Effect of Cooking Conditions on Creatinine Formation in Cooked Ham

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The aim of this study was to evaluate the effect of different cooking procedures on the concentrations of creatine and creatinine and the ratio of creatinine/creatine in cooked ham. Two cooking methods (constant temperature and increasing temperature, constant T and ΔT, respectively) were tested on different locations in porcine longissimus dorsi muscle and ham (semimembranosus, biceps femoris, and gluteus muscles). The results showed larger creatine conversion into creatinine in the surface layer than in the core as well as higher creatinine/creatine ratio values when applying the ΔT in comparison to the constant T method. A correlation between the creatinine/creatine ratio and the heat treatment was established, and 15 samples of commercial cooked hams were analyzed to support these results. This creatinine/creatine ratio analyzed in the surface of the ham could be used as a rapid and nondestructive indicator to determine the effectiveness of the heat treatment in cooked ham processes.

INTRODUCTION

Cooked ham is a well-known meat product with high consumption levels all over the world. It contains a large number of compounds of interest including creatine and creatinine. Creatine is an important component of the energy delivery process in several tissues, particularly those implicated in high energy demand. Creatine exists in equilibrium with phosphocreatine in skeletal muscle, where the reversible conversion of creatine into phosphocreatine is catalyzed by creatine kinase and involves the transfer of the γ-phosphate group of adenosine triphosphate (ATP) onto the guanidine group of creatine. Thus, creatine plays an important role in the energy metabolism of skeletal muscle, providing the necessary energy for vigorous muscle contraction. Creatine is produced in the liver, kidneys, and pancreas but can also be obtained from dietary supplements and from foods such as meat and fish. Supplements of creatine have been shown to improve muscle performance in high-intensity exercise (*1–3*).

Creatine turns into creatinine in muscle due to a nonenzymatic conversion by the removal of water and the formation of a ring structure. This nonenzymatic conversion takes place easily under heating conditions such as meat cooking. Some authors have established a correlation between creatinine content and the flavor of cooked meat (*4, 5*). On the other hand, the addition of creatine to broths has also been strongly recommended because of its contribution to the full flavor of meat extracts (*6, 7*).

The presence of creatine and creatinine in cooked meat has also been associated with negative aspects, because creatine and creatinine can constitute important precursors of heterocyclic amines (HAs) formed on the surface of meat when it is cooked at high temperatures such as roasting, frying, and grilling (*8*).

It is important to consider the study of the cooking step as a critical part of the cooked ham process in relation to its safety and quality. Critical (indicator) microorganisms and acid phosphatase (ACP) activity have been used to assess the extent of heat treatment in the central area of canned hams and meat products for several decades (*9–12*) despite their disadvantages because they are destructive determinations.

The main objective of the present study was to evaluate the effect of cooking temperature on creatine and creatinine concentration as well as establish a correlation between this ratio and the applied heat treatment.

MATERIALS AND METHODS

Chemicals and Reagents. All chemical and chromatographic reagents were of HPLC grade. Acetonitrile, acetone, ammonium acetate, and glacial acetic acid were purchased from Scharlau (Barcelona, Spain). Creatine, creatinine, and carnosine standards were from Fluka Chemie AG (Buchs, Switzerland). Sodium carbonate anhydrous and sodium hydrogen carbonate used in phosphates analysis were from Panreac (Spain).

Materials. In a first set of experiments, longissimus dorsi muscle, excised from four carcasses of 6-month-old female pigs at 24 h post-mortem, was used to detect differences in creatine and creatinine amounts by varying time and temperature conditions according to the cooking technique established. The use of just one type of muscle such

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11280 *J. Agric. Food Chem.,* Vol. 56, No. 23, 2008

Mora et al.

Table 1. Effect of Different Cooking Methods on Cooking Efficiency, Weight Loss, and Yield in Longissimus Dorsi Muscle and Cooked Ham

cooking method	sample type	cooking efficiency (min/°C)	process loss (%)	yield (%)	cooking time (min)
constant *T*	longissimus dorsi[a]	0.54	19.9	83.43	43
	cooked ham	4.90	15.7	101.55	325
Δ*T*	longissimus dorsi[a]	0.83	30.3	76.75	69
	cooked ham	6.96	13.6	105.05	455

[a] Longissimus dorsi muscle was not brine injected.

