: pH ≥ 4.8 , proteins not less than 15%, fat between 32 and 42%, moisture/protein ratio ≤ 3.1 , fat/protein ratio ≤ 2.8 . The only exception was fat/protein ratio that was slightly lower than 32% at T1 and T2, excluding T0, when the values were not to be applied.

A_w value also dropped throughout the ripening from expected values of raw salted minced meat (over 0.95) to values below 0.87 recorded at T4 with no differences between the samples of the two groups.

The TVBN values obtained are reported in

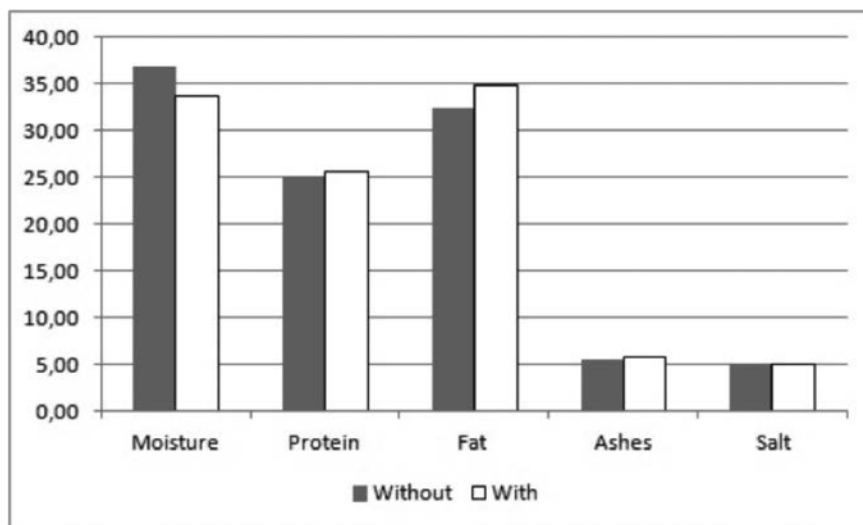


Figure 1. Chemical composition (%) of Protected Geographical Indication *Ciauscolo* salami made with and without starter cultures (*Lactobacillus plantarum* and *Staphylococcus xylosus*) at 60 days of ripening.

Figure 3. Differences were registered from T2 to T4 with significantly higher values in *Ciauscolo* salami made with the addition of starter culture than those made without starters: 52.98 vs 42.84 mg/100 g at T2 ($P < 0.05$), 66.41 vs 55.71 mg/100 g at T3 ($P < 0.001$); 76.37 vs 69.30 mg/100 g at T4 ($P > 0.001$), respectively.

The results of microbial analyses are reported in Figure 4. No significant differences were recorded between the two groups of samples for all the microbial parameters considered with the exception of *Staphylococcus* spp. count at T0 when values of 4.18 and 3.08 Log CFU/g were detected for salami made with and without starters, respectively ($P < 0.05$). Also, the count of mesophilic aerobic and lactic bacteria followed the guidelines provided for PGI *Ciauscolo* salami that fix a lower limit of 7 log CFU/g.

The BA contents determined in the two groups of salami are reported in Table 1 and in Figures 5 and 6. The differences detected were significant for all BAs when the ripening factor was considered ($P < 0.001$). Considering the starter addition as a fixed factor, significance was observed for PUT ($P = 0.006$), CAD ($P < 0.001$) and SPM ($P = 0.003$). Considering both time and presence of starters, the differences were only significant ($P = 0.049$) for HIS.

The total amount of BAs throughout the study increased progressively showing significant differences among the different sampling times as expected. With the exception of samples at T0, control products (made without starters) showed slightly higher amounts of BAs than samples with starters added at each sampling time but these differences were never significant. The concentration of total BAs at T0 in both groups of products was mostly due to the presence of SPM but PUT was also present. The most evident increase of total BA concentration was observed between T0 and T1, which corresponded to the lowest pH values observed. This was mainly due to TYR, which was the most represented BA at each sampling time [as observed by Rea *et al.* (2005b) in other kinds of traditional Italian salami] and to PUT and CAD to a lesser extent. In any case, this increase was due to BAs considered as *generic* and without a relevant increase of HIS, which is the most important BA from a toxicological point of view. This significant progressive increase between T0 and T1, such as the increase observed throughout the study, was real and not related to the progressive decrease of moisture because the values referred to dry matter (data not reported) showed an identical trend.

