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# STORAGE OF SELECTED SORT OF PROCESSED MEAT PRODUCT AT CRYOSCOPIC TEMPERATURE — AN ATTEMPT AT ENERGY CONSERVATION. CHANGES IN PROTEINS, AMINO ACIDS BALANCE AND IN VITRO DIGESTIBILITY OF CURED SMOKED RAW PORK-LOIN

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Key words: cured raw pork-loin, storage, cryoscopic temperature, changes in proteins

The effect of storage at -3°C of cured smoked raw pork-loin on changes in proteins, amino acids balance and in vitro digestibility was investigated. Long-term storage of the experimental product at near-cryoscopic temperature does not substantially affect the amino acids content. In vitro digestibility was found to improve during storage. Two new electrophoretically separated fractions of protein after 8 and 12 weeks of pork-loin storage were determined, this being regarded as an interesting finding reflecting both qualitative and quantitative changes in the experimental product's protein.

## INTRODUCTION

Food raw materials and finished foodstuffs are today preserved with a variety of methods, and freezing, especially of food of animal origin, is widely used on a large industrial scale. The freezing of meat, semi-finished and finished processed meat products and the long-term freeze-storage that usually follows, lead to many unfavourable and undesirable changes, including substantial mass losses, despite the generally satisfactory preservation effect. Moreover, this technology is energy-consuming and hence expensive [2-5, 19, 20]. Accordingly, a search for means of limiting the freezing of selected finished food products of animal origin seems to be fully justified.

Our preliminary survey of the subject matter indicates that the replacement of freezing of cured semi-processed or processed meat products by their storage at temperatures close to those at which the tissue juices of the given product sort begin to freeze, effectively limits the adverse effects of freeze-storage, eliminating them altogether with regard to the majority of sensory characteristics [21].

One can and indeed should assume that the storage of processed meats at temperatures close to cryoscopic will sufficiently reduce the dynamics

of endo-and exogenous enzymatic processes, will significantly diminish mass losses due to storage, denaturation changes in proteins and damage of muscale tissue structure, and will also enhance commercial and organoleptic characteristics [21].

Accordingly, we decided to launch studies of the effectiveness of storage of selected sorts of processed meats in deep-chilled state. The results reported here represent only a fragment of the so far performed studies of the effect of long-term storage of smoked raw pork loin at cryoscopic temperature, and they illustrate the observed changes in proteins, in amino acid balance and in vitro digestibility.

## MATERIAL

Experiments were performed with pork-loins produced in industrial conditions according to the technology schematically illustrated in Fig. 1.

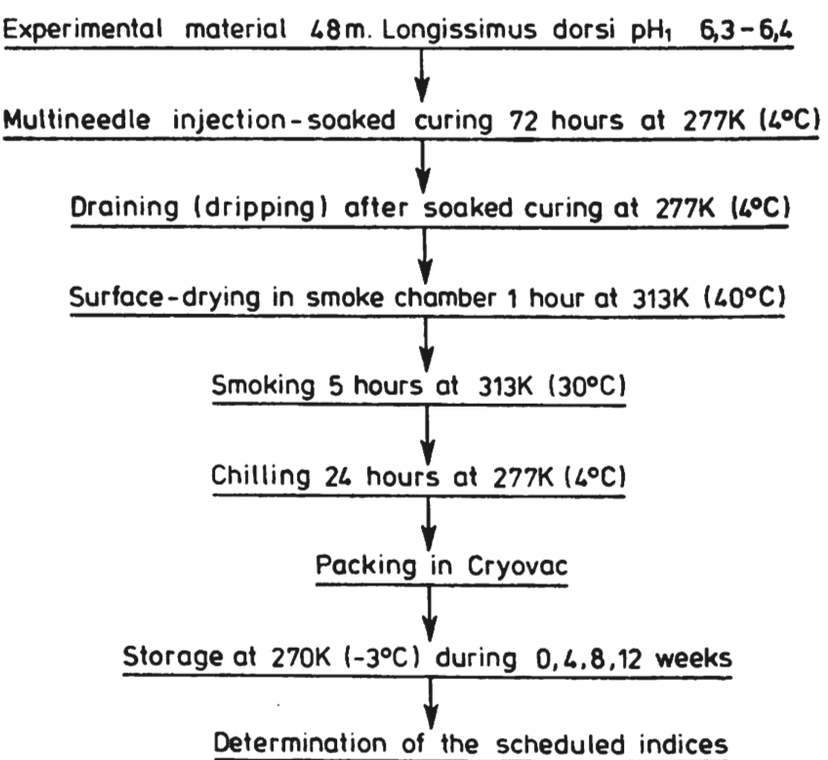


Fig. 1. Scheme of experimental product processing

## METHODS

Changes in the muscle tissue proteins of stored pork-loins were analyses on the basis of the folowing characteristics:

— myofibrillar fragmentation index (MFI) [12];
— electrophoretic analysis of proteins on polyacrilamide gel [22];
— determination of amino nitrogen content [18];
— analysis of amino acids composition [10, 11, 17];
— determination of peptic digestibility of proteins in vitro [7, 8].

