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Article

Influence of Dietary Vitamin E Supplementation on Fatty Acid Composition of the *Biceps Femoris* Muscle and Cooked Ham during Storage

V. L. F. Souza,*[a] R. S. S. F. Silva[b] and N. Bragagnolo[c]

[a]*Departamento de Zootecnia, Universidade Estadual de Maringá, 87020-900 Maringá-PR, Brazil*

[b]*Departamento de Ciência e Tecnologia de Alimentos, Universidade Estadual de Londrina, 86051-990 Londrina-PR, Brazil*

[c]*Departamento de Ciência de Alimentos, Faculdade de Engenharia de Alimentos, Universidade Estadual de Campinas, 13083-862 Campinas-SP, Brazil*

O objetivo deste trabalho foi avaliar a influência da suplementação de dietas para suínos com diferentes níveis de vitamina E sobre a composição de ácidos graxos do *bíceps femoris* músculo e do presunto processado com o mesmo músculo durante 60 dias de estocagem a 5 °C. Em adição, foi realizada análise sensorial para verificar a influência das dietas suplementadas com Vitamina E no sabor e aroma do presunto cozido. Dezesseis suínos (oito machos castrados e oito fêmeas) foram divididos em quatro grupos. Cada grupo recebeu uma dieta controle (sem vitamina E), e dietas formuladas com 100, 200 e 400 mg de vitamina E/kg de ração. Não foi observado diferença significativa nos níveis de ácidos graxos entre o *bíceps femoris* músculo e o presunto cozido e entre os diferentes tratamentos com vitamina E. Entretanto, foi observada diferença significativa entre os níveis de C18:1n9 de machos castrados e fêmeas. Durante a estocagem foram observadas perdas de C16:1n7 e C18:1n9 nas amostras controle e suplementadas com 100 mg de vitamina E/kg de ração. A composição de ácidos graxos nas amostras proveniente de suínos alimentados com dietas contendo 200 mg de vitamina E/kg de ração ou mais não foi alterada durante 60 dias de estocagem a 5 °C e não houve modificação do sabor e aroma.

The objective of this work was to evaluate the influence of feeding pork with different levels of vitamin E on the fatty acids composition of *biceps femoris* muscle and processed ham, during 60 days at 5 °C. To verify off flavors proveked by suplementation of vitamin E in the pig diets, sensory analysis of cooked ham was carried out. Sixteen pigs (eight barrows and eight gilts) were divided in four groups. Each group received a control diet, and diets formulated with 100, 200 and 400 mg of vitamin E/kg of feed. No significant difference in the fatty acids composition was observed between *biceps femoris* muscle and cooked ham samples, and between different treatments with vitamin E. However, it was observed significative difference between the C18:1n9 contents of barrows (49.0 ± 1.7%) and gilts (45.0 ± 1.5%). During storage were observed loss of C16:1n7 and C18:1n9 in the control samples and samples supplemented with 100 mg vitamin E/kg of feed. However, the composition of fatty acids in the samples supplemented with 200 and 400 mg vitamin/kg of feed at 5 °C during 60 days was not modified, and it was not observed the appearance of off flavors.

Keywords: fatty acids, oxidation, vitamin E, antioxidative**,** storage, sensory analysis, pigs

Introduction

Dietary monounsatured fat has received increasing attention from medical and scientifical communities because of its promising health benefits. In certain areas of Mediterranean region, the typical diets, rich in olive oil, that contains high levels of oleic acid, can be related to the low incidence of heart disease.[1] Inclusion of oleic acid in the diet has been shown to decrease the level of the undesirable plasma lipid, and low density lipoprotein-cholesterol, without decreasing the desirable plasma lipid, and high density lipoprotein-cholesterol.[2]

Cardiovascular chronic diseases have been related to the ingestion of diets with high contents of saturated fatty acids as in some types of meat.[3] The composition of fat of monogastric meat animals shows the highest content of

*e-mail: vlfsouza@uem.br

monounsaturated fatty acids mainly oleic acid. However, unsaturated lipids are particularly susceptible to oxidation during meat processing and storage.[4]

One way to increase the oxidative stability of lipids and cholesterol in foods is to increase the amount of natural antioxidants such as α-tocopherol (vitamin E) or β-carotene in the diet. Feeding diets supplemented with vitamin E to animals like chickens, cows, and pigs resulted in vitamin accumulation in the animal muscle, and higher oxidative stability under prooxidative condition, such as in cooking and storage.[5,6] Few studies have been done so far on lipid oxidative process in processed produts, therefore this research was focused on the protective effect of vitamin E on the fatty acid profiles of the bíceps femoris muscle and cooked ham lipids during storage at 5 °C for 60 days. In addition, sensory evaluation of cooked ham was carried out to find off flavors provoked by suplementation of vitamin E in the pig diet.

