Influence of storage time and packaging system on free amino acids content from *Longissimus thoracis* of "Cachena" calves

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Abstract- The effect of time of storage and packaging system on free amino acids amount from LT was studied. Six male veal calves of the "Cachena" breed from intensive livestock production system were used. Samples were packed under vacuum and MAP and after 5, 9 and 14 days of storage were analyzed. The content of FAA increase significantly (P<0.05) during storage time reached final values of 609 and 517 mg/100g fresh meat for vacuum packaging and MAP, respectively. Not significance differences (P>0.05) have been observed on content of FAA between both packaging systems. We observed a increase for all amino acids studied during storage time in both packaging systems except for glutamic acid under MAP packed. Packaging system affected the content of glutamic acid after 5, 9 and 14 days of storage and aspartic acid, isoleucine and phenylalanine after 14 days of packaging.

Keywords: **"Cachena" breed, Free amino acids, Storage time, Packaging system**

I. INTRODUCTION

The role of food packaging in the food industry is being increasingly recognised as it has multiple functions and is very important in terms of increasing product shelf life by retarding food quality degradation and ensuring food safety. Furthermore, packaging of fresh red meat is carried out to avoid contamination, delay spoilage, permit some enzymatic activity to improve tenderness, reduce weight loss, and where applicable, to ensure a cherry-red colour in red meats at retail or consumer level (Kerry, O´Grady, & Hogan, 2006). Vacuum and modified atmosphere packaging (MAP) techniques are used in the food industry to extend the product shelf-life.

Shelf life and quality of fresh beef are strongly influenced by initial meat quality, package parameters, and storage conditions (Zhao, Wells, & McMillan, 1994). MAP is widely used to extend the shelf life and quality of chill stored beef.

The aim of this study was to assess the effect of packaging system and days of store on content of free amino acids in meat from "Cachena" calves.

II. MATERIALS AND METHODS

II.1. Animals and treatment of the samples

Six male veal calves of the "Cachena" breed from the experimental herd of BOAGA **(**Bovine Autochthonous Galician Association) were used for this study. Animals were born in winter of 2007 and were together in a single group, in spring pasture. Three months, before slaughtered (at 8-9 months)

animals had access a commercial concentrate "*ad libitum*". Animals were conventionally slaughtered at a commercial abattoir and carcasses were ageing during seven days at 4 ºC in a cold chamber. At this point, the *Longisimus thoracis* (LT) muscle was extracted from the left half of each carcass, between the first and the twelve ribs.

II.2. Preparation of samples and package conditions

Samples were taken immediately to the laboratory, under refrigerated conditions. LT was cut aseptically in 20 steaks of 1.5 cm of thickness with a surface areas of about 150 cm^2. Steaks were individually packed under vacuum (98%) (V) and in modified atmosphere protective (MAP) conditions (M1 [20%CO_2-80 %O_2] in a LAR13/pn T-VG-R-SKIN. The trays (a transparent and thermo-sealing film of propylene and polyethylene 126 with a water vapour permeability of 10 g/m^2/24h/bar at 38°C and oxygen permeability of 110 ml/m2127 /24h/bar at 23 °C was used. Traits were displayed on a refrigerated chamber at 4 ºC dark conditions. Analyses were carried out for 0, 5, 9 and 14 days of exposure time.

II.3. Analytical methods

II.3.1. Chemicals and chromatographic instrumentation

AccQ.Fluor reagent kit (AQC, borate buffer) and AccQ.Tag Eluent A concentrate were acquired from Waters (Milford, MA, USA). Acetonitrile (MeCN), disodium ethylenediaminetetraacetic acid (EDTA), phosphoric acid, sodium acetate trihydrate, and sodium azide were from Baker (Phillipsburg, PA, USA); triethylamine (TEA) was purchased from Aldrich (Milwaukee, WI, USA). Amino acid standards and taurine and hydroxyproline were from Sigma (St. Louis, MO, USA).

HPLC systems used were a Waters system Alliance 2695 with a 2475 scanning fluorescence detector. Empower 2 software (Waters, Milford, MA, USA) was used to control system operation and results management.