The concentration of single BAs were almost similar in control samples than in those with added starters. Higher values of PUT, HIS, TYR, SPD and SPM were constantly observed in control samples than in samples with starters

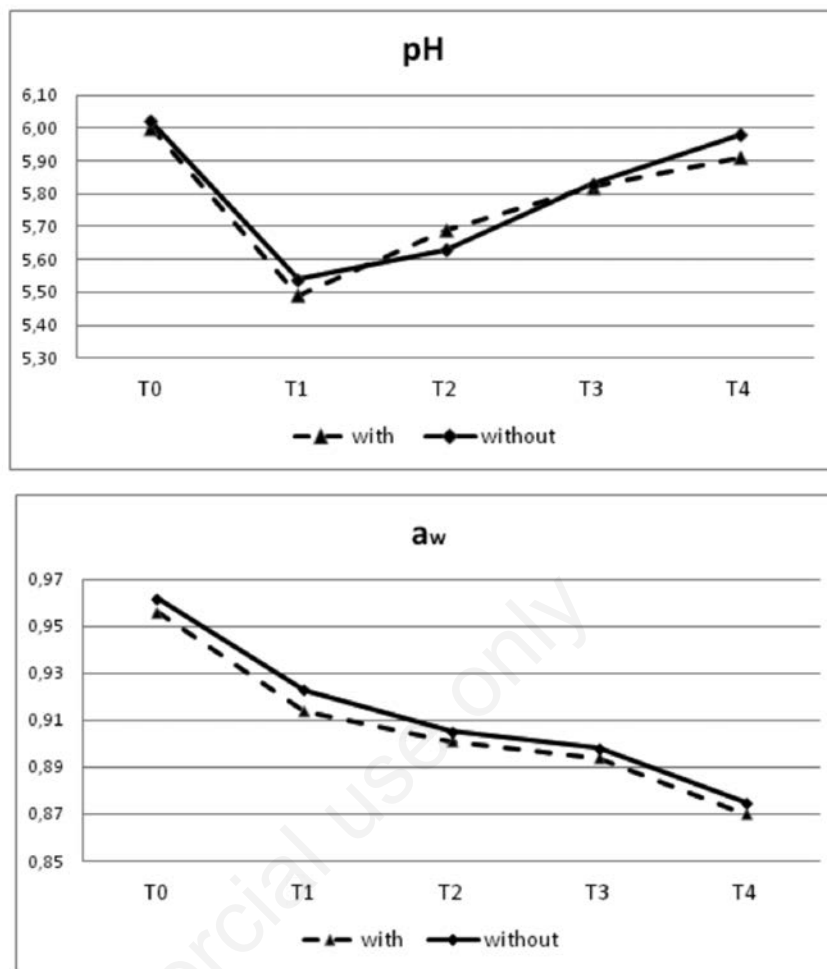


Figure 2. pH and water activity evolution in Protected Geographical Indication *Ciauscolo* salami with and without starter cultures (*Lactobacillus plantarum* and *Staphylococcus xylosum*) at different times of ripening. T0=after casing; T1=15 days; T2=30 days; T3=45 days; T4=60 days.

