ELSEVIER

Meat Science 61 (2002) 61–65

MEAT SCIENCE

www.elsevier.com/locate/meatsci

Fatty acid and triglyceride profiles of intramuscular and subcutaneous fat from fresh and dry-cured hams from Hairless Mexican Pigs

G.L. Delgado[a], C.S. Gómez[a], L.M.S. Rubio[b,*], V.S. Capella[a], M.D. Méndez[b], R.C. Labastida[a]

[a]*Departamento de Química Analítica de la Facultad de Química de la Universidad Nacional Autónoma de Mexico, Circuito Universitaria, Coyoacán, 14400, Mexico D.F.*

[b]*Meat Science Laboratory, Facultad Medicina Veterinaria y Zootecnia de la Universidad Nacional Autónoma de Mexico, Ciudad Universitaria, Coyoacán, 14400, Mexico*

Received 12 April 2001; received in revised form 18 July 2001; accepted 18 July 2001

Abstract

The study was designed to compare the fatty acid profiles and triglyceride composition of subcutaneous and intramuscular fat of raw and cured hams from Hairless Mexican Pigs. Curing (180 days) was developed throughout several ordered steps: salting, stabilization, drying and ripening. For the salting step, hams were rubbed with nitrate salt, and kept on salt 1 day per kilogram at 0–4 °C and at relative humidity of 80–85%. The rest of the process consisted of small increments of temperature (from 0–24 °C) and small decrements of relative humidity (from 82–68%). The curing process decreased ($P<0.05$) significantly the proportion of unsaturated fatty acids (raw: 59.2±0.3% and cured: 55.3±0.4%) and increased the saturated ones (raw: 34.4±0.3% and cured: 39.4±0.5%). Subcutaneous and intramuscular fat had similar ($P>0.05$) percentages of unsaturated (57.60±0.3 and 56.88±0.4%, respectively) and saturated fatty acids (37.5±0.33 and 38.3±0.4%, respectively). With the exception of the monopalmitate, the curing process decreased ($P<0.05$) the levels of all acylglycerols studied.

Keywords: Hairless Mexican Pigs; Dry-cured ham; Intramuscular and subcutaneous fat; Fatty acids; Tryglicerides

1. Introduction

Hairless Mexican Pigs (HMP) have the ability to deposit great quantities of fat in and on their carcasses, which reduces their value at market. For this reason, Mexican swine producers have lost their interest in producing HMP; therefore, the numbers of HMP have declined considerably to the point of extinction (FAO, 1993). However, the ability of HMP to deposit fat is a remarkable feature, especially in the production of high quality, high priced cured meat products, such as "Serrano Ham" (De Anda, Rubio, Santillán, & Méndez, 1999). Fat contributes to the technological and sensory qualities of dry-cured ham (Antequera, López-Bote, Córdoba, García, Asensio, & Ventanas, 1992). Fat contributes to the juiciness of dry-cured hams (Girard, Goutefongea, Monin, & Touraille, 1986) and due to the lipolytic and oxidative processes that occurs during the curing treatment, it also influences the development of the aroma (López, Hoz, Cambero, Gallardo, Reglero, & Ordóñez, 1992). Fat composition changes during the curing process due to the salt added and the evaporative losses. Considerable research has been conducted to discern the role of fat in the quality of cured products, in particular high-priced loins and hams from Iberian pigs (ancestor of the HMP); however, no research has been conducted in cured products from HMP. Because fat plays an important role in the development of chemical and sensory characteristics of dry-cured products, the objective of this study was to compare the fatty acid profile and triglyceride composition of subcutaneous and intramuscular fat from raw and dry-cured hams from HMP.

* Corresponding author at present address: Cruz Blanca 286, San Miguel Toplilejo, Tlalpan 14050, Mexico D.F. Tel.: +52-5-8480515; fax: +52-5-8480514.

E-mail address: msalud@servidor.unam.mx (L.M.S. Rubio).