II.3.2. Derivatization of standards and samples and chromatographic analysis

10 µl of sample was buffered to pH 8.8 (AccQ.Flour borate buffer) to yield a total volume of 100 µl. Derivatization was initiated by the addition of 20 µl of AccQ-Fluor reagent (3 mg/ml in MeCN). Reaction of the AQC with all primary and secondary amines was rapid and excess reagent was hydrolyzed within 1 min. Completion of hydrolysis of any tyrosine phenol modification was accelerated by heating for 10 min at 55 °C.

Separations were carried out using a Water AccQ-Tag column (3.9 mm x 150 mm with a 4 µm of particle size) with a flow-rate of 1.0 $mlmin^{-1}$ and performed at 37 ºC. The gradient profile and composition of the mobile phase was adapted from methodology developed by Vandelen & Cohen, (1997). Detection was accomplished by fluorescence with excitation at 250 nm and emission at 395 nm. Amino acids were identified by retention time using an amino acid standard to which taurine and hydroxiproline were added.

II.4. Statistical analysis

For the statistical analysis of the results, data were analyzed using the SPSS (version 15.0, USA). One-way analysis of variance (ANOVA) was used to analyze the effect of package type and displays days

on free amino acids content studied in the work. The least squares mean (LSM) were separated using Duncan's t-test. All statistical test of LSM were performed for a significance level $\alpha<0.05$.

III. RESULTS AND DISCUSSION

The content of FAA among this study is shown in Table 1. The content of FAA increase significantly ($P<0.05$) during storage time reached final values of 609 and 517 mg/100g fresh meat for vacuum packaging and MAP, respectively. Not significance differences ($P>0.05$) have been observed on content of FAA between both packaging systems. These results are agree with those reported by Feidt, Petit, Bruas-Reignier, & Brun-Bellut, (1996), who found higher amount of FAA in samples vacuum packed from Friesian bulls after 14 days of storage and those showed by Watanabe, Ueda, & Higuchi, (2004), who reported higher values in samples vacuum packaged after 10 days of storage.

We observed a increase for all amino acids studied during storage time in both packaging systems except for glutamic acid under MAP packed, which decrease after 14 days of storage although this decrease was not significantly ($P>0.05$). A similar result was found for valine after 14 days under vacuum packed samples from Friesian bulls (Feidt et al., 1996) and for aspartic acid and taurine after 10 days under vacuum packaged samples (Watanabe et al., 2004).

Packaging system affected the content of glutamic acid after 5, 9 and 14 days of storage and aspartic acid, isoleucine and phenylalanine after 14 days of packaging. In all cases amount of these amino acids were higher in samples under vacuum packaged.

In our study, we found a large increment on arginine, threonine, alanine and histidine in both packaging systems. The large increase on alanine and histidine reported in this work was in agreement with observed by (Feidt et al., 1996).

IV. CONCLUSIONS

Storage time affected significantly on total content of FAA, however packaging system did not affect on total free amino acids amount. Arginine, threonine, alanine and histidine were amino acids which showed a large increased. Packaging system only affected the amount of four amino acid (glutamic acid, aspartic acid, isoleucine and phenylalanine) of total FAA studied after 14 days of storage.

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Table 1. *Content of free amino acids of Longisimus thoracis muscle from "Cachena" calves packed under vacuum and MAP*

