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# Creatinine, creatine and protein in cooked meat products

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The accuracy of the results obtained with a flow injection (FI) system for the simultaneous determination of creatinine and creatine in cooked meat products was evaluated in 30 samples by comparison with those obtained by reference methods. The FI method was then applied to 52 different samples (cooked ham, 14; frankfurters, 11; wieners, 9; chopped, 10; mortadella, 8) and the protein content was also determined. From % creatinine of total creatine (creatine + 1.159 creatinine) an estimation of the heat treatment applied in the processing was made. Cooked ham, mortadella and chopped had the highest values, indicating that the cooking conditions were more severe for these products than those used in the sausages. The ratio of total creatine/protein permitted an estimation of muscular protein to be made. According to the mean value for this ratio, the products were ordered as: cooked ham, 21.9 > chopped, 17.7 > wieners, 15.9 > mortadella, 13.0 > frankfurters, 12.3 mg total creatine per gram of protein.


## INTRODUCTION

Creatine and creatinine are characteristic constituents of muscle tissue and their assays are used to detect the presence of meat extract in a food product. Raw meat has a high creatine content (0.3–0.6% in fresh beef), whereas the creatinine content is low (about 6% of that of creatine) (Belitz and Grosch, 1987); therefore the former is appropriate to be used as an index of meat quality (Dvorák, 1981). However, when meat is cooked, the creatine content decreases whilst the creatinine content increases (Macy *et al.*, 1970; Campero *et al.*, 1992), and the total creatine content (creatine + 1.159 creatinine) might therefore be a better indicator of quality for cooked meat products.

Besides meat proteins, most of the products processed from meat contain other proteins from vegetables, milk or eggs. They are added to improve the technological process and to cheapen the production cost. It is customary to determine the total protein as an indicator of quality, but the additional determination of total creatine should allow the protein from muscle tissue, which generally is the most expensive protein, to be estimated.

Previous studies on meat cuts from pork, beef and lamb, have shown that the values of the ratio creatine in muscle tissue to contractile muscle protein fall on a narrow range (20.5–25.9 mg total creatine per gram of contractile muscle protein) (Dahl, 1963; Kahn and Cowen, 1977; Dvorák, 1981). On this basis, the ratio of total creatine/protein could be of practical importance for cooked meat products, since their value would allow the muscular protein of the product to be estimated by comparison with reference values obtained from regulated meat products, e.g. cooked ham.

Creatinine and creatine are commonly determined by the Folin method, based on the reaction that takes place between creatinine and alkaline picrate (Jaffé reaction). The creatine is previously converted into creatinine by prolonged heating in acid medium. This technique forms the basis of some official methods for the determination of creatine and creatinine in meat (AOAC, 1984). Another method for creatine determination is based on the reaction of creatine with 1-naphthol and biacetyl, which has been applied with satisfactory results to muscle tissue samples (Mussini *et al.*, 1984; Dvorák, 1988).

Recently we developed a method for simultaneous determination of creatinine and creatine by flow injection (FI) based on the Jaffé and the 1-naphthol-biacetyl reactions which was tested on broth cubes (Del Campo *et al.*, 1995). The main objectives of the present study were to evaluate the accuracy of the cited FI method to determine both components in cooked meat products, checking the results against standard methods and to

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determine creatinine, creatine and protein, as well as the ratio total creatine/protein on a variety of commercially prepared cooked meat products, in order to evaluate the practical merit of measuring this ratio.

## MATERIAL AND METHODS

### Samples

The 52 samples studied were purchased (a lot each week) from different markets and were wrapped in aluminium foil and placed in a refrigerator at 4°C until their analysis (maximum 3 days). They included 14 samples of 'extra' cooked ham, 20 samples of sausages (11 samples of frankfurters, and 9 of wieners), 10 samples of chopped and 8 samples of mortadella. The samples of 'extra' cooked ham were considered as the reference group, given that Spanish regulations do not allow the addition of protein in this product.