## DISCUSSION OF RESULTS

During storage of meat and meat products there take place proteolytic changes in the muscular tissue structure. Among manifestations of these changes are decompositions of boundary line Z and line M. In the course of long-term storage these lines were found to disappear altogether, and some myofibrils were seen to decompose down to individual sarcomers [9]. The extent of these changes is indicated by the myofibrillar fragmentation index (MFI).

In the experimental material, MFI tended to rise starting from the 8th week of storage (Fig. 2). After 12 weeks of storage the MFI of muscular tissue of pork-loin

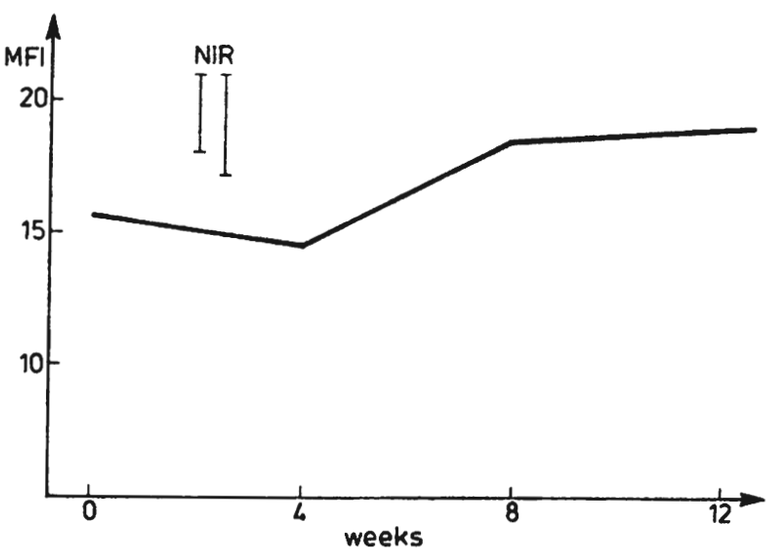


Fig. 2. Changes of the myofibril fragmentation index of cured smoked raw pork-loin during storage at cryoscopic temperature. NIR — least statistically significant difference (P = 0.05 and/or P = 0.01)

was significantly different from the corresponding value in the just-processed material, despite the fact that the intensity of changes expressed by this characteristic decreased in the final period of experimental storage.

The qualitative and quantitative changes of proteins of pork-loin muscular tissue were also assessed on the basis of electrophoretic separation of protein fractions in polyacrylamide (PAA) gel with SDS. The separated protein fractions were identified on the basis of their molecular mass and literature data [9, 13, 16].Quantitative changes of the separated fractions were determined densitometrically.

A total of 18 protein fractions were determined in the experimental pork-loins (Tab. 1, Fig. 4), including 16 fractions in the freshly processed materials and in pork-loins stored for four weeks (Fig. 3). The protein fraction with the greatest molecular mass (200 000 daltons) was myosin in spite of the fact that the actual molecular mass of this protein is 470-500 thousand daltons (Fig. 3). However, denaturation or the action of proteolytic enzymes reduced its molecular mass by half, since the myosin monomer splits into subunits of smaller molecular mass [9]. The least mobile bands during electrophoretic separation were two heavy chains of myosin of ca 200 000 daltons, and two-three bands of alkaline protein with

Table 1. Identification of SDS-PAGE electrophoretically separated striated muscle proteins of cured smoked raw pork-loin stored at cryoscopic temperature