Amino acids	Vacuum packaging				SEM	MAP [20%CO_2-80 %O_2]			SEM
	Storage time (days)					Storage time (days)			
	Raw material	5	9	14		5	9	14	
Hydroxiproline	0.96±0.37^{a}	1.40±0.51^{a}	1.26±0.56^{a}	0.98±0.80^{a}	0.11	1.00±0.72^{a}	1.19±0.53^{a}	1.33±0.56^{a}	0.11
Aspartic acid	0.37±0.18^{a}	1.45±1.07^{ab}	1.90±0.74^{b}	3.46±1.61^{c*}	0.30	1.13±0.56^{a}	1.45±1.44^{a}	1.26±1.55^{a}	0.22
Serine	9.71±1.67^{a}	11.43±3.53^{a}	12.18±3.07^{a}	17.81±5.35^{b}	0.94	9.59±1.61^{a}	9.31±2.65^{a}	12.68±3.09^{b}	0.53
Glutamic acid	8.71±2.74^{a}	9.39±2.52^{a*}	11.05±4.63^{ab*}	15.35±5.91^{b*}	0.96	6.74±1.12^{ab}	5.78±1.06^{b}	8.21±1.33^{a}	0.41
Glycine	8.26±1.10^{a}	10.56±2.29^{ab}	11.16±1.88^{b}	13.03±2.53^{b}	0.52	9.93±1.44^{ab}	9.39±1.25^{b}	10.60±2.07^{b}	0.34
Histidine	31.62±6.99^{a}	39.12±7.92^{a}	41.33±6.01^{a}	42.35±12.01^{a}	1.84	38.98±8.93^{a}	40.04±10.34^{a}	42.12±12.17^{a}	2.04
Taurine	4.89±0.89^{a}	10.80±2.79^{b}	10.59±3.70^{b}	12.19±4.81^{b}	0.86	12.00±4.13^{b}	13.73±4.29^{b}	14.52±5.22^{b}	1.09
Arginine	206.02±22.00^{a}	265.97±58.42^{b}	283.64±65.74^{b}	291.95±40.72^{b}	11.77	263.32±38.88^{b}	250.40±37.14^{b}	257.99±44.56^{b}	8.41
Threonine	27.71±3.02^{a}	42.32±11.53^{ab}	46.26±16.26^{b}	51.66±14.45^{b}	3.00	41.31±6.32^{b}	41.17±11.53^{b}	41.97±4.41^{b}	1.83
Alanine	27.67±4.12^{a}	33.89±7.04^{a}	28.94±14.71^{ab}	42.43±10.80^{b}	2.25	33.81±5.69^{ab}	31.98±6.00^{ab}	38.58±9.00^{b}	1.47
Proline	4.12±0.80^{a}	5.86±1.32^{a}	6.19±1.33^{ab}	8.06±2.60^{b}	0.43	5.69±0.79^{ab}	5.26±0.88^{bc}	6.78±1.82^{c}	0.30
Cysteine	8.17±2.85^{a}	12.63±5.91^{ab}	13.46±5.29^{ab}	18.59±7.24^{b}	1.31	12.04±2.89^{ab}	11.95±4.62^{ab}	15.01±3.29^{b}	0.83
Tyrosine	6.24±1.19^{a}	8.39±4.39^{ab}	9.72±2.94^{ab}	12.62±6.00^{b}	0.91	8.07±1.13^{ab}	8.02±2.49^{ab}	9.73±3.52^{b}	0.51
Valine	6.22±1.28^{a}	9.19±3.04^{ab}	9.61±2.98^{ab}	12.91±7.65^{b}	0.97	7.36±1.11^{a}	7.26±2.22^{a}	10.51±2.54^{b}	0.49
Methionine	3.90±0.53^{a}	3.93±1.47^{a}	4.01±1.44^{a}	6.34±2.69^{b}	0.39	3.09±0.82^{a}	3.09±1.25^{a}	4.28±0.90^{a}	0.20
Lysine	8.59±1.88^{a}	10.48±3.07^{a}	11.11±3.51^{a}	16.21±5.00^{b}	0.89	9.00±0.90^{a}	8.70±2.21^{a}	11.59±2.83^{b}	0.47
Isoleucine	5.00±0.95^{a}	7.54±2.23^{a}	7.71±2.24^{a}	11.87±3.26^{b*}	0.68	5.90±0.85^{a}	5.91±1.80^{a}	6.22±1.76^{b}	0.36
Leucine	9.24±1.60^{a}	13.15±4.15^{a}	13.43±4.14^{a}	20.47±5.88^{b}	1.17	10.63±1.50^{a}	10.36±3.24^{a}	14.61±3.12^{b}	0.64
Phenylalanine	4.96±0.65^{a}	6.93±2.10^{a}	7.03±2.14^{a}	11.09±3.09^{b*}	0.62	5.47±0.70^{a}	5.44±1.72^{a}	7.84±1.51^{b}	0.33
Total amino acid	**382.42±37.55^{a}**	**504.52±94.37^{b}**	**530.66±113.64^{b}**	**609.45±122.23^{b}**	**25.19**	**485.14±42.57^{b}**	**470.51±75.77^{b}**	**517.76±83.13^{b}**	**15.90**

$^{a-b}$ Different letters in the same row show significant differences ($p<0.05$, Duncant test)

***** Significant differences ($P<0.05$) between packaged system