### Reagents and solutions

All the solutions were prepared with doubly-distilled water and all chemicals were of analytical-reagent grade, except creatinine, which was 'for determination of creatinine in blood'. Trichloroacetic acid, creatinine, creatine, 1-naphthol and biacetyl were purchased from Merck (Darmstadt, Germany), and selenium dioxide from Carlo Erba (Milan, Italy). The remaining materials were supplied by Panreac (Barcelona, Spain).

### Analytical methods

*Protein*

Total extractable nitrogen was determined using the method described by the AOAC (1984), but modified by the addition of $SeO_2$ and $CuSO_4$ as catalysts instead of HgO. Percentage nitrogen was multiplied by the protein factor for meat products (6.25) to obtain percentage protein.

*Creatinine and creatine*

Five grammes of the milled sample were homogenized for approximately 20 min with 100 ml of 30 g litre$^{-1}$ trichloroacetic acid and, after filtration of precipitated protein and fat, the colourless extract was used for analysis on the same day.

Both species were simultaneously measured in triplicate by means of FI analysis using a simple procedure: the sample containing both analytes was continuously merged with a picrate stream and mixed through a reactor, resulting in a continuous signal proportional to the creatinine concentration. The mixture of biacetyl and 1-naphthol (in alkaline medium) was then injected and a transient signal was obtained proportional to the creatine concentration. The manifold used has been previously described (Del Campo *et al.*, 1995).

When the same extracts were analyzed by the reference methods, creatinine was determined by the standard method of the AOAC (1984) and creatine was determined colorimetrically at 520 nm by the 1-naphthol-biacetyl reaction (Dvorák, 1988).

All determinations were repeated at least once. Results were calculated as the average of the respective replicates.

### Statistical analysis

Comparison of results obtained by the FI and reference methods was performed using the Student *t*-test for paired samples and a linear regression analysis with the 'Stat-Works'® Macintosh program.

## RESULTS AND DISCUSSION

### Comparison of methods for the determination of creatinine and creatine

The results obtained for creatinine and creatine by the FI analysis methodology are compared with those obtained by the reference methods for 30 samples (Table 1). A paired samples comparison *t*-test showed no significant difference among them for both species at the 95% confidence level. Regression graphs were obtained from Table 1 by plotting the concentration data obtained by FI method vs reference methods for creatinine and creatine. The results of these paired analyses are summarized in Table 2. Since the confidence interval ($p < 0.05$) for the intercept includes zero and that the confidence interval for the slope includes 1, neither background nor systematic errors were detected. Moreover, the high values of the correlation coefficient ($r$) indicate low random errors. These results show that the FI method could be used as an advantageous alternative to simultaneously determine creatinine and creatine in cooked meat products, with a considerably reduced analysis time compared to batch methods. Hence, this method was applied to carry out the remaining routine determinations.

### Creatinine, creatine and protein

From creatinine and creatine contents, the total creatine (creatine + 1.159 creatinine) was calculated. The relationship of total creatine to protein in cooked ham, frankfurters, wieners, chopped and mortadella is illustrated in Fig. 1. Table 3 shows the mean values and the relative standard deviations (rsd) for % creatinine of total creatine, protein, total creatine, and the ratio of total creatine/protein, calculated for each class of samples. A statistical comparison of these results was carried out by using the *F*-test ($p < 0.05$) for the comparison of variances and *t*-test ($p < 0.05$) for the comparison of the means.

**Table 1. Results obtained in the determination of creatinine and creatine in 30 samples of cooked meat products, by the flow injection (FI) and the reference methods (RM)**