| Protein | Rf | Molecural weight (daltons) | % indyvidual protein of stored products (weeks) | | | |
|---|---|---|---|---|---|---|
| | | | 0 | 4 | 8 | 12 |
| Myosin heavy chain | 0.025 | 200 000 | 14.2 | 13.5 | 12.6 | 12.1 |
| Unidentified | 0.040 | 186 000 | 4.2 | 4.1 | 3.6 | 3.4 |
| M-protein | 0.060 | 172 000 | 3.2 | 3.2 | 3.2 | 3.2 |
| Unidentified | 0.080 | 161 000 | 5.8 | 5.7 | 5.6 | 4.7 |
| C-protein | 0.120 | 140 000 | 9.2 | 9.2 | 9.0 | 9.0 |
| α-actinin | 0.220 | 100 000 | 4.1 | 3.9 | 3.4 | 3.4 |
| Unidentified | 0.280 | 80 000 | 2.7 | 2.7 | 2.7 | 2.7 |
| β-actinin | 0.320 | 70 000 | 1.0 | 1.1 | 1.1 | 1.2 |
| Unidentified | 0.360 | 60 000 | 3.2 | 3.6 | 4.1 | 4.8 |
| Actin | 0.440 | 45 000 | 7.4 | 7.2 | 7.3 | 7.8 |
| Troponin-T | 0.500 | 37 000 | 14.3 | 13.8 | 13.7 | 12.5 |
| Tropomyosin | 0.520 | 35 000 | 11.2 | 11.1 | 10.9 | 10.6 |
| Myosin light chain-1 | 0.610 | 25 000 | 6.1 | 5.9 | 5.9 | 5.7 |
| Troponin-I | 0.640 | 23 000 | 4.8 | 5.4 | 5.5 | 5.5 |
| Troponin-C | 0.680 | 20 000 | 3.1 | 3.1 | 3.0 | 3.6 |
| Myosin light chain-2 | 0.760 | 16 000 | 5.3 | 6.4 | 6.5 | 7.5 |
| Myosin light chain-3 | 0.810 | 13 000 | — | — | 1.2 | 1.5 |
| Unidentified | 0.880 | 10 000 | — | — | 0.9 | 1.3 |

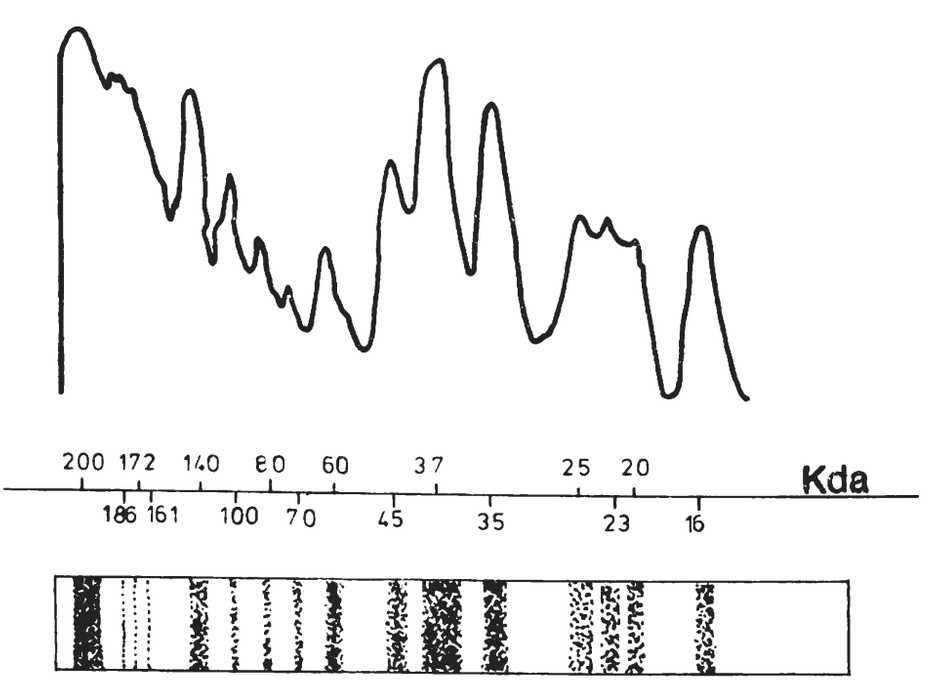


Fig. 3. SDS-PAGE electrophoregram of proteins of cured smoked raw pork-loin characteristic for freshly processed material and material stored for four weeks at cryoscopic temperature

molecular weights ranging from about fifteen to twenty odd thousand daltons. The contents of myosin decreased systematically in the subsequent study periods, and after eight and 12 weeks of storage these contents were already significantly different ($P = 0.05$ and $P = 0.01$) from those in the just processed material.

The content of light myosin chains M-1 and M-2 (26 and 16 thousand daltons, respectively) also tended to decrease, but significant changes were observed only

