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NUTRITIONAL, MICROBIOLOGICAL AND SENSORY ATTRIBUTES OF AN ITALIAN LOCAL MEAT-PRODUCT

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The study provides a picture of the compositional figure, nutritional value, sensory and safety attributes of the *Sausage* from *Monte San Biagio*. typical Italian local meat product. The dry fermented sausages were prepared with salt and natural ingredient (coriander seeds, hot and sweet pepper), as natural microbial growth inhibitors. Proximate composition showed differences among manufacturers mainly dependent on formulation of the batter (type of muscle, fat, ingredients). The unsaturated fatty acids accounted for 66% of the total lipids, among these oleic acid was the most represented, being 44.9% of total lipids, followed by palmitic acid. The P/S ratio (0.52), used as criteria to evaluate the nutritional quality of fat, was in the recommended range. Samples were similar in minerals content, differences were detected mainly for calcium and sodium (added as preservative) content. Trace elements, like zinc (from 3.9 to 5.8 mg/100 g), iron (from 2.15 to 2.71 mg/100 g), manganese (from 72 to 98 mg/100 g), selenium (from 26 to 28 mg/100 g) were well represented. Nitrate was about 5.9 mg/Kg, low compared in industrially dry-*sausages* B group vitamins showed high level of Niacin and Thiamin (mean values 7.16 and 0.44 mg/100 g, respectively). Microbial assessment showed absence of pathogens in any samples. The quantitative-descriptive sensory analysis displayed a sensory profile very similar among the manufactures.

Keywords: Local meat-product, Nutrients, Sensory attributes, Microbiological features

INTRODUCTION

Rural development is one of the major objectives of the European Union policies, and the strategies applied to achieve it often focus on traditional local products (Marescotti, 2003). Agri-food traditional products are usually linked to both territory and cultural tradition with local specific know-how and distinct sensory properties.

The Italian diet is strongly characterized by the consumption of a variety of typical foods produced in a *local dimension*, namely with specific geographical identity and produced by a small number of manufactures. This is especially true for meat products, some of which are strictly linked to a specific geographical area, with a moderate production but of major relevance in the local economy. A significant part of these traditional meat products is represented by dry-fermented sausages. Traditional dry-fermented sausages are produced on basis of specific know-how, made of autochthonous pig breeds, using different meat cuts (chopped or minced), fat, a variety of ingredients

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not always present (spices and additives), stuffed in casings. Obviously, the final nutritional and sensorial properties of these products are strongly affected by the composition of the batter as well by the size and ripening process (Ordóñez *et al.*, 1999). In addition, fermentation process often relies on natural contamination that takes place from the slaughtering to the production process. These microorganisms colonize the environment of the meat plants constituting the so-called "house-flora" (Santos *et al.*, 1998), which is in a continuous symbiotic exchange with the sausages. This microflora is often more competitive than starter cultures (Leroy *et al.*, 2006) and produce fermented sausages of higher quality, with distinct flavour characteristics (Moretti *et al.*, 2004).

An example of Italian typical dry-fermented sausage is the *Sausage from Monte San Biagio*, produced at local scale in Monte San Biagio (Lazio region, center Italy). In formulation of the sausages no synthetic preservatives have been used, by contrast only salt and natural ingredients, as coriander seeds (*Coriandrum sativum* L.) locally grown and hot and sweet pepper, have been added as natural microbial growth inhibitors. The batter is stuffed into natural pork casings. In addition, the drying phase is enhanced by smoking with mastic and myrtle smoke. As in most traditional dry-fermented sausages starter cultures are not used, so the fermentation is due to the colonization of the "house flora", completely adapted to the environment of the meat plants and the batter. The different characteristics of the ingredients utilised and the ripening process has contributed to the particular flavour of the *Sausage from Monte San Biagio*.

The aim of this study was to provide the full characterization of the fermented *Sausage from Monte San Biagio*. The nutritional profile (moisture, protein, lipid, fatty acids, minerals and trace elements, hydrosoluble and liposoluble vitamins), sensory properties and microbiological safety were analysed. Furthermore, the presence of salt, utilised in the manufacture of the sausages as preservative, and the possible presence of nitrate and nitrite was also investigated.

