PHYSICAL AND CHEMICAL CHANGES IN PROTEINS DURING THE MATURATION OF PARMA HAM.
I. BIOCHEMICAL AND FUNCTIONAL CHANGES

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Investigations on chemical and physical phenomena responsible for the maturation of Parma ham have been mainly centred on the study of muscular proteins breakdown.

Ambanelli et al.(1968,1969) observed, with maturing, a progressive increase of non proteic and - amino nitrogen together with free amino acids. Intense proteolytic break-down has been confirmed by Cantoni at al. (1970 a,b; 1972) and by Giolitti et al. (1971) who reported an enhanced catheptic activity, cathepsins appear to be able to attack connective tissue proteins as well (Frati e Ambanelli, 1972 a,b). Maggi et al. (1973,1977) studied water soluble proteins and peptides using polyacrylamide gel electrophoresis, with and without SDS, showing proteolytic degradation of sarcoplasmic proteins.

It is therefore accepted that Parma ham maturation is associated with proteolytic phenomena but very little is known of physical and chemical changes of muscular proteins and of their functional state. A deeper knowledge of these aspects, though, could be particularly useful from a technological point of view and for a nutritional evaluation of dry-cured pork products.

This paper reports the results of studies dealing with some chemical and functional parameters of biceps femoris muscles taken from swine legs employed for the production of Parma ham. Biceps femoris muscle has been chosen because, due to its deep position, it receives salt only by slow inward diffusion and loses moisture gradually over a long period of time.

For these reasons it can be considered truly representative of the effects that the production technology of Parma ham has on muscular proteins.

METHODS AND MATERIALS

Twenty swine legs were chosen at the slaughterhouse from a group of pigs belonging to the same breeding unit. The legs, which met the requirements to be classified as suitable for Parma ham production, were handed over to a plant to be processed in a traditional way. The lot was divided into 5 groups of 4 to fit in the following sampling scheme:

- pre-salting (F): the hams were held, after slaughter, for 48h at 0°C;
- post-salting (S): the hams were sprinkled with coarse salt (NaCl only), at 48h post mortem and 7 days later, and left at 0-4°C up to 28 days post-mortem (p.m.);
- post-resting (R): the hams, brushed to free them from salt left on their surface, were kept at 0-5°C up to 83 days (MS) p.m.;
- half-maturing (MS): the hams, at the end of the previous phase, were washed with lukewarm water, dried and left to mature at 13-15°C up to 6 months p.m.;
- end of maturing (ST): the hams were left to mature up to 12 months p.m., always at 13-15°C.

16 more legs of the same group of pigs were cured with a lower amount of salt to evaluate possible effects linked with salt concentration. Such legs, though, were kept at refrigeration temperatures for longer, i.e. 104 days instead of 83, to reduce the risk of bacterial growth. Sampling times were therefore modified as follows:

end of curing: 21 days p.m.;
end of resting: 104 days p.m.

The chemical and functional parameters studied were measured, as outlined in fig.1, in the following way:

- pH: samples were homogenized with iodoacetic acid 0,01 M, pH 7.0;
- proximate composition: water, total nitrogen (TN), ash and salt were determined following official AOAC methods. Salt was measured as chloride by titration with A_gNO_3-NH_4CNS; the samples were ashed in muffle furnace increasing the temperature very slowly, especially between 250 and 350°C, up to a maximum of 500°C at which temperature they were kept until the ashes had become completely white;
- sarcoplasmic proteins (total extractable nitrogen-TEN): 50g of muscle (30g for the samples of the last two sampling times to take into account the moisture they had lost) were homogenized with 200 ml of distilled water which, for the first three samples, was added with NaCl 0.9% (pre-curing), 0,6% (post-curing) and 0,5% (post-resting) (nitrogen was determined by Kjeldahl);
- non-protein extractable nitrogen (NPEN) : samples of TEN extracts were treated with an equal volume of TCA 10% and filtered through Whatman No 2 (nitrogen by Kjeldahl);
- -SH groups (total and non-protein) were determined on TEN extract as indicated by Sedlak and Lindsay (1968);
- myofibrillar proteins extraction was performed as Ikeuchi et al.(1980) and the extract employed for the evaluation of ATPase activity both with and without Ca^{2+} (Ikeuchi et al.,1980).

