

Seasonal variations in fatty acids composition of Istrian and Dalmatian prosciutto

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ABSTRACT

In this study, seasonal variations in fatty acid composition of saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids present in Istrian and Dalmatian prosciutto, were investigated. Prosciutto samples, produced according to traditional recipes and using traditional technologies by different producers from Istria (n=43) and Dalmatia (n=30), were sampled on the annual fairs during three-year period of production (2012-2014). Fatty acid methyl esters were analysed using gas chromatography-flame ionisation (GC-FID) after the extraction of total fat using the standardized Soxhlet method. In both types of prosciutto, the results showed the highest representation of oleic acid (C18:1n-9), followed by palmitic (C16:0), stearic (C18:0) and linoleic (C18:2n-6) acid, representing fatty acid composition typical for pork meat products. Statistically significant differences between Istrian and Dalmatian prosciutto in individual fatty acids during three year production period were not determined. In Istrian prosciutto according to year of production, significant differences ($p < 0.05$) in SFA, MUFA, PUFA n-6 and PUFA n-3 groups and their ratios with exception PUFA/SFA were observed. However, at the same timeframe in samples of Dalmatian prosciutto significant difference was observed only for MUFA content. It could be concluded that season of production could significantly influence the dry-cured meat products fatty acid profile, especially in the case of Istrian prosciutto.

Keywords: fatty acid composition, seasonal variations, Istrian prosciutto, Dalmatian prosciutto, SFA, MUFA and PUFA

INTRODUCTION

Dalmatian and Istrian prosciutto are products protected on the Croatian national level by protected geographical indication (Dalmatian prosciutto) and protected designation of origin (Istrian prosciutto). Products specification defines that an Istrian prosciutto is produced exclusively from heavy pigs (>160 kg) of the precious meaty breeds (except for Pietrain) and their hybrids in any combination. The pigs have to be dusted and fattened in the geographical area of production, while the Dalmatian prosciutto allowed the use of fresh hams of all commercial meaty breeds and their crosses, without limitation (geographical origin, breeding methods, nutrition, etc.). Unlike the Dalmatian prosciutto, Istrian prosciutto is made from hams with removed skin and fat, not smoked and with brine mixture used in pro-

duction that consists of the sea salt and spices (black pepper, bay leaves, rosemary and garlic) that gives a specific and distinctive taste and smell of mature product while Dalmatian prosciutto undergoes exclusively dry salting with sea salt.

Opposed to the Dalmatian prosciutto, Istrian prosciutto during the ripening is overgrown by colonies of grey moulds (Comi et al., 2004), whose exuberance and later spidery remain represent specific indicator of its proper process of drying and ripening. Due to the application of various technological processes during prosciuttos production lipids undergo a series of transformations which include hydrolytic processes, the release of short-chained fatty acids, and the latter oxidation of the acids together with the formation of peroxides and volatile compounds, contributing to the aroma of the

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final product (Jiménez-Colmenero et al., 2001, Siciliano et al., 2013, Barbir et al., 2014).

However, lipids are nowadays under special attention in terms of nutritional quality of food. Consumers are advised to reduce consumption of fatty food including meat and meat products which are generally rich in fat and saturated (SFA) acids, representing a significant source of fat and SFA in human nutrition. One of the characteristics of the modern diet is an excessive intake of fat, particularly SFA and, at the same time, a disturbed balance of polyunsaturated fatty acids (PUFA) intake in terms of an increased intake of n-6 as compared to n-3 fatty acids. Research results at the same time have shown that fat mass fraction and fatty acid composition are affected by many factors, starting from the animal breed selection and feeding and farming practices to technological processes and parameters used during production. The pork and pork food products generally characterize a high proportion of SFA and lower proportion of monounsaturated fatty acids (MUFA) and PUFA (Wood et al., 2004; Wood et al., 2008; Woods and Fearon, 2009).