MATERIAL AND METHODS

Sausage Production

Dry-fermented sausages were prepared according to the traditional procedure followed in the *Monte San Biagio* area from three local manufacturers (Colabello, Monticellano, SVPT). Commercial hybrid heavy pigs (live weight higher than 160 kg) were used. The batter was composed by meat from different pork cuts (ham, loin, bacon, etc.), up to 25% fat and common salt (2%). The following spices were added: 0.5-1% coriander seeds, 0.15-0.8% red hot pepper, 0.16-0.3% red sweet pepper. The batter was kept at 0×2 ºC for 24 hours and then stuffed in natural pork casings. Unripened sausages were smoked using mastic and myrtle branches: cold smoke produced from branches and leaves in the proportion 300 g/500 kg fresh sausages was applied for 2 hours. Sausages were then kept at moderately low relative humidity (60%) for three days and at 79-80% relative humidity until the end of the process (21 days overall).

Each of the three manufacturers follows three "family recipes" that may be different in the choice of the anatomical parts (muscles) of the pig, and in variations in the amount of spices and salt used.

Sampling

Sampling was conducted according to the guidelines proposed by Greenfield and Southgate (2003). A total of 21 fermented sausages were provided by the three most representative local manufacturers, 5 units from each producer were destined to nutrient and sensory analyses and 2 to microbiological analysis. Samples were stored in under-vacuum package at 5 °C until analyses. For chemical analyses the sausages were chopped and minced together in order to obtain a homogeneous pool. Moisture, vitamins, nitrite and nitrate content and fatty acid profile were quantified on the obtained pool. Proteins, lipids and mineral and trace elements content was quantified on the lyophilized pools. Each analysis on each pool was carried out in triplicate. For sensory evaluations the sausages were removed from the package and brought to room temperature. For the individual portions, slices of constant thickness of about 1 cm were obtained, using a special knife and the casing removed before evaluation.

Microbiological analyses were carried out on two sausages collected from each manufacturer.

Chemicals

All the reagents used were of analytical grade purity. The reagents used in the chemical composition and lipid oxidation analyses were supplied by Sigma–Aldrich (Milan, Italy) and Carlo Erba (Milan, Italy). The standards of the fatty acid methyl esters were supplied by Sigma-Aldrich (Milan, Italy).

Proximate Analysis

Moisture, protein, lipid and salt content were determined according to the AOAC methods (2012).

Determination of Fatty Acid Profile

Fatty acids profiling was carried out after fat extraction according to the method of Bligh and Dyer (1959). Fatty acid methyl esters were prepared through acid-catalysed trans-esterification with methanolic hydrogen chloride (5%) according to Christie (1993). The fatty acid methyl esters were analyzed using an Agilent 7890A gas chromatograph, equipped with both Flame Ionization Detector (FID) and Mass Spectrometer (Agilent 5975C). The split ratio between FID and MS was 1:2. Separation was carried out on a polyethylene glycol capillary column (Mega-Wax-10, Mega) (30 m length, 0.32 mm id, 0.25 µm film thickness). Standard Reference Material: Beef-Pig Fat Blend (BCR 163, Community Bureau of Reference, Brussels) and FAME Mix C4-C24 (Supelco, Bellofonte PA, USA) were analysed as a control of the accuracy of the analysis (Lucarini *et al.*, 2018). Individual fatty acid methyl ester peaks were identified also by comparison of the mass spectra with those reported in the Willey and Nist libraries.

Mineral Composition

Analyses were performed by ICP-Plasma (Perkin-Elmer Optima 8000, Norwalk, CT, USA). Samples were previously ashed (4 ml HNO_3 + 1 ml H_2O_2) in an Ethos One high performance microwave digestion system (Mileston). Bovine muscle (BCR 184, Community Bureau of Reference, Brussels) and Bovine liver (NBS 1577, National Bureau of Standards, Gaithersburg, MD, USA) Standard Reference Materials were analysed as a control of the analysis' accuracy.

B Vitamins

Thiamine and riboflavin were separated and quantified by HPLC after acidic and enzymatic (Takadiastase) hydrolysis of the samples, following the procedure described by Arella *et al.* (1996). Niacin was quantified following the method described by Lahély *et al.* (1999).

Vitamin E and t-Retinol

Vitamin E and t-retinol were separated and quantified by HPLC following the method by Albalà-Hurtado *et al.* (1997).

Nitrate and Nitrite Content

The determination of residual nitrate and nitrite was carried out following the procedure described by Ferreira and Silva (2008). Separation and quantification of nitrate and nitrite ions was performed by ion-pair reverse phase chromatography. The HPLC system (Waters, Milford, USA) was equipped with photodiode array detector (DAD). Separation was carried out on a column Luna Phenomenex C18 (5 µm; 250 mm length, 4.6 mm diameter) (Torrance, CA, USA). After injection (10 µl) isocratic elution with 0.01 M *n*-octylamine-5 mM tetrabutylammonium hydrogenosulphate, pH 6.5 was applied.