The samples were in part freeze-dried, powdered in a mortar, defatted with cold diethyl ether and sieved with a 80 mesh sieve. The powder was utilised for the following tests:

- total amino acids (TAA): samples were hydrolysed with HCl 6 N for 18h in open flasks under reflux and analysed with a Carlo Erba 3A29 amino acid analyser;
- free amino acids (FAA): samples of the freeze- dried muscle powder were cold extracted with ethyl alchool (ETOH) 75% and the extract subjected to amino acid analysis without pre-digestion;
- ethanol extractable nitrogen (ETOH-EN): nitrogen content of ETOH extract for free amino acids was ascertained by micro-Kjeldahl.

Finally the freeze-dried muscle powder was dialysed with Visking tubing (18/32", retaining substances with molecular weights over 14,000) for 3 days against distilled water (3 water changes) at 0°C to get rid of salt, free amino acids and peptides of low molecular weight. The residue was again freeze-dried and employed for moisture adsorption isotherms determination. This test was carried out by pre-drying the freeze-dried samples over P_2O_5 for 7 days and exposing them afterwards for 14 days to 10 environments of varying relative humidities controlled through saturated solutions.

RESULTS AND DISCUSSION

Results are summarised in Table 1.

Proximate composition and pH values are in line with known Parma ham standards (Baldini and Raczynski, 1979).

pH increases gradually to just over 6 at the end of processing suggesting that proteolysis do occur but in the absence of putrefactive microorganisms. Salt/water ratio grows up to the half-maturing phase and, from then on, remains constant.

The second group of hams has lower salt/water ratios as a consequence of the different curing technique employed but the processing pattern is the same.

ATPase activity and -SH groups decrease regularly from the beginning to the end but ATPase is already not measurable

at half-maturation while-SH groups diminish to about 50-60% of their initial values at the end of maturation. Both parameters behave therefore in a similar way in accordance with the hypothesis that a relation exists between ATPase activity and -SH groups condition (Arakawa et al., 1970). -SH group decrease can probably be ascribed to oxidative processes which are made possible by the higher ionic strength of muscular sarcoplasm consequent to the increased salt/water ratio.

The process of maturation has therefore the effect of gradually impairing the most important muscular function, that is muscle contractile properties, clearly linked with ATPase activity. -SH groups certainly play an important role in this process as two active sites involved in the actin-myosin interaction have thiol groups present ,or near to their active centres (Briskey e Fukazawa,1971).

Further information on the functional state of muscular proteins can be inferred from moisture adsorption isotherm (fig.2) and in vitro digestibility assays, carried out on dialysed samples. In vitro digestibility values (see for full report Chizzolini et al., 1983) do not change over the entire period of maturation while sorption isotherms show a moderate increase in water holding capacity after curing, an increase which remains unchanged till the end of processing.

The latter event is so limited, though, that it cannot by itself suggest that physical or chemical changes in proteins have taken place, on account also of the unchanged digestibility values and of the increase of soluble nitrogen. Sorption isotherms could be tentatively explained here as a stable swelling of protein filaments following chloride ions addition.

Data on soluble nitrogen and free amino acids (Table 1) confirm the occurence of proteolytic phenomena but show, on the other hand, that protein breakdown takes place after the initial period of processing, i.e. the refrigerated phase, and has an inverse relation with salt concentration. It has been observed that differences between group 1 and 2 are in this regard significant (P=0.05), raising some questions on the effect of the second technique employed (milder curing and longer refrigeration) on the appearance of typical Parma ham quality traits. It is interesting also to note the apparent

inverse relation between proteolysis and salt concentration although it would be necessary to investigate more thoroughly the separate effects of cold storage and salt concentration.