Producers are therefore trying to modify meat products fatty acids profile in order to get them closer to nutritionally acceptable values (Muguerza et al., 2004; Pelser et al., 2007; Fernandez et al., 2007; Valencia et al., 2006). Jiménez-Colmenero et al. (2001) pointed out that modification of carcass composition, the manipulation of meat raw materials and reformulation of meat products as three fundamental strategies to achieve healthier meat and meat products. Modification of the fatty acid profile of pork products is mostly achieved with genetic selection in combination with feeding practices. Partial substitution of pork backfat with PUFA oils from animal and vegetable origin (preemulsified soy, linseed oils and deodorised fish oil) is also often implemented (Ansorena and Astiasaran, 2007).

The aim of this study was to investigate the differences in composition of SFA, MUFA and PUFA fatty acids present in Istrian and Dalmatian prosciutto and to compare obtained profiles according to seasons during the three years production period.

MATERIALS AND METHODS

Sampling and sample preparation

Istrian and Dalmatian prosciutto samples were taken during the period 2012-2014 from the annual fairs in Istria and Dalmatia (Table 1). Prosciuttos were produced according to traditional recipes and technologies by different producers located in Istria and Dalmatia region. Production of both types of prosciuttos takes at least a year, and is characterized by a process of long-term maturity with the finished products water activity (a_w)

below 0.93 and the mass fraction of salt to a maximum of 7.5 (Dalmatian prosciutto) and 8% (Istrian prosciutto). Since both types of prosciuttos are protected at national level their production technology are described in details in Product Specifications of Istrian prosciutto and Dalmatian prosciutto.

Samples representative for analysis were prepared in accordance with ISO3100-1:1991 by homogenization for 15 s at 6000 rpm and use of Grindomix GM 200 (Retch, Germany). Before determination of the fat content and fatty acid composition samples were stored at +4°C.

Table 1. Year of production and number of Istrian and Dalmatian prosciutto samples used in study

Year of production	N (Istrian prosciutto)	N (Dalmatian prosciutto)
2012	11	10
2013	12	9
2014	20	11
Total	43	30

Standards and reagents

Standard solution of fatty acids methyl esters (FAME), concentration of 10 mg/mL, was prepared by dilution of 100 mg of standard Supelco™ 37 Component FAME Mix (Bellefonte, Pennsylvania, SAD) in 10 mL of hexan. Obtained solution was stored in a freezer at -20°C and used for identification of FAME with each analysis.

Hexane and methanol used in the analysis of fatty acids were HPLC grade (JT Baker Derventer, Netherland). Ultra-pure water with electrolytic conductivity of ≤ 0.05 S/cm was obtained using Milipore Direct-Q 3UV (Merck, Darmstadt, Germany). All other chemicals used in the analysis were analytical grade (Kemika, Zagreb, Croatia).

Determination of total fat

Total fat content was determined by Soxhlet method (EN ISO 1443:1999) which involves sample digestion in acid conditions followed by fat extraction with petroleum ether in Soxtherm 2000 Automatic device (Gerhardt, Königswinter, Germany) and drying in the oven Epsa 2000 (Ba-Ri, Velika Gorica, Croatia). Results of the total fat were expressed as the mean value of two parallel determinations, in percentage (%) of weight, with an accuracy of 0.01%. Verification of the method for determination of total fat content was performed with each analyses using certified reference material CRM T0149 (FAPAS, York, England).

Determination of fatty acids

Fatty acid methyl esters were prepared from the extracted fat according to EN ISO 5509:2000. One hundred mg of the extracted fat were dissolved in 10 mL of hexan and shaken on HS260 control (IKA, Königswinter, Ger-

many). In addition, 200 µL of 2N-methanolic potassium hydroxide solution were added and the samples were shaken for 30 seconds. The samples were then centrifuged in a 320AR centrifuge (Hettich, Tuttlingen, Germany) for 15 min at 3,000 rpm and the temperature of 15 °C. Two hundred mL of each sample were filtered through a PTFE filter into vials to be analyzed.