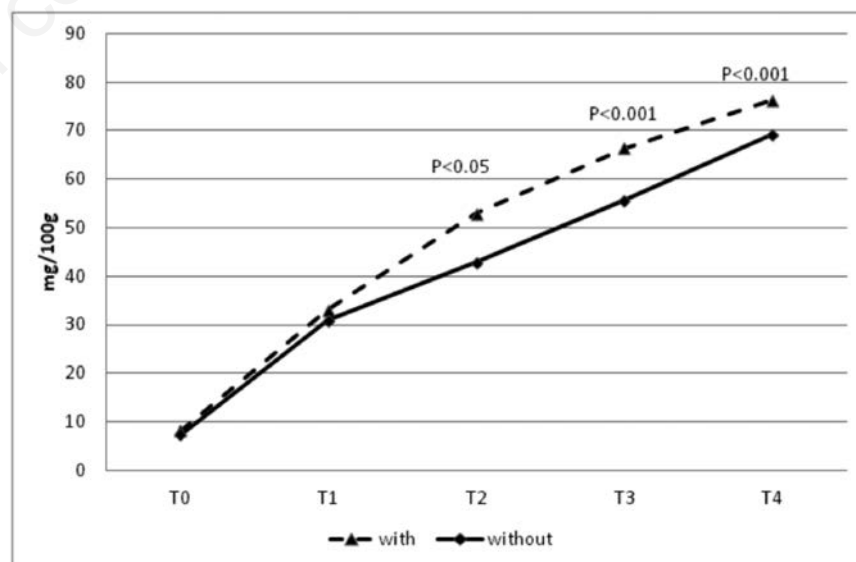


Figure 3. Total volatile basic nitrogen evolution in Protected Geographical Indication *Ciauscolo* salami with and without starter cultures (*Lactobacillus plantarum* and *Staphylococcus xylosum*) at different times of ripening. T0=after casing; T1=15 days; T2=30 days; T3=45 days; T4=60 days.

added, even at very low extent, with the exception of TYR and SPD at T3 when the values were lower in control samples. However, the differences between the two groups were significant only for PUT at T1 and T4 and for HIS and SPM at T4. Lower values in control samples compared to starter-added samples were constantly observed for B-PHE and CAD with the exception of both BAs at T1, but the differences were significant only for CAD at T3 and T4.

Discussion

The pH values recorded in both starter-added and control *Ciauscolo* salami were slightly higher than those found by other authors (Rea *et al.*, 2005b; Trani *et al.*, 2010; Ranucci *et al.*, 2013) in the same products even though the LAB count increased from T0 to T1 up to values over 8 Log CFU/g. The microbial dynamics and subsequently the pH evolution, particularly in the first part of fermentation, may be due to the different ripening temperatures adopted. The effect of proteolysis could therefore be responsible for the pH evolution from T1 to T4. The use of the selected starter culture did not seem to alter pH evolution even if proteolysis was higher in starter-added samples than in control ones. Although the artisanal production could be responsible for differences in microbial population, the evolution of the microbial growth between the two groups was similar: as the LAB increased the rest of bacterial population, including *Staphylococcus* spp., decreased.

Despite a higher degree of proteolysis observed, the use of selected starter culture had no effect on BA content of the products. The production of BAs in dry sausages is often related to the proteolytic activity during fermentation and ripening (Suzzi and Gardini, 2003) but no direct correlation has been found

between proteolytic activity of *Staphylococcus xylosum* and BA production (Bover-Cid *et al.*, 1999).

In the present study, the most evident increase of total BA concentration was observed between T0 and T1 and may be related, beside the normal catabolism of nitrogenous compounds carried out by the microflora during ripening, to the possible protective activity of BAs against the increase of micro-environmental acidity that has been hypothesized by several authors about BA producing bacteria (Silla Santos, 1996; Suzzi and Gardini, 2003; Wunderlichova *et al.*, 2014). As already reported, such an evident increase in both groups of samples between T0 and T1 was mainly due to TYR and to PUT and CAD to a

lesser extent. HIS was observed at higher concentrations in control samples than in starter-added ones where the microorganisms are selected. However, these differences were never significant, with the exception of values at T4. The progressive slight increase of HIS has been consistently observed in fermented sausages by several authors (Rea *et al.*, 2005b) and also the presence of this BA in the latest stages of ripening has been consistently detected in the same and other kinds of Italian salami (Rea *et al.*, 2005a).