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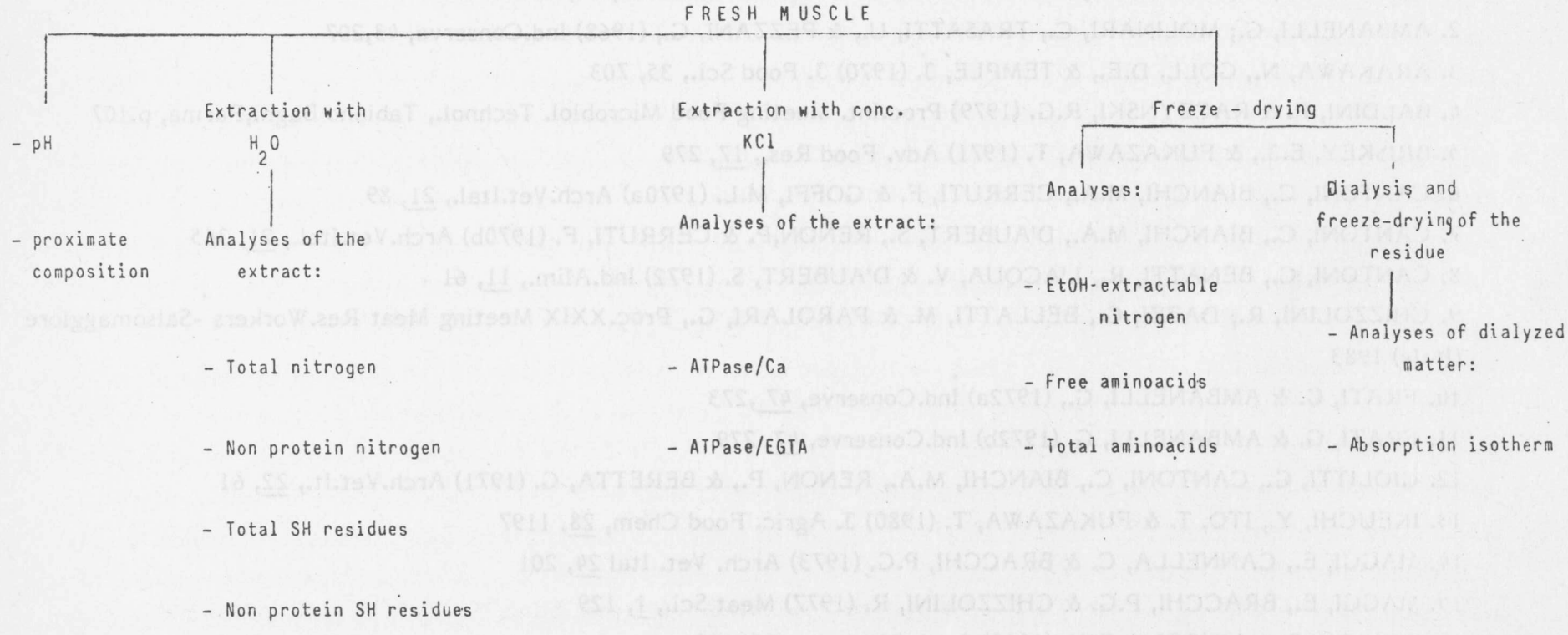
Fig. 1 - Flow-chart showing the analyses performed on biceps femoris muscles