0309-1740/02/$ - see front matter
PII: S0309-1740(01)00163-2

2. Materials and methods

A group of eight castrated HMP males from Mizantla (Veracruz, Mexico), fed commercial cereal diet and with an average slaughter weight of 115 ± 34 kg were slaughtered to obtain their hams. After slaughter, hams were refrigerated for 24 h and then kept frozen for 60 days. After that, four hams were submitted to analysis of their fat and four hams were cured and the analyzed.

2.1. Cured hams production

Curing was developed throughout several ordered steps: salting, stabilization, drying and ripening. For the salting period, hams were rubbed with nitrate salt, and kept on salt one day per kg. Chamber temperature for the salting period was 0–4 °C and the relative humidity was 80–85%. After the first 2 days, hams that were at the bottom of the pile of salt were positioned on the top and vice-versa. Finally, superficial salt was removed from the hams using pressured hot water. During stabilization, hams were kept 40 days at 0–6 °C and 82–85% relative humidity. After that, hams were moved to a room where temperature gradually increased from 6 to 16 °C and humidity dropped to 75–80%, the drying process was initiated; hams where kept under these conditions for a total of 60 days. After this, and during 60 days, temperatures raised gradually from 16 to 22 °C at a relative humidity of 72–78%. Finally, hams were kept at 22–24 °C and a 65–68% of relative humidity for 20 days for the ripening period. Sampling was stopped at 180 days due to the rancid aroma that hams presented after this time.

2.2. Fatty acid determination

On each ham, the subcutaneous fat, with no skin, was trimmed off the ham to be analyzed. To study intramuscular fat, a homologous sample of all the muscle of the ham was obtained. To extract intramuscular fat 40 ml of hexane was added to 25 g of sample. The mix was submitted to ultrasound for 20 min. The organic phase (hexane) was then separated and another 40 ml of hexane were added and submitted to 20 min of ultrasound. Again the hexane was separated. The fat extracts (from each extraction) were mixed and the hexane was evaporated using nitrogen. To continue with hydrolysis, 15 mg of the fat extracted were added with 1.5 ml of a solution of 5% KOH in methanol. The vial was heated to 80 °C for 30 min. The solution was then cooled for 30 min at 18 °C. To obtain the methyl esters, 1.5 ml of a solution 10% HCl in methanol and 0.1 ml of BCl_3 in methanol were added to the vial. The mix was heated for 30 min at 80 °C and then cooled for 30 min at 18 °C. To dissolve the potassium salts, 1 ml of water was added. Two extractions were made using 2 ml of hexane. Then, hexane was used to complete the 5-ml vial, and 1 μl of this solution was injected in the gas chromatograph.

The equipment used to obtain the fatty acid profiles was: a gas chromatograph with flame ionization detector and Split-Splitless injector (Hewlett Packard 5890; Hewlett-Packard Co., Avondale, Pennsylvania, USA, 19311), an integrator (Hewlett Packard P3396A) and a capillary column of fused-silica SPTM-1000 (Supelco) of 30 m in length and 0.32 mm internal diameter, a thickness stationary phase of 0.25 μm. The column temperature was programmed as follow: 1 min at 150 °C, then increasing at 10 °C/min, until 220 °C, and then it was kept at 220 °C temperature for 15 min. The detector and injector temperatures were 220 °C. The carrier gas was hydrogen at a flow of 1 ml/min.

To identify and quantify the fatty acids, standards of each fatty acids were used: miristic (C14), palmitic (P; C16), palmitoleic (C16:1), stearic (S; C18), oleic (O; C18:1), linoleic (L; C18:2) and linolenic (C18:3). Identification of fatty acids on the samples was accomplished comparing retaining times for standards and samples. Data was reported as the total area of the injected methyl esters. The area was expressed as percentages.