Generally, TYR, PUT, CAD and HIS are the most important amines detected in both artisanal and industrial fermented sausages (Latorre-Moratalla *et al.*, 2010). In the present study, these BAs were abundant in both groups

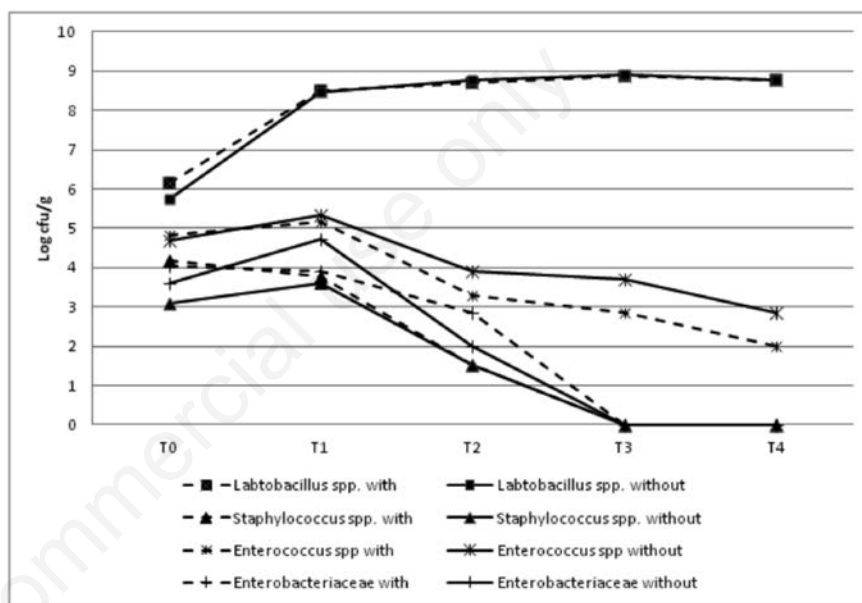


Figure 4. Microbial growth in Protected Geographical Indication *Ciauscolo* salami made with and without starter cultures (*Lactobacillus plantarum* and *Staphylococcus xylosum*) at different times of ripening. T0=after casing; T1=15 days; T2=30 days; T3=45 days; T4=60 days.

Table 1. Biogenic amine content (ppm, mean±standard deviation) in *Ciauscolo* salami made with and without starter cultures.

Time	Sample	B-Phenylethylamine	Putrescine	Cadaverine	Histamine	Tyramine	Spermidine	Spermine	Total
T0	With	0	0	17.09±0.01 ^a	0	0	0	28.10±0.01 ^a	45.19±0.01 ^a
	Without	0	0	9.60±1.07 ^a	0	0	0	30.81±6.30 ^a	40.41±7.37 ^a
T1	With	0 ^a	40.24±1.25 ^a	54.62±2.55 ^b	15.35±1.41 ^a	113.86±0.43 ^a	1.51±2.06 ^a	31.52±2.24 ^a	257.11±4.77 ^b
	Without	7.16±9.08 ^a	69.85±7.97 ^b	56.69±1.17 ^b	22.59±3.06 ^a	117.53±2.11 ^a	2.64±2.93 ^a	33.28±0.68 ^a	309.75±3.93 ^b
T2	With	26.83±2.94 ^b	91.35±12.20 ^c	75.12±9.83 ^c	30.24±5.09 ^b	162.73±23.66 ^b	0 ^a	35.31±1.02 ^a	421.59±49.14 ^c
	Without	24.15±12.53 ^b	101.58±30.86 ^c	70.01±6.52 ^c	35.17±15.38 ^b	163.33±24.16 ^b	3.64±3.10 ^a	35.39±1.15 ^a	433.27±85.76 ^c
T3	With	30.91±1.24 ^{bc}	125.76±17.48 ^d	91.48±4.52 ^e	36.88±5.73 ^{bc}	208.18±1.08 ^c	5.40±0.52 ^b	35.75±1.95 ^b	534.37±12.32 ^d
	Without	30.61±2.86 ^{bc}	141.51±30.99 ^d	82.20±7.13 ^d	46.31±6.45 ^c	195.01±26.66 ^c	4.71±2.33 ^b	37.48±1.41 ^b	537.83±72.05 ^d
T4	With	37.71±2.41 ^c	164.43±14.20 ^e	104.40±4.05 ^f	69.89±5.14 ^d	230.16±5.06 ^d	5.55±0.88 ^b	33.57±1.88 ^a	645.69±19.50 ^e
	Without	34.37±6.53 ^c	195.15±36.12 ^f	96.95±4.94 ^e	81.94±17.29 ^e	238.05±5.06 ^d	5.84±0.66 ^b	36.88±2.90 ^b	689.69±73.69 ^e
P value	Time	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Starter	0.921	0.006	<0.001	0.241	0.964	0.094	0.003	0.216
	Time×Starter	0.245	0.495	0.140	0.049	0.539	0.055	0.456	0.426

