Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 103 (2007) 1159–1167
Food Chemistry
www.elsevier.com/locate/foodchem

Fatty acid and volatile compounds from salami manufactured with yerba mate (*Ilex paraguariensis*) extract and pork back fat and meat from pigs fed on diets with partial replacement of maize with rice bran

R.M.L. de Campos [a], E. Hierro [a,*], J.A. Ordóñez [b], T.M. Bertol [c], N.N. Terra [d], L. de la Hoz [a]

[a] *Departamento de Nutrición, Bromatología y Tecnología de los Alimentos, Facultad de Veterinaria, Universidad Complutense de Madrid, 28040 Madrid, Spain*
[b] *Instituto de Ciencia y Tecnología de la Carne, Universidad Complutense de Madrid, Avda. Puerta de Hierro s/n, 28040 Madrid, Spain*
[c] *Embrapa Suínos e Aves, RB 153. Km 110, CEP: 89700-000 Concórdia/SC, Brazil*
[d] *Departamento de Tecnologia e Ciencia dos Alimentos, Universidade Federal de Santa Maria, Santa Maria/RS, Brazil*

Received 1 June 2006; received in revised form 24 July 2006; accepted 9 October 2006

Abstract

Four batches of salami were manufactured. Back fat and meat from pigs fed on diets with maize or maize partially substituted with rice bran (maize/rice bran, 62/38, w/w) were used to prepare two different batches, M and M/RB, respectively. The other two batches (M-YM or M/RB-YM) were prepared as batch M or M/RB, added a yerba mate (*Ilex paraguariensis*) extract. Salamis did not show differences in percentual general composition, texture analysis or sensory features. The TBAR values were affected by the storage time. The use of yerba mate extract controlled the lipid oxidation.

In general, salamis M/RB and M/RB-YM showed higher concentrations of C18:2 $n-6$, C18:3 $n-3$ and polyunsaturated $n-6$ and $n-3$ fatty acids. Salamis M and M-YM showed high C18:1 $n-9$, and saturated and monounsaturated fatty acid contents. Volatile compounds from lipid oxidation were the most abundant in salamis M/RB and M, while volatiles from fermentation were dominant in salamis M-YM and M/RB-YM.

Keywords: Salami; Pork; Fatty acids; Maize; Rice bran; Yerba mate

1. Introduction

Rice bran is a by-product of rice industry containing about 87% dry matter, 11–15% crude protein, 15–20% lipids and 6.5–10% ash (Juliano & Bechtel, 1994; Rostagno et al., 2000). Its fat is characterised by a fatty acid composition of 34–37% oleic, 36–42% linoleic, 21–23% palmitic, 1.7% stearic and 1.2% linolenic (Juliano, 1994; Miyazawa, Tazawa, & Fujino, 1978). It also contains proteins with a good amino acid balance for monogastric animals (EMBRAPA, 1991). The low price of rice bran permits the reduction of the final cost of pig rations. Research work published so far with this by-product shows that it is possible to use about 40% of defatted rice bran in swine feeds without negatively affecting either animal performance or carcass quality (Borin, Gai, & Silveira, 1988).

The fatty acid composition of pork muscle and adipose tissue reflects the fat diet composition (Hoz et al., 2003; Kouba & Mourot, 1999; Wiseman & Agunbiade, 1998). Dietary fatty acids are absorbed without modification from the intestine and are incorporated into tissue lipids. The effect of diet fatty acids is mainly observed in the polyunsaturated fatty acids, being less marked in saturated and monounsaturated (Wood, 1984). For this reason it is possible that the dietary inclusion of rice bran in pig feed could

* Corresponding author. Tel.: +34 91 3943946; fax: +34 91 3943743.
E-mail address: hierro@vet.ucm.es (E. Hierro).

0308-8146/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2006.10.018

modify the fatty acid composition of pork back fat and meat. The rice bran lipids are characterised by a high content of linoleic acid (Juliano, 1994; Miyazawa et al., 1978). The accumulation of this fatty acid, with the concomitant increase in unsaturation, in both pork and meat products manufactured with this ingredient, could lead to an increase of susceptibility to oxidation with a clear decrease of meat and meat products quality (Allen & Foegeding, 1981; Gurr, Harwood, & Frayn, 2002).

The objective of this work was to evaluate the use, in salami manufacture, of pork back fat and meat from pigs fed with diets in which a partial replacement of maize with rice bran was made. To control the possible salami high lipid susceptibility to oxidation, an ethanolic extract of yerba mate (*Ilex paraguariensis*) was used. *I. paraguariensis* is the most commercialized plant of South America. The antioxidant properties of this plant have been reported by several authors (Bracesco et al., 2003; Gugliucci, 1996; Schinella, Troiani, Dávila, de Buschiazzo, & Tournier, 2000; VanderJagt, Ghattas, VanderJagt, Crossey, & Glew, 2002). VanderJagt et al. (2002), who analyzed the antioxidant capacity of 30 aqueous extracts prepared from medicinal plants of New Mexico, showed that *I. paraguariensis* had the highest antioxidant value. Infusions of yerba mate are traditionally consumed in South America as a mild stimulant beverage with a bitter taste. Some of the therapeutic properties of this plant (choleretic, hypocholesteremic, hepatoprotective) are attributed to the phenolic constituents of the leaves and are conserved when the infusion is prepared. (Filip, Lotito, Ferraro, & Fraga, 2000; Filip, López, Giberti, Coussio, & Ferraro, 2001).

2. Materials and methods

2.1. Experimental design

Twenty castrated crossed male pigs (MS 60 male, based on Duroc, Large White and Pietrain × female F1, based on Large White and Landrace) (Fávero, 2000) of approximately 25 kg, were randomly distributed and located in individual cages and fed a conventional pig diet until they weighed around 40 kg. Ten pigs were randomly assigned to each experimental diet. All pigs were fed *ad libitum* for 80 days and then weighed. Two diets were formulated; one was based on maize (M) and a second had maize partially substituted with rice bran (maize/rice bran, 62/38, w/w) (M/RB).

Ingredients, chemical composition and fatty acids of tested diets are shown in Table 1. Determination of feed composition was carried out according to the Association of Official Analytical Chemists procedures (A.O.A.C., 1995).

2.2. Slaughter, sample collection and chemical analysis

Animals were stunned, slaughtered and exsanguinated at a local slaughterhouse at Concordia, Santa Catarina State (Brazil). At 24 h *post-mortem*, pork from the whole legs of the right side of the carcass and the back fat were removed.

