# Influence of Tumbling and Dextrose on Furosine Content in Cooked Ham

Carlo Pompei* and Alessandro Spagnolello

Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche, Sezione di Tecnologie Alimentari, Università degli Studi di Milano, Via Celoria 2, I-20133 Milano, Italy

In this study the influence of two technology phases on furosine content in cooked ham has been examined. The furosine content in semimembranosus muscles separated from legs tumbled for 25, 45, and 65 h and then subsequently heat treated at 65, 75, and 85 °C for different times is not dependent on tumbling time. Raw muscles with various quantities of dextrose added (between 0.25 and 1.50 g/100g) after heat treatment at 80 °C for different time periods display a quantity of furosine that is directly correlated to the amount of added dextrose. A highly significant correlation ($P < 0.001$) between the furosine index (FI = milligrams of furosine per 100 g of protein divided by percent dextrose) and the intensity of the heat treatment, expressed in terms of $C_0$ (equivalent time at 80 °C), was noted for semimembranosus muscle heated for different lengths of time and in various temperature conditions.

**Keywords:** *Furosine; cooked ham; heat damage*

## INTRODUCTION

Furosine (ϵ-*N*-2-furoylmethyl-L-lysine), formed by acid hydrolysis of ϵ-*N*-deoxyfructosyl-L-lysine (fructose-lysine) and ϵ-*N*-deoxylactulosyl-L-lysine (lactulose-lysine), is a product of Amadori rearrangement of glucose and ϵ-*N*-2-lysine (Erbersdobler, 1986). It is detected, after acid hydrolysis, through the use of different chromatographic techniques such as gas chromatography (Buser and Erbersdobler, 1985), ion exchange chromatography (Erbersdobler et al., 1987; Desrosiers et al., 1989), reversed phase HPLC (Chiang, 1983; Watanabe et al., 1995; El Zeany et al., 1995), ion-pair reversed phase HPLC (Resmini et al., 1990a,b; Resmini and Pellegrino, 1991; Pellegrino, 1994; Pellegrino et al., 1995; Hidalgo et al., 1995; Henle et al., 1995), and capillary electrophoresis (Tirelli and Pellegrino, 1995; Corradini et al., 1996).

Furosine detection has been used for the evaluation of heat treatment intensity in different food products such as milk (Erbersdobler and Dehn, 1989; O'Brien and Morrisey, 1989; Resmini et al., 1990b; Pellegrino, 1994; Pellegrino et al., 1995; Watanabe et al., 1995; Tirelli and Pellegrino, 1995; Corradini et al., 1996; Meissner and Erbersdobler, 1996), pasta products (Resmini et al., 1990a), eggs (Hidalgo et al., 1995), and cooked ham (Pompei and Spagnolello, 1997).

In relation to cooked ham it has been demonstrated that the furosine content, in the case that production technologies do not differ, can be traced back to the intensity of cooking, expressed in terms of $C_0$, the "equivalent time" of treatment at a reference temperature.

The objective of this work is to evaluate the influence of two phases involved in the production of cooked ham—tumbling and dextrose content in brine injection—on furosine formation, to make the latter an index of general use.

## MATERIALS AND METHODS

The hind legs of French hogs, weighing between 130 and 140 kg (Large White × Landrace hybrid), were used in the experiments. After slaughter, the legs were kept refrigerated (1 °C) for about 130−140 h.

For the tumbling tests, the legs were injected with 30% (w/w) brine containing 8.8% NaCl and 3.6% dextrose; this operation was performed in a commercial plant. Tumbling was carried out in a small scale vacuum tumbler that could treat eight legs per cycle. Three tumbling treatments were carried out, for 25, 45, and 65 h, respectively; 45 h represents the standard tumbling time used by the company at which the tests were carried out. After the completion of the tumbling operation, four legs were chosen at random from each treatment, and the semimembranosus muscles were manually extracted and transferred, at a temperature of 5 ± 1 °C, to our laboratory.

The four semimembranosus muscles from each treatment were minced and mixed. Fifteen gram samples of minced muscle were then placed in cylindrical glass vials (i.d. = 13.5 mm) and cooked in water baths at 65, 75, and 85 °C. Vials were maintained at each temperature for 35, 65, and 95 min. After cooking, the vials were immediately cooled by dipping them into cold water. Furosine and total nitrogen were detected in the samples.