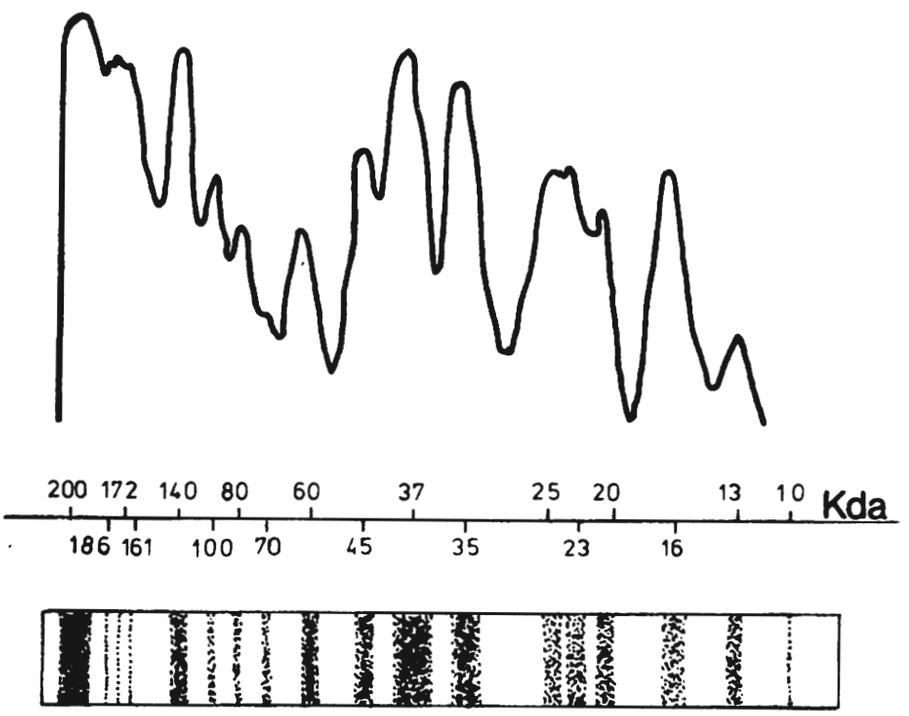


Fig. 4. SDS-PAGE electrophoretogram of proteins of cured smoked raw pork-loin characteristic for material stored for eight and 12 weeks at cryoscopic temperature

in the case of the light myosin chain M-2 after 12 weeks of pork-loin storage.

The M-3 light myosin chain was one of the two fractions determined only after eight and 12 weeks of storing the pork-loins.

The other myofilament structural protein, viz. actin, did not exhibit linear changes during storage. The per cent content of this protein either increased or decreased in the various experimental periods but in a statistically non-significant manner.

Protein C, β-actinin and protein M did not change much in the separate experimental periods, and their contents remained fairly stable in the stored pork-loins. On the other hand, the content of troponin T was seen to decrease systematically, while the initial contents of troponin I and troponin C increased slightly after 12 weeks of storing the smoked meat products.

The determined contents of α-actinin and tropomyosin decreased slightly in the experimental products with the increase of storage time.

Also isolated were unidentified proteins with molecular masses of 186, 161, 80, 60 and 10 thousand daltons. The per cent contents of the first two of these proteins decreased as storage time increased. The per cent content of the 80 000-dalton protein remained unchanged while the content of the 60 000--dalton fraction increased systematically. The 10 000-dalton protein fraction, similarly as the light myosin chain M-3, was isolated only after eight and 12 weeks of storage (Fig. 4).

The balance of amino acids was studied in the experimental products alongside changes in proteins. The level of most of the determined exogenous amino acids remained largely unchanged during storage. Only in the case of leucine, isoleucine, valine and tryptophane there was a permanent increase of content with the increase of storage time (Fig. 6), a phenomenon which is hard to

