Losses of available lysine in canned beef as influenced by the severity of processing *)

Z. ZIEMBA and Y. MÄLKKI

It is generally known that the free epsilon group of lysine bound in proteins, and accordingly the so-called available lysine, decreases during heat treatments. However, quantitative data regarding the extent to which this change occurs in canned meat, with regard to the severity of the processing, are scarce, and within the range of commercial processing conditions they are lacking.

The following study has been made in order to elucidate this relationship. Presuppositions found necessary were a simulation of the conditions existing during the commercial heat processing of cans, a uniform record the commercial heat processing of cans, a uniform record of temperature throughout the whole sample heated, and a sufficiently precise method for the chemical determination of available lysine.

EXPERIMENTAL

Pilot plant experiments and process calculation

The fresh raw beef meat was taken from neck cuts of bulls about 1 year old. It was frozen two days after slaughtering, then cut preliminarily by a meat-cutting band saw, mixed, stored at −30° C for about 4 months, and, before processing, thawed at +4° C for 16 hrs. Details on the properties and uniformity of the meat used for canning are given in Table 1.

*) This work was done at the State Institute for Technical Research, Laboratory for Food Research and Technology, Otaniemi, Finland, by a member of the staff, Dr. Y. Mälkki, and by a visiting scientist from Poland, Dr. Z. Ziemba, whose present address is: Technological University Politechnika Gdańska, Department of Animal Food Products Technology, Gdańsk 6, Poland.

Table 1. *The properties and uniformity of meat used for canning.*

	Water %	*Fat %*	*Protein % (N×6.25)*	*pH*
No. of samples	3	3	12	3
Mean	75.53	2.64	21.27	5.57
Maximum	75.59	2.99	21.69	
Minimum	75.43	2.46	20.50	
Standard Deviation	0.09	0.31	0.37	
Coeff. of Variation	0.12	11.74	1.74	

Non-lacquered tinplate 99 × 60 mm round cans were packed with 324 g of beef, cut into cubes of about 2 cm, 5.4 g of salt was added, and the can made up to 360 g net weight with distilled water. The headspace was about 9 millimetres, equalling 16.6 % of the can volume.

The heat processing was effected in a Labor-Rotomat pilot-plant scale autoclave, manufactured by Messrs. Mittelhäuser & Walter, Hamburg, West Germany. The rotating drum was packed with fifty cans for each experiment, a number of them being ballast cans with 9 % bentonite suspension. According to preliminary experiments, 30 rpm end-over-end rotation was chosen as a practical optimum for heat penetration. Before being packed in the autoclave, the cans were equilibrated in a water bath for one hour to an initial temperature of 25° C. The sterilizing formula was 8 + 12 + X + 15 min, the times representing holding the temperature of the retort at 100° C, heating up to 121° C, holding at 121 °C, and cooling to 40° C respectively. Subsequently the cooling was continued until a temperature of 35° C in the centre of each can was reached.

To record the heat penetration, two thermocouple needles were inserted radially from the side of each can tested, one hot junction being at the geometrical centre (c), the other at a point (λ) 8 millimetres from each of the cover and the can body. The recording was effected by a Honeywell Multipoint Recorder, the time interval for each point of a thermocouple being 72 seconds.

The F_o values for the points c and λ were calculated using a numerical variant of the improved general method (Patashnik, 1953). The ratio $F_{o\lambda}/F_{oc}$ was used as a index for the heating uniformity of the contents of the can. Characteristics of the thermal processes are given in Table 2.

Table 2. *Thermal process evaluation and uniformity*

Experimental group	*I*	*II*	*III*	*IV*	V
Process time/min. at 121° C	6	10	20	40	80
$F_o\lambda/F_{oc}$ ratio [x]	1.16	1.09	1.02	1.05	1.08
F_{oc}	5.17	8.45	17.40	34.45	67.29
$F_o\lambda$	5.99	9.25	17.67	36.06	72.88

[x] maximal ratio found for which F_{oc} and $F_o\lambda$ values are given in the table.

After sterilization, the canned samples were stored at +4° C, and the determination of available lysine were made over a fortnight.

Determination of available lysine

In earlier determinations of available lysine from materials of animal origin, Carpenter's method (Carpenter, 1960), including a treatment with methoxycarbonyl chloride, was used (Czeremski and Jarzabek, 1964, Janicki and Skupin, 1964, Dvořàk and Vognarovà, 1965). Later modifications including an ion-exchange column chromatographic separation (Rao *et al.*, 1963, Blom *et al.*, 1967) offer a much better reproducibility of determinations.

A method based on that of Rao *et al.* (1963) was used in this study. Canned meat, while, at +4° C, was ground twice with a meat grinder, then a small portion of it was triturated in a mortar before weighing. As it was found by Blom *et al.* (1967) that the yield of available lysine decreases markedly with an increasing amount of sample, nearly constant weight samples, equal to about 900 mg of raw meat, were subjected to hydrolysis after dinitrophenylation.