The effect of dextrose concentration in brine on furosine formation was studied according to the following procedure. Semimembranosus muscles extracted from chilled legs were minced and mixed. Dextrose was added to the minced meat aliquots of 100 g in the following quantities: 0.25, 0.50, 0.75, 1.00, 1.25, and 1.50 g. After mixing, the samples were kept overnight at 5 ± 1 °C. Samples of 15 g of minced meat were then cooked according to the procedure described above in a water bath, at 80 °C for 33, 63, and 93 min. After cooling in cold water, furosine and total nitrogen were detected in the samples.

To verify the influence of dextrose content in muscle on furosine formation, after cooking, whole semimembranosus muscles were tested in the following manner. Semimembranosus muscle (weighing 1.2−1.3 kg) was separated from the hog legs injected with brine and tumbled in a commercial plant. Muscle was pressed in the aluminum molds, of about 60 mm diameter, normally used for commercial cooking of salami. Through a hole in the lid, a needle thermocouple was inserted (Ellab, Model 1001, Copenhagen) to measure temperature in the thermal center. Cooking tests were conducted by dipping the molds in water baths at a temperature ranging between 70 and 90 °C. To simulate distinct heat treatments, different cooking times at the various temperatures were used. After cooking, the molds were cooled in running water and then kept overnight at the temperature of 5 ± 1 °C.

S0021-8561(97)00116-7 CCC: $14.00 

The sample used for evaluation was a cylinder of muscle, 10 cm long and of 15 mm diameter, obtained by means of coring the cooked muscle along its central axis; obviously, the ends were discarded. After mincing and mixing, the quantities of furosine, total nitrogen, and dextrose in the sample were measured.

Protein content was determined as total nitrogen multiplied by 6.25 and expressed as percentage (w/w) of the sample. The digestion of the sample was carried out with Maxidigest MX4350 (Prolabo, Paris), while subsequent distillation was performed following the traditional method (AOAC, 1990).

Furosine content was determined by HPLC following the method proposed by Resmini et al. (1990b); however, it was slightly modified, as reported by Pompei and Spagnolello (1997).

Dextrose was detected using the enzymatic method (Boehringer Mannheim, code no. 716 251). A 1 g sample of minced meat was carefully homogenized with 60 mL of distilled water and heated for 15 min in a water bath at 75 °C. For protein precipitation 5 mL of Carrez 1 solution [3.60% potassium hexacyanoferrate(II) in water; w/v], 5 mL of Carrez 2 solution (7.20% zinc sulfate in water; w/v), and 10 mL of 0.1 M NaOH were added. After cooling at room temperature, the mixture was kept at 5 ± 1 °C for at least 1 h and then filtered (Whatman No. 4). The filtrate was collected in a 100 mL flask and brought to volume with the distilled water used for washing the filter; 0.1 mL of this solution was used for the enzymatic determination. Results are expressed in grams of dextrose per 100 g of the sample.

Repeated tests carried out on six aliquots of the same sample showed for furosine, total nitrogen, and dextrose percent variation coefficients of 3.8, 3.2, and 4.7, respectively, on a mean value of 92.0 mg/100 g of protein for furosine, 2.51 g/100 g for total nitrogen, and 0.38 g/100 g for dextrose.

All analytical results obtained are the average of at least two replicates.

Statistical data were processed using Systat for Windows (SYSTAT, 1990).

## RESULTS AND DISCUSSION

The quantity of furosine in heat-treated food products depends on both the intensity of the heat treatment and the chemical availability of the compounds that are responsible for Maillard reaction. A direct linear correlation between furosine and the cooking index, $C_0$, has been demonstrated in cooked ham (Pompei and Spagnolello, 1997).

In cooked ham production technology, two factors may influence the chemical availability of the compounds responsible for Maillard reaction: The first is tumbling, whose purpose is to cause, by "massaging" or "bumping", the release of the proteins, which are subsequently coagulated by heat. The second factor is the quantity of dextrose that is injected in ham during brine injection.

With regard to tumbling, the effect of time on it has been studied. Figure 1 illustrates furosine content in samples of semimembranosus leg muscle tumbled for 25, 45, and 65 h and subsequently heat-treated, as described under Materials and Methods, for 35, 65, and 95 min at temperatures of 65, 75, and 85 °C. The figure shows that furosine formation kinetics are practically not influenced by tumbling time. Therefore, it can be inferred that this stage of the process is not related to the presence of furosine content in the end products.