Table 2. Levels of Creatinine (Cn), Creatine (Cr), and Creatinine/Creatine Ratio in Fresh and Cooked Pork Longissimus Dorsi Muscle and Ham, According to the Type of Cooking and Location[a]

	cooking method	location	creatinine		creatine		ratio
			mean	SD	mean	SD	(Cn/Cr)
longissimus dorsi[b]	no cooking		8.78a	0.71	354.56ab	17.56	0.02a
	constant *T*	core	22.68b	0.55	387.09a	6.58	0.06b
		half	23.06bc	0.72	354.06ab	11.91	0.06bc
		surface	44.57cde	1.59	340.92b	8.87	0.13bc
		exudate	0.010f	0.0003	0.025c	0.0002	0.40de
	Δ*T*	core	22.34bc	0.88	338.13b	13.05	0.07b
		half	23.96bc	0.87	328.86b	7.60	0.07bc
		surface	43.81bcd	1.48	319.52b	6.46	0.14bc
		exudate	0.011f	0.0003	0.027c	0.0005	0.41de
cooked ham	no cooking		4.94g	0.70	351.99ab	7.24	0.01a
	constant *T*	core	35.12bcd	0.47	243.70de	13.44	0.14bc
		half	39.50bc	2.75	232.44de	14.36	0.17bc
		surface	83.30h	9.58	212.04df	4.82	0.39de
		exudate	270.27i	16.43	183.98f	47.85	1.53f
	Δ*T*	core	56.07de	10.82	256.10e	34.79	0.22bcd
		half	65.38eh	4.36	256.93e	27.81	0.25cd
		surface	107.88j	7.18	234.15de	22.54	0.46e
		exudate	230.24k	31.97	99.25g	21.21	2.35g

[a] Results expressed as means of three samples (in mg/100 g of muscle) and standard deviation. Different letters within the same column mean significant differences ($p < 0.05$). [b] Longissimus dorsi muscle was not brine injected.

as longissimus dorsi allowed the evaluation of creatine conversion to creatinine according to the location and the cooking method in a homogeneous sample, avoiding the variability in the amounts of these compounds that could exist in a mixture of muscles (*13*).

In a second approach, semimembranosus, biceps femoris, and gluteus muscles were chosen from four carcasses of 4-month-old female pigs at 15 h post-mortem and immediately prepared and processed to obtain cooked ham. These three muscles were selected for being the main muscles of the pork leg. Controlled muscles were needed to analyze the variability in creatine and creatinine amounts in different cooked ham muscles.

Finally, five different classes of commercial boneless cooked ham from different brands were purchased from different supermarkets. Two top-quality ("extra") cooked hams, a tinned cooked ham, a ham cooked in its juice, and a cold-cut sandwich shoulder (*14*) were selected to obtain the highest variety of results in creatine and creatinine contents. Three samples of each quality, each from different batches, were obtained.

Sample and Standards Preparation. *Standards Preparation.* The calibration ranges for the assayed compounds were established using a duplicate set of standards. Carnosine and creatinine calibration standards were prepared by diluting a stock solution of 1 mg/mL, whereas creatine standards were prepared from a stock solution of 0.3 mg/mL. All dilutions were done using 0.01 N HCl/acetonitrile (25:75, v/v).