The procedure for dinitrophenylation and separation of the components was further modified, based on the works of Blom *et al.* (1967), Bujard *et al.* (1967) and Roch *et al.* (1967). Details will be published elsewhere. In brief, the dinitrophenylation took place in centrifuge tubes, followed by washing operations with ethanol (once), and diethyl ether (twice), centrifuging at 3000 rpm for 15 minutes at 0° C. The residual aqueous solutions were then transferred to 500 ml round-bottomed long-necked flasks and subjected to further procedures acc. to Rao *et al.* (1963). The resin used for the chromatographic separation was Amberlite CG 120, Type II, 200 mesh, (BDH Ltd., England). The absorbance readings were made at 435 nm, using a Beckman Spectrophotometer Model G 2400, and 10 millimeter cells.

Pure epsilon-DNP-L-lysine hydrochloride, prepared acc. to Porter and Sanger (1948) and dissolved in the 1: 3 mixture of methyl ethyl ketone and 3

N aqueous HCl was used for calibrations, and Beer's law applied throughout the range of concentrations from 5 to 50 mg per litre.

The recovery of epsilon-DNP-lysine from columns, as indicated in Table 3, was sufficient, a recovery of 97.1 % being the minimum.

Table 3. *The recovery of epsilon-DNP-lysine hydrochloride added to the Amberlite CG 120 columns.*

Added, mg	0.100	0.200	0.300	0.400	0.500	0.600
Recovery, %	102.2	98.2	98.3	97.6	97.2	97.1

The available lysine concentration was expressed, according to the results of total nitrogen determinations for each group of samples, as grams of lysine per 16 grams of nitrogen.

Statistics

The differences found in the experiments were statistically calculated using Tukey's simplified procedure, as described by Mahoney *et al.* (1957). The regression equations were computed from mean values of the available lysine determinations.

RESULTS AND DISCUSSION

Results of changes in available lysine during thermal processing are given in Table 4. The Table also indicates the statistical significance of the differences observed.

Table 4. *Changes in available lysine in beef protein during canning (6 replicates*) as g./16 g. N*

Experimental group	*raw material*	*I*	*II*	*III*	*IV*	V
Mean	6.50	6.47	6.43	6.33	6.13	5.83
Maximum	6.62	6.55	6.50	6.42	6.25	5.90
Minimum	6.43	6.37	6.34	6.25	6.05	5.75
Standard Deviation	0.11	0.10	0.09	0.09	0.10	0.08
Coeff. of Variation	1.69	1.55	1.40	1.42	1.63	1.37
Losses, %	0.0	0.6	1.1	2.7	5.8	10.3
Stat. signif. of diff. (P level) compared with raw material	—		—	0.05	0.01	0.01
Stat, signif. of diff. (P. level) compared with the former group	—		—	—	0.01	0.01

*) Three hydrolysates, each separated twice.

As indicated in Table 4, the available lysine in beef is virtually affected by the heat treatment. The losses of 2.7 % are just significant.

When the retention of available lysine is plotted against the severity of the heat treatment, a linear correlation is found (Fig. 1), like that for thiamine, which is well-known. (Jackson *et al.*, 1945). The following regression equations were found to be valid:

(1) Available lysine (%) = $100.1 - 0.158\ F_{oc}$

or

(2) Available lysine (%) = $100.1 - 0.146\ F_{o\lambda}$

the retention of available lysine being expressed as a per centage of the lysine found in the samples before the heat treatment.

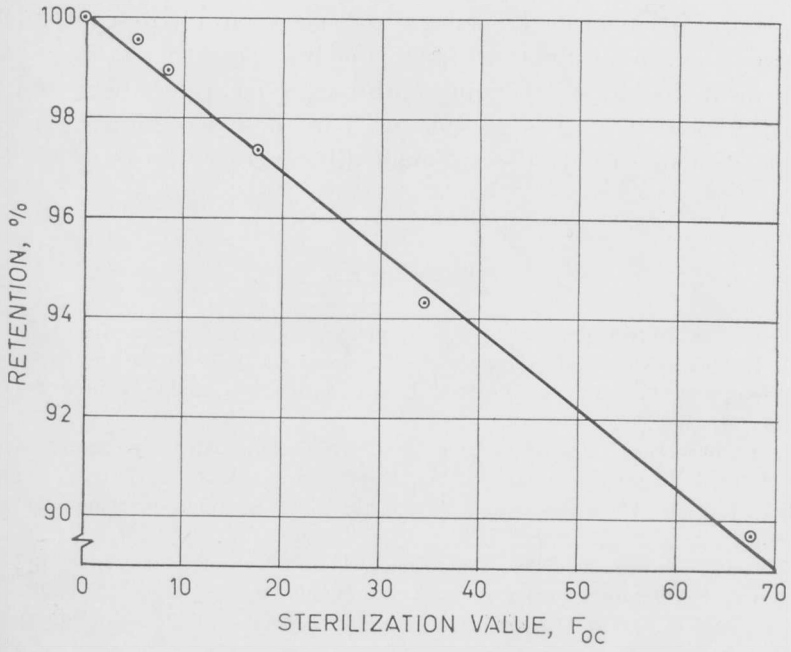

Fig. 1. *Correlation between losses of available lysine in beef during heat processing and its sterilization value.*

When the regression lines are extended to the heating time range used in the experiments of Dvořák and Vognarová (1965), the points estimated from their data fall on both sides of these lines, although deviations are greater than those in our experiments. This may prove a rather general validity of this reaction pattern when beef meat is heated, at least in the absence of significant amounts of other compounds such as carbohydrates or intermediates of browning reactions, which might participate in this reaction. The deviations

of the last-mentioned authors' results may be partly due to differing heating conditions, and partly to the different analytical method used.