The influence of dextrose on furosine formation was studied in cooking tests; these tests were carried out on raw muscles cooked at 80 °C to which different quantities of dextrose were added. Results are reported in Figure 2. When plotting the slopes of the curves

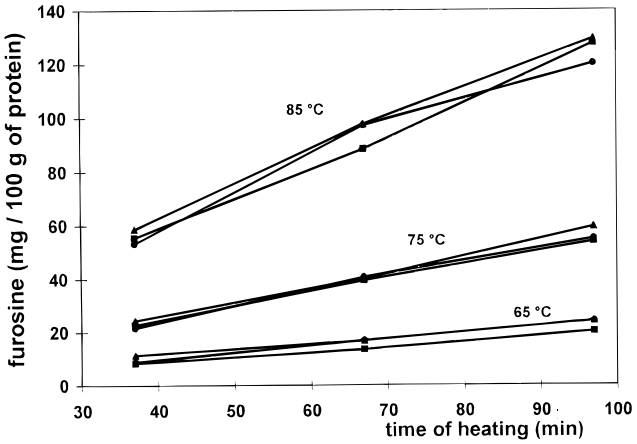


**Figure 1.** Furosine content in semimembranosus muscles obtained from legs tumbled for 25 (■), 45 (●), and 65 (▲) h and heat treated at temperatures of 65, 75, and 85 °C for different lengths of time.

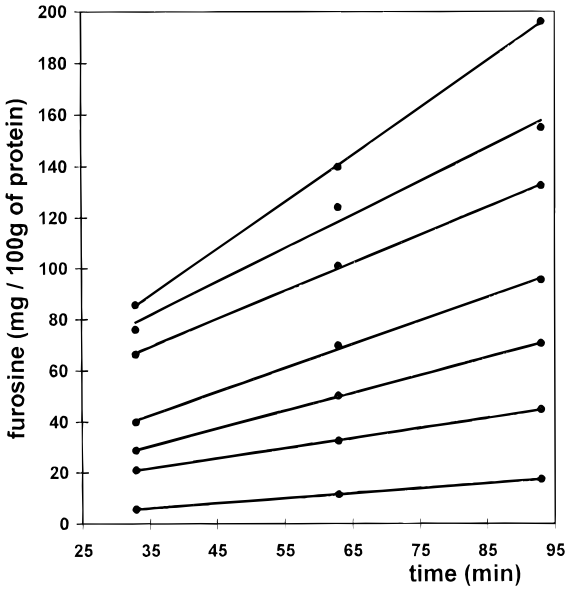


**Figure 2.** Influence of dextrose content on furosine formation. The curves are related to muscle added with increasing dextrose quantities (0.0, 0.25, 0.50, 0.75, 1.00, 1.25, and 1.50 g/100 g, bottom to top) and heat treated at 80 °C.

versus the quantity of dextrose added (Figure 3), a direct linear correlation ($r^2 = 0.976$; $P < 0.001$) between furosine formation kinetics and the added dextrose is apparent. Therefore, the quantity of furosine in cooked ham rules out the possibility of tracing back to the heat treatment intensity, since the quantity of dextrose, added during brine injection, can vary in cooked hams of different qualities produced by different companies.

To use a "furosine index" (FI) as an indicator of heat treatment, dextrose content must be considered. Since in end products of unknown origin the only information available about dextrose is the one pointed out at the time of analysis, cooking tests on semimembranosus muscles, separated from legs injected in a commercial plant with different quantities of dextrose, were carried out.

Temperature was measured during the different cooking tests and, for each test, the time−temperature profile of the heating and cooling phases was determined. On the basis of such profile the value of $C_0$ was

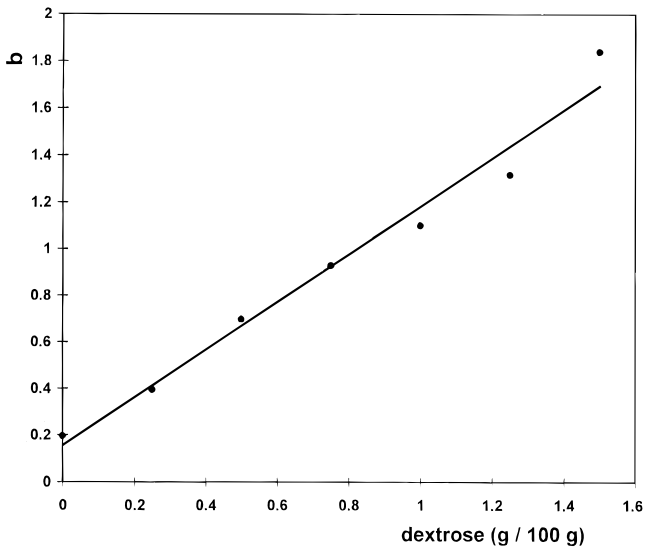


**Figure 3.** Correlation between furosine formation kinetic ($b$ = slope of the curves of Figure 2) and dextrose added to the muscle.