T0, after casing; T1, 15 days; T2, 30 days; T3, 45 days; T4, 60 days. ^{a-f}Different letters in the same column show significant differences (P<0.05).

of samples at each sampling time, with the exception of SPM whose presence is considered physiological and often normal. Indeed, polyamines SPD and SPM are almost always present in raw meat and the presence in raw fermented sausages is mostly related to the drying process involved in the manufacture of the product (Martuscelli *et al.*, 2000). This reason may explain the irregular presence and/or trend of these two compounds during the ripening of products as already observed in dry fermented sausages (Cecchini *et al.*, 2004; Rea *et al.*, 2005a, 2005b, 2008). The results obtained in the present study showed that the concentration of SPM apparently increased as for all the other BAs but if the values referred to total matter were reported to dry matter, the trend was almost the same for all BAs (obviously at higher values) with the exception of SPM. SPM showed a gradual and constant decrease during the ripening process both in control and in starter-added samples: from 78.51 to 58.42 mg/kg and from 68.54 to 50.60 mg/kg, respectively (data not shown).

Moreover, sometimes B-PHE is related to high concentrations of TYR due to non-specificity of tyrosine decarboxylase even though in the present study B-PHE was lower or around 30 ppm, which is generally considered the limit of acceptance for this BA (Shalaby, 1996; Rea *et al.*, 2005a, 2005b). PUT and CAD showed quite high levels but noticeably lower than those observed by Rea *et al.* (2005a) in the same products. TYR was always the most represented amine as observed by different authors (Rea *et al.*, 2005b) and at levels similar to those detected by Rea *et al.* (2005a) in the same type of salami at 0, 25 and 60 days of ripening. However, TYR was never found by those authors at levels close to the range 100-800 ppm considered compatible with the usual good manufacturing practices (Shalaby, 1996; Rea *et al.*, 2005a, 2008). As for the limits of toxicity for PUT, CAD and TYR, higher limits than those cited have been reported by Naila *et al.* (2010) and the values detected in the present study for these BAs were much lower than such limits: 2000 mg/kg as oral toxicity level for PUT; more than 2000 mg/kg as acute toxicity level for TYR and CAD; 2000 mg/kg as no observed adverse effect level (NOAEL) for TYR, PUT and CAD.

The BAs in food are mainly produced by microbial activity especially in fermented raw products which have undergone lactic acid fermentation (Suzzi and Gardini, 2003; Rea *et al.*, 2005a, 2005b). Indeed, amino acid decarboxylases are enzymes present in many genera of microorganisms of food concern (*Bacillus*, *Pseudomonas*, *Photobacterium*, *Enterobacteriaceae*, *Staphylococcus*, *Micrococcus*), including many LAB (*Lactobacillus*, *Enterococcus*, *Carnobacterium*, *Pediococcus*, *Lactococcus*, *Leuconostoc*), some of which are

used as starter cultures (Leuscher and Hammes, 1998; Suzzi and Gardini, 2003; Rea *et al.*, 2005a). Many fermented products are obtained from raw material containing high amounts of proteins that provide the substrate for decarboxylase activity of both starter culture and contaminating microflora during ripening (Shalaby, 1996; Silla Santos, 1996;

Avellini *et al.*, 1999; Mascaro *et al.*, 2010), especially meat products like dry fermented sausages (Suzzi and Gardini, 2003). For this reason the selection of starter cultures is particularly important in fermented food, where the use of short fermentations with carefully selected starter cultures may prevent uncontrolled fermentation and the formation of BAs