Table 3. Levels of Creatinine (Cn), Creatine (Cr), and Creatinine/Creatine Ratio on the Surface of the Cooked Ham Main Muscles According to the Type of Cooking[a]

cooking method	muscle	creatinine		creatine		ratio
		mean	SD	mean	SD	(Cn/Cr)
no cooking	semimembranosus	4.32a	0.28	346.77a	29.23	0.012a
	biceps femoris	4.79a	0.48	348.95a	23.04	0.014a
	gluteus	5.70a	0.38	360.27a	19.68	0.016a
constant *T*	semimembranosus	90.69b	11.54	222.79b	26.44	0.407b
	biceps femoris	82.12bc	8.95	193.05c	3.88	0.425bc
	gluteus	103.95d	5.32	217.20bc	23.33	0.479c
Δ*T*	semimembranosus	95.88cd	14.61	215.90bc	20.07	0.444bc
	biceps femoris	94.61cd	6.87	220.71bc	17.65	0.429bc
	gluteus	125.68e	23.91	233.22b	28.45	0.539d

[a] Results expressed as means of 3 samples (in mg/100g muscle) and standard deviation (SD). Different letters within the same column mean significant differences ($p < 0.05$).

Pork Loin Processing. Four pieces of pork loin, weighing approximately 250 g each, were cooked in a bath until an internal temperature of 72 °C. The effect of two cooking methods on creatine and creatinine amounts was tested. Pork loin pieces were cooked using a constant one-stage cooking cycle (cooked at 85 °C to a core temperature of 72 °C) and a Δ*T* cycle (maintaining a constant difference of 35 °C between water bath and core temperature until the bath reached 85 °C, whereupon the core was allowed to rise to 72 °C). Both assays were done using a digital sensor thermometer to control the core temperature of the pieces. After cooking, a traditional water immersion method (at 0 °C) was used to cool all samples to 4 °C at the core. Brine solution was not injected into longissimus dorsi muscle in order to avoid its possible influence in creatine conversion to creatinine.

Cooked Ham Preparation and Processing. A study about how the creatine conversion to creatinine can be influenced by the cooking method has been carried out as a way to assess the effectiveness of the heat treatment. Two cooking methods, constant *T* and Δ*T*, were selected as representative of different cooking processes, and their effect on creatinine formation was studied in pork loin pieces and ham.

The individual muscles excised from four carcasses and weighing approximately 2 kg (biceps femoris and semimembranosus muscles) and 1.5 kg (gluteus muscle) were injected manually to reach 120% of their green weight with a brine solution formulated to give the following concentrations of ingredients in the injected meat: 2.34% of sodium tripolyphosphate, 11.69% of NaCl, 0.03% of nitrites ($NaNO_2$), and finally, 1.47% of glucose and 0.13% of sodium ascorbate (*15*). After brine injection, the samples were tumbled manually for 24 h at 4 °C at 3 h intervals. After tumbling, the muscles were formed by hand and vacuum packaged in cooking bags. Hams were cooked using the two cooking methods previously described under Pork Loin Processing. For each cooking treatment, weights of pork loin and cooked ham before cooking and after cooling were recorded to calculate the weight losses during the process as follows:

$$\text{process wt loss (\%)} = \frac{\text{wt before cooking} - \text{wt after cooling}}{\text{wt before cooking}} \times 100$$

The weights of hams before brine injection were also recorded to calculate the yield of the complete process:

$$\text{yield (\%)} = \frac{\text{wt after cooling}}{\text{wt before brine injection}} \times 100$$

After cooking, a traditional water immersion method (at 0 °C) was used to cool all samples to 4 °C at the core.

Cooking efficiency was calculated as the slope of the heating curve (time versus temperature in the core of the ham) of the cooking process.

Commercial Cooked Ham. A cut cross slice 15 mm thick from the geometrical middle was cut from each cooked ham, and three

Creatinine Formation in Cooked Ham

J. Agric. Food Chem., Vol. 56, No. 23, 2008 **11281**

samples from each slice were excised to analyze the content of creatine, creatinine, and carnosine in different random muscles from each type of cooked ham. The identification of the different muscles was not necessary, having proved that nonsignificant differences in creatinine/ creatine ratios exist between the main ham muscles (semimembranosus, biceps femoris, and gluteus).

