# PHYSICAL AND CHEMICAL CHANGES IN PROTEINS IN THE MATURATION OF PARMA HAM. III. NUTRITIONAL ASPECTS

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One of the aspects of Parma ham technology which has aroused the interest of research workers and of dieticians is the evaluation of its effects on nutritional values. Such a technology is merely restricted to establishing minimum requirements for preservation without acting directly with additives or special treatments on the production of typical quality attributes (maturing).

For this reason only limited amounts of salt are employed with the backing of refrigeration at the beginning of processing (2-3 months) and of a restricted degree of dehydration up to consumption. This makes the technique the mildest form of preservation and therefore it is considered not to impair meat nutritional properties.

Investigations specifically devoted to assess the nutritional value of Parma ham have been limited in the past to approximate digestibility assays and to the quantitative evaluation of some B vitamins. Minoccheri & Cantoni (1971) have shown that vitamin  $B_{12}$  levels remain constant over the entire maturing period, while Massi (1964) and Cantoni et al.(1971) have observed an increase of pepsin digestibility of matured hams relative to fresh pork.

No research has been carried out on the effect of maturing on essential amino acids, especially on those which more than others can be damaged by processing. It is the case of tryptophan, cystine and available lysine which are often used as indices of processing damage (Lawrie, 1979).

In addition to the determination of these three essential aminoacids it has been considered worthwhile to reassess the digestibility of Parma ham proteins as this parameter, besides the amino acid profile, is the most important determinant of the availability of amino acids. Most tests on proteins nutritional value and digestibility are carried out with rats in vivo assays but such tests are time-consuming and expensive. For this reason several alternative in vitro digestibility methods have been proposed all based on enzymic digestion with proteolytic enzymes (Saunders et al., 1973; Rhinehart 1975, Floridi & Fidanza, 1975).

Most of these in vitro methods, though, are not completely satisfactory because the procedures are complicated and time-consuming. Recently a multyenzyme method has been proposed (Satterlee et al., 1979) which requires a very limited time and, moreover, has been compared with digestibility values in humans with significant correlations (Bodwell et al., 1980).

### METHODS AND MATERIALS

Samples have been prepared as previously described (Bellatti et al., 1983). Total amino acids were determined on freeze-dried samples of fresh and matured muscles. Digestibility was assessed on freeze-dried and on freeze-dried and dialysed samples from each of the sampling times, i.e. pre-salting (F), post-salting (S), post-resting (R), half-maturing (MS) and end of maturing (ST) (Satterlee et al., 1979).

Tryptophan has been determined after alkaline hydrolysis in the following way:samples of 7-10 mg N<sub>2</sub> were mixed with 3.2g of barium hydroxide and 5 ml of distilled water. Oxygen was eliminated by boiling and the samples cooled in a freezer. After cooling hydrolysis was carried out at 110°C for 22h; the hydrolysed samples were brought to pH 4 with sulphuric acid 5N and centrifuged to get rid of barium sulphate. The remaining solution was rotary evaporated to dryness, dissolved in 20 ml of sodium citrate buffer pH 2.2 and analysed with a Carlo Erba 3A29 amino acid analyser.

Available lysine was assayed following Carpenter's method (1960), based on the reaction of amino groups with 1-fluoro-2,4-dinitrobenzene (FDNB), and estimated as the difference between total lysine before and after reaction with FDNB (Vervack et al.1976). Cystine was determined as indicated by Moore (1962).

### RESULTS AND DISCUSSION

The results of tryptophan, available lysine and cystine as well as of the other aminoacids (Table 1) give an interesting piece of information on Parma ham processing technology. All aminoacids are remarkably constant from beginning to end of maturing, thus confirming the view that this type of processing does not reduce meat nutritional value.

It would have been interesting to compare tryptophan values here reported with similar data on pork products prepared with other curing technologies. Unfortunately no such data are available and comparison is thus possible only with processing methods such as those based on heat treatment. Thermal processing, even if it isn't sterilisation, causes a variable, but constant, destruction of tryptophan and in some instances such a loss continues during storage (Bender, 1978) Lawrie, 1979; Rechcigl, 1982).

The influence of processing techniques on the availability of lysine has been more thoroughly investigated. As with tryptophan heat processing causes a loss of available lysine both in pork and in beef, frequently due to a Maillard reaction (Heller et al., 1961; Donoso et al., 1962; Dvorak & Vognarova, 1965; Osner & Johnson, 1968). Available lysine, though, has been determined also in some cured and smoked pork products, with results rather different from those reported in this work.

Dvorak and Vognarova (1965) have observed a decrease of available lysine both in salted pork added with nitrite and in smoked pork. Salted pork with nitrite would show a loss of available lysine because in the acid medium of meat some of the nitrite ions react with free amino groups such as lysine amino groups. The reaction is proportionate to nitrite concentration. Similarly in smoked pork a loss of available lysine is due to a reaction between formaldehyde present in smoke and lysine e-amino groups. Such a bonding is as firm as that with sugars in the case of a Maillard reaction. This report has been confirmed by De-Vuyst et al. (1973) for marinated and smoked hams. The former had a 61% of available lysine while the latter had a slightly higher value, i.e. 72%.

Analogous considerations can be made for cystine which is affected to a variable degree by most processing methods but which does not seem to suffer during Parma ham maturation.

Digestibility studies have given a more complex picture.