| | | Creatinine (mg $g^{-1}$) | | Creatine (mg $g^{-1}$) | |
|---|---|---|---|---|---|
| Sample | Source | FI | RM | FI | RM |
| 1 | Cooked ham | 0.66 | 0.65 | 3.15 | 3.15 |
| 2 | | 0.51 | 0.50 | 2.64 | 2.65 |
| 3 | | 0.76 | 0.67 | 2.31 | 2.31 |
| 4 | | 0.74 | 0.73 | 3.26 | 3.25 |
| 5 | | 0.33 | 0.32 | 3.01 | 2.90 |
| 6 | | 0.46 | 0.49 | 2.90 | 2.96 |
| 7 | Frankfurters | 0.18 | 0.18 | 1.16 | 1.31 |
| 8 | | 0.075 | 0.075 | 0.88 | 0.86 |
| 9 | | 0.22 | 0.22 | 1.34 | 1.35 |
| 10 | | 0.12 | 0.11 | 1.45 | 1.42 |
| 11 | | 0.14 | 0.16 | 2.64 | 2.74 |
| 12 | | 0.27 | 0.25 | 0.85 | 0.81 |
| 13 | Wieners | 0.22 | 0.21 | 1.63 | 1.70 |
| 14 | | 0.23 | 0.23 | 2.06 | 2.03 |
| 15 | | 0.15 | 0.14 | 2.12 | 2.12 |
| 16 | | 0.14 | 0.14 | 1.43 | 1.41 |
| 17 | | 0.15 | 0.14 | 1.34 | 1.31 |
| 18 | | 0.12 | 0.13 | 2.39 | 2.34 |
| 19 | Chopped | 0.31 | 0.31 | 1.95 | 2.10 |
| 20 | | 0.43 | 0.42 | 2.14 | 2.13 |
| 21 | | 0.28 | 0.21 | 2.12 | 2.20 |
| 22 | | 0.59 | 0.59 | 2.51 | 2.48 |
| 23 | | 0.13 | 0.10 | 2.15 | 2.00 |
| 24 | | 0.080 | 0.080 | 1.41 | 1.46 |
| 25 | Mortadella | 0.33 | 0.39 | 1.55 | 1.56 |
| 26 | | 0.21 | 0.20 | 1.17 | 1.30 |
| 27 | | 0.10 | 0.11 | 1.36 | 1.32 |
| 28 | | 0.16 | 0.13 | 1.01 | 0.99 |
| 29 | | 0.33 | 0.31 | 1.58 | 1.22 |
| 30 | | 0.14 | 0.11 | 2.42 | 2.42 |

**Table 2. Results of the statistical treatment performed on the pairs of data shown in Table 1. Tabulated values of *t* were obtained at the 95% confidence level.**

| | Student's *t*-test | | Linear regression. FI v.s. RM | | |
|---|---|---|---|---|---|
| | $t_{calc.}$ | $t_{tab.}$ | Intercept | Slope | *r* |
| Creatinine | 1.824 | 2.045 | 0.005 ± 0.008 | 1.018 ± 0.024 | 0.993 |
| Creatine | 0.253 | 2.045 | 0.033 ± 0.053 | 0.985 ± 0.026 | 0.991 |

The percentage creatinine of total creatine increases with heating (temperature and/or time) (Macy *et al.*, 1970; Campero *et al.*, 1992). This increase may be due to conversion of creatine to creatinine under the slightly acid conditions of meat which catalyzes the removal of a molecule of water from creatine to form creatinine (Edgar and Shiver, 1925). Consequently, the percentage creatinine of total creatine may provide additional information on the heat treatment suffered by these products. Cooked ham had significantly more percentage creatinine (15.1) than sausages (frankfurters, 11.0

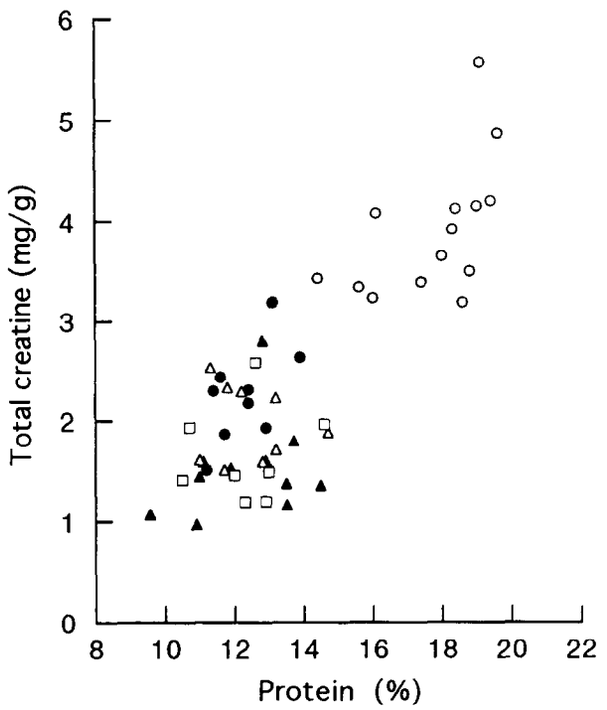


**Fig. 1.** The relationship between total creatine and protein in different samples of ○ cooked ham, ▲ frankfurters, △ wieners, ● chopped and □ mortadella.