Methyl esters of fatty acids were analyzed according to Pleadin et al. (2014) by GC-FID method and use of gas chromatograph 7890 BA (Agilent Technologies, USA) with the capillary column HP88 of 100 m length, internal capillary diameter 0.25 mm and thickness of stationary phase of 0.20 µm (Agilent Technologies, USA). The components were detected by a flame ionization detector at temperature of 280°C, a hydrogen flow was 40 mL/min, air flow was 450 mL/min and nitrogen flow was 30 mL/min. The column temperature program was: initial column temperature was 120 °C for 1 min, then at 10 °C/min to 175 °C, maintaining for 10 min, then at 5 °C/min to 210 °C and maintaining for 5 min, then at 5 °C/min to final temperature of 230 °C which was maintained for 5 min. A sample (1 µL) was injected in split-splitless injector with temperature of 250 °C with split ratio 1:50. Carrier gas was helium (99.9999%) with a constant flow of 2 mL/min. FAME were identified by comparison of their retention times with retention times of FAME of the standard mixture (10 mg/mL, Supelco™ 37 Component FAME Mix) analyzed under the same conditions. Results are expressed as a percentage (%) of total fatty acids, with accuracy of 0.01%. In determination of fatty acids composition with each run of analysed samples CRM BCR163 (Institute for Reference Materials and Measurements, Belgium), with assigned content of seven individual fatty acids, was used and analyzed in the same way as the samples.

Statistical data analysis

Statistical analysis was performed using computer program SPSS 20.0 (SPSS Inc., USA). Results are expressed as mean±SD. Shapiro Wilks test was conducted to determine whether the results of the analyzed parameters have a normal distribution (p >0.05). Since, for determining the difference between the groups in the share of fats and fatty acids, one way ANOVA and Kruskal Wallis test were used, with significance defined at p < 0.05.

RESULTS AND DISCUSSION

According to recommendations, daily intake of fat should not exceed estimated 15–30% of total energy intake, among which SFA should represent up to 10%, PUFA 6–10% (n-6: 5–8%; n-3: 1–2%), MUFA 10–15%, and trans fatty acids less than 1% of total daily energy intake (Whitney and Rolles, 2005). Studies presented that dietary fat has health im-

plications on humans resulting from the presence of SFA, MUFA and PUFA n-6 and n-3 fatty acids and their proportions. Ratio of n-6/n-3 is associated with disorders of a number of physiological processes that increase the incidence of so-called chronic diseases related to diet, primarily heart disease and disease of cardiovascular system (Cordain et al., 2005). It is also known that the main fatty acids in dry cured meat products are MUFA (41-59%), followed by SFA (30-45%) and the lowest PUFA (9-18%), and that many factors such as animal breed selection, feeding and farming practices are varying to affect fatty acids composition of the final product and to contribute to the PUFA/SFA and n-6/n-3 ratios characteristic for healthier diet (Jiménez-Colmenero et al., 2001; Siciliano et al., 2013; Barbir et al., 2014).

In this study, influence of season on fatty acid composition and differences in proportions of SFA, MUFA and PUFA of Istrian and Dalmatian prosciutto were investigated during three years production period.

To investigate fat content and fatty acid composition applied analytical methods were verified by determination of trueness parameter and use of CRMs in each set of samples, respectively. Obtained values of CRMs, compared with the criteria defined for validation of analytical methods and interpretation of the results (OG 2/2005) as also with criteria of repeatability defined in the used ISO standards for both methods respectively, are shown to be acceptable. Obtained verification results are presented in Table 2.

Table 2. Results of analytical methods verification for total fat and fatty acids determination

Analytical parameter	Assigned value (%)	Obtained value ^c (%)
Total fat	2,12–2,87 ^a	2,44±0,07
C14:0	2,29±0,04 ^b	2,27±0,04
C16:0	25,96±0,30 ^b	26,49±0,31
C16:1	2,58±0,16 ^b	2,34±0,08
C18:0	18,29±0,17 ^b	19,22±0,21
C18:1n-9c	38,30±0,40 ^b	37,64±0,27
C18:2n-6c	7,05±0,17 ^b	7,12±0,09
C18:3n-3	0,86±0,14 ^b	0,75±0,09

^a assigned value of CRM for fat is given as a range

^b assigned values of CRM for the fatty acids are expressed as mean ± standard deviation

^c obtained values are given as mean values of all performed analyses determined in this study

Results of determined total fat content in Istrian and Dalmatian prosciutto samples according to year of production are presented in Table 3.

