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ZYGMUNT NIEDZIELSKI
LUCJAN KRALA

EFFECT OF THE COMPOSITION OF GAS MIXTURES ON THE PROLONGATED STORAGE OF BEEF

Department of Chemical Food Technology, Technical University, Łódź

Key words: storage of meat, gas mixtures, beef meat

The effect of three different gas mixtures on the period of fresh beef storage was investigated. The quality of samples being tested was assessed on the basis of a complex physico-chemical analysis (10 discriminants in total) and a microbiological analysis. A mixture of gas containing 20% CO_2, 79% N_2, 1% O_2 proved to be the best.

In addition to a temperature close to cryoscopic, natural gases and mixtures of gases with different compositions are ever more often used in tests as factors prolonging the period of storage of fresh meat. Studies on the possibilities of using gases for an improvement of the standard of meat have considerably gained in intensity in the last few years [1, 4, 13, 16, 18, 19, 20, 21, 24]. The works published point to the large variety of the techniques and gases used for the tests. Meat samples are stored in containers or gas-tight packages with a continuous flow of gases and at a single filling of the containers.

There are differences of opinions concerning the effect of particular gases on the standard of meat. This concerns especially the effect of carbon dioxide. For example, Brooks and Ledward [14] are of the opinion that the optimum concentration of CO_2 in gas mixtures should amount to 20%; these authors consider any higher concentration of CO_2 to be harmful because of the undesired change of colour. These observations have been confirmed by Stefanovič [30], Jasineckij [11] and O'Keeffe [19]. On the other hand, Partmann and Bomar [23] and Pohja and Niinivaara [28] consider that carbon dioxide of a concentration of even up to 100% causes a brightening of the surface of meat stored. Taking into account the fact that CO_2 is a strong inhibitor of the growth of yeasts, moulds and aerobiopsychrophylic bacteria, this gas would be the best suited for

the prolongation of the time of the availability of meat for use. However the absence of works concerning a broad experimental material and of reports on the use of high CO_2 concentrations on a large production scale does not confirm the rightness of this hypotheses. The investigations conducted in the Institute of the Chemical Technology of Food [17] have shown that a high concentration of the CO_2 even at a small amount of oxygen (below 1%) cause that the meat surface turns brown. As the time of storage gets longer, browning spreads to ever deeper layers of the tissue.

The considerable difficulties related with the obtaining and maintenance of aerobic conditions over a comparatively long period of time, have induced researches to look for possibilities of prolonging the time of meat storage by means of using multi-component gas mixtures comprizing small amounts of oxygen. The largest number of reports published concern gas mixtures composed of carbon dioxide, nitrogen and oxygen. The works of Partmann [20, 21, 22, 23, 24], Golovkin and Kondratev [9] and Pohja and Niinivaara [28] show that like in the case of using-componential atmospheres, the views on the effect of various mixtures on the meat standard are very differentiated.

Data from literature also show that the various experiments were not conducted in the same conditions which makes it additionally difficult to compare the results, to establish the optimal composition of the gas mixture, to comprehensively evaluate the quality of meat and to fix the time of storage. None of the studies takes into account the possibility of a simultaneous use of temperature close to cryoscopic.

MATERIAL AND METHODS

The material used for the tests was the bicipital muscle of the thigh taken from the first grade heifers. Random samples were obtained from the slaughterhouse in Łódź, 24 hours after slaughter. Samples weighing approx. 2 kg were put in gas-tight containers (Fig. 1) which were placed in a cooling chamber at -1+0°C. The cross-section of a container is shown on Fig. 2. The containers were filled with one of the three gas mixtures used in the tests: A — 10% CO_2+89% N_2+1% O_2; B — 20% CO_2+79% N_2+1% O_2; C — 30% CO_2+69% N_2+1% O_2. The relative humidity of the gas mixtures was kept at the level of 95%.

The main problem when filling the containers with gas mixtures was the removal of air (oxygen). This problem was solved by two methods:

a) by repeated blowing through of the containers with nitrogen or carbon dioxide,

b) by an evacuation of air using a vacuum pump.

In case when air (oxygen) was evacuated by means of nitrogen, the closed containers were filled with this gas up to an absolute pressure of 130 kPa. After a thorough mixing of the gases (using a fan), the pressure in the containers was levelled out to air pressure. Next, the content of oxygen in the containers was measured by means of a gas analyser. The containers were then refilled with nitrogen to the same pressure, the pressure was levelled out and the content of oxygen was measured again. These operations were repeated six-times, the content of oxygen in the containers was reduced to 0.65%. The initial content of oxygen in the containers amounted to 20.8%. After the evacuation of air, the containers contained nitrogen and a small amount of oxygen. In order to achieve a gas mixture of a composition eg B, carbon dioxide to an amount corresponding to a pressure of 125 kPa was fed into the container. After mixing the gases and levelling out pressure to atmospheric pressure,