Sensory Analysis

Sensory evaluations were performed by a 9-members professional panel with extensive training in descriptive sensory evaluation (ISO, 2012) of several foods, including meat products, and constantly monitored for reliability and consistency in evaluations. The products were evaluated in triplicate by quantitative descriptive analysis according to ISO guidelines (ISO, 2016). Evaluation were performed in standard sensory laboratory with individual booths (ISO, 2007), at room temperature under white light for visual attributes. For odour, taste, texture and flavour evaluations 1-cm slices were presented to the panellists in end-cupped polypropylene containers, under red light, and in a sequential monadic way following a Latin square design generated by the FIZZ software (Biosystemes, Couternon, France). None information was provided about products' identity. One of the products (*Colabello)* was used as reference to facilitate the alignment of the panellists in the use of the evaluation scale before evaluations, and then presented anonymously in the sample set. Descriptors were scored on a continuous rating scale ranging from 0 to 9, anchored at both ends with extremes for each attribute (weak, strong). *Fat cuts size* (visual) and *Cartilage* (texture) were scored on categorical scales (*prevailing small fat cuts, mixed small-medium, prevailing medium, mixed medium-large, prevailing large; none cartilage, few, many*). Bottled mineral water at room temperature and white unsalted bread was used as palate cleanser to avoid carry-over effects and adaptation to sensory stimuli.

Microbiological Analysis

Microbiological analysis was focused on the search of the pathogens *Listeria monocytogenes, Salmonella* spp. and *E.coli O157*. The analyses were performed by Enzyme Linked Fluorescent Assay (ELFA) with miniVidas (Biomerieux), validated protocols by AOAC and NF validation (AFNOR). Briefly was carried out analysis according to the manufacturer's instruction for detection of

L. monocytogenes (VIDAS® *L. monocytogenes* Xpress-LMX); *E.coli* O157 (VIDAS® UP *E. coli* O157 -ECPT) and *Salmonella* (VIDAS® UP *Salmonella*-SPT). All samples were analyzed together with positive and negative controls provided in each Kit.

Statistical Analysis

Results were presented as Mean±Standard Deviation of three replicates for each manufacturer. The analysis of variance (ANOVA) and post hoc Tukey's test (HSD) were performed. For category variables, the frequency for each category (contingency tables) was calculated and significance of the differences estimated through a Chi-square analysis (χ^2). Threshold for statistical significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Substantial differences in macronutrients content among the *Sausage from Monte San Biagio* samples provided by the three manufacturers were observed (Table 1). Moisture

Table 1: Macronutrients, Minerals, Trace Elements, Vitamins and Nitrate Content in Dry-Fermented Monte San Biagio Sausages (f.w.)

		Manufacturers			Mean Value
		Colabello	Monticellano	SVPT	
Macronutrients					
Moisture	g/100 g	24.71±0.47a	32.22±0.19b	22.86±1.02a	26.6 ± 4.96
Protein	g/100 g	29.64±0.86a	34.53±0.83b	32.83±1.83b	32.33 ± 2.49
Lipid	g/100 g	36.85±2.39	30.56±1.02	37.85±1.63	35.08 ± 3.95
Minerals					
Ca	mg/100 g	45.2±3.8b	29.49 ± 3.67a	48.9±11b	41.20 ± 10.3
P	mg/100 g	296±6.6	322 ± 60	336±39	318.27 ± 20.1
Mg	mg/100 g	42.3±4.8	37.55 ± 5.8	41.6±5.9	40.49 ± 2.57
K	mg/100 g	550±5.1	630 ± 102	508±76	562.89 ± 61.9
Na	mg/100 g	1439±20a	1782 ± 31b	1451±21a	1557 ± 194
Trace Elements					
Fe	mg/100 g	2.15 ± 0.09	2.71 ± 1.2	2.64 ± 0.69	2.49 ± 3.03
Zn	mg/100 g	3.98 ±0. 22	5.84 ± 1.79	3.91 ± 0.74	4.58 ± 10.9
Cu	mg/100 g	0.15 ± 0.019	0.19 ± 0.075	0.17 ± 0.078	0.17 ± 0.019
Mn	mg/100 g	0.09 ± 0.002	0.07 ± 0.017	0.09 ± 0.018	0.08 ± 0.014
Se	μg/100 g	26 ± 0.1	27 ± 0.9	28 ± 0.10	28 ± 0.03
Vitamins					
Thiamin	mg/100 g	0.36±0.06	0.52±0.76	0.46±0.02	0.44±0.08
Riboflavin	mg/100 g	0.11±0.02	0.08±0.02	0.14±0.04	0.11±0.04
Niacin	mg/100 g	8.16±3.15	7.18±1.65	6.14±1.48	7.16±2.11
Ions					
Nitrate	mg/Kg	5.51±1.98	6.27±0.25	5.95±1.39	5.91±0.38
Nitrite	mg/Kg	nd	nd	nd	nd