2.3. Triglycerides determination

To determine triglycerides, samples were treated according to the following method: 10 mg of fat were dissolved in 10 ml of hexane and 1 ml of this solution was mixed with 200 μl of 1,1,3,3,3-hexamethyl disilazane and 100 μml of trimethyl cholorosilane. The mix was heated to 80 °C for 30 min. After that, it was cooled to 18 °C for 30 min. Finally, 1 ml of isoctane was added and 1-μ was injected in the gas chromatograph.

To obtain triglycerides profile, a gas chromatograph with flame ionization detector and injector type On column (Hewlett Packard 5890 Serie II Plus, Hewlett-Packard Co., Avondale, Pennsylvania, USA, 19311) connected to a computer (with a database: CHENN station of Hewlett-Packard) and a capillary column of fused-silica 5% fenil methyl silicone (Supelco) of 10 m of length, 0.25 mm internal diameter and 0.25 μm on film thickness was used. The column temperature was programmed as followed: 1 min at 60 °C and increasing by 15 °C/min until 350 °C, and then kept at 350° for 30 min. The detector temperature was 350 °C, using hydrogen as carrier gas at constant pressure.

To identify and quantify the results, standards of fatty acids, monoglycerides (1-monopalmitate and 1-monosterate), diglycerides [diesterato (SS, C36) and dioleato (OO, C36:1)], and triglycerides [trimiristate C42 (MMM), tripalmitate C48 (PPP), tristearate C54 (SSS), and trioleate C54:1 (OOO)] were submitted to the same process as the rest of the samples. To identify and quantify triglycerides in the samples, standards retention time

comparison was used. The chromatograms obtained were compared with the standards to know the fatty acids, mono-di- and triglycerides. Then chromatograms were analyzed to quantify the area of each dependent variable. This area was reported as percentage.

Results of fatty acid profile and triglycerides composition were analyzed using the SAS. To understand the effect of curing and location on fat composition, an analysis of variance, using type of ham and type of fat as the independent variables, was run for the results. The statistical method Lsmeans was used to generate and separate the means. Interactions between type of fat and type of ham were also included in the model.

3. Results and discussion

3.1. Fatty acid profile of raw and cured Hairless Mexican Pig hams fat

Table 1 shows the means and standard errors of the percentage of major fatty acids found in the fat of raw and cured HMP hams, in subcutaneous and intramuscular fat and the interactions between both independent variables.

Curing increased ($P<0.05$) the percentage of myristic, palmitic and steraric acids and decreased ($P<0.05$) the percentage of oleic and linoleic acids. Martín, Córdoba, Ventanas, and Antequera (1999) found that saturated fatty acids (SFA) increased and monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) decreased during the curing process. Oleic acid is considered to be responsible for the changes that fat suffers during the curing process, because it degrades to aldehydes and compounds that are responsible for the fine and special aroma in the final product. Linoleic acid has great influence on the rancidness process during curing, and it is also considered an indicator of the feeding regime of pigs (Vázquez, Menaya, Benito, Ferrera, Fallola, & García-Casco, 1996).

The enzymatic hydrolysis that occurs during the curing process leads to the increase of free fatty acids. Unsaturated fatty acids (UFA) are considered the precursors of aroma because they are susceptible to chemical and enzymatic oxidation to generate volatile compounds (Toldrá, 1998). Therefore, a higher percent of SFA was expected at the end of the curing process due to enzymatic hydrolysis. However, by the end of the curing process, UFA had been converted to other compounds making the final concentration lower than initial values.

Fatty acids percentages vary depending on breed, feeding (De Pedro & Pires, 1992; Ventanas et al., 1997) weight, sex, castration, exercise (Ventanas et al., 1997), type of tissue and anatomical location (Villegas, Henrick, Veum, McFate, & Baley, 1973; Vázquez et al., 1996). Subcutaneous fat had a higher ($P<0.05$) percentage of MUFA than intramuscular fat (sub: 48.0 ± 0.29, int:46.5 ± 0.36). Furthermore, percentages of PUFA, SFA and total UFA were similar ($P>0.05$) between subcutaneous and intramuscular fat. Vázquez et al. (1996) studying fat composition in different location of Iberian pig carcasses found that intramuscular fat of *biceps femoris* had the highest percent of MUFAS and the lowest percent of PUFAS compared with subcutaneous fat (external and internal). Timón, Ventanas, Carrapiso, Jurado, and García (2001) found that lipolytic and oxidative processes occur more intensively in subcutaneous than in intermuscular fat. Vázquez et al. (1996) found lower percentages of palmitic and palmitoleic acids