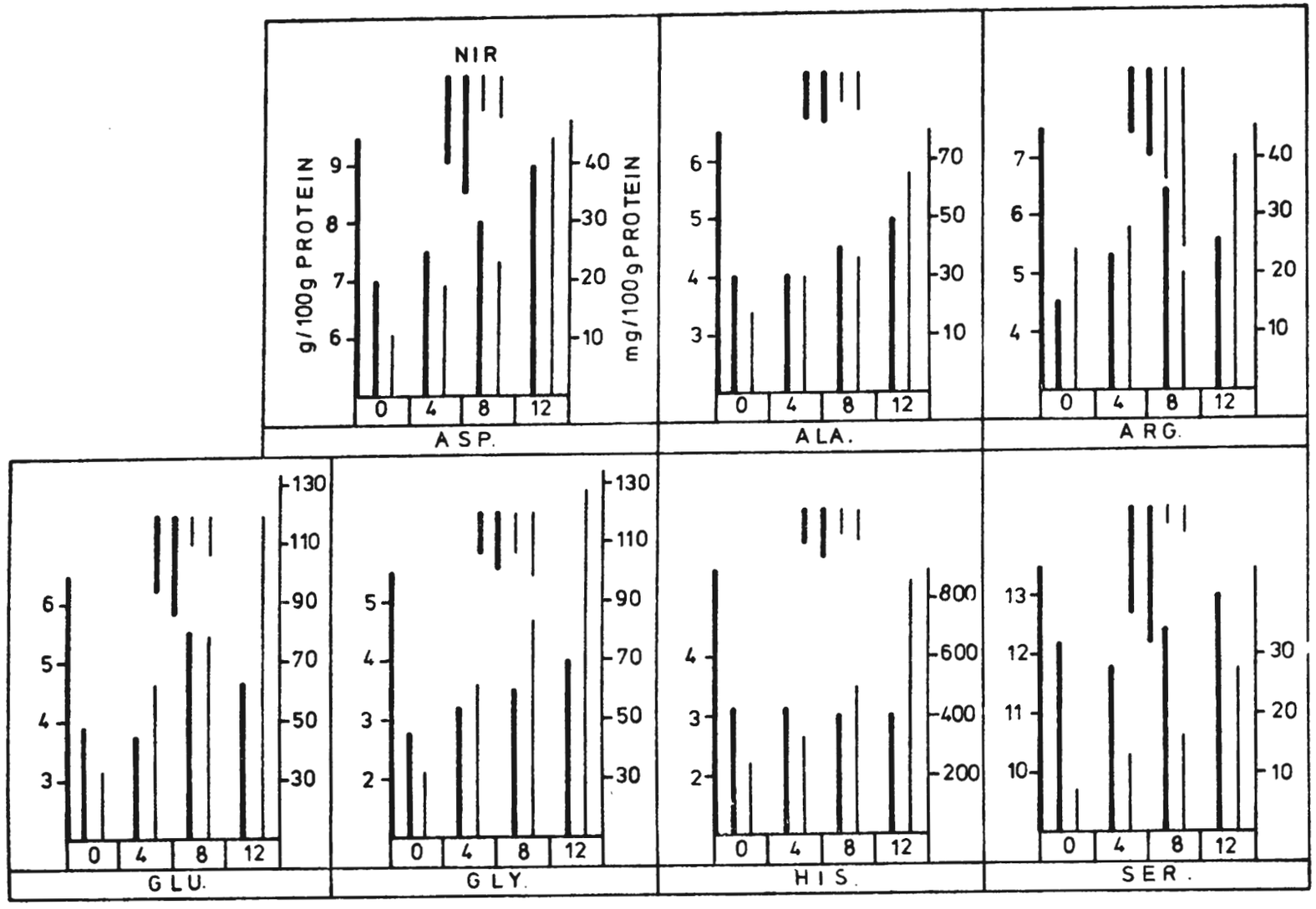


Fig. 5. Changes of non-essential amino acids contents during storage of cured smoked raw pork-loin for four, eight and 12 weeks at cryoscopic temperature. Thick line — total non-essential amino acids; thin line — non-essential free amino acids