Creatine, Creatinine, and Carnosine Analysis of Longissimus Dorsi Muscle and Cooked Ham. The temperature profile in the core of the pieces of loin and cooked ham is a factor that differs among the various cooking techniques. For this reason, after cooking and cooling, all pieces were half-cut, and three samples from each piece were taken to analyze differences in creatine and creatinine amounts between the core, half, and surface. All samples were immediately processed for further analysis following the method described by Aristoy and Toldrá (*16*) with some minor changes. Briefly, 5 g of sample tissue was homogenized with 20 mL of 0.01 N HCl in a stomacher (Seward Laboratory) for 15 min and further centrifuged in the cold (4 °C) at 10000 rpm for 20 min. Supernatant was filtered through glass wool, and 20 mL of this solution was deproteinized by adding 3 volumes of acetonitrile, standing at 4 °C for 20 min. Finally, the sample was centrifuged (10000 rpm) for 10 min at 4 °C and the supernatant directly analyzed.

Chemical Analysis. Measurements of pH were taken in semimembranosus, biceps femoris, and gluteus muscles before brine injection and after cooling. For this purpose, 5 g of each muscle was taken, triturated, and homogenized in 10 mL of bidistilled water using a Polytron PT2100 (Kinematica, Inc.). The pH measurement was taken directly on the homogenized solution with an electrochemical sensor (238000/09 Hamilton). Moisture content was determined by oven-drying to constant weight at 100 °C (ISO R-1442). Phosphates were analyzed using an ion analyzer with a chemical conductivity detector (Metrohm). The chromatographic separation was developed in a Metrosep A Supp 5 column (4.0 × 250 mm, 5 μm) from Metrohm. The mobile phase contained 3.2 mM sodium carbonate anhydrous and 1 mM sodium hydrogen carbonate in water, and 30 mL of acetone was added per liter of solution. The chromatographic separation was isocratic, and it was developed at a flow rate of 0.7 mL/min.

Description of the Chromatographic Method. Creatine, creatinine, and carnosine were analyzed by hydrophilic interaction chromatography (HILIC) under the conditions described by Mora et al. (*17*).

Statistical Analysis. The ANOVA procedure was used to determine significant differences between longissimus dorsi muscle and the different cooked ham muscles as well as between the five classes of commercial cooked ham analyzed using the software Statgraphics Plus (v 5.1). Each statistical analysis and the normality of the data were tested before application of the ANOVA procedure.

RESULTS AND DISCUSSION

Cooking stage is a critical part of the cooked ham processing. Differences in cooking bath temperature and time of immersion influence the conversion of creatine to creatinine as well as the efficiency, water loss, and yield. In this way, the constant T cooking method, which consists of cooking at 85 °C, is shorter than the ΔT method, which increases the water bath temperature in a progressive way. The effect of the cooking technique on cooking efficiency is shown in **Table 1**. As can be observed, constant T cooking shows the highest cooking efficiency (0.54 and 4.90 min/°C in longissimus dorsi and cooked ham, respectively), probably due to the shorter times needed for this type of cooking.

With regard to water losses in cooked ham samples, the constant T cooking method produces higher losses (15.7%) compared with the ΔT cooking method (13.6%). These results fully agree with those reported by other authors, who established that the ΔT cooking method significantly reduced cooking losses by 2−3% in biceps femoris and semimembranosus pork muscles with respect to constant T cooking (*18*). However, process losses obtained in longissimus dorsi muscle were much higher in the samples cooked using the ΔT method. This could be due to the

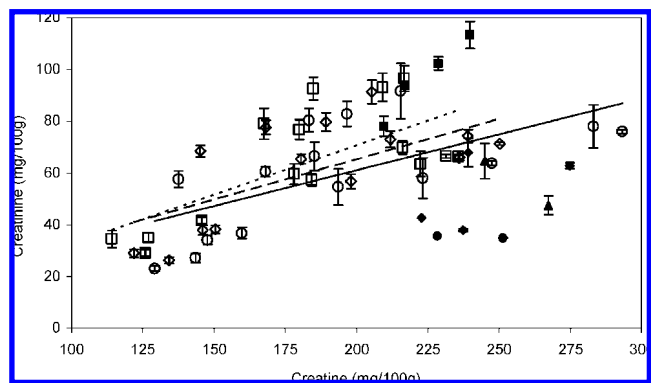**Figure 1.** Relationship between creatine and creatinine amounts (mg/ 100 g) in cooked ham samples. Regression lines represent core (—), half (− − -), and surface (- - -) according to the sample location on the slice. Open symbols correspond to commercial cooked hams samples (○, ◇, and □ for core, half, and surface, respectively), and solid symbols correspond to core (●), half (◆), and surface (■) of hams cooked under constant T and ΔT conditions.