Table 1
Ingredients, chemical composition and calculated metabolizable energy of diets

	Diets	
	Maize/rice bran	Maize
Ingredients (g kg^{-1} of diet)		
Maize	496	782
Soybean meal	172	188
Rice bran	300	–
Sodium chloride	3	3
Dicalcium phosphate	2	8
Lysine supplement	1.6	1.6
Vitamin and mineral premix[a]	2	2
Antiparasite	0.1	0.1
Methionine	–	0.3
Threonine	0.4	0.3
Choline chloride	0.4	0.4
Kaolin ($Al_2O_7Si_2 \cdot 2H_2O$)	8	3
Limestone ($CaCO_3$)	11.6	7.8
Adsorbent (SiO_2)	3	3
Zinc bacitracin	0.2	0.2
Calculated energy (MJ kg^{-1})	13.5	13.5
Proximate analysis		
Dry matter (DM g kg^{-1} feed)	887.5	876.4
Crude protein (g kg^{-1} WM[b])	144	131
Crude fat (g kg^{-1} WM[b])	57.0	29.9
Crude fiber (g kg^{-1} WM[b])	65.2	22.9
Crude ash (g kg^{-1} WM[b])	85.3	43.8
Fatty acid composition (g kg^{-1} WM[b])		
C14:0	0.0307	0.0119
C16:0	3.16	1.69
C18:0	0.435	0.347
C18:1 $n-9$	7.79	3.91
C18:2 $n-6$	8.75	5.61
C18:3 $n-3$	0.518	0.198
C22:6 $n-3$	0.546	0.258
SFA[c]	3.62	2.05
MUFA[c]	7.81	3.91
PUFA[c]	9.81	6.07
Total fatty acids (g kg^{-1} WM)	21.2	12.0

[a] Vitamin and mineral premix provided (per kg of diet): vitamin A, 8000 IU, vitamin D_3, 2000 IU, vitamin E, 10 IU, menadione, 0.7 mg, vitamin B_1, 0.6 mg, vitamin B_2, 3.0 mg, niacin, 15 mg; pantothenic acid, 7 mg, vitamin B_6, 1 mg, vitamin B_{12}, 12 μg, choline, 100 mg, Fe, 60 mg; Cu, 7 mg, Zn, 45 mg, Mn, 5.0 mg, Se, 0.15 mg, I, 0.2 mg.
[b] WM: wet matter.
[c] SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

2.3. Preparation of yerba mate extract

Yerba mate extract (YM) was obtained from dried and powdered leaves of *Ilex paraguariensis*. One hundred grammes of these leaves were homogenised in 400 ml of a mixture of ethanol 95%/water (4/1, v/v) for 3 min and left for 1 h at room temperature. After that, the homogenate was filtered through a Whatman No. 1 filter paper. Then, the solid phase was twice reextracted using ethanol

as solvent. Ethanolic extracts were collected all together and solvent was eliminated in a Büchi Rotavapor R-200. One hundred grams of dried and powdered yerba mate leaves gave rise to 89.3 ml of extract. The extraction was done using amber glass. Samples were maintained at 4 °C and protected from light.

2.4. Preparation of salamis

The back fat and the meat from three or four pigs on the same diet were used to prepare a lot of sausage. All the sausages were manufactured on the same day, using the same technology, ingredients and formulation, which were: (a) raw material (% w/w): pork meat (90), back fat (10); (b) additives and other ingredients (g/kg): NaCl (25), $NaNO_3$ (0.12), $NaNO_2$ (0.08), ascorbic acid (2.5), glucose (4.0), sucrose (6.0), powdered black pepper (2.0), garlic (5.0), nutmeg (2.0), monosodium glutamate (1.0); and (c) a microbial starter (0.05%) of *Lactobacillus sakei*, *Kocuria varians* and *Staphylococcus xylosus* in the same proportions.

Three lots of sausages were manufactured for each diet (M/RB or M). Meat and back fat were processed in a mincer equipped with an adjustable plate set at a hole diameter of 5 mm, and then, inoculated with the starter culture. After the addition of additives and other ingredients, the mixture was divided in two parts; one was treated (5 ml/kg) with the yerba mate extract (YM) and the other was treated with 5 ml of water/kg. Following this procedure, four different batches of salami were obtained: M/RB, M/RB-YM, M and M-YM. The final mixture was stuffed into synthetic casings (70 mm in diameter) and left to ripen in a Menoncin ripening cabinet (Menoncin, Erechim/RS, Brazil). The sausages were fermented at 25 °C and 90% relative humidity (RH) for 24 h. Then, the temperature and RH were slowly reduced to reach 15 °C and 75% RH, respectively, after 25 days. At that time, the final product was vacuum-packed and maintained at room temperature.

Samples were taken at the end of the ripening process (just before vacuum packing, time of storage 0 days) and after 30 and 60 days of vacuum storage. Samples were vacuum-packed, frozen at −18 °C and stored until analysis.

2.5. Chemical analysis

Protein (Kjeldahl nitrogen), moisture (oven air-drying method) and ash (muffle furnace) were analysed following A.O.A.C. (1995) procedures. Water activity (a_w) was determined using a Decagon CX1 hygrometer (Decagon Devices Inc., Pullman, WA) at 25 °C.

For lipid analysis, the methods of Hanson and Olley (1963) (lipid extraction), Hartman and Lago (1973) (methylation), and Firestone (1998) (analysis of fatty acid methyl esters) were used. Fatty acids were analysed using 0.5 μl of sample in a Konik mod. HRGC 4000 gas chromatograph (Barcelona, Spain) equipped with a flame ionization detector and a capillary column, CP Sil 88 Tailor Made FAME (Chrompak). The chromatographic conditions were as follows: the initial column temperature was 180 °C, which was kept for 2 min, then raised to 225 °C at 3.0 °C/min and finally held for 15 min; injector and detector temperatures were 270 °C. Helium was used as carrier gas, at a flow rate of 2 ml/min. Fatty acid methyl esters were identified by comparison with standards run previously, alone or together with samples. Fatty acid methyl esters were quantified as percentage of total methyl ester weight.