Table 1
Effect of curing and type of fat on fatty acid profile from HMP hams

Fatty acids[a]	Type of ham (H)[b]		Type of fat (F)[b]		Interaction[c]
	Raw (mean±S.E.)	Cured (mean±S.E.)	Intramuscular (mean±S.E.)	Subcutaneous (mean±S.E.)	H×F ($P<0.05$)
SFA	34.37a±0.30	39.41b±0.45	38.26±0.41	37.52±0.33	ns
Myristic (C14)	1.20a±0.53	1.53b±0.08	1.33±0.07	1.38±0.06	*
Palmitic (C16)	23.63a±0.23	26.30b±0.35	26.24a±0.32	23.44b±0.26	ns
Steraric (C18)	9.57a±0.13	11.84b±0.19	10.70±0.16	10.70±0.15	ns
MUFA	48.71a±0.26	45.82b±0.38	46.48a±0.36	48.00b±0.29	ns
Palmitoleic (C16:1)	3.19±0.09	2.94±0.14	3.39a±0.13	2.74b±0.11	ns
Oleic (C18:1)	45.51a±0.24	42.88b±0.36	43.09a±0.34	45.31b±0.26	ns
PUFA	10.51±0.29	9.46±0.43	10.40±0.40	9.56±0.32	ns
Linoleic (C18:2)	10.41a±0.28	9.41b±0.42	10.40±0.39	9.42±0.32	ns
Linolenic (C18:3)	0.10±0.02	0.04±0.03	0a±0	0.14b±0.03	ns
Total unsaturated	59.21a±0.29	55.27b±0.43	56.88±0.40	57.60±0.33	ns

[a] SFA = saturated fatty acids, MUFAS = monounsaturated fatty acids, PUFAS = polyunsaturated fatty acids.
[b] a, b, Means with different letter in the same row within main effects are different significantly ($P<0.05$).
[c] *, Significant interaction; ns, not significant interaction

Table 2
Effect of curing and type of fat on the acylglycerides profile

	Type of ham (H)[a]		Type of fat (F)[a]		Interaction[b]
Acylglycerides[c]	Raw (mean±S.E.)	Cured (mean±S.E.)	Intramuscular (mean±S.E.)	Subcutaneous (mean±S.E.)	H×F ($P<0.05$)
Monopalmitate	23.86±3.19	23.81±4.38	22.61±4.05	25.10±3.60	ns
SS (C36)	28.91[a]±3.13	8.94[b]±4.29	28.44[a]±3.97	9.41[b]±3.52	*
OO (C36:1)	1.92[a]±0.21	0.59[b]±0.28	1.89[a]±0.26	0.63[b]±0.23	*
PPO (C50)	4.03[a]±0.44	1.25[b]±0.60	3.96[a]±0.55	1.31[b]±0.49	*
POO (C52)	3.59[a]±0.39	1.11[b]±0.53	3.53[a]±0.49	1.69[b]±0.44	*
OOO (C54:1)	6.17[a]±0.67	1.9[b]1±0.91	6.07[a]±0.84	2.01[b]±0.75	*
Free Fatty Acids					
Palmitic (P)	9.22[a]±1.00	2.85[b]±1.37	9.07[a]±1.27	3.00[b]±1.12	*
Oleic (O)	6.03[a]±0.65	1.87[b]±0.90	5.94[a]±0.83	1.97[b]±0.73	*

[a] a,b, Means with different superscript in the same row within the main effects are different significantly ($P<0.05$)
[b] *=Significant interaction ($P<0.05$); ns, not significant interaction
[c] Acylglycerides: S= stearic; P= palmitic, O= oleic acids.