Table 3. Total fat content in Istrian and Dalmatian prosciutto determined per year of production

Year of production	Total fat / Mean value ± SD (%)	
	Istrian prosciutto	Dalmatian prosciutto
2012	15,78±5,66	18,37±5,81
2013	19,03±8,42	17,30±4,82
2014	20,13±4,51	21,08±6,33

The average fatty acid composition determined in Istrian and Dalmatian prosciutto during whole period of production is presented in Table 4. Figure 1 shows proportions of SFA, MUFA and PUFA obtained also during whole period of three years production for each type of prosciutto, respectively.

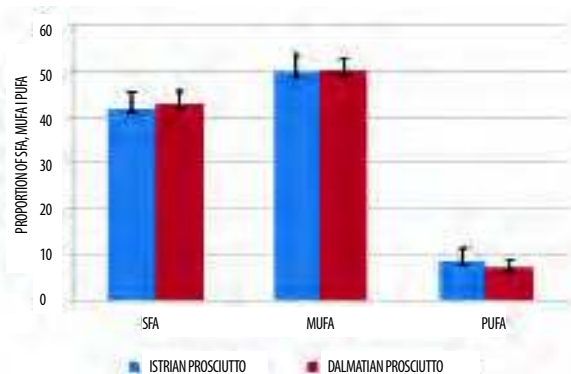
Table 4. Average fatty acid composition of the Istrian and Dalmatian prosciutto during three year production period

Fatty acids	Mass fraction of fatty acids* Mean value ± SD (%)	
	Istrian prosciutto	Dalmatian prosciutto
C10:0	0,10±0,02	0,11±0,03
C12:0	0,10±0,04	0,10±0,03
C14:0	1,42±0,26	1,43±0,12
C16:0	25,95±1,98	26,38±1,63
C17:0	0,29±0,12	0,25±0,13
C18:0	13,51±1,71	14,03±1,52
C20:0	0,24±0,08	0,29±0,14
C16:1	2,85±0,67	2,92±0,66
C18:1n-9t	0,05±0,08	0,15±0,06
C18:1n-9c	46,25±3,58	46,77±2,21
C20:1	0,59±0,45	< 0,10
C21:0	0,10±0,16	0,33±0,19
C22:1n-9	0,03±0,07	0,11±0,11
C18:2n-6c	7,51±2,99	6,15±1,91
C18:3n-6	0,07±0,11	0,19±0,08
C20:2n6	0,26±0,22	< 0,10
C20:4n6	0,10±0,09	< 0,10
Σ n-6	7,94±2,99**	6,34±1,92**
C18:3n-3	0,57±0,30	0,82±0,15
Σ n-3	0,58±0,30**	0,82±0,15**
n-6/n-3	17,04±9,11**	8,38±4,59**
PUFA/SFA	0,21±0,08	0,17±0,05
MUFA/SFA	1,21±0,17	1,17±0,13

* mass fraction of fatty acid is expressed as the total proportion of fatty acids

** statistically significant difference (p<0.05)

SFA=saturated fatty acids, MUFA=monounsaturated fatty acids, PUFA=polyunsaturated fatty acids



SFA=saturated fatty acids, MUFA=monounsaturated fatty acids, PUFA=polyunsaturated fatty acids

Figure 1. Proportion of SFA, MUFA and PUFA obtained in Istrian and Dalmatian prosciutto during production period 2012-2014

Statistically significant differences between Istrian and Dalmatian prosciutto in individual fatty acids, during three year (from 2012 to 2014) production period, were not determined (p>0.05). Notably, Istrian prosciutto had significantly (p<0.05) higher average portion of n-6 but lower average portion of n-3 fatty acids in comparison to Dalmatian prosciutto. Consequently, the n-6/n-3 ratio was significantly (p<0.05) higher in Istrian prosciutto samples during the entire period of investigation.