Experimental

Vitamin E in the from of α-tocopherol was supplied by Hoffmann-LaRoche, Nertley, NJ, USA.

Animal management and treatments

Sixteen crossbred pigs (*Large white X Landrace X Pietran*), eight barrows and eight gilts, with an average initial weight of 24 kg were individually penned. The pigs were alloted to one of the four treatment groups, each treatment consisting of four pigs (two barrows and two gilts).

The four treatments consisted of a control diet without supplementary vitamin E, and diets additioned of 100, 200 and 400 mg of vitamin E/kg of feed. Pigs were fed *ad libitum* and were housed in an environmental controlled pig pen. The feeding trial was divided into a growing phase (65 to 123 days) and a finishing phase (124 to 181 days). The feeding period was completed in 116 days.

Slaughter and processing procedures

At the end of the feeding period, pigs were weighed and feed was removed approximately 12 h before slaughter. The average weight of the pigs was around 110 kg. Pigs were transported from Marechal Cândido Rondon city to Medianeira city, where they were humanly slaughtered at a commercial slaughterhouse. After a 24 h chilling period, the *biceps femoris* muscles were removed from the carcass to produce the cooked ham that were further analysed. Before processing the cooked ham, samples of *biceps femoris* were taken from each ham and stored at −20 °C prior to analysis.

Cooked ham processing

The cooked hams were produced in industrial unit; the samples were deboned and membranes, tendons, fatty tissue, and rind removed. Brine was evenly injected into the ham muscles with a Retus Inject-O-mat type multineedle brine injecton. The cooked ham was manufactured with 64.4% of deboned ham and 35.6% of brine. The composition of the brine (in % v/v) was salt (4.78%), sodium erythorbate (0.13%), mono-sodium glutamate (0.30%), maltodextrin (5.48%), phosphate (2.44%), nitrate (2.14%), carageenan (1.52%), protein isolate (4.48%), sugar (2.39%), cochineal dye (0.03%) and water (76.31%). For distribution of the curing ingredients throughout the entire product each ham was tumbled for 40 min and stored for 12 h at 2 °C. After that time the hams were tumbled again for 40 min, wrapped in polyethilene film, vacuum pressed, and heated at 62 °C for 30 min. The cooked hams were stored at 5 °C for two months and analysed after 0, 30 and 60 days.

Analytical determination

Slices of *biceps femoris* were thawed and homogeinazed in a blender. The extration and determination of total lipids were undertaken according to the method of Folch *et al.*[7] The lipids were transesterified to methyl esters according to the Hartman and Lago's[8] method and analysed by gas chromatography (Philips PU 4550) using a CP-Sil 88 column of length 50 m, i.d. 0.25 μm and 0.20 μm film thickess of cyanoalkyl polysiloxane and equipped with a flame ionisation detector and a work station (Borwin, France). The injector temperatures was maintained at 270 °C and the detector at 300 °C. Column oven temperature was maintained at 180 °C (isothermic). Hydrogen carrier gas flow was 2.25 mL min^{-1} and "make-up" gas was nitrogen with 30 mL min^{-1}. The individual fatty acid peaks were identified by comparing retention times with standard fatty acid mixtures. A total of 36 saturated, monounsaturated and polyunsaturated fatty acid standards (Sigma and Polyscince, USA) were used. The fatty acids profile of cooked ham samples were analysed in the same way as described above, after 0, 30 and 60 days of storage.

Statistical analysis

The statistical significance of the difference between the fatty acids profile in *biceps femoris* muscle and cooked ham was determinad by ANOVA. Significance of the difference between means was determined by Tukey test. Statistical

analysis of the fatty acids profile from cooked ham between treatments and sex during 60 days period (0, 30 to 60 days) was tested in a split-plot design.[9] The principal variables were treatments and sex. Time was the split variables. All data were analyzed using the General Linear Model procedure of SAS.[10] The tests of the multiple comparision were performed by Tukey ($P < 0.05$). The panel of variance analysis is in the Table 1.