Lipid oxidation of the sausages was determined using the 2-thiobarbituric acid method (TBARs) described by Salih, Smith, and Dawson (1987). Samples (5 g) and 15 ml of 0.38 M $HClO_4$ were homogenized for 3 min using a Polytron probe in a glass vessel immersed in an ice bath. To avoid further oxidation, 0.5 ml of a 0.19 M BHT ethanolic solution was added. The homogenate was centrifuged (3000*g*, 5 min, 5 °C) and filtered through Whatman No. 54 paper. An aliquot (0.7 ml) was mixed with the same volume of a 0.02 M TBA solution and heated at 100 °C for 30 min. After cooling in an ice water bath, the mixture was centrifuged at 3000*g* for 15 min at 5 °C. Finally, the absorbance was measured at 532 nm. A stock solution of 1×10^{-7} M 1,1,3,3 tetraethoxypropane (TEP) in distilled water was used to prepare dilutions ranging from 1×10^{-8} to 8×10^{-8} mol TEP. Two millilitres of each TEP dilution were mixed with 2 ml of 0.02 M TBA solution, incubated, and absorbance at 532 nm was used to plot a standard curve. Results were expressed as mg malondialdehyde/kg sample.

2.6. Texture analysis

The texture profile analysis (TPA) was used to evaluate the sausage texture (Bourne, 1978; Szczesniak, 1986), using the Stable Micro System Mod. TA-XTZi Texture Analyzer (Stable Micro Systems, Godalming, England) equipped with a cylindrical P/25 probe. This procedure involved cutting samples approximately 1.5 cm thick and 2.5 cm wide after discarding the external layer (2 cm) of the sausage piece. Samples were allowed to reach room temperature and then compressed, twice, to 50% of their original thickness. The following parameters were determined: hardness (N), maximum force required to compress the sample (H); springiness (m), ability of the sample to recover its original shape after the deforming force was removed (S); adhesiveness ($N \times s$), area under the abscissa after the first compression; cohesiveness, extent to which the sample could be deformed prior to rupture ($A2/A1$, $A1$ were the total energy required for the first compression and $A2$ the total energy required for the second compression); gumminess (N), force required to disintegrate a semisolid meat sample for swallowing ($H \times$ cohesiveness); chewiness (J), work required to masticate the sample before swallowing ($S \times$ gumminess).

2.7. Volatile compound analysis

The extraction of the volatile compounds was performed using solid-phase microextraction (SPME). A SPME

device (Supelco, Bellefonte, PA, USA) containing a fused-silica fibre (10 mm length) coated with a 75 μm layer of Carboxen-PDMS (polydimethylsiloxane) was used. Salami was ground with a commercial grinder and 1 g was transferred to a 4 ml vial. The vial was screw-capped with a laminated Teflon-rubber disk. The needle of the SPME holder was inserted into the sample vial through the septum and then the fibre was exposed to the headspace. The fibre was conditioned prior to analysis by heating it in a gas chromatograph injection port at 300 °C for 60 min. Extraction was performed at 35 °C for 30 min. Before extraction, samples were equilibrated for 15 min at the same temperature as used for extraction. Once sampling was finished, the fibre was withdrawn into the needle and transferred to the injection port of the gas chromatograph–mass spectrometer (GC–MS) system. Duplicate analysis was conducted.

Analyses were performed on a Hewlett-Packard 5890 Series II gas chromatograph fitted with a HP5972 mass spectrometer and a G1034 Chemstation (Hewlett-Packard, Palo Alto, CA, USA). A split/splitless injection port, held at 250 °C, was used to thermally desorb the volatiles from the SPME fibre onto the front of a CP-Sil 8 CB low bleed/MS fused silica capillary column (60 m × 0.25 mm i.d., 0.25 μm film thickness, Chrompack, Middelburg, The Netherlands). The injection port was in splitless mode, the split valve opening after 3 min. During a desorption period of 5 min, volatile compounds were cryofocussed by immersing 15 cm of column adjacent to the heater in a solid CO_2 bath while the oven was held at 40 °C. After desorption, the bath was then removed and chromatography achieved by holding at 40 °C for 2 min, followed by a programmed rise to 280 °C at 4 °C/min, and holding for 5 min. A series of *n*-alkanes (C6–C22) (Sigma) was analysed under the same conditions to obtain linear retention index (LRI) values for the aroma components.

The mass spectrometer was operated in electron impact mode with an electron energy of 70 eV and an emission current of 50 μA. Compounds were identified by first comparing their mass spectra with those contained in the HP Wiley 138 Mass Spectral Database and then comparing the LRI values with either those of authentic standards or with published values.

2.8. Sensory analysis

The salamis were evaluated by a panel of fifteen tasters selected among the members of the Centro Nacional de Pesquisa de Suinos e Aves (Embrapa, Concordia/SC), which were previously trained in the sensory assessment of meat products. The evaluations were performed in individual booths built according to the criteria of the International Standards Organisation (ISO, 1981a). The tasters were given unsalted crackers and room temperature water between samples.

The assessors evaluated the following attributes of the sausages on a non-structured 5 cm hedonic scale: odour, colour, texture and taste (0 = very unpleasant to 5 = very pleasant), juiciness (0 = very dry to 5 = very juicy) and taste quality (sweet, bitter, sour, salty and meaty impression; 0 = not detected to 5 = very strong). The global quality was calculated from the expression: overall quality = (Colour × 0.1) + (Texture × 0.25) + (Odour × 0.15) + (Flavour × 0.5). This expression was calculated taking into account the opinion of the 18 tasters who, in a study on commercial fermented sausages, had been asked to assess the relative importance of the different sensory characteristics (Bruna, Fernández, Hierro, Hoz, & Ordóñez, 1999).

Additionally, a triangle test (ISO, 1981b) was conducted, after the former two sessions, to determine if there were sensory differences between dry-fermented sausage batches. To reduce fatigue, panel members conducted three sessions per day (two of the hedonic rating and one of the triangular test) with a minimum of 1 h between sessions.

2.9. Statistical analysis

Data were analysed using the SAS General Linear Model (1999–2001). An individual batch of sausages was the experimental unit for analysis of all data. Dietary treatment and time of storage were considered as independent variables. The comparative analysis between means was conducted using the Duncan multiple range test. Data were presented as the means of each group and the standard deviation (SD) of the mean.