Obtained ratios of PUFA/SFA and n-6/n-3 were 0.21±0.08 and 17.04±9.11 in Istrian and 0.17±0.05 and 8.38±4.59 in Dalmatian prosciutto, respectively, what could be comparable with results of other prosciutto studies and generally those obtained for pork meat products. In previous studies determined PUFA/SFA and n-6/n-3 values were ranged from 0.2 to 0.3 and from 12.9 to 16.6 in Istrian prosciutto, respectively (Karolyi, 2006; Marušić et al., 2013). In Dalmatian prosciutto PUFA/SFA and n-6/n-3 ratios were 0.2 and 14.7, respectively (Marušić et al., 2013). Also in studies of some other similar pork products these values ranged from 0.2 to 0.6 for PUFA/SFA and from 7.6 to 39.9 for n-6/n-3, pointing to significantly high variations in ranges per type of product (Santos et al., 2008, Jiménez-Colmenero et al., 2010, Campo and Sierra, 2011; Pugliese, 2009; Fernández et al., 2007; Ventanas et al., 2007; Pastorelli et al., 2003; Lo Fiego et al., 2005; Gandemer, 2009). Published data have shown that generally in traditional fermented pork products the largest share of fatty acids belongs to oleic acid (C18: 1n-9c), followed by palmitic (C16:0), stearic (C18:0) and linoleic (C18:2n-6) regardless of the geographical origin of the product (Casaburi et al., 2007; Visessanguan et al., 2006; Marušić et al., 2013; Pleadin et al., 2014). The results of this study are also comparable with literature data that revealed that generally the main fatty acids in dry cured hams are monounsaturated fatty acids (41-59%), then saturated (30-45%) and at least are polyunsaturated (9-18%). Data also shown that the most prevalent MUFA is oleic fatty acid (C18:1n-9c) (40-50%) and that the principal SFA components are palmitic (C16:0) (23-26%) and stearic C18:0 (10-15%) acid (Fernández et al., 2007; Pleadin et al., 2014). The main PUFA component shown to be linoleic acid C18:2n-6(c) with shares of 6-10% in dry cured hams (Moretti et al., 2004; Jiménez-Colmenero et al., 2010; Karolyi, 2006; Jurado et al., 2008).

Pugliese and colleagues (2015) concluded that the semimebranosus (SM) and the biceps femoris (BF) muscles fatty acid levels may differ due to different content of intramuscular fat as well as higher lipolytic activity of SM muscle resulting in higher fatty acid content. Results also shown that an intense lipolysis takes place during the first five months of hams curing (salty and lower water activity environment are favourable for acid muscle lipases) and that the lipids undergo a series of transformations during

ripening which include hydrolytic processes, releasing of fatty acids (those of short chain intervene in a direct way in the development of the aroma), and oxidation of these fatty acids with the formation of peroxides and volatile compounds contributing to the aroma of the final product (Toldrá, 1998; Pleadin et al., 2014; Pugliese et al., 2015).

The nutritional properties of fermented meat products are generally assessed with PUFA/SFA and the n-6/n-3 PUFA ratios. The PUFA/SFA values above 0.4-0.5 and of n-6/n-3 below 4 are recommended (Wood et al., 2004; Wood et al., 2008). Studies data revealed that according to these recommendations, dry-cured hams would not be within the desirable limits for both the PUFA/SFA and n-6/n-3 ratios (Santos et al., 2008; Jiménez-Colmenero et al., 2010; Campo and Sierra, 2011; Pugliese, 2009; Fernández et al., 2007; Ventanas et al., 2007; Gandemer, 2009).

In this study fatty acid composition determined in Istrian and Dalmatian prosciutto per year during three years investigation period are shown in Table 5 and Table 6, respectively.

Significant differences ($p < 0.05$) in content of SFA, MUFA, PUFA n-6 and PUFA n-3 and their ratios in Istrian

prosciutto, according to year of production, were observed for all groups of fatty acids and their ratios except for PUFA/SFA ratio whereas in Dalmatian prosciutto significant seasonal variation was ($p < 0.05$) was observed only for MUFA.

The difference between Istrian and Dalmatian prosciutto samples was the most obvious in samples from 2013, in which measured n-6/n-3 ratio was more than double in Istrian prosciutto in comparison with samples of Dalmatian prosciutto from the same year (16.94 vs 6.98). Mentioned difference in n-6/n-3 ratio is due to the significantly lower portion of n-3 fatty acids Istrian prosciutto from 2013 compared to Dalmatia prosciutto (0.58 vs 0.86).