and wieners, 8.2), but the comparison with chopped (13.0) and mortadella (13.3) showed no significant differences. In the processing, frankfurters and wieners were only precooked since they will have had to be heat treated before consumption, but cooked ham, chopped and mortadella are ready to consume directly and the cooking conditions must be more severe than those used in the sausages, justifying the higher proportion of creatinine found. Wieners had significantly less % creatinine than the other products except frankfurters, which were not significantly different. Frankfurters showed an intermediate value of percentage creatinine and was significantly lower only than that of cooked ham. Moreover, this group had the highest rsd (49), possibly due to differing heat treatments applied to the different samples. In contrast, cooked ham and wieners showed the lowest rsd (24 and 28, respectively), indicating the application of similar heat treatments for each group of samples.

The variation of total creatine/protein between the different groups was parallel to that of total creatine. The cooked ham had the highest mean values for all the parameters considered and showed significant differences with respect to the total creatine, protein contents and their ratio compared to frankfurters, wieners, chopped and mortadella. These last four groups did not differ significantly with respect to protein content.

Cooked ham had a mean value of 21.9 mg total creatine per gram of protein. In this class of samples, the connective tissue protein is low (about 10%), and the

**Table 3. Group means and relative standard deviations (rsd) for percentage creatinine of total creatine, protein, total creatine and ratio total creatine/protein**

| | Cooked ham ($n=14$) | | Frankfurters ($n=11$) | | Wieners ($n=9$) | | Chopped ($n=10$) | | Mortadella ($n=8$) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | rsd | Mean | rsd | Mean | rsd | Mean | rsd | Mean | rsd |
| Percentage creatinine of total creatine | 15.1 | 24 | 11.0 | 49 | 8.2 | 28 | 13.0 | 37 | 13.3 | 37 |
| Protein (%) | 17.8 | 9.0 | 12.3 | 12 | 12.4 | 9.7 | 12.4 | 7.1 | 12.3 | 11 |
| Total creatine (mg $g^{-1}$) | 3.90 | 17 | 1.51 | 32 | 1.96 | 19 | 2.19 | 24 | 1.65 | 29 |
| Total creatine/ protein (mg $g^{-1}$) | 21.9 | 14 | 12.3 | 30 | 15.9 | 23 | 17.7 | 22 | 13.0 | 32 |

majority of the protein is net muscle protein. This value agrees with those reported for different meat cuts of various mamals by some authors (Dahl, 1963; Kahn and Cowen, 1977; Dvorák, 1981), who suggested that the ratio total creatine/net muscle protein is independent of the age and geographic origin of the animal. The values obtained in the present work, although limited to ham, support this opinion. A comparison of all samples indicated that the ratio of total creatine/protein of cooked ham was significantly higher than that of other products. Chopped also had significantly more total creatine (and a higher ratio of total creatine/protein) than frankfurters and mortadella, and wieners more than frankfurters, whilst the differences found between frankfurters and mortadella and between wieners and chopped or mortadella were not significant.

Some conclusions can be drawn from Fig. 1 and Table 3. The samples of the reference group (cooked ham) had the highest values for the ratio of total creatine per gram of protein and they formed the most homogeneous group (rsd = 14) and were clearly differentiated from the other groups (Fig. 1). The samples of frankfurters and mortadella had the lowest values for this ratio (12.3 and 13.0 mg total creatine per gram of protein, respectively) and they showed the highest dispersion (rsd: 30 and 32, respectively). Wieners and chopped had intermediate values for both parameters (15.9 and 17.7 mg total creatine per gram of protein; rsd, 23 and 22, respectively), chopped being the product closest to cooked ham. This similarity indicated that, in general, the protein used for the production of chopped had a higher content of muscular protein than that used for the manufacture of sausages and mortadella.

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