3. Results and discussion

The dry matter, fat, protein, water activity (a_w) and pH of salamis manufactured with pork tissues of animals fed with rice bran and maize (M/RB) or maize (M) are shown in Table 2. Also, values of sausages treated with the yerba mate extract (M/RB-YM and M-YM) are included (Table 2). No differences were observed ($P > 0.05$) among the different sausages for values of protein, fat or ash (g/100 g D.M.) at different periods of vacuum storage. The fat and protein contents were close to those described by Zanardi, Dorigoni, Badiani, and Chizzolini (2002) and can be considered as common for this kind of meat product.

The fatty acid (FA) composition of salami is shown in Table 3. Two different FA profiles were observed, the first corresponding to those products manufactured with pork tissues of animals fed with rice bran and maize (M/RB and M/RB-YM) and the second to sausages prepared from pigs fed with maize (M and M-YM). On the other hand, the use of the yerba mate extract as ingredient did not show any influence on the salami fatty acid composition. In all sausages, the major FA was C18:1 $n-9$, ranging from 41% in M/RB-YM to 48% in M. In salami (Table 3), significant differences ($P < 0.05$) among dietary treatments were found for six fatty acids (C16:0, C16:1 $n-7$, C18:0, C18:1 $n-9$, C18:2 $n-6$ and C18:3 $n-3$). The use of pork from animals fed with rice bran (M/RB, M/RB-YM) gave rise to a significant increase ($P < 0.05$) in the C18:2 $n-6$, C18:3

Table 2
Chemical composition, a_w and pH of salami

	Dietary treatment[a]			
	M/RB	M/RB-YM	M	M-YM
Dry matter (%)	62.85 ± 2.66	63.07 ± 3.32	63.30 ± 2.17	64.73 ± 1.33
Fat (% DM[b])	53.04 ± 2.39	53.23 ± 1.31	53.27 ± 2.39	53.77 ± 2.19
Protein (% DM[b])	39.5 ± 1.19	39.0 ± 1.55	38.9 ± 1.87	38.7 ± 1.99
Ash (% DM[b])	7.11 ± 0.22	7.55 ± 0.73	7.57 ± 0.59	7.38 ± 0.55
a_w	0.856 ± 0.006	0.841 ± 0.007	0.853 ± 0.002	0.852 ± 0.003
pH	5.45 ± 0.12	5.12 ± 0.14	5.51 ± 0.16	5.20 ± 0.21

Values are means of data from different vacuum storage times (0, 30 and 60 days).
No significant differences were found ($P > 0.05$).
[a] Salami manufactured with back fat and meat of pigs fed diets containing maize and rice bran (62/38, w/w) (M/RB) or maize (M). Salami M/RB treated with 0.5% of an ethanolic extract of yerba mate (M/RB-YM) and salami M treated with 0.5% of an ethanolic extract of yerba mate (M-YM).
[b] DM: dry matter, g/100 g dry product.

Table 3
Fatty acid composition (% of total methyl esters) of salami manufactured with pork back fat and meat from animals fed with different diets

Fatty acid	Dietary treatment[a]			
	M/RB	M/RB-YM	M	M-YM
C12:0	0.06 ± 0.01	0.07 ± 0.001	0.06 ± 0.01	0.06 ± 0.001
C14:0	1.05 ± 0.01	1.19 ± 0.10	1.16 ± 0.01	1.15 ± 0.05
C16:0	20.9 ± 0.58a	22.1 ± 0.62a	23.0 ± 0.57b	23.4 ± 0.82b
C16:1 $n-7$	0.95 ± 0.08a	1.02 ± 0.04a	1.66 ± 0.05b	1.97 ± 0.09b
C17:0	0.25 ± 0.03	0.25 ± 0.02	0.27 ± 0.03	0.31 ± 0.04
C18:0	10.5 ± 0.67a	10.9 ± 0.46a	12.6 ± 0.61b	13.1 ± 0.70b
C18:1 $n-9$	41.8 ± 0.49a	40.5 ± 0.27a	48.1 ± 0.62b	46.4 ± 0.66b
C18:2 $n-6$	20.6 ± 0.57b	20.3 ± 0.55b	9.88 ± 0.33a	9.98 ± 0.54a
C18:3 $n-3$	1.18 ± 0.08b	1.11 ± 0.05b	0.47 ± 0.03a	0.46 ± 0.02a
C20:1 $n-11$	0.93 ± 0.08	0.84 ± 0.05	0.86 ± 0.09	0.91 ± 0.07
C20:0	0.84 ± 0.07	0.70 ± 0.10	0.66 ± 0.04	0.90 ± 0.08
C20:4 $n-6$	0.42 ± 0.06	0.40 ± 0.05	0.40 ± 0.05	0.38 ± 0.07
C20:5 $n-3$	0.05 ± 0.001	0.04 ± 0.002	0.04 ± 0.002	0.05 ± 0.001
C22:4 $n-6$	0.29 ± 0.02	0.31 ± 0.03	0.30 ± 0.05	0.32 ± 0.08
C22:5 $n-3$	0.03 ± 0.001	0.04 ± 0.002	0.04 ± 0.001	0.03 ± 0.002
C22:0	0.03 ± 0.001	0.04 ± 0.001	0.04 ± 0.001	0.03 ± 0.001
C22:6 $n-3$	0.01 ± 0.001	0.01 ± 0.002	0.01 ± 0.001	0.01 ± 0.001
Total SFA	33.6 ± 0.96a	35.2 ± 0.19a	37.8 ± 0.30b	39.0 ± 0.23b
Total MUFA	43.7 ± 0.47a	42.4 ± 0.30a	50.6 ± 0.87b	49.3 ± 0.45b
Total PUFA	22.6 ± 0.63b	22.2 ± 0.58b	11.1 ± 0.36a	11.2 ± 0.12a
PUFA $n-6$	21.3 ± 0.57b	21.0 ± 0.55b	10.6 ± 0.33a	10.7 ± 0.42a
PUFA $n-3$	1.27 ± 0.08b	1.21 ± 0.05b	0.56 ± 0.03a	0.55 ± 0.03a
$n-6/n-3$	16.8 ± 0.98	17.4 ± 0.59	18.85 ± 0.68	19.3 ± 1.84

Values are means of data from different vacuum storage times (0, 30 and 60 days).
a,b: means of the same row with different letters are significantly different ($P < 0.05$).
[a] Salami manufactured with back fat and meat of pigs fed diets containing maize and rice bran (62/38, w/w) (M/RB) or maize (M). Salami M/RB treated with 0.5% of an ethanolic extract of yerba mate (M/RB-YM) and salami M treated with 0.5% of an ethanolic extract of yerba mate (M-YM).