Table 4. Levels of Creatinine, Creatine, and Creatinine/Creatine Ratio in Five Different Qualities of Commercial Cooked Ham According to Sample Location[a]

cooked ham class[b]	location	creatinine		creatine		
		mean	SD	mean	SD	ratio (Cn/Cr)
I	core	75.99ab	9.14	189.15ab	13.32	0.40ab
	half	74.45ab	7.17	181.09abc	10.58	0.41ab
	surface	73.61abc	13.77	171.93ac	21.40	0.43b
II	core	56.94d	16.77	237.36d	65.92	0.25cde
	half	55.92d	14.63	204.64b	34.87	0.27defg
	surface	54.55b	13.25	177.73abc	33.54	0.30g
III	core	66.01ce	8.86	254.61d	31.63	0.2def
	half	67.67bce	2.67	240.27d	7.74	0.28efg
	surface	65.46ce	2.90	229.82d	14.00	0.29fg
IV	core	65.99de	13.35	170.15ce	34.81	0.39a
	half	78.11a	10.22	196.14ab	37.90	0.41ab
	surface	88.45f	11.42	186.80abc	40.18	0.48h
V	core	29.68g	5.24	141.46ef	15.16	0.21c
	half	31.07g	5.60	134.03ef	11.65	0.23cd
	surface	33.22g	3.31	123.02f	8.24	0.27defg

[a] Results expressed as means of three samples (in mg/100 g of muscle) and standard deviation (SD). Different letters within the same column mean significant differences ($p < 0.05$). [b] I, the most expensive top-quality cooked ham; II, the cheapest top-quality cooked ham; III, tinned cooked ham; IV, cooked ham cooked in its juice; V, cold-cut sandwich shoulder.

lack of brine solution injection because phosphates clearly influence the muscle absorption of water. On the other hand, some authors also established that cooking losses should not be extrapolated between muscles cooked under standard conditions (*19*). In fact, cooking losses in biceps femoris were found to be independent of the cooking technique, whereas, in the case of longissimus dorsi, differences in cooking losses were detected when different cooking techniques were used (*20*). **Table 1** also shows that cooking ham with the ΔT method could obtain the highest yield compared with the constant T cooking method. The lowest yield values obtained during longissimus dorsi cooking could be due to the absence of brine solution injection, which caused higher losses of water and, thus, a decrease in the percentage of yield.

The amounts of creatine and creatinine and also the creatinine/ creatine ratios in the two different processes studied are shown

Table 5. Mean (*n* = 3) and Standard Deviation of pH, Carnosine, and Phosphates Amounts, Moisture Percentage, and Creatinine/Creatine Ratio in the Surface of Cooked Hams[a]

cooked ham quality[c]	pH		carnosine		phosphates		moisture		ratio (Cn/Cr)[b]	
	mean	SD	mean (mg/100 g)	SD	mean (ppm)	SD	mean (%)	SD	mean	SD
I	6.20ab	0.03	165.43a	32.98	4119.92a	16.36	74.99ab	1.35	0.43a	0.08
II	6.44b	0.22	227.53b	8.16	5263.90b	167.28	72.98bc	1.34	0.30b	0.02
III	6.22ab	0.23	252.48b	12.97	6159.26c	630.61	76.99a	1.59	0.29b	0.01
IV	6.23ab	0.10	143.92a	26.55	6071.11cd	43.17	71.98c	1.69	0.48c	0.06
V	6.26ab	0.25	94.52c	11.13	3956.28a	452.96	72.42bc	0.97	0.27b	0.03
VI	6.07a	0.05	261.48b	15.68	5474.40bd	419.67	72.80bc	2.39	0.46c[d]	0.06