Table 1. Panel of Variance analysis

Source of variation	Degree of freedom
Treatments (T)	(4 – 1) = 3
Sex (S)	(2 – 1) = 1
T x S (a)	(3 x 1) = 3
Time (t)	(3 – 1) = 2
T x t	(3 x 2) = 6
Resídue (b)	(47 – 15) = 32
Total	(48 – 1) = 47

Sensory analysis

The objective of the sensory analysis was to detect possible development of flavour by the addition of the vitamin E in the pigs diet. Samples of the cooked ham with 30 days of storage were judged through a ranking test. The panel consisted of the employees of the Institute of Food Technology (Center of Meat Technology, Campinas, Brazil), 60% women and 40% men in the range of the 21 to 52 years. Results were analysed using the Newell & McFarlane Table.[11] In addition, flavor, characteristic flavor, aroma and texture were judged using a 7 points hedonic scale (1 = disliked very much; 7 = liked very much). Results generated by purched intention scale were evaluated through the Dunnett Test (Figure 1). Eight samples of the barrows' cooked ham were analysed first.

Results and Discussion

The fatty acid composition was not affected by the different stages of processing, which did not present significant difference ($P > 0.05$) between the *biceps femoris* muscle and cooked ham processed with the same muscle (Table 2).

The different levels of vitamin E supplementation in the diet did not influence the fatty acid composition ($P > 0.05$), the averages are present in Table 2. Similar results were obtained by Lauridsen *et al.*[12] in the *psoas mayor* muscle of swines and by De Winne and Dirinck[13] in the chest and thigh of chickens. However, Onibi *et al.*[14] have observed

Table 2. Effect of the processing on the profile of fatty acids of biceps femoris muscle and cooked ham[a]

Fatty acids (%)	Biceps femoris muscle	Cooked ham	CV (%)
C10:0	0.09[a]	0.11[a]	1.24
C12:0	0.06[a]	0.07[a]	1.87
C14:0	1.24[a]	1.18[a]	2.41
C15:0	0.85[a]	0.92[a]	1.63
C16:0	23.15[a]	23.89[a]	2.90
C16:1n7	3.56[a]	3.42[a]	2.17
C18:0	11.48[a]	11.72[a]	3.04
C18:1n9	46.62[a]	46.17[a]	3.42
C18:2n6	9.89[a]	9.12[a]	2.81
C18:3n3	0.48[a]	0.61[a]	3.87
C18:4n3	1.06[a]	1.12[a]	1.43
C20:0	0.12[a]	0.09[a]	2.24
C20:2n6	0.08[a]	0.10[a]	1.69
C20:4n6	1.32[a]	1.48[a]	2.42
ΣSFA[b]	36.59[a]	37.98[b]	3.71
ΣMUFA[c]	50.18[a]	49.59[b]	2.53
ΣPUFA[d]	12.83[a]	13.77[b]	3.98
PUFA/SFA	0.35[a]	0.36[a]	3.17
Total Lipids	1.90[a]	1.95[a]	2.15

[a]Means obtained among the four treatments, 2 sexes and true repetitions; [b]Total saturated fatty acids; [c]Total monounsaturated fatty acid; [d]Total polyunsaturated fatty acid; Different letters in the same line are significantly different ($P < 0.05$).

reduction in the amount of saturated and polyunsaturated fatty acids, and an increase in the monounsaturated, when compared the *swine's longissimus dorsi* fatty acid contents, fed with control diet to the ones fed with diet supplemented with 200 mg of vitamin E/kg diet. Shortland *et al.*[15] observed reduction in the levels of C12:0, C14:0 and C16:0 and increase in the levels of C18:0, in the muscles of veal fed with 500 mg of vitamin E/kg of feed. These results strengthen the theory that the vitamin E promotes the chain extension of C12:0, C14:0 and C16:0 to C18:0. These chain extensions occur in the mitochondria, with the addition of acetyl-CoA, or in the microsomes with malonyl-CoA as source of the acetyl groups.[16]

The principal fatty acids found in *biceps femoris* muscle and cooked ham were: C18:1n9 (46.39 ± 1.59%), C16:0 (23.52 ± 0.68%), C18:0 (11.60 ± 0.35%), C18:2n6 (9.50 ± 0.27%), C16:1n7 (3.49 ± 0.07%), C20:4n6 (1.48 ± 0.02%) and C14:0 (1.18 ± 0.03%). These results agree to the ones obtained by Lauridsen *et al.*[12] in *psoas mayor* muscle of swine, except for C14:0 and C18:3n3 which were higher and C18:0 lower than the results obtained in this work (Table 3). On the other hand, Bragagnolo and Rodriguez-Amaya[17] found lower values for C18:1n9, C18:0, C16:1n7 and C18:4n3 and higher for C14:0, C18:2n6 and C20:4n6 in fresh ham meat than the results obtained in this work.