$n-3$ and PUFA contents while salami from animals fed on diets based on maize (M, M-YM) showed high C18:1 $n-9$, saturated and monounsaturated FA contents. The salami enrichment in C18:2 $n-6$ and C18:3 $n-3$ (batches M/RB and M/RB-YM) was not concomitant with a modification in the $n-6/n-3$ ratio which was about 17–19 in all salami. The PUFA contents (Table 3) of M/RB dietary treatments (mean value about 22) were higher than those reported by Chan, Brown, Church, and Buss (1996), Zanardi et al. (2002), Warnants, Van Oeckel, and Boucque (1998) for salami, who described values of approximately 12, 13.5 and 16.3, respectively.

The FA composition of dry-fermented sausages remains generally unchangeable after processing and ripening (Hoz, D'Arrigo, Cambero, & Ordóñez, 2004; Kristinsson, Jonsdottir, Valdimarsdottir, & Thorkelsson, 2001). Therefore, the FA profile of these meat products is similar to that of raw material (pork back fat and meat). In pigs, dietary FAs are absorbed unchanged from the intestine and incorporated into tissue lipids. The PUFA linoleic and linolenic acids cannot be synthesised *in situ*; thus tissue concentrations respond rapidly to dietary changes. However, saturated and monounsaturated FA are *de novo* synthesised; hence their concentrations are less readily influenced by

the diet (Wood, 1984; Rosenvold & Andersen, 2003). Bearing these ideas in mind it appears that the salami fatty acid composition is a clear image of the pig feed FA.

An increase in the content of PUFA may lead to "soft" meat and meat products of inferior quality (Jorgensen, Jensen, & Eggum, 1996; Warnants et al., 1998). Lack of adipose tissue firmness can cause an obturation of the meat grinder and, moreover, the storage of meat and meat products could also be more difficult with a reduced shelf life when unsaturated fatty acids are present in large amounts due to their high susceptibility to oxidation (Mourot & Hermier, 2001; Allen & Foegeding, 1981). In this respect, and to avoid problems during the transformation of pork, it is reported that adipose tissue of good quality should contain less than 12% linoleic acid and at least 12% stearic acid (Girard, Bout, & Salort, 1988). The salami from the M/RB batches did not cover the requirements for linoleic and stearic acids.

The TBARs values (Fig. 1) of salami (M/RB, M/RB-YM, M and M-YM) were affected by the dietary treatment ($P < 0.05$) and the storage period ($P < 0.05$). Always, batches treated with the yerba mate extract (M/RB-YM and M-YM) showed significantly ($P < 0.05$) lower values than did products manufactured without the extract (M/RB and M). These data indicate a control of the lipid oxidation (antioxidant activity) by the yerba mate extract (Fig. 1). All TBARs values were moderated, indicating a low lipid oxidation which could not induce a rancid taste (Chizzolini, Novelli, & Zanardi, 1998; Hoz et al., 2004). Zanardi et al. (2002) found very similar TBARs values, also close to 1 mg malondialdehyde/kg after 60 days in vacuum storage.

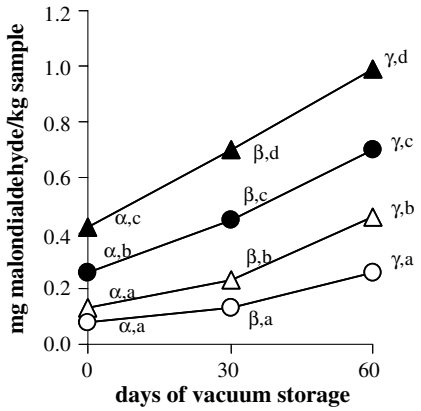

Fig. 1. Changes in TBARs values during the storage of experimental salamis prepared: (▲) from back fat and meat of pigs fed diets containing maize and rice bran (62/38, w/w) (batch M/RB); (●) from back fat and meat of pigs fed diets containing maize (batch M); (△) batch M/RB added with 0.5% of an ethanolic extract of yerba mate (M/RB-YM); and (○) batch M added with 0.5% of an ethanolic extract of yerba mate (M-YM). α, β, γ: values of the same batch with different letters are significantly different ($P < 0.05$); a, b, c, d: values at the same day of storage with different letters are significantly different ($P < 0.05$).

Although the low stearic and high linoleic acid level could induce salami softness, no significant differences in the results of the texture profile analysis (TPA) were found (Table 4). The mean values were close to those of conventional dry- fermented sausages (Bruna et al., 2001).

In total, 42 volatile compounds were identified using a SPME-GC/MS method. Table 5 shows the mean areas of the identified compounds, grouped according to their chemical class. They included 7 aldehydes, 6 acids, 6 ketones, 6 terpenes, 4 esters, 4 sulphur compounds, 3 alcohols, 3 hydrocarbons, 2 pyrazines and 1 furan.

There is a clear pattern when the volatile compounds of the different batches are compared. The lipid oxidation group was significantly higher ($P < 0.05$) in the salamis manufactured without the yerba mate extract, representing around 47% of the total chromatographic area. This fact is mainly due to their higher levels of straight-chain saturated aldehydes (C6–C9), hydrocarbons (C5–C7) and some ketones (2-pentanone and 2-heptanone) and is in total agreement with the high TBARs value (Fig. 1) found in batches M/RB and M.

Volatiles from carbohydrate fermentation also showed significant ($P < 0.05$) differences among batches. Acetic acid and some ketones, such as 2,3-butanedione, 2-butanone and 3-hydroxy-2-butanone, were responsible for these differences. This group of compounds was the most abundant in batches prepared with the yerba mate extract. This profile suggests that the herb extract could promote the growth of lactic acid bacteria. The stimulatory effects of manganese and magnesium ions on the growth of *Lactobacillus* are widely known (Kandler, 1983; Zaika & Kissinger, 1984) and it has been reported that both ions are present at high levels in yerba mate (Tenorio & Torija, 1991). Coventry and Hickey (1993) have reported that the inclusion of