It can be noted first that non dialysed samples (Table II) show a slight but constant and significant decrease in digestibility.

The cause of such a phenomenon could be attributed to:

- 1. the concentration of salt, a possible inhibitor of enzyme activity, which increases with maturing;
- 2. changes in the buffering capacity of muscles due to the presence of higher amounts of free aminoacids and peptides of low molecular weight (Bellatti et al. 1983);
- 3. proteins denaturation.

As for the first possible cause assays with salt concentrations equalised in all samples, the results of which have not been included in the table, did not indicate changes in the digestibility values, showing that salt, at the concentrations of these tests, does not inhibit the activity of the proteolityc enzymes employed. Hsu et al.(1977), though, underlined the

possibility that strong buffering substances could affect the results. All samples therefore, including the pre-salting ones, were dialysed against distilled water with Visking tubing (18/32") retaining substances with molecular weight over 14.000. Dialysed samples gave the surprising result (Table II) of constant digestibility values over the entire processing period showing in this way that low molecular weight compounds could limit pH fall during digestibility assays.

At the same time digestibility tests with dialysed samples indicate that muscle proteins are not significantly touched by processing, at least to a degree sufficient to make them less susceptible to proteolytic attack. This agrees with what has been observed in a previous work (Bellatti et al., 1983) and with what has been reported by Bender (1978) concerning the lack of effect of denaturation on protein nutritional value. It should also be remarked that the increase of small protein and non-protein nitrogenous compounds in matured hams (Bellatti et al., 1983), responsible for the lower digestibility values of non dialysed samples, could have a nutritional significance of its own. Such compounds could be important both as partially pre-digested proteins and as flavor precursors, yet their role is difficult to evaluate.

It appears therefore that Parma ham nutritional quality, as it has been evaluated in this research, is not impaired by the processes of salting and maturing since the decreased digestibility of non dialysed samples can be attributed to inadequacy of the method. Indeed the clear difference observed between the digestibility values of non-dialysed versus dialysed samples suggests the need for amendments to the method employed in this study. Bodwell et al. (1980), on the other hand, have already reported that this method under-values digestibility of animal proteins, a fact which has received support in this investigation and which requires further research.

Table I - Amino acid composition and available Lysine content of fresh and matured Parma hams (as % crude protein). In parenthesis the perc.standard deviation of four independent analyses.

Amino acid	Fre	Fresh		Matured	
Isoleucina	4.8	(6.0)	4.5	(5.0)	
Leucine	7.7	(5.1)	7.8	(3.5)	
Lysine	10.8	(5.0)	10.4	(3.5)	
Available Lysine	10.6	(4.9)	10.4	(4.3)	
Methionina	2.7	(7.0)	3.0	(2.3)	
Cystine	1.4	(11.0)	1.4	(12.3)	
Phenylalanine	3.6	(6.6)	3.8	(3.8)	
Threonine	4.4	(9.9)	4.6	(10.9)	
Tryptophan	1.5	(2.6)	1.5	(533)	
Valine	4.5	(5.4)	4.4	(1.8)	
Arginine	7.3	(5.1)	7.3	(3.0)	
Histidine	5.8	(6.8)	5.9	(1.4)	
Alanine	5.3	(4.4)	5.4	(3.0)	
Aspartic Acid	8.7	(5.9)	8.8	(5.8)	
Glutamic Acid	15.0	(5.1)	15.8	(3.1)	
Glycine	3.8.	(5.3)	3.9	(2.3)	
Proline	3.1	(6:0)	3.1	(5.7)	
Serine	4.1	(8.9)	4.0	(5.4)	
Tyrosine	3.4	(7.0)	3.5	(3.2)	

Table II.

Digestibilities of freeze-dried, dyalised (FD) and non-dyalised (FND) hams.

Abbreviations: F=Fresh: S=post-salting; R=post-resting; MS=half-maturing; ST=end of maturing. Each value is the mean of four independent analyses.

	F	S	R	MS	ST Suk
FD	102.3	101.5	103.8	102.9	101.9
END	00.0	01.6	mell ves less	00.0	IT & Sec o
FND	92.9	91,6	91.5	86.8	86.2

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## MODIFICAZIONI FISICHE E CHIMICHE DELLE PROTEINE NELLA MATURAZIONE DEL PROSCIUTTO DI PARMA III. ASPETTI NUTRIZIONALI

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Sono state seguite le variazioni degli amminoacidi totali (inclusi il triptofano, la cistina e la lisina disponibile) e della digeribilità enzimatica nella maturazione del Prosciutto di Parma. I risultati mostrano che non esiste differenza significativa nel contenuto di amminoacidi è di lisina disponibile all'inizio della lavorazione e alla fine della stagionatura. - Anche la digeribilità, misurata per via enzimatica sulle proteine private della frazione a peso molecolare inferiore a 14.000, rimane costante, e conferma che il processo di stagionatura non influisce negativamente sulle carateristiche nutrizionali di questa derrata.

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Changes in total aminoacid content (including triptophan, cystine and available lysine) were followed throughout the maturation of Parma ham. Results indicate no significant difference in the aminoacid composition and in the available - lysine content at the beginning and at the end of maturing. Digestibility, measured with the 4-enzyme method on the proteins made free of components with M.W. \( \alpha \)14.000, remained constant, too, thus confirming that maturing does not impair Parma ham nutritional properties.