and higher percentages of oleic and linoleic acids in subcutaneous fat compared with intramuscular fat (Vázquez et al., 1996). Similar changes in palmitic and stearic acids were observed by Vázquez et al. (1996), who concluded that palmitic acid compensates the low stearic concentration in intramuscular fat. They also found a high variability (6 intramuscular to 11% subcutaneous) in the linoleic percentage, which has been related to the oxidation processes during the curing process. The percentage of linoleic acid found in this study was around 10%. Other studies support the high variability in the percentage of linoleic acid that pigs could have, even when they belong to the same breed or even if they have been under the same feeding regime (Cava, Ruiz, Ventanas, & Antequera, 1999; Flores, Biron, Izquierdo, & Nieto, 1988; Isabel, López-Bote, Rey, & Sanz, 1999).

The only significant interaction between the type of fat and type of ham was found for miristic acid. The percent of miristic in raw subcutaneous fat was similar to that of the cured subcutaneous fat but different to the other type of fats.

3.2. Trygliceride profile of raw and cured Hairless Mexican Pig hams fat

Monoglycerides, diglycerides, triglycerides and free fatty acids percentages found in raw and cured HMP hams, in subcutaneous and intramuscular fat and the interactions between both independent variables are shown on Table 2. Curing caused a reduction in the percentage of di- and triacylglicerols; however, monopalmitate unchanged.

The subcutaneous fat had lower acylglycerols, with the exception of the monoacylglycerol monopalmitate. Díaz and García (1997) found that subcutaneous fat had higher percentage of POO and PLL, and lower percentages of POL + POPO and OOO than intramuscular fat.

Significant interactions were found for all the acylglycerols between type of ham and type of fat, with the exception of monopalpitate. The differences for all the variables were found in the raw intramuscular fat (higher) with respect to the other types.

As mentioned earlier, the lypolitic process that fat undergoes during the curing process affects fat composition in the final product and this is reflected on the acylglycerols analysis, because free fatty acids are found in cured ham, which come, in part, from derivative activity (lipases) over triglycerides. Triglycerides from adipose tissue undergo an intense lipolysis during the curing process, especially during salting and post-salting stages (Toldrá, 1998). In this study, the cured ham presented lower percentage of free fatty acids than those in raw ham, due to their high susceptibility to oxidation when in the free form, which results on the oxidized compounds that act as flavor precursors of a great number of volatile compounds (Toldrá, 1998).

A study from Vázquez et al. (1996), mentioned that the mayor triglycerides in both white and Iberian pig fat are: PPO, POS, POO, POL, SOO, OOO and OOL; in the fat from HMP the triglycerides PPO, POO and OOO where the more abundant but in a lower percentage than those in other pigs. These differences could be due to the feeding regime (Toldrá, Flores, Aristoy, Virgili, & Paralari, 1996). HMP were fed commercial cereal diet, which is different from the acorn-based diet of the white and Iberian pigs.

The reduction of the acylglycerols found in the subcutaneous fat can be attributed to its external location, which allows a high exposition to lypidic degradation.

4. Conclusions

The determination of fatty acids showed that the curing process promotes the reduction of unsaturated fatty

acids and increases the percentage of saturated fatty acids. Subcutaneous fat had a higher percentage of monounsaturated fatty acids than intramuscular fat, and the percentages of PUFA and SAF were similar among fat depots. The curing process also promoted a reduction in the percentage of acylglycerols with the exception of monopalmitate.

Acknowledgements

Thanks to the CONACYT project 2385PB for the financial support in the realization of this project. Authors also liked to thank Sr. Ricardo Vergara (Cinta de Oro) for his support with the equipment to cure the experimental hams.

References

Antequera, T., López-Bote, C. J., Córdoba, J. J., García, C., Asensio, M. A., & Ventanas, J. (1992). Lipid oxidative changes in the processing of Iberian hams. *Food Chemistry*, *45*, 105–110.