**Table 1. Values of $C_0$, Furosine, and Dextrose in Semimembranosus Muscles Separated from Legs Injected in a Commercial Plant and Heat Treated at Various Temperatures for Different Lengths of Time**

| test | $C_0$ (min) | furosine (mg/100 g of protein) | SD[a] | dextrose (g/100 g of sample) | SD |
|---|---|---|---|---|---|
| 1 | 80.8 | 33.1 | 0.5 | 0.40 | 0.020 |
| 2 | 115.8 | 65.5 | 2.5 | 0.52 | 0.015 |
| 3 | 135.7 | 55.2 | 7.1 | 0.32 | 0.005 |
| 4 | 190.8 | 89.0 | 6.5 | 0.35 | 0.014 |
| 5 | 232.3 | 186.9 | 4.0 | 0.54 | 0.032 |
| 6 | 275.0 | 204.8 | 4.7 | 0.51 | 0.024 |

[a] SD, standard deviation.

calculated using the formula

$$C_0 = \int_0^t \frac{dt}{10^{(\vartheta^* - \vartheta)/z}}$$

where $\vartheta^*$ is the reference temperature (80 °C) and $\vartheta$ is the temperature at time $t$. The value of $z$, representing a temperature increase (°C) that causes a 10-fold increase in the reaction rate, was 29.2 °C (Pompei and Spagnolello, 1997). $C_0$, the so-called cooking effect, allows the comparison, with reference to a heat damage reaction, of heat treatments performed at different time−temperature conditions, in terms of "equivalent time" at a reference temperature. In this case, the reference temperature was 80 °C.

Table 1 reports, for the six tests, the values of $C_0$, furosine, and dextrose. Assuming as a FI, furosine content (milligrams per 100 g of protein) divided by dextrose concentration (grams per 100 g of sample), and plotting such index versus $C_0$, the correlation curve reported in Figure 4 is obtained. The tests that we have carried out, which reproduce the whole range of industrial heat treatments, clearly show the existence of a highly significant linear correlation ($r^2 = 0.994$; $P < 0.001$) between FI and heat treatment:

$$FI = 1.7C_0 - 61.51$$

Therefore, it is possible to trace back to the intensity of the heat treatment at a certain point (for example, the thermal center of a cooked ham), by measuring, at that point, both furosine and residual dextrose and determining the furosine index. The latter is not influenced by tumbling intensity after brine injection.

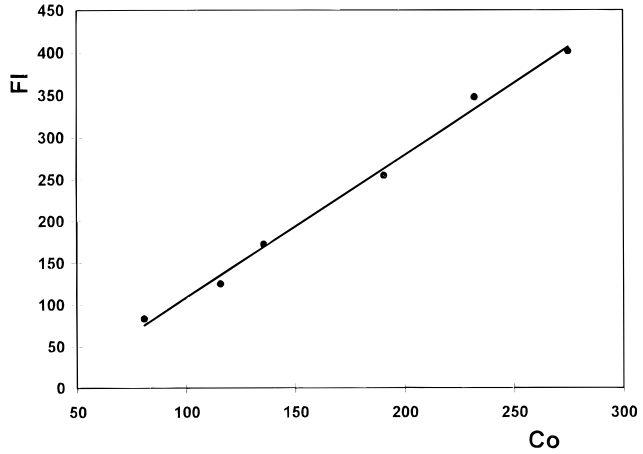


**Figure 4.** Correlation between FI and heat treatment intensity, expressed in terms of $C_0$ (equivalent time at 80 °C), in heat-treated semimembranosus muscles.

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Received for review Feburary 10, 1997. Revised manuscript received June 13, 1997. Accepted June 16, 1997.[⊗]

JF970116N

[⊗] Abstract published in *Advance ACS Abstracts,* August 1, 1997.