Both the destruction of thiamine and the loss of availability of lysine are suggested to be effected by browning reactions (Lyman, 1966, de Lange and Mijll Deker, 1954). The destruction of thiamine, however, becomes apparent at lower Fo levels, and there is no direct evidence that the reactive compounds would be the same.

Nutritionally, the losses of available lysine observed have as such no significance in commercially canned meat, especially because lysine is not the limiting amino acid in meat. This is also in conformity with the earlier experiments using biological methods, reviewed by Bender (1960, 1966). However, as shown by Dvořák and Vognarová (1965), the content of available lysine in meat is in indirect proportion to the content of connective tissue, and is decreased also by microbial spoilage, salting with nitrite-containing salt, and smoking. The determination of available lysine can thus serve as a quality index for meat, its value depending both on the meat cuts used and on the technological processes. The method used in these experiments proves to be reliable enough to detect even small differences or losses.

REFERENCES

Bender, A. E. 1960. Processing damage to protein foods. Food Process. Packaging 29, 323.

Bender, A. E. 1966. Nutritional effects of food processing. J. Food Technol. 1, 261.

Blom, L., Hendricks, P. and Caris. J. 1967. Determination of available lysine in foods. Anal. Biochem. 21, 382.

Bujard, E., Handwerck, V. and Mauron, J. 1967. The differential determination of lysine in heated milk. I. In vitro methods. J. Sci. Food Agric., 18, 52.

Carpenter, K. J., 1960. The estimation of the available lysine in animal-protein foods. Biochem. J. 77, 604.

Czeremski, K. and Jarzabek, A. 1964. Badanie zmian wartośći biologicznej bialka mięsa poddawanego wysokiej temperaturze w róznych warunkach: na podstawie oznaczeń dostępnej lizyny za pomoca FDNB: (Investigation of the changes in protein biological value during meat thermal processing under different conditions measured by available lysine determined with FDNB). Przemysl Spożywczy, 18, 714. (in Polish).

Dvòrák, Z. and Vognarová, I. 1965. Available lysine in meat and meat products. J. Sci. Food Agric., 16, 305.

Jackson, J. M., Feaster, J. F. and Pilcher, R. W. 1945. The effect of canning procedures on vitamins in foods. Proc. Inst. Food Technol., 81. Quoted in Ball, C.O. and Olson, F.C.W. 1957. »Sterilization in Food Technology», pp. 511—514. McGraw Hill New York and London.

Janicki, J. and Skupin, S. 1964. Adaptacja metody Carpentera do ilośćiowego oznaczania lizyny przyswajalnej w macze rybnej. (Adaptation of Carpenter's method for quantitative determination of the available lysine in fish meal). Roczniki Technologii i Chemii Żywnośći, 10, 69. (In Polish).

De Lange, P. and v.d. Mijll Deker, L. P. 1954. A browning reaction between thiamine and glucose. Nature, 173, 1040.

Lyman, C. H. 1966. Effect of processing on nutritional value of foods. Paper 72, presented at the 26th Annual Meeting, Institute of Food Technologists, Portland.
Mahoney, Ch. H., Stier, H. L. and Crosby, E. A. 1957. Evaluating flavor differences in canned foods. II. Fundamentals of the simplified procedure. Food Technol. II No. 9, Symposium Insert, p. 37.
Patashnik, M. 1953. A simplified procedure for thermal process evaluation. Food Technol., 7, 1.
Porter, R. R. and Sanger, F. 1948. The free amino groups of haemoglobins. Biochem. J. 42, 287.
Rao, S. R., Carter, F. L. and Frampton, V. L. 1963. Determination of available lysine in oilseed meal proteins. Anal. Chem. 35, 1927.
Roach, A. G., Sanderson, P. and Williams, D. R. 1967. Comparison of methods for determination of available lysine value in animal and vegetable protein sources. J. Sci. Food Agric. 18, 274.

ACKNOWLEDGEMENTS

Thanks are given to Drs. A. Ruiter, the Institute for Fishery Products T. N. O., Ymuiden, The Netherlands, for a gift of his preparation of epsilon-DNP-L-lysine hydrochloride used in this study.

The senior author is grateful to Prof. Dr. O. E. Nikkilä and all other members of the Laboratory for Food Research and Technology for the facilities granted and the friendly atmosphere during his stay in Finland.