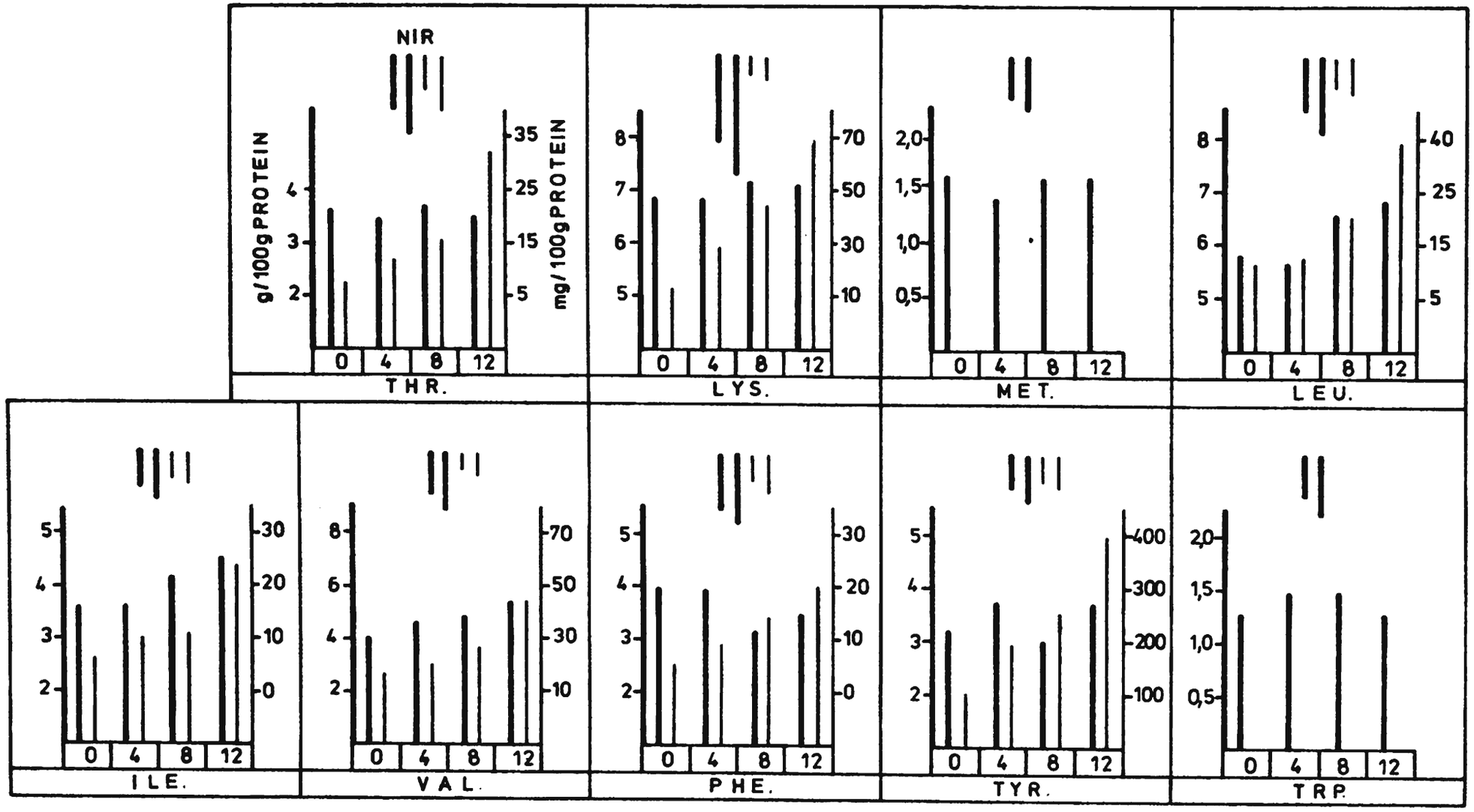


Fig. 6. Changes of essential amino acids contents during storage of cured smoked raw pork-loin for four, eight and 12 weeks at cryoscopi temperature. Thick line — total essential amino acids; thin line — essential free amino acids

account for. Likewise among endogenous amino acids (Fig. 5): aspartic acid, glycine, alanine and arginine also tend to increase in value during storage of pork-loins, while the contents of glutamic acid, histidine and serine remained constant. The pork-loins are characterized by a considerable content of essential amino acids, ranging from 45.5 to 48.5% (Tab. 2). Worth noting is that 12-week deep-chilled storage of smoked meat products does not lead to a reduced content of exogenous amino acids which determine the products' nutritive value.

The relatively low levels of methionine and cystine (determined only in trace amounts in the experimental meat products), which are usually limiting amino acids for meats and meat products, may be due to partial oxidation of these amino acids during acid hydrolysis [11, 17]. Accordingly, the limiting amino acid index (SC) calculated for the experimental pork-loins is relatively low (Tab. 2). A

Table 2. The biological value of raw pork-locin proteins as influenced by storage at cryoscopic temperature

| | Chilling storage (weeks) | | | |
|---|---|---|---|---|
| | 0 | 4 | 8 | 12 |
| Total essential amino acids (%) | 46.9 | 47.1 | 48.5 | 45.5 |
| CS (Chemical score) | Met + Cys 29.1 | Met + Cys 27.3 | Met + Cys 29.1 | Met + Cys 29.1 |
| EAA index (esential amino acid index) | 61.4 | 63.3 | 65.3 | 67.3 |

characteristic feature of the stored pork-loins is the value of the integrated index of exogenous amino acids (EAA) increasing with the increase of storage time (from 61.41 to 67.34 after 12 weeks of storage). This EAA value, lower from that determined by Rakowska et al. [15] for fresh loin (74.9), may be the results of the destructive effect of technological processing on exogenous amino acids and of partial decomposition of methione and cystine during acid hydrolysis.

The content of free amino acids in smoked meat products stored at near cryoscopic temperature tends to rise (Fig. 5, 6). In pork-loins stored for 12 weeks at-3°C we found statistically significant (at $P = 0.01$) several-fold increases of the content of these amino acids. A tendency to rise was also noted in the case of contents of exogenous (Fig. 6) and endogenous (Fig. 5) amino acids. Increases in the content of free amino acids in stored pork-loins are characteristic for maturing meat products, being caused by proteolytic processes occurring in these products. The increased level of free amino acids in stored meat and meat products was observed universally and is frequently reported in the literature [1,6].

The proteolytic changes in proteins of experimental meat products due to storage, manifest in the increased degree of myofibrilar fragmentation (MFI), differentiation of the electrophoretic separation of protein fractions, and the

increased level of free amino acids, are also confirmed by accumulation of amino nitrogen (Fig. 7). Such changes in the amino nitrogen level in maturing meat and meat products are also documented in the literature [14]. In the experimental material the amount of amino nitrogen was found to have increased at the end of storage in a statistically significant manner ($P = 0.01$) from the initial figure of 271.50 mg% to 306.83 mg%. The increments of the amino nitrogen levels in the successive experimental periods were almost in direct proportion to the time of storage.