Istrian prosciutto in average contained more PUFA and higher average values of PUFA/SFA and MUFA/SFA. For Istrian prosciutto it could be mention increased values for SFA, PUFA and n-6 fatty acids but decreased values for MUFA and fatty acids from n-3 group ($p < 0.05$). Consequently the following ratios of fatty acids increased: n-6/n-3, PUFA/SFA while MUFA/SFA decreased ($p < 0.05$). Generally speaking, the very similar seasonal trends were observed in samples of Dalmatian prosciutto with the notable exception of

Table 5. Fatty acids composition of Istrian prosciutto per year of production

Year of production	Statistic parameter	SFA (%)	MUFA (%)	PUFA/n-6 (%)	PUFA/n-3 (%)	n-6/n-3	PUFA/SFA	MUFA/SFA
2012	Mean	39,21	52,74	7,11	0,93	8,38	0,20	1,35
	SD	1,67	2,77	1,66	0,18	3,27	0,04	0,12
	Min	35,40	47,27	4,55	0,66	3,76	0,13	1,08
	Max	43,77	58,41	10,1	1,30	15,30	0,27	1,65
2013	Mean	41,83	50,61	6,98	0,58	16,94	0,18	1,22
	SD	2,14	1,95	2,34	0,26	9,77	0,06	0,10
	Min	37,24	45,22	3,48	0,16	4,23	0,09	1,01
	Max	46,45	54,27	11,05	0,99	29,10	0,30	1,38
2014	Mean	42,77	47,93	8,88	0,42	20,99	0,22	1,13
	SD	2,66	3,03	2,42	0,14	5,28	0,07	0,12
	Min	36,04	41,08	2,67	0,12	2,67	0,05	0,80
	Max	54,14	55,94	17,07	0,67	32,28	0,43	1,44
p-value		0,043	0,001	0,015	$0,23 \times 10^{-9}$	$1,01 \times 10^{-8}$	0,096	0,003

SFA=saturated fatty acids, MUFA=monounsaturated fatty acids, PUFA=polysaturated fatty acids

Table 6. Fatty acids composition of Dalmatian prosciutto per year of production

Year of production	Statistic parameter	SFA (%)	MUFA (%)	PUFA/n-6 (%)	PUFA/n-3 (%)	n-6/n-3	PUFA/SFA	MUFA/SFA
2012	Mean	43,20	50,12	5,87	0,80	7,59	0,16	1,16
	SD	1,56	1,59	1,16	0,08	2,21	0,03	0,07
	Min	40,38	47,39	4,05	0,61	4,57	0,11	1,02
	Max	46,80	53,03	7,88	1,03	12,54	0,20	1,31
2013	Mean	41,43	51,67	6,05	0,86	6,98	0,17	1,26
	SD	2,52	1,45	2,12	0,06	2,13	0,06	0,10
	Min	38,46	48,97	3,65	0,74	4,01	0,10	1,05
	Max	46,47	54,54	8,94	0,92	9,72	0,26	1,35
2014	Mean	43,52	48,42	7,22	0,81	10,49	0,19	1,12
	SD	2,69	2,39	1,92	0,15	4,69	0,05	0,13
	Min	37,10	45,25	4,75	0,35	4,94	0,14	0,99
	Max	46,99	52,26	10,14	1,02	24,57	0,30	1,39
p-vrijednost		0,182	0,019	0,316	0,751	0,300	0,592	0,059

SFA=saturated fatty acids, MUFA=monounsaturated fatty acids, PUFA=polysaturated fatty acids

samples originated from 2013 year. Interestingly, Dalmatian prosciutto from 2013 had the lowest values of SFA and n-6/n-3 but the highest values of MUFA, n-3 and MUFA/SFA. The values of n-6 and PUFA/SFA continuously increased during the three years period.

CONCLUSION

In Istrian and Dalmatian prosciutto, the highest representation of oleic acid (C18:1n-9C), followed by palmitic (C16:0), stearic (C18:0) and linoleic (C18:2n-6) acid were observed, representing fatty acid composition typical for pork meat products. Statistically significant differences between Istrian and Dalmatian prosciutto in individual fatty acids during three year production period were not determined. According to year of production, differences in SFA, MUFA, PUFA n-6 and PUFA n-3 groups and their ratios were observed, particularly for Istrian prosciutto, pointing out that season of production could significantly influence on the dry-cured meat products fatty acid profile.

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