Cava, R., Ruiz, J., Ventanas, J., & Antequera, T. (1999). Oxidative and lipolytic changes during ripening of Iberian hams as affected by feeding regime: extensive feeding and alpha-tocopheryl acetate supplementation. *Meat Science*, *52*, 165–172.

De Anda, S. A., Rubio, L. M. S., Santillán, V. M. C., & Méndez, M. R. D. (1999). Análisis descriptivo cuantitativo del jamón tipo "serrano" elaborado a partir del cerdo pelón mexicano. *Alimentaria*, *306*, 29–35.

De Pedro, E., & Pires, J. (1992). Effect de la raza y la alimentación en la composición en grasos de productos del cerdo ibérico: 1. lomo. Segunda Coloquio de Cerdo Mediterráneo Badajoz, España.

Díaz, I., & García, R. (1996). Triglycerides composition of fresh ham fat from Iberian pigs produced with different systems of animal nutrition. *Food Chemistry*, *55*(4), 383–387.

FAO. (1993). World watch list. In L. Ronan, & S. Beate (Eds.), *For domestic animal diversity*. (1st Ed.). Rome: Food and Agriculture Organization of the United Nations.

Flores, J., Biron, C., Izquierdo, L., & Nieto, P. (1988). Characterization of green hams from Iberian pigs by fast analysis of subcutaneous fat. *Meat Science*, *23*, 253–262.

Girard, J. P., Goutefongea, R., Monin, G., & Touraille, C. (1986). Qualities des viands de porc. In J. M. Perz, P. Mornet, & A. M. Rerat (Eds.), *Le Porc et son elevage* (pp. 461–480).

Isabel, B., Lopez-Bote, C. J., Rey, A. I., & Sanz, A. R. (1999). Influence of dietary α tocopheryl acetate supplementation of pigs on oxidative deterioration and weight loss in sliced dry-cured ham. *Meat Science*, *51*, 227–232.

López, M. O., de la Hoz, L., Cambero, M. I., Gallardo, E., Reglero, G., & Ordóñez, J. A. (1992). Volatile compounds of dry hams from Iberian pigs. *Meat Science*, *31*, 267–277.

Martín, L., Córdoba, J. J., Ventanas, J., & Antequera, T. (1999). Changes in intramuscular lilpids during ripening of Iberian dry-cured ham. *Meat Science*, *51*, 129–134.

Timón, M. L., Ventanas, J., Carrapiso, A. I., Jurado, A., & García, C. (2001). Subcutaneous and intermuscular fat characterisation of dry-cured Iberian hams. *Meat Science*, *58*, 85–91.

Toldrá, F. (1998). Desarrollo de las características de textura y flavor; contribución enzimatica. El jamón curado: Tecnología y análisis de consumo. In *Proceedings of 44th International Congress of Meat Science and Technology* (pp. 42–54), 1 September 1998, Barcelona, Spain.

Toldrá, F., Flores, M., Aristoy, M. C., Virgili, R., & Paralari, G. (1996). Patterns of muscle proteolytic and lipolytic enzymes from light and heavy pigs. *Journal of Science Food Agriculture*, *71*, 124–128.

Vázquez, C., Menaya, C., Benito, J., Ferrera, J. L., Fallola, A., & García Casco, J. M. (1996). Perfil de ácidos grasos en distintas localizaciones del tejido adiposo del cerdo ibérico. *Eurocarne*, *52*, 17–22.

Ventanas, J., Ruiz, J., Antequera, T., Martín, L., García, C., Tejeda, J. F., & Cava, R. (1997). Características de la materia prima y del producto que determinan la calidad del jamón ibérico: composición de la grasa. *Eurocarne*, *62*, 35–42.

Villegas, F. J., Henrick, H., Veum, T. L., McFate, K. L., & Baley, M. (1973). Effect of diet and breed on fatty acid composition of porcine adipose tissue. *Journal of Animal Science*, *36*, 663–669.