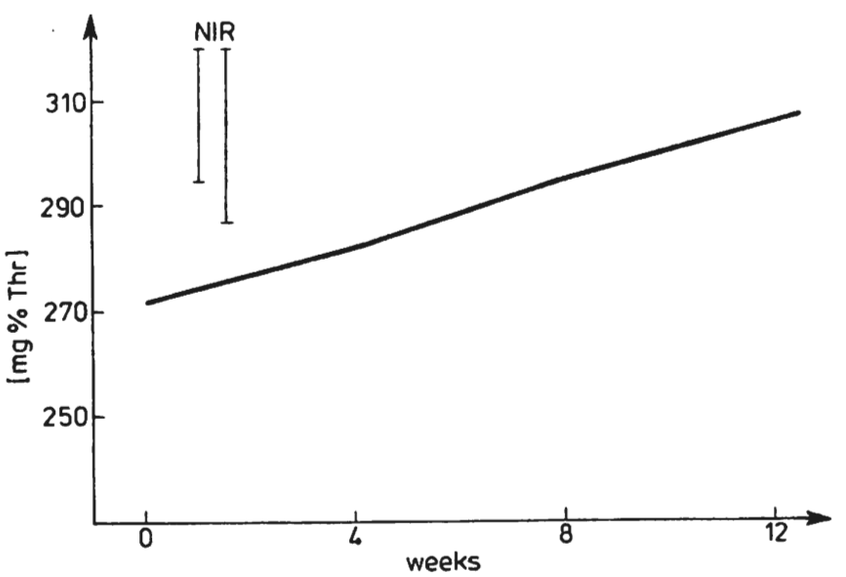


Fig. 7. Changes in amino nitrogen content during storage of cured smoked raw pork-loin at cryoscopic temperature

The changes occurring during storage of pork-loins had no serious effect on the digestibility of protein (Fig. 8). A slight decrease of in vitro digestibility was noted only after four weeks of storing the experimental material at cryoscopic temperature. However, after longer storage the digestibility of pork-loin proteins

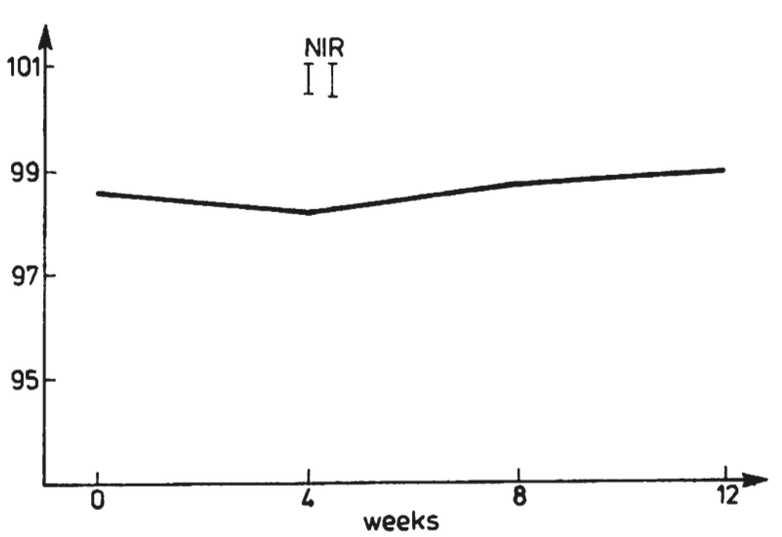


Fig. 8. Digestibility (in vitro) of protein of cured smoked raw pork-loin during storage at cryoscopic temperature

even increased somewhat in comparison to the initial digestibility, and after 12 weeks it was 99.03%. The observed differences were not statistically significant.

The increasing time of storage had a constantly increasing adverse effect on the general organoleptic properties of the pork-loins (Fig. 9). Statistically significant deterioration ($P = 0.01$) was determined already after four weeks of cryoscopic storage. The overall organoleptic index of pork-loins steadily worsened as storage time increased, but the assessments of the individual organoleptic characteristics as well as the overall organoleptic index remained above or around good at all time of storage.