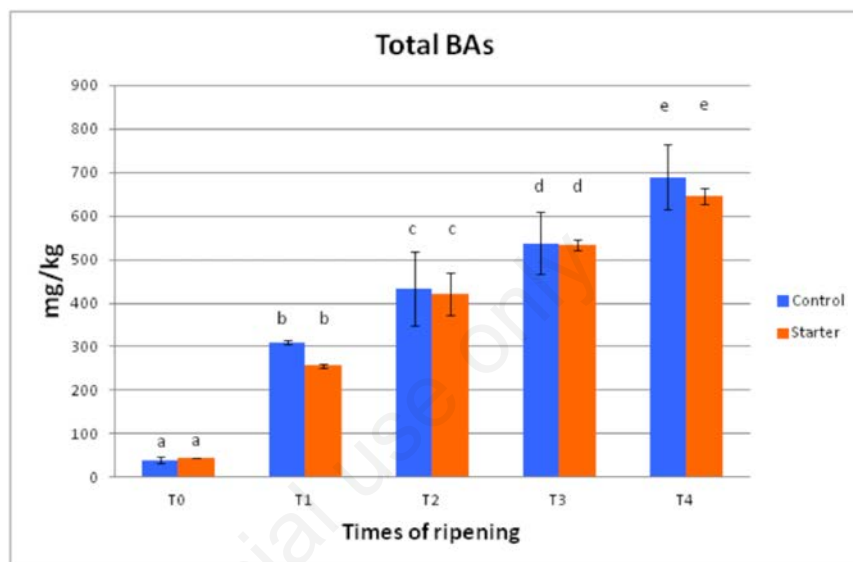


Figure 5. Total amounts of biogenic amines in Protected Geographical Indication Ciauscolo salami made with (starter) and without (control) starter cultures (*Lactobacillus plantarum* and *Staphylococcus xylosum*) at different times of ripening. T0=after casing; T1=15 days; T2=30 days; T3=45 days; T4=60 days. Different letters in the same column show significant differences.

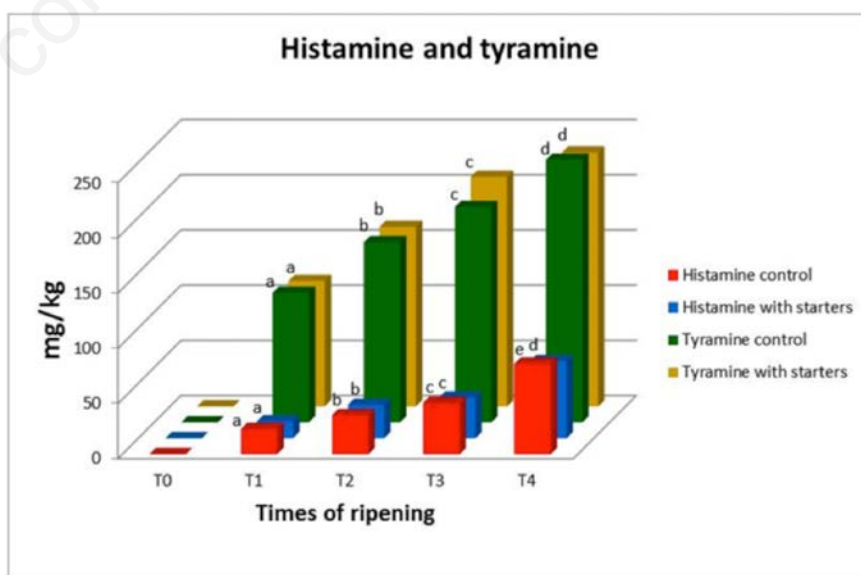


Figure 6. Amounts of histamine and tyramine in Protected Geographical Indication Ciauscolo salami made with and without (control) starter cultures (*Lactobacillus plantarum* and *Staphylococcus xylosum*) at different times of ripening. T0=after casing; T1=15 days; T2=30 days; T3=45 days; T4=60 days. Different letters in the same column show significant differences.

(Silla Santos, 1996). However, variable amounts of BAs have been reported for processed meat, particularly for fermented dry and semi-dry sausages even using BA-negative starter cultures that may limit the growth of natural microflora. This variability can be due to several factors such as ripening process time, residue decarboxylase activity of the natural microflora and/or starter culture, manufacturing process, type and quality of raw meat and other ingredients used, and general compliance with hygiene principles (Maijala *et al.*, 1995; Shalaby, 1996; Martuscelli *et al.*, 2000).