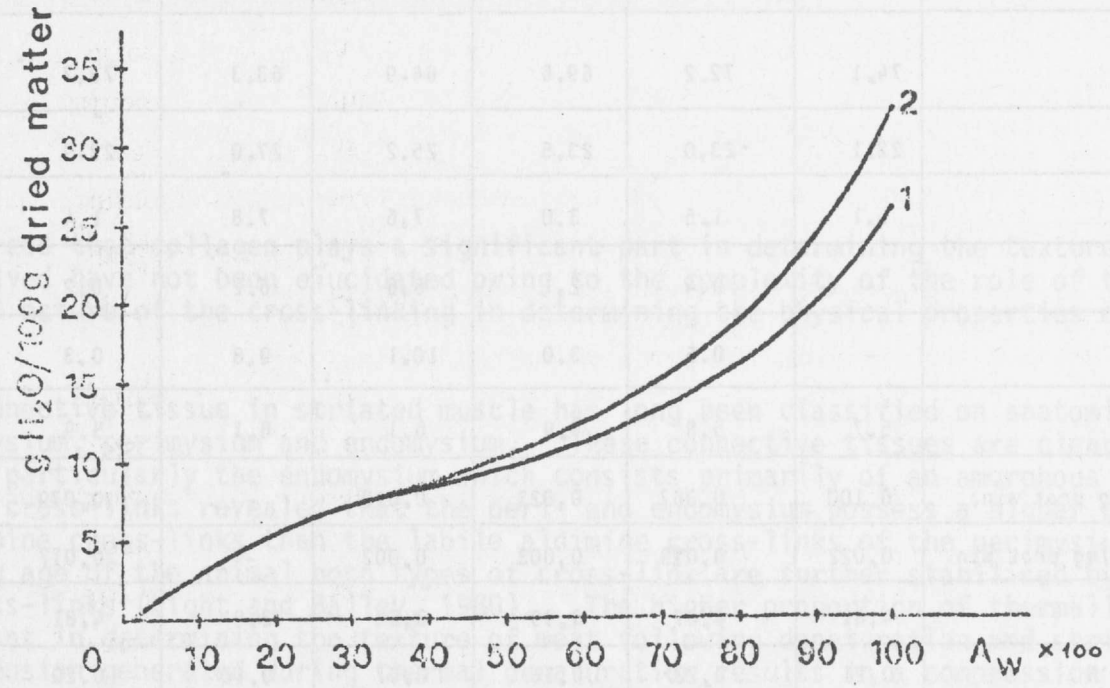


Fig. 2 - Sorption isotherms of fresh hams (curve 1) and of hams post-salting, post-resting and fully-matured (curve 2).

Table I - Results of the analyses of hams prepared with traditional (1) and low (2) salt. Sampling phases: F= fresh; S=post salting; R=post resting; MS= half-maturing; ST= end of maturing. Each result is the mean of two replicates.

	F	S1	R1	MS1	ST1	S2	R2	MS2	ST2
H_2O %	74,1	72,2	69,6	64.9	63.3	73,2	70,1	65,6	64.5
TN x 6,25%	22,1	23,0	23,5	25.2	27,0	22.9	23,2	24.8	26,8
Ash %	1,1	1,5	3.0	7,6	7.8	1,4	2,7	6,0	6.7
NaCl %	-	0.4	2,1	6,6	6.2	0,2	1,6	5,1	5,3
$NaCl/H_2O$ %	-	0.5	3.0	10,1	9.8	0,3	2.3	7.7	8.2
pH	5.7	5.8	5,9	6.1	6,1	5,9	6,0	6,1	6,0
ATP ase/Ca μ moles P/mg prot min.	0,100	0,067	0,023	0,010	-	0,079	0,023	0,009	-
ATP ase/EGTA μ moles P/mg prot min.	0,022	0,019	0,003	0,002	-	0,017	0,002	0,001	-
TOT SH moles. 10^{-4} g N	4,81	4,87	3,99	3,63	2,9	4,81	4,29	3,61	2,6
NP SH moles . 10^{-4} g N	0,21	0,20	0,11	0,07	0,10	0,20	0,13	0,08	0,09
NP SH/T SH	4,3	3,8	2,6	1,9	3,3	4,2	2,8	2,1	3,3
TEN/TN %	30.6	29,7	29,9	23,1	39,8	n.d.	n.d.	25,7	38,6
ETOH-EN/TN%	8,5	9,1	9,9	20,2	23,7	n.d.	n.d.	n.d.	21,7
FAA/TAA %	0,4	1,0	2,1	11,3	14.9	n.d.	n.d.	n.d.	13.7
NPEN/TN %	11,1	13,9	13,5	13,8	26,6	n.d.	n.d.	14,7	26,0