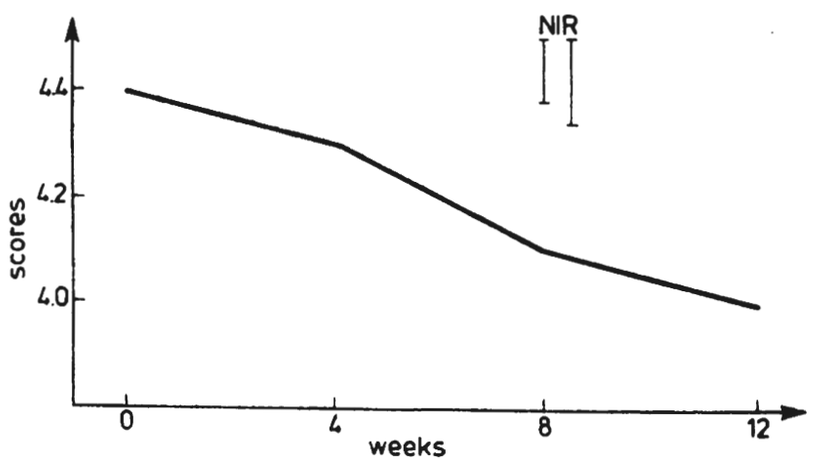


Fig. 9. Changes of overall organoleptic satisfaction of cured smoked raw pork-loin stored at cryoscopic temperature for 12 weeks

## CONCLUSIONS

1. The quantitative and qualitative changes occuring in proteins of smoked meat products stored at near-cryoscopic temperature are reflected by myofibrils fragmentation, the appearance of two new protein fractions in the electrophoretogram after eight and 12 weeks of storage, and the constantly increasing contents of free amino acids and amino nitrogen.
2. The storage of raw pork-loins in deep-chilled temperature for 12 weeks did not lead to a significant decrease of total amino acids content.
3. Several-week-long maturing of pork-loins during their cryoscopic storage improves the in vitro digestability of proteins.

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PRZECHOWYWANIE WYBRANEGO ASORTYMENTU WYROBU MIĘSNEGO W TEMPERATURZE KRIOSKOPOWEJ. PRÓBA OCENY MOŻLIWOŚCI ZMNIEJSZENIA ENERGOCHŁONNOŚCI MAGAZYNOWANIA PRZETWORÓW MIĘSNYCH. ZMIANY W BIAŁKACH, BILANSIE AMINOKWASÓW I W STRAWNOŚCI IN VITRO PEKLOWANYCH, WĘDZONYCH, SUROWYCH POLĘDWIC

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Streszczenie

Przedmiotem badań były zmiany w białkach, składzie aminokwasowym i strawności in vitro zachodzące w surowych, peklowanych, wędzonych plędwicach wieprzowych, przechowywanych w stanie głębokiego schłodzenia, tj. w temperaturze bliskiej punktowi krioskopowemu soków tkankowych charakterystycznemu dla tego asortymentu przetworu mięsnego. Materiałem doświadczalnym było 48 polędwic wyprodukowanych w warunkach przemysłowych, w trzech powtórzeniach doświadczalnych, z mięśni najdłuższych grzbietu (*Longissimus dorsi*) o wyjściowym pH = 6,3-6,4.

Po ukończeniu procesu produkcyjnego polędwice opakowano w woreczki z termokurczliwego tworzywa syntetycznego (Cryovac) i przechowywano w temp. 270K (−3°C) przez 0,4, 8 i 12 tygodni.

Ocenę endo- i egzogennych proteolitycznych zmian przechowalniczych frakcji białkowej polędwic oparto na oznaczeniach: indeksu miofibrylarnej fragmentacji (MFI), jakościowych i ilościowych przemian elektroforetycznie rozdzielanych frakcji białek w żelu poliakrylamidowym z SDS, składu aminokwasowego i dynamiki nagromadzenia się wolnych aminokwasów i azotu niebiałkowego, strawności białek in vitro oraz ocenę organoleptyczną.

Wśród 16 elektroforetycznie rozdzielonych frakcji białek zmiany statystycznie istotne stwierdzono jedynie w odniesieniu do frakcji o cięż. cząst.: 200 000, 161 000, 37 000 i 16 000 daltonów. Po 8

tyg. przechowywania polędwic oznaczono dwie nowe frakcje białek, tj. o cięż. cząst. 13 000 i 10 000 daltonów. Ilościowe i jakościowe proteolityczne degradacyjne zmiany przechowalnicze przejawiały się postępującą fragmentacją włókienek mięśniowych i nagromadzaniem się wolnych aminokwasów i azotu aminowego. Nie obserwowano istotnych zmian w ogólnej ilości aminokwasów. Przechowywanie polędwic przez 12 tyg. w temperaturze krioskopowej nieznacznie zwiększyło strawność białek in vitro.

Dane doświadczalne wskazują, że długoterminowe przechowywanie finalnych peklowanych, wędzonych, surowych przetworów mięsnych w temperaturze bliskiej krioskopowej dla danego asortymentu, w miejsce praktykowanego zamrażania i zamrażalniczego magazynowania może przyczynić się do znacznych oszczędności energii i zmniejszenia kosztów produkcji przy zachowaniu dobrej jakości składowanych przetworów.