Table 4
Textural profile analysis of salami

Salami[a]	Parameter				
	Hardness (*N*)	Springiness (*m*)	Cohesiveness	Gumminess (*N*)	Chewiness (*J*)
M/RB	125.73 ± 13.6	0.0070 ± 0.002	0.47 ± 0.02	71.8 ± 6.2	50.2 ± 11.1
M/RB-YM	135.10 ± 14.5	0.0066 ± 0.001	0.45 ± 0.01	69.1 ± 8.1	53.5 ± 8.9
M	117.80 ± 19.1	0.0072 ± 0.001	0.43 ± 0.01	73.6 ± 4.7	55.3 ± 7.6
M-YM	131.57 ± 13.4	0.0069 ± 0.002	0.46 ± 0.00	74.3 ± 5.2	54.2 ± 12.3

Values are means of data from different vacuum storage times (0, 30 and 60 days).
No significant differences were found ($P > 0.05$).
[a] Salami manufactured with back fat and meat of pigs fed diets containing maize and rice bran (62/38, w/w) (M/RB) or maize (M). Salami M/RB treated with 0.5% of an ethanolic extract of yerba mate (M/RB-YM) and salami M treated with 0.5% of an ethanolic extract of yerba mate (M-YM).

Table 5
Volatile compounds found in the headspace of salami manufactured with pork back fat and meat from animals fed with different diets

LRI[a]	Compound	Origin[b]	Dietary treatment[c]				Method of identification[d]
			M/RB	M/RB-YM	M	M-YM	
	Alcohols		29,456	38,427	27,138	34,916	
503	Ethanol	F	25,046	34,352	23,217	30,597	ms + lri
740	3-Methyl-1-butanol	AC	4065	3654	3564	3987	MS + LRI
744	2-Methyl-1-butanol	AC	345	421	357	332	MS + LRI
	Aldehydes		48,872b	11,418a	52,263b	12,006a	
654	3-Methylbutanal	AC	2174	4165	3505	3460	MS + LRI
662	2-Methylbutanal	AC	1564	2357	1151	1662	MS + LRI
802	Hexanal	LO	40,771b	2233a	43,040b	4154a	MS + LRI
902	Heptanal	LO	1547b	372a	1462b	590a	MS + LRI
972	Benzaldehyde	AC	123	142	196	210	MS + LRI
1005	Octanal	LO	775	603	588	482	MS + LRI
1105	Nonanal	LO	1918	1547	2320	1448	MS + LRI
	Hydrocarbons		30,524b	3189a	40,382b	2729a	
500	Pentane	LO	22,929b	1703a	28,765b	867a	MS + LRI
600	Hexane	LO	4138b	1249a	4909b	1517a	MS + LRI
700	Heptane	LO	3457b	237a	6708b	345a	MS + LRI
	Furans		336b	74a	225b	59a	
994	2-Pentyl furan	LO	336b	74a	225b	59a	MS + LRI
	Ketones		9069a	65,196b	16,974a	47,895b	
	2-Propanone	MI	3838a	15,222b	6652a	13,980b	ms
587	2,3-Butanedione (*diacetyl*)	F	1117a	6091b	1292a	4756b	ms + lri
604	2-Butanone	F	902a	2304b	887a	2711b	MS + LRI
683	2-Pentanone	LO	406	305	599	228	MS + LRI
711	3-Hydroxy-2-butanone (*acetoin*)	F	1479a	41,101b	5875a	26,044b	MS + LRI
898	2-Heptanone	LO	1327b	173a	1669b	176a	MS + LRI
	Esters		2579	2210	2907	2300	
615	Ethyl acetate	ME	1945	1746	2195	1801	MS + LRI
846	Ethyl 2-methylbutanoate	ME	160	190	418	147	MS + LRI
849	Ethyl 3-methylbutanoate	ME	381	192	141	240	MS + LRI
877	3-Methylbutyl acetate	ME	93	82	153	112	ms + lri
	Acids		17,503a	37,885a,b	26,259a	51,427b	
649	Acetic acid	F	13,495a	32,831b	20,970a	45,687b	MS + LRI
782	2-Methylpropanoic acid	AC	263a	611b	360a	816b	ms + lri
818	Butanoic acid	F	255	151	504	459	MS + LRI
857	3-Methylbutanoic acid	AC	1762	2562	2725	2778	ms + lri
868	2-Methylbutanoic acid	AC	777	819	816	906	ms + lri
1005	Hexanoic acid	MI	951	911	884	781	MS + LRI
	Pyrazines		370	418	599	678	
912	2,6-Dimethylpyrazine	MI	291	298	354	458	MS + LRI
1014	Trimethylpyrazine	MI	80	120	245	220	MS + LRI
	Terpenes		4313	5441	5824	5918	
934	α-Pinene	S	857	660	673	599	ms + lri
989	β-Myrcene	S	196	249	386	300	ms + lri
1018	α-Phellandrene	S	2068	2689	3240	3387	ms + lri
1012	α-Terpinene	S	381	662	527	477	ms + lri
1031	Limonene	S	195	330	315	482	MS + LRI
1033	1,8-Cineole	S	616	853	684	673	ms + lri
	Sulphur compounds		19,570	19,233	21,091	20,566	
712	3-Methylthio-1-propene	S	12,091	11,129	13,248	11,504	ms + lri
864	3,3′-Thiobis-1-propene	S	4586	5246	4143	5119	ms + lri
910	Methyl 2-propenyl disulfide	S	925	832	1334	1256	ms + lri
1065	Di-2-propenyl disulfide	S	1969	2026	2367	2687	ms + lri
	Total volatiles		162,593	183,491	193,663	178,495	

Values are means (total area counts $\times 10^4$) of three salamis at the end of the ripening period.
a,b: values in the same row with different letters are significantly different ($P < 0.05$).
[a] Linear retention index on a CP-Sil 8 CB low bleed/MS column.
[b] Origin: F (carbohydrate fermentation); AC (amino acid catabolism); LO (lipid oxidation); ME (microbial esterification); S (spices and condiments); MI (miscellaneous: contaminants, unknown).
[c] Salami manufactured with back fat and meat of pigs fed diets containing maize and rice bran (62/38, w/w) (M/RB) or maize (M). Salami M/RB treated with 0.5% of an ethanolic extract of yerba mate (M/RB-YM) and salami M treated with 0.5% of an ethanolic extract of yerba mate (M-YM).
[d] MS + LRI, mass spectrum and LRI agree with those of authentic compounds; ms + lri, mass spectrum and LRI in agreement with the literature; ms, mass spectrum agrees with spectrum in the HP Wiley 138 Mass Spectral Database.

Table 6
Acceptance test of some sensory attributes and overall quality of salami

Salami[a]	Characteristic				
	Colour	Odour	Flavour	Texture	Overall quality[b]
M/RB	3.8 ± 0.25	3.3 ± 0.34	3.7 ± 0.30	3.6 ± 0.22	3.6 ± 0.24
M/RB-YM	3.6 ± 0.22	3.8 ± 0.29	3.8 ± 0.25	3.7 ± 0.21	3.7 ± 0.20
M	4.0 ± 0.11	3.4 ± 0.16	3.2 ± 0.13	3.4 ± 0.16	3.3 ± 0.10
M-YM	3.8 ± 0.20	3.5 ± 0.17	3.7 ± 0.21	3.8 ± 0.20	3.7 ± 0.16

Values are means of data from salamis at the end of the ripening period.
No significant differences were found ($P > 0.05$).
[a] Salami manufactured with back fat and meat of pigs fed diets containing maize and rice bran (62/38, w/w) (M/RB) or maize (M). Salami M/RB treated with 0.5% of an ethanolic extract of yerba mate (M/RB-YM) and salami M treated with 0.5% of an ethanolic extract of yerba mate (M-YM).
[b] Overall quality = (colour × 0.1) + (odour × 0.15) + (flavour × 0. 5) + (texture × 0.25).

appropriate levels of manganese in the salami mixture via the starter culture preparation was critical in order to obtain optimal fermentation rates. Therefore, the addition of the yerba mate extract in the manufacture of salamis M-RB/YM and M-YM could be equivalent to this recognised effect.

Volatiles arising from spices made up around 14% of the total chromatographic area in all batches. They were mainly sulphur compounds and, to a lesser extent, terpenes, derived from garlic and black pepper, respectively. Different levels of these compounds have also been reported in salami by other authors (Meynier, Novelli, Chizzolini, Zanardi, & Gandemer, 1999; Procida, Conte, Fiorasi, Comi, & Gabrielli Favreto, 1999), depending on the amount of spices formulated.

The salami sensory analysis did not show differences (Table 6) among the salamis. These results are in total agreement with the TBARs values and the results of TPA analysis found in the different batches of salami.

4. Conclusions

From this study it can be concluded that it is possible to use pork and back fat from pigs fed on diets with partial substitution of maize with rice bran to manufacture salami without general compositional or sensory modifications. The fatty acid pattern was profoundly modified, with significant increases of both linolenic acid and PUFA. The use of a yerba mate extract as an ingredient of salami controlled the lipid oxidation since lower TBARs values and volatiles from lipid oxidation were detected.

Acknowledgement

R.M.L. de Campos is a beneficiary of a predoctoral grant from the Brazilian Government (CAPES).

References

Allen, C. E., & Foegeding, E. A. (1981). Some lipid characteristics and interactions in muscle foods- a review. *Food Technology, 35*, 253–257.
A.O.A.C. (1995). Official methods of analysis (16th ed.). Washington, DC: Association of Official Analytical Chemists.
Borin, J. R. H., Gai, J. N., & Silveira, J. C. G. L. (1988). Efeitos da adição de diferentes níveis de farelo de arroz desengordurado em rações para suínos nas fases de crescimento e terminação. *Revista Brasileira de Zootecnia, 17*, 553–563.
Bourne, M. C. (1978). Texture profile analysis. *Food Technology, 32*, 62–72.
Bracesco, N., Dell, M., Rocha, A., Behtash, S., Menini, T., Gugliucci, A., et al. (2003). Antioxidant activity of a botanical extract preparation of *Ilex paraguariensis*: prevention of DNA double-strand breaks in *Saccharomyces cerevisiae* and human low-density lipoprotein oxidation. *Journal of Alternative and Complementary Medicine, 9*, 379–387.
Bruna, J. M., Fernández, M., Hierro, E., Hoz, L., & Ordóñez, J. A. (1999). Effect of the combined use of Pronase E and a fungic extract (*Mucor racemous* forma *Sphaerosporus*) on the ripening of dry fermented sausages. *Food Science and Technology International, 5*, 327–338.
Bruna, J., Hierro, E., Hoz, L., Mottram, D. S., Fernández, M., & Ordóñez, J. A. (2001). The contribution of *Penicillium aurantiogriseum* to the volatile composition and sensory quality of dry fermented sausages. *Meat Science, 59*, 97–107.
Chan, W., Brown, J., Church, S. M., & Buss, D. H. (1996). *Sixth supplement to the fifth edition of McCance and Widdowson's. The composition of food*. London: The Royal Society of Chemistry, Information Service (The Royal Society of Chemistry and Ministry of Agriculture, Fisheries and Food, Meat Products and Dishes), p. 149.
Chizzolini, R., Novelli, E., & Zanardi, E. (1998). Oxidation in traditional Mediterranean meat products. *Meat Science, 49*, S87–S99.
Coventry, M. J., & Hickey, M. W. (1993). The effect of spices and manganese on meat starter culture activity. *Meat Science, 33*, 391–399.
EMBRAPA Empresa Brasileira De Pesquisa Agropecuária (1991). *Tabela de composição química e valores energéticos de alimentos para suínos e aves* (3rd ed.). Concórdia: EMBRAPA, CNPSA, p. 97.
Fávero, J. A. (2000). Evolução das características de desempenho e de carcaça da linha sintética Embrapa MS 58. In: *Congreso Mercosur de Producción Porcina* (p. G3). Buenos Aires, Argentina: no. 01.
Filip, R., López, P., Giberti, G., Coussio, J., & Ferraro, G. (2001). Phenolic compounds in seven South American *Ilex* species. *Fitoterapia, 72*, 774–778.
Filip, R., Lotito, S. B., Ferraro, G., & Fraga, C. G. (2000). Antioxidant activity of *Ilex paraguariensis* and related species. *Nutrition Research, 20*, 1437–1446.
Firestone, D. (1998). Official methods and recommended practices of the American Oil Chemists Society, (fifth ed., vol. I – II). Champaign: (MÉTODOS Ce 1–62; Ce 1b–89; Ce 1c–89).
Girard, J. P., Bout, J., & Salort, D. (1988). Lipides et qualités des tissus adipeux et musculaires de porc, facteurs de variations. *Journées de la Recherche Porcines France, 20*, 255–278.
Gugliucci, A. (1996). Antioxidant effects of *Ilex paraguariensis*: induction of decreased oxidability of human LDL in vivo. *Biochemical and Biophysical Research Communications, 224*, 338–344.

Gurr, M. I., Harwood, J. L., & Frayn, K. N. (2002). *Lipid biochemistry. An introduction* (5th ed.). Oxford: Blackwell.

Hanson, S. W. F., & Olley, J. (1963). Application of the Bligh and Dyer method of lipid extraction to tissue homogenates. *Biochemical Journal, 89*, 101P–102P.

Hartman, L., & Lago, R. C. A. (1973). Rapid preparation of fatty acid methyl esters from lipids. *Laboratory Practice, 22*, 475–476.

Hoz, L., D'Arrigo, M., Cambero, I., & Ordóñez, J. A. (2004). Development of an $n-3$ fatty acid and α-tocopherol enriched dry fermented sausage. *Meat Science, 67*, 485–495.

Hoz, L., López-Bote, C. J., Cambero, M. I., D'Arrigo, M., Pin, C., Santos, C., et al. (2003). Effect of dietary linseed oil and (α-tocopherol on pork tenderloin (*Psoas major*) muscle. *Meat Science, 65*, 1039–1044.

ISO. (1981a). Analyse sensorielle guide pour l'implantation d'un local destiné aux analyses sensorielles. (ISO-DP 6658). International Organization for Standardization. Genéve, Switzerland.

ISO. (1981b). Analyse sensorielle. Methodologie. Essai Triangulaire. (*ISO/TC 34/SC 12*). International Organization for Standardization. Genéve, Switzerland.

Jorgensen, H., Jensen, S. K., & Eggum, B. O. (1996). The influence of rapeseed oil on digestibility, energy metabolism and tissue fatty acid composition in pigs. *Acta Agriculturae Scandinavica Section A-Animal Science, 46*, 65–75.

Juliano, B. O. (1994). Polysaccharides, proteins and lipids of rice. In *Rice chemistry and technology* (pp. 59–174). St. Paul, Minnesota, USA: The American Association of Cereal Chemists, Inc.

Juliano, B. O., & Bechtel, D. B. (1994). The rice grain and its gross composition. In *Rice chemistry and technology* (pp. 17–57). St. Paul, Minnesota, USA: The American Association of Cereal Chemists, Inc.

Kandler, O. (1983). Carbohydrate metabolism in lactic acid bacteria. *Antoine van Leeuwenhoek, 49*, 209–224.

Kouba, M., & Mourot, J. (1999). Effect of feeding linoleic acid diets on lipogenic enzyme activities and on the composition of the lipid fraction of the fat and lean tissues in the pig. *Meat Science, 52*, 39–45.

Kristinsson, H. G., Jonsdottir, R., Valdimarsdottir, T., & Thorkelsson, G. (2001). Oxidative stability of pork pepperoni during processing and different packing and storage conditions as influenced by fat source. *Journal of Muscle Foods, 12*, 301–315.

Meynier, A., Novelli, E., Chizzolini, R., Zanardi, E., & Gandemer, G. (1999). Volatile compounds of commercial Milano salami. *Meat Science, 51*, 175–183.

Miyazawa, T., Tazawa, H., & Fujino, Y. (1978). Molecular species of triglyceride in rice bran. *Cereal Chemistry, 55*, 138–145.

Mourot, J., & Hermier, D. (2001). Lipids in monogastric animal meat. *Reproduction Nutrition Development, 41*, 109–118.

Procida, G., Conte, L. S., Fiorasi, S., Comi, G., & Gabrielli Favreto, L. (1999). Study on volatile components in salami by reverse carrier gas headspace gas chromatography–mass spectrometry. *Journal of Chromatography A, 830*, 175–182.

Rosenvold, K., & Andersen, H. J. (2003). Factors of significance, for pork quality – a review. *Meat Science, 64*, 219–237.

Rostagno, H. S., Albino, L. F. T., Donzele, J. L., Ferreira, A. S., Oliveira, R. F., & Lopes, D. C. (2000). In H. S. Rostagno (Ed.), *Tabelas Brasileiras para aves e suinos; composicao de alimentos e exigencias nutricionais. Vicosa UFV* (p. 141).

Salih, A. M., Smith, D. M., & Dawson, L. E. (1987). Modified extraction of 2-thiobarbituric acid method for measuring lipid oxidation in poultry. *Poultry Science, 66*, 1483–1488.

SAS Institute Inc. (1999–2001). System for Microsoft Windows, Release 8.2 [CD-ROM], Cary, NC, USA.

Schinella, G. R., Troiani, G., Dávila, V., de Buschiazzo, P. M., & Tournier, H. A. (2000). Antioxidant effects of an aqueous extract of *Ilex paraguariensis*. *Biochemical Biophysical Research Communications, 269*, 357–360.

Szczesniak, A.S. (1986). Sensory Texture Evaluation Methodology. In: Reciprocal Meat Conference Proceedings, (Vol. 39, pp. 86–96).

Tenorio, M. D., & Torija, M. E. (1991). Mineral elements in mate herb (*Ilex paraguariensis* St H.). *Archivo Latinoamericano de Nutrición, 41*, 441–454.

VanderJagt, T. J., Ghattas, R., VanderJagt, D. J., Crossey, M., & Glew, R. H. (2002). Comparison of the total antioxidant content of 30 widely used medicinal plants of New Mexico. *Life Sciences, 70*, 1035–1040.

Warnants, N., Van Oeckel, M. J., & Boucque, C. V. (1998). Effect of incorporation of dietary polyunsaturated fatty acids in pork backfat on the quality of salami. *Meat Science, 49*, 435–445.

Wiseman, J., & Agunbiade, J. A. (1998). The influence of changes in dietary fat and oils on fatty acid profiles of carcass fat in finishing pigs. *Livestock Production Science, 54*, 217–227.

Wood, J. D. (1984). Fat deposition and the quality of fat tissue in meat animals. In J. Wiseman (Ed.), *Fats in animal nutrition* (pp. 407–415). London: Butterworth.

Zaika, L. L., & Kissinger, J. C. (1984). Fermentation enhanced by spices: identification of active component. *Journal of Food Science, 49*, 5–9.

Zanardi, E., Dorigoni, V., Badiani, A., & Chizzolini, R. (2002). Lipid and colour stability of Milano-type sausages: effect of packing conditions. *Meat Science, 61*, 7–14.