Many BAs are physiologically degraded mainly through oxidative deamination catalyzed by amino oxidases DAO and MAO which are very important for BA metabolism, ubiquitous in plants and also found in animal and human cells – particularly DAO at intestinal, hepatic and renal level (Zaman *et al.*, 2010) as well as MAO in mitochondria of brain cells (Wunderlichova *et al.*, 2014). These enzymes were also observed in several strains of bacteria such as *Enterobacteriaceae* (*Klebsiella*, *Enterobacter*, *Escherichia*, *Salmonella*, *Serratia*, *Proteus*), *Micrococcus*, *Lactobacillus* (*L. plantarum*, *L. sakei*, *L. pentosus*), *Staphylococcus xylosum*, *Bacillus* (Leuschner *et al.*, 1998). In particular *L. plantarum* amino oxidase positive for HIS (but also for TIR, PUT and CAD) was observed in sauerkraut, *Staph. xylosum* amino oxidase positive for HIS and TYR in salted fermented anchovies (Zaman *et al.*, 2010) and Italian artisanal fermented sausages (Martuscelli *et al.*, 2000), and *Bacillus* positive for HIS degradation (Zaman *et al.*, 2010) was found in salted fermented anchovies. These enzymes catalyze the oxidative deamination of amines with production of aldehydes, hydrogen peroxide and ammonia thus allowing many microorganisms to use amines as nutrient sources (Leuschner *et al.*, 1998; Martuscelli *et al.*, 2000; Wunderlichova *et al.*, 2014). In fact it was observed for B-FEN in *E. coli* K12 (Parrott *et al.*, 1987) and for TYR in *Kocuria* (once *Micrococcus*) *varians* (Leuschner and Hammes, 1998; Leuschner *et al.*, 1998) again at the conditions present during sausage fermentation but with a retarded decrease rate in the center of products compared to the peripheral parts (Leuschner and Hammes, 1998). Moreover, a low potential for BA degradation was observed among lactobacilli by Leuschner *et al.* (1998). Another pathway of degradation peculiar to HIS, which represents the primary of the two pathways according to some authors (Stratton *et al.*, 1991), is the methylation process catalyzed by histamine N-methyltransferase (HNMT) present in endothelial, hepatic and gastric cells of animals to form N-methylhistamine which is then oxidized by MAO to form methyl imidazoleacetic acid. Many bacteria capable of degrading BAs belong to halotolerant or halophilic groups but

the studies on BA degradation are often performed in buffer systems and probably their activity may be not the same as when transferred to complex systems which are found in salted and fermented foods (Zaman *et al.*, 2010). Moreover, sometimes the reduction in BA concentration by bacterial strains capable of reducing the production of BAs is not the same when raw material of a different hygienic quality is used for sausage manufacturing (Latorre-Moratalla *et al.*, 2010). Actually, it is not only the inoculation of starters and/or the modification of formulation but also the optimal hygienic conditions of raw materials and processing procedures that contribute to the enhancement of the starter performance and to the reduction of the aminogenesis during traditional fermented sausage manufacture (Latorre-Moratalla *et al.*, 2010). Despite different authors referring to the effects of *Staphylococcus xylosum* and *Lactobacillus plantarum* on the BA production or degradation (Martuscelli *et al.*, 2000; Gardini *et al.*, 2002; Ammor and Mayo, 2007), no differences were noticed in this study. The results obtained confirm that these commercial starter cultures cannot prevent BA production absolutely. Indeed, products made using commercial starters sometimes show similar or even higher levels of these compounds than traditional fermented meat products manufactured without starter cultures (Latorre-Moratalla *et al.*, 2010) and also reported by Parente *et al.* (2001) in different types of Italian dry sausages.

Conclusions

The use of selected starter cultures in PGI *Ciauscolo* salami has limited effects on the chemical and physical characteristics of the products as well as on the quantity of BAs. Therefore, the use of these starters could be avoided especially if products are consumed after 20 days of ripening. Further studies on the possible effects of different starter culture addition on the sensory properties of the products could be of utmost interest for the producers to define a possible intervention strategy alternative to the strictly artisanal production. From the opposite point of view, the correct compliance with good manufacturing practices and the use of high quality raw materials and ingredients may guarantee high standards of production even in artisanal manufacturing of dry fermented sausages without using standardised starter cultures that sometimes may reduce or hide the peculiarities of such valuable products.

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