Note: Values are the M±SD of three determinations. Values in the same line followed by different letters are significantly different p<0.05; nd = not quantifiable.

content ranged from 22.8 to 32.2 g/100 g showing a high variability among the manufacturers, this influenced both protein and lipid content accordingly (Table 1). The highest protein content (34.5 g/100 g) and the lowest lipid content (30.56 g/100 g) was found in the Monticellano sausages. By contrast Colabello sausages showed the lowest protein content 29.6 g/100 g and a high lipid content similar to that found in the SVPT sausages, SVPT showed also the intermediate protein content (Table 1). These differences among the manufactures might be due to different cuts selected in the batter's formulation, the choice of leaner tissues could be also the basis of the highest moisture content found in the Monticellano sausages compared to the other two samples studied. The Cholesterol content was about 99 mg/100 g in all the meat-products (data not shown). These results are close to those reported in previous studies for traditional Italian dry-fermented sausages both industrially and locally produced (*Salumi Italiani: aggiornamento dati nutrizionali,* 2011; Moretti *et al.*, 2004) and for Spanish *salchichon* (Rubio *et al.*, 2008).

Minerals and trace elements content in the *Sausage from Monte San Biagio* is also reported in Table 1. The dry-sausages studied are a rich source of minerals, marked differences among the manufacturers were detected mainly for calcium and sodium contents, Monticellano showing the lowest calcium content and the highest potassium and sodium content (Table 1). Compared to a previous study carried out on Italian industrial dry-sausages (*Salumi Italiani: aggiornamento dati nutrizionali,* 2011), these local meat products showed about twice the content in Ca and Mg and a slightly higher P content. *Sausage from Monte San Biagio* was found to be also an important source of trace elements. All the products provided very high amount especially of zinc (from 3.9 to 5.8 mg/100 g) and iron (from 2.15 to 2.71 mg/100 g), and also of manganese (from 72 to 98 µg/100 g) and selenium (from 26 to 28 µg/100 g) (Table 1). The trace elements content of the *Sausage from Monte San Biagio* was higher than that found in similar industrial Italian meat-products (*Salumi Italiani: aggiornamento dati nutrizionali,* 2011): about twice for both Fe and Zn, four times for Mn and 0.7 times higher for Se; this could be ascribed to differences in both rearing and feeding systems utilised. B vitamins content in the samples studied are also reported in Table 1. Samples showed a Thiamin content ranging from 0.36 to 0.52 mg/100 g, values close to those reported in the database of Italian POD cured meats (*Salumi Italiani: aggiornamento dati nutrizionali,* 2011). The mean Riboflavin content was of 0.11 mg/100 g, values lower than those found in other Italian POD Salumi. As regards Niacin the values found in the *Monte San Biagio* sausages were found steadily higher than those detected in the Italian POD cured meats (*Salumi Italiani: aggiornamento dati nutrizionali,* 2011). Salt, together with coriander seeds and red pepper, was utilized as preservative in the sausages studied. The medium content of salt, calculated utilizing the convertion factor of 2.5, resulted almost homogeneous among the three manufacturers being 3.9 g/100 g, a value similar to that reported in other Italian sausages (Meynier *et al.*, 1999; Moretti *et al.*, 2004; Di Cagno *et al.*, 2008; and *Salumi Italiani: aggiornamento dati nutrizionali,* 2011). Even if nitrate was not added as additive in the *Monte San Biagio* sausages, the natural level we found ranged from 5.5 mg/Kg in Colabello to 6.2 mg/Kg found in Monticellano. A level very low compared to that reported for the industrially produced Italian dry-sausages (Di Cagno *et al.*, 2008; and *Salumi Italiani: aggiornamento dati nutrizionali,* 2011) and probably coming from the spices used in the batter formulation. As concerns the fatty acids profile of the sausages analysed, the percentages of SFA, MUFA and PUFA was almost homogeneous among the three manufacturers (Figure 1). Among Saturated Fatty Acids (SFA), accounting for 34.67% of total lipids, palmitic (21.6%) and stearic (11.18%) acids were found to be the dominant ones, while the others SFA were minor components. In all the samples the monounsaturated fatty acids (MUFA) represented the prominent group being 50.32% of total lipids and oleic acid showed the highest amount (mean value 46%). The unsaturated fatty acids accounted for 65% of the total fat. The most abundant polyunsaturated fatty acids (PUFA) was linoleic acid (13.15%). These four above mentioned fatty acids accounted together for more than 95% of total fatty acids.