[a] Different letters within the same column mean significant differences (*p* < 0.05). [b] Results also shown in **Table 4**. [c] I, the most expensive top-quality cooked ham; II, the cheapest top-quality cooked ham; III, tinned cooked ham; IV, cooked ham cooked in its juice; V, cold-cut sandwich shoulder; VI, cooked hams cooked under controlled conditions of temperature and time. [d] Mean of the ratio values obtained in both cooking conditions in semimembranosus, biceps femoris, and gluteus muscles (shown in **Table 3**).

in **Table 2**. As can be observed, cooking produces a remarkable increase in creatinine amounts, probably due to the conversion of creatine to creatinine. Creatine contents before and after cooking in both pork loin and ham are very similar, although a slight decrease is observed after cooking, especially in the case of ham. A considerable increase in creatinine/creatine ratio values has been shown in samples after cooking (0.02 and 0.01 in noncooked longissimus dorsi and ham samples, respectively, vs 0.13 and 0.39 in the constant *T* method and 0.14 and 0.46 in the Δ*T* method, all for surface location). Our results agree with those obtained by other authors, who demonstrated that cooking brought about a decrease in the amounts of creatine and an increase of creatinine, presumably due to the heat-induced conversion of creatine to creatinine (*4, 21, 22*). As can be observed with creatine and creatinine values obtained after cooking, increases in creatinine levels did not fully account for decreases in creatine levels. In fact, it has been established that as a result of thermal processing, creatine levels in pork meat preparations decreased by 22%, whereas creatinine levels increased by 390% (*23*).

Creatine conversion takes place more rapidly on the surface than in the core of the samples, independent of the brine solution and type of muscle as observed from results shown in **Table 2**. In fact, creatinine amounts in the surface increase up to about 2 times the core values obtained in both longissimus dorsi and ham in both methods. These results agree with those obtained with commercial cooked ham as will be discussed later.

To determine the extent to which the differences in creatine and creatinine contents between core and surface were due to losses of these compounds in the exudate generated after cooking or conversion of creatine to creatinine, the effect of the cooking technique on exudates was also studied. In this way, creatinine and creatine amounts analyzed in the exudate of longissimus dorsi are almost negligible (0.010 and 0.025 mg/100 g in the constant *T* method and 0.011 and 0.027 mg/100 g in the Δ*T* method for creatinine and creatine, respectively); however, their ratio values are considerably higher than those obtained in pork loin pieces (0.4 using both methods). This could be possibly due to differences in solubility of creatine and creatinine compounds and/or the greater heat transfer suffered by the exudate because of its direct contact with the hot water. Curiously, despite the higher loss values obtained in longissimus dorsi muscle compared to ham (see **Table 1**), it is interesting to highlight the higher amounts of creatinine and creatine found in the exudate of ham samples. These high values could be due to the effect of brine solution and tumbling on cooked ham muscles, which would allow creatine and creatinine solubilization and, thus, an easier mobilization of these compounds to the exudate.

Different samples were also taken from the surface of the cooked hams to obtain creatinine and creatine amounts as well as their ratio values in semimembranosus, biceps femoris, and gluteus muscles and to compare these results with their corresponding values before cooking (**Table 3**). The lower creatinine values presented in **Table 3** for noncooked semimembranosus, biceps femoris, and gluteus muscles in comparison to those published previously by us (*13*) could be due to differences in post-mortem time at the moment of analysis. It has been observed that during the first hours post-mortem creatinine values increase (results not shown), important differences appearing in the amounts of this compound in samples collected between 15 and 24 h post-mortem. Ratio values presented in **Table 3** for cooked muscles show slightly higher values in muscles corresponding to hams cooked with the Δ*T* method, although all creatinine/creatine ratio values were above 0.4 at the surface for all muscles.