Name/Age: Occupation/hour/data:

1. Taste the sample and circle the scale at that point which reflects your judgment about the flavor.
   (7) Like very much
   (6) Like moderately
   (5) Like slightly
   (4) Neither like or dislike
   (3) Dislike slightly
   (2) Dislike moderately
   (1) Dislike very much
2. Describe what do you like very much and dislike very much about the flavor.

   _____
3. Indicate how much the flavor of sample comes close to the characteristic.
   (3) much to strong of the standard
   (2) moderately strong of the standard
   (1) slightly strong of the standard
   (0) just about right (standard)
   (-1) slightly weak of the standard
   (-2) moderately weak of the standard
   (-3) much to weak of the standard
4. Smell the sample and circle the scale at the point which reflects your judgment about the aroma.
   (7) Like very much
   (6) Like moderately
   (5) Like slightly
   (4) Neither like or dislike
   (3) Dislike slightly
   (2) Dislike moderately
   (1) Dislike very much
5. Evaluate the texture and circle the scale at the point which reflects your judgment about it.
   (7) Like very much
   (6) Like moderately
   (5) Like slightly
   (4) Neither like or dislike
   (3) Dislike slightly
   (2) Dislike moderately
   (1) Dislike very much
6. Describe what do you like very much and dislike very much about the texture.

   _____ _____
7. If this product was avaible at marked, would you:
   (1) certainly purchase
   (2) probability purchase
   (3) perhaps purchase/perhaps not purchase
   (4) probability not purchase
   (5) certainly not purchase
8. Order the samples according to the most agreeable color to the less agreeable.

   _____
   (most agreeable) (less agreeable)

Figure 1. Ballot illustrating the sensory analysis of the cooked ham at 30 days of storage.

However, the discrepancies observed among the fatty acids can be explained by the differences between breed,[18] feed,[19] climate,[20] sex[21] or sampling. Most references give the fatty acid composition of meat *in nature*; however, the results obtained by Isabel *et al.*,[22] are among the few to present the composition of the fatty acids in processed ham, although the differences observed were not significant ($P > 0.05$); the results of Table 3 showed a small increase in the total amount of polyunsaturated fatty acids, to the ones that received diets supplemented with 200 mg of vitamin E/kg feed.

The composition and the quality of the meat are influenced by the sex, due to differences of hormone, with barrows presenting higher saturated fatty acids contents than the gilts.[21] In fact, difference in oleic acid contents (C18:1n9) was significant ($P < 0.05$) in the samples of *biceps femoris* muscle between sex, and as consequence also in the samples of cooked ham. The barrows exhibited

Table 3. Comparing the fatty acids composition of mentioned references

Fatty acids (%)	Ham[17]	Cooked Ham[22]	Ham[12]	Ham[a]	Cooked Ham[b]
C10:0	0.20	0.00	0.00	0.09	0.11
C12:0	0.30	0.00	0.00	0.06	0.07
C14:0	2.30	6.00	1.6	1.24	1.18
C16:0	24.10	20.0	23.3	23.15	23.89
C16:1n7	3.00	1.70	3.30	3.56	3.42
C18:0	9.60	14.00	9.70	11.48	11.72
C18:1n9	38.80	44.50	47.20	46.62	46.17
C18:2n6	13.00	12.80	10.1	9.89	9.12
C18:3n3	0.50	0.40	2.30	0.48	0.61
C18:4n3	0.50	0.00	0.00	1.06	1.12
C20:0	0.00	0.18	0.22	0.12	0.09
C20:2n6	0.02	0.90	0.38	0.08	0.10
C20:4n6	2.10	0.60	0.17	1.32	1.48
∑SFA[c]	36.50	40.18	34.82	36.59	37.98
∑MUFA[d]	41.80	46.20	50.50	50.18	49.59
∑PUFA[e]	16.2	14.70	12.95	12.83	13.77
PUFA/SFA	0.44	0.36	0.37	0.35	0.36