This profile presents an unsaturation level higher than that of industrial heavy pigs farmed in Italy (Renaville *et al.*, 2013), this could be ascribed to a high unsaturated fatty acid content in the pigs' diet, reflecting the free range rearing of pigs. To assess the nutritional properties of fat, the PUFA/SFA ratio (P/S) was considered. The analysed sausage showed a P/S ratio of 0.52, highlighting the high PUFA content. The recommended P/S value for healthy foods and diets ranges in fact from 0.40 to 0.65 (Simopoulos, 2004).

Sensory Analysis

Results of the quantitative-descriptive sensory analysis evidenced small inter-product variability, with variances

Table 2: Sensory Attributes of Dry-Fermented Monte San Biagio Sausages

	Colabello		Monticelliano		SVPT		F	p-value
	Mean	SD	Mean	SD	Mean	SD		
Colour intensity[1]	6.2[b]	0.96	7.8[a]	0.78	6.6[b]	1.05	14.67	**<0.0001**
Amount of fat	7.3	0.79	6.8	0.89	6.8	1.36	1.273	0.288
Odour intensity	7.7	0.52	7.4	0.53	7.6	0.56	1.862	0.165
Animal odour	3.6	1.09	3.6	0.89	4.3	1.42	2.873	0.065
Smoke odour	4.3	1.23	5.2	1.06	4.7	1.04	2.911	0.063
Overall spicy odour	6.3	0.99	6.1	0.78	6	1.15	0.389	0.679
Coriander odour	4.1	0.91	4.1	0.93	3.9	0.99	0.375	0.689
Red pepper odour	5.5[a]	1.02	4.4[b]	1.19	5.3[a]	1.24	4.685	**0.013**
Salty	4.4	1.06	4.9	1.34	4.3	0.99	1.818	0.172
Sweet	3.8	0.86	3.5	0.78	3.8	0.96	0.642	0.53
Flavour intensity	7.8	0.54	7.6	0.66	7.4	0.62	2.263	0.113
Overall spicy flavour	6.7	1.18	6.4	0.93	6.3	1.16	0.536	0.588
Coriander flavour	4.2	0.89	4.9	1.02	4.2	0.92	2.998	0.058
Red pepper flavour	6.1[a]	1.18	4.9[b]	1.29	5.6[ab]	1.09	5.222	**0.008**
Cured flavour	6.9	0.87	6.7	0.56	7	0.73	1.157	0.322
Hot flavour	7.1[a]	0.95	6.6[ab]	0.95	5.8[b]	1.2	8.823	**0**
Hardeness	6.2	0.93	5.9	1.12	6.6	0.73	2.823	0.068
Batter cohesiveness	5.1	1.15	5.2	1.01	4.4	1.29	2.927	0.062
Fat mouth feeling	5.7	0.71	5.6	0.98	5.5	1	0.245	0.783
Hot flavour persistence	6.9[a]	0.97	6.7[a]	1.21	5.6[b]	1.21	7.016	**0.002**
Smoke aftertaste	3.3[b]	1.09	4.2[a]	1.14	3.5 [ab]	1.06	3.696	**0.031**