It has been shown that different cooking methods applied to pork meat can influence the amount of creatine and creatinine present at the end of the treatment, depending on time, temperature, and initial amounts of creatine. To study the conversion of creatine to creatinine in hams that have been cooked under real industrial cooking conditions, the analysis of 15 samples from five different qualities of commercial cooked ham has been performed. Thus, a correlation between creatine amounts and creatinine formation after cooking has been obtained with the analysis of the cooked ham samples (*13*). As can be observed in **Figure 1**, hams with the highest content of creatine are also those with the greatest amounts of creatinine generated after the cooking process. Regression lines corresponding to the three different locations, core, half, and surface, show higher slopes as they move away from the core of the sample. This higher slope value at the surface compared to the core confirms that the greatest conversion of creatine to creatinine occurs in this location.

Creatine and creatinine amounts obtained according to the market quality of the cooked ham and the location of the sample are shown in **Table 4**. Although very little literature about creatine and creatinine contents in cooked hams has been found, our results are on the order of those obtained by other authors (*24, 25*). As can be observed, creatine, creatinine, and ratio values confirm results obtained in **Table 3**. Commercial cooked ham samples also showed that the cooking treatment brought about a reduction in creatine and an increase in creatinine levels, as well as the lower ratio values obtained in the core in comparison to the surface.

Table 5 reports the average value and the standard deviations of pH, carnosine, phosphates, and moisture, as well as a summary of the creatinine/creatine ratios that appear in **Tables**

Creatinine Formation in Cooked Ham

J. Agric. Food Chem., Vol. 56, No. 23, 2008 **11283**

3 and **4** to facilitate their comparison between cooked hams. According to the literature, opposite results concerning the effect of cooking on carnosine amounts have been found. Whereas some authors have reported that histidine dipeptides, such as carnosine, are unaffected by cooking (*26*), others obtain relatively small differences in carnosine amounts between uncooked and cooked beef meat (*27*). In our case, nonsignificant differences in the amounts of carnosine have been found between uncooked and cooked hams (results not shown). Carnosine is a peptide present only in animal tissues. The amount of this compound is much higher in the skeletal muscle than in other tissues, especially in muscles with glycolytic metabolism such as the main muscles of pork ham. Thus, this characteristic together with its resistance after cooking makes carnosine very useful in the knowledge of the amount of animal protein present in cooked ham samples.

It seems that higher creatinine/creatine ratio values could be correlated to the more intense heat treatments and, for this reason, it is possible to assume that cooked hams I and IV have experienced the highest temperature and/or longest time conditions. The lowest carnosine contents present in these hams would be indicative of the lower animal protein amounts contained in them, so water retention would be lower and a longer cooking would be needed to reach 70 °C in their core. In fact, higher carnosine amounts obtained in cooked ham III also correspond to high values of moisture content and were also in coincidence with the lowest ratio amounts and, therefore, the smoothest heat treatment. The lowest ratio creatinine/creatine of ham V would be perfectly justified because cold-cut sandwich shoulder is presented at supermarkets as a rectangular bar with lower distance to the core and, thus, a minor heat treatment is necessary to reach an adequate temperature in the core.

On the basis of the obtained results, the creatinine/creatine ratio could be used as an index of the heating efficiency of cooked ham because it gives a good estimation of the magnitude of the heat treatment experienced by these products. Thus, according to the results shown in **Table 2**, pieces with a weight of 5.5 kg and injected with controlled brine solution would need a ratio above 0.4 on the surface to ensure adequate heat treatment of 70 °C in the core of the ham. Therefore, these measurements could be easily assayed in a small sample of the surface of the ham, minimizing the damage to the pieces as a type of nondestructive assay.

In summary, the tested cooking processes (constant T and ΔT) brought about a decrease of creatine and an increase of creatinine contents. In this way, slightly higher creatinine/creatine ratio values were obtained when the ΔT method was applied in comparison to the constant T method. On the other hand, it has been observed that creatine conversion takes place more rapidly on the surface than in the core of the samples, probably due to the longer heating time and the higher heat transfer values corresponding to this location. Thus, after demonstrating the increase of the creatinine/creatine ratio with cooking, we can state that this ratio could be used to determine the extent of the applied heat treatment during the processing of cooked ham. When ham piece weights, brine solution composition, and cooking method conditions are taken into account in the determination of the critical ratio, this procedure would be a rapid and nondestructive technique, requiring only a small sample of the cooked ham surface to perform the assay.

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