[a]Average of the hams on the 4 treatments; [b]Average of cooked ham at time zero of storage on the 4 treatments; [c]Total saturated fatty acids; [d]Total monounsaturated fatty acid; [e]Total polyunsaturated fatty acid.

higher values than those presented by the gilts, 49.0 ± 0,8% and 45.2 ± 0,7%, respectively. LesKamich *et al.*,[23] also observed influence of the diet in the fatty acid composition of the swine meat; however, the oleic acid levels (C18:1n9) were higher in the gilts (36.7%), when compared to the males (35.3%).

Table 4 shows the averages obtained during the treatments, of cooked hams fatty acid contents, during the 60 days of storage under refrigeration.

In all samples it was observed no significant differences ($P > 0.05$) among the fatty acids contents in the first 30 days of storage; however, after 60 days, a significant reduction in the contents of C18:1n9, C16:1n7, C18:3n3 and C18:4n3 and increase of C16:0, C18:0, C20:0, C10:0, C12:0, C14:0, C15:0 and C20:4n6 was verified in the control as well in the samples of pigs feed with diet containing 100 mg of vitamin E/kg of the diet. Since the swine meat is considered one of the greatest sources of C18:1n9, a monounsaturated fatty acid that influences the reduction of the cholesterol levels, its loss is considered a public health problem.[3,24,1] Labuza,[4] also found reduction in the monounsaturated fatty acids content.

The fatty acid composition of the cooked ham in swines that received diets supplemented with 200 and 400 mg of vitamin E/kg of feed, did not present significant differences ($P > 0.05$) in all samples during storage (Table 4). The maintenance of the fatty acid lipid profile in cooked ham, during 60-day storage at 5 °C, demonstrates the anti-oxidation effect of vitamin E when incorporated in the diet, in levels equivalent or higher than 200 mg kg^{-1} of feed (Table 4).

It was not observed significant difference ($P > 0.05$) in the total lipid contents between *biceps femoris* muscle and cooked ham, which presented the average of 1.91 ± 0.04 g/100g; Bragagnolo and Rodriguez-Amaya[17] found higher values of lipid contents in ham meat (3.5 ± 1.4 g/100g) and in swine loin (2.4 ± 0.8 g/100g).

The sensory analysis of ham showed that the different levels of vitamin E supplementation did not cause significant differences ($P > 0.05$) in the texture, odor and flavor in ham. However, the results of TBARS (data not shown) had significant difference between hams with different levels of vitamin E. When the panelists were questioned about the intention of purchase, 75% answered "certainly" or "probably" without significant difference ($P > 0.05$). In contrast, De Winne and Dirinck,[25] under different conditions, found that ham with and without supplementation of vitamin E, showed significant difference between the control and supplemented samples; besides, the sensorial analysis confirmed the results obtained in the chemical analyses, which indicated a reduction in substances responsible by the rancid aroma in the samples with vitamin E supplementation, maintaining fresh product characteristic, after 16 days of storage at 6 °C.

The ranking test analysis indicated that the barrow ham presented the best color, differing significantly ($P < 0.05$) from the gilts ham. No significant differences in flavor, aroma and texture were observed ($P \geq 0.05$) between the cooked ham

Table 4. Fatty acid composition (%) of cooked ham during storage at 5 °C for 60 days[a]

Fatty Acids (%)	Control			100 mg/kg of Vitamin E			200 mg/kg of Vitamin E			400 mg/kg of Vitamin E		
	0 days	30 days	60 days	0 days	30 days	60 days	0 days	30 days	60 days	0 days	30 days	60 days
C10:0	0.09[b]	0.09[b]	0.15[a]	0.10[a]	0.10[a]	0.13[a]	0.11[a]	0.10[a]	0.11[a]	0.11[a]	0.11[a]	0.10[a]
C12:0	0.07[b]	0.07[b]	0.13[a]	0.08[a]	0.08[a]	0.10[a]	0.08[a]	0.07[a]	0.07[a]	0.08[a]	0.06[a]	0.07[a]
C14:0	1.21[b]	1.13[b]	1.78[a]	1.32[b]	1.19[b]	1.57[a]	1.20[a]	1.23[a]	1.16[a]	1.15[a]	1.17[a]	1.20[a]
C15:0	0.98[b]	1.00[b]	1.89[a]	1.12[b]	1.08[b]	1.54[a]	0.96[a]	1.00[a]	0.90[a]	0.91[a]	0.89[a]	0.90[a]
C16:0	23.51[b]	23.37[b]	25.78[a]	22.97[b]	23.02[b]	25.53[a]	23.64[a]	24.06[a]	23.98[a]	23.95[a]	23.34[a]	23.43[a]
C16:1n7	3.28[a]	3.38[a]	1.23[b]	3.26[a]	3.33[a]	1.89[b]	3.28[a]	3.63[a]	3.27[a]	3.39[a]	3.67[a]	3.28[a]
C18:0	12.03[b]	12.17[b]	14.20[a]	11.89[b]	11.48[b]	13.07[a]	12.48[a]	11.47[a]	12.07[a]	11.95[a]	12.20[a]	11.75[a]
C18:1n9	46.84[a]	46.38[a]	38.15[b]	46.87[a]	47.01[a]	39.97[b]	46.03[a]	45.98[a]	45.62[a]	45.58[a]	46.12[a]	47.00[a]
C18:2n6	9.04[b]	9.37[b]	11.41[a]	9.32[b]	9.34[b]	11.23[a]	9.07[a]	8.97[a]	9.31[a]	9.51[a]	9.03[a]	8.83[a]
C20:0	0.11[b]	0.12[b]	2.37[a]	0.15[b]	0.16[b]	1.67[a]	0.54[a]	0.59[a]	0.63[a]	0.60[a]	0.65[a]	0.65[a]
C18:3n3	0.57[a]	0.63[a]	0.13[b]	0.61[a]	0.64[a]	0.47[b]	0.98[a]	1.20[a]	1.17[a]	1.08[a]	1.13[a]	1.16[a]
C18:4n3	0.98[a]	0.96[a]	0.23[b]	0.97[a]	1.03[a]	0.34[b]	0.10[a]	0.08[a]	0.09[a]	0.09[a]	0.09[a]	0.10[a]
C20:2n6	0.10[a]	0.09[a]	0.08[a]	0.11[a]	0.12[a]	0.09[a]	0.11[a]	0.09[a]	0.10[a]	0.10[a]	0.09[a]	0.11[a]
C20:4n6	1.19[b]	1.24[b]	2.47[a]	1.23[b]	1.33[b]	2.40[a]	1.42[a]	1.53[a]	1.52[a]	1.50[a]	1.45[a]	1.42[a]
∑SFA[b]	38.00[b]	37.95[b]	46.30[a]	37.63[b]	37.21[b]	43.61[a]	38.57[a]	38.52[a]	38.92[a]	38.75[a]	38.42[a]	38.10[a]
∑MUFA[c]	50.12[a]	49.76[a]	39.38[b]	50.13[a]	50.34[a]	41.86[b]	49.31[a]	49.61[a]	48.89[a]	48.97[a]	49.79[a]	50.28[a]
∑PUFA[d]	11.88[b]	12.29[b]	14.32[a]	12.32[b]	12.46[b]	14.53[a]	12.12[a]	11.79[a]	12.19[a]	12.28[a]	11.79[a]	11.62[a]
PUFA/SFA	0.31[a]	0.32[a]	0.31[a]	0.33[a]	0.33[a]	0.32[a]	0.31[a]	0.30[a]	0.31[a]	0.31[a]	0.30[a]	0.30[a]
total lipids	1.90[a]	1.95[a]	1.95[a]	1.90[a]	1.85[a]	1.90[a]	1.85[a]	1.85[a]	1.90[a]	1.90[a]	1.85[a]	1.85[a]

[a]Means obtained among the four treatments, 2 sexes and true repetitions; [b]Total fatty acids saturated; [c]Total fatty acid monounsaturated; [d]Total fatty acid polyunsaturated; Different letters in the same row are significantly different ($P < 0.05$).

samples, since the obtained average responses was *I liked moderately*. As for the intention of purchasing, the samples of cooked ham reached the level of 75%, or *I would probably buy*, not presenting significant difference ($P > 0.05$).

Conclusions

Supplementation of pigs with vitamin E at the levels of 200 mg/kg diet or higher in the diet, supplied during the 116 days before slaughter, keep the fatty acid profile of the cooked ham unchanged during 60 days of cold storage, without incorporating other flavors or tastes.

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