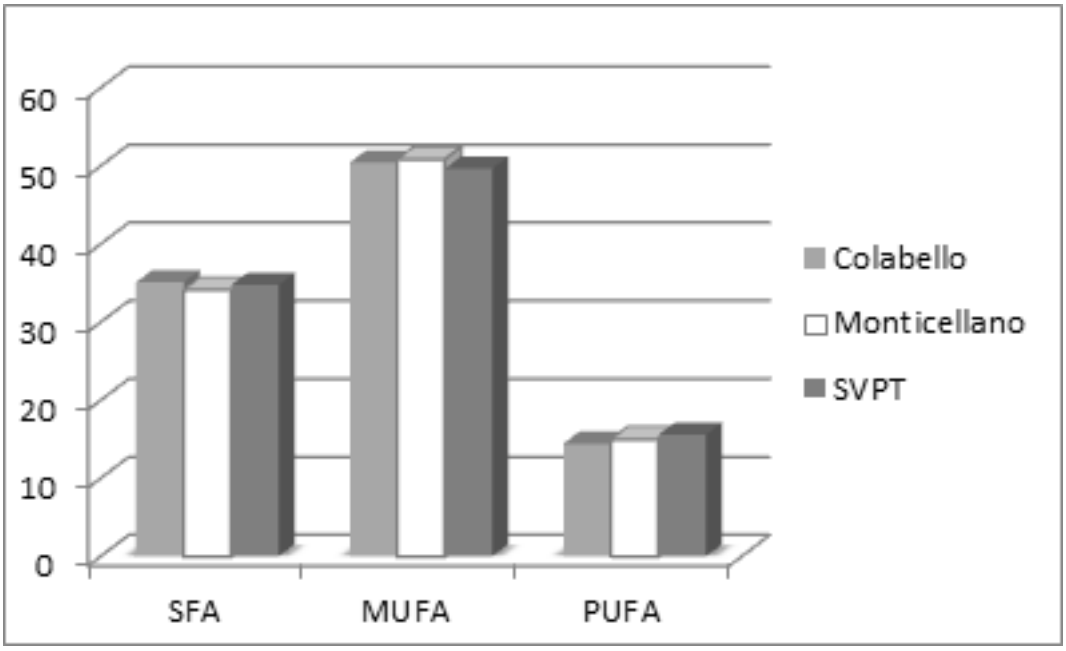
Note: [1] Red brick = 0, red plum = 9. Values in the same line followed by different letters are significantly different at p<0.05.

mainly related to a different dosage of spices. Significant differences among manufactures were related to *red pepper* odour and flavour, with Monticellano presenting lower scores (Table 2). This may be related to a lower pepper amount on the batter formulation as well as to the higher moisture content of Monticellano products. On the other hand, hot pepper flavour and hot flavour persistence were significantly lower (p<0.001) in SVPT sausages, possibly due to a lower ratio between hot and sweet dosed peppers. In addition, Monticellano had a more intense colour and smoked aftertaste. At last, for category descriptors, fat cuts size was significantly lower in Monticellano ($\chi^2 = 24.006$; $p = 0.002$), and the presence of cartilage perceived during chewing, did not show difference among the products ($\chi^2 = 6.153$; $p = 0.188$).

Microbiological Analysis

Our data indicated absence of pathogens in any analyzed samples in comply with Regulation (EC) no 2073/2005, as amended Regulation (EC) n.1441/2007. Meat is a natural substrate for bacteria growth, resulting from primary or secondary contamination (spices and handling). Good

Figure 1: Percentages of SFA, MUFA and PUFA in Sausage from Mosnte San Biagio



slaughtering and handling practices appear to strongly influence the microbiology quality, therefore to maintain in long time this product, is necessary to adopt conservation strategy to guarantee the food safety. Strict adherence to GMP Good Hygiene Practices (GHP) and a strict adherence to Good Manufacturing Practices (GMP) can improve the microbiological quality and guarantee safe of this kind of meat products (De Giusti *et al.*, 1992; and López Dýìaz *et al.*, 2002).

CONCLUSION

The compositional profile of the dry-fermented *Sausage from Monte San Biagio*, confirmed the local dimension of the products analysed, each one corresponding to the single manufacturer's recipe. This was of great relevance in that the nutritional profile of these meat-products could also differed from the others. The high variability found in macronutrients content of the samples analysed well describe the not standardized sausage manufacture. Furthermore, the high unsaturated fatty acids level of these meat products further confirms the free range rearing methods adopted. Our findings also show the dry-sausages as a rich source of trace elements as Zn, a portion of sausage (50 g) provides about 20% of the Italian Recommended Daily Allowance for this trace elements, as well for B Vitamins, also in this case a portion of sausage (50 g) provides about 20% of the Italian Recommended Daily Allowance of Niacin (SINU, 2014). Meat-products are widely appreciated for their sensory characteristics and, furthermore, the traditional salting, curing and drying technologies used since ancient times to extend the shelf life of this meat products, nowadays attract the interest of consumer and producers are even more encouraged to use safer preserving methods. The microbiological results showed that a careful use of spices can replace nitrates and nitrites without affecting product safety. The dry-sausages anlysed in this study are an example of how the combination of the use of spices and adequate technological practices can substitute the use of preservatives in the production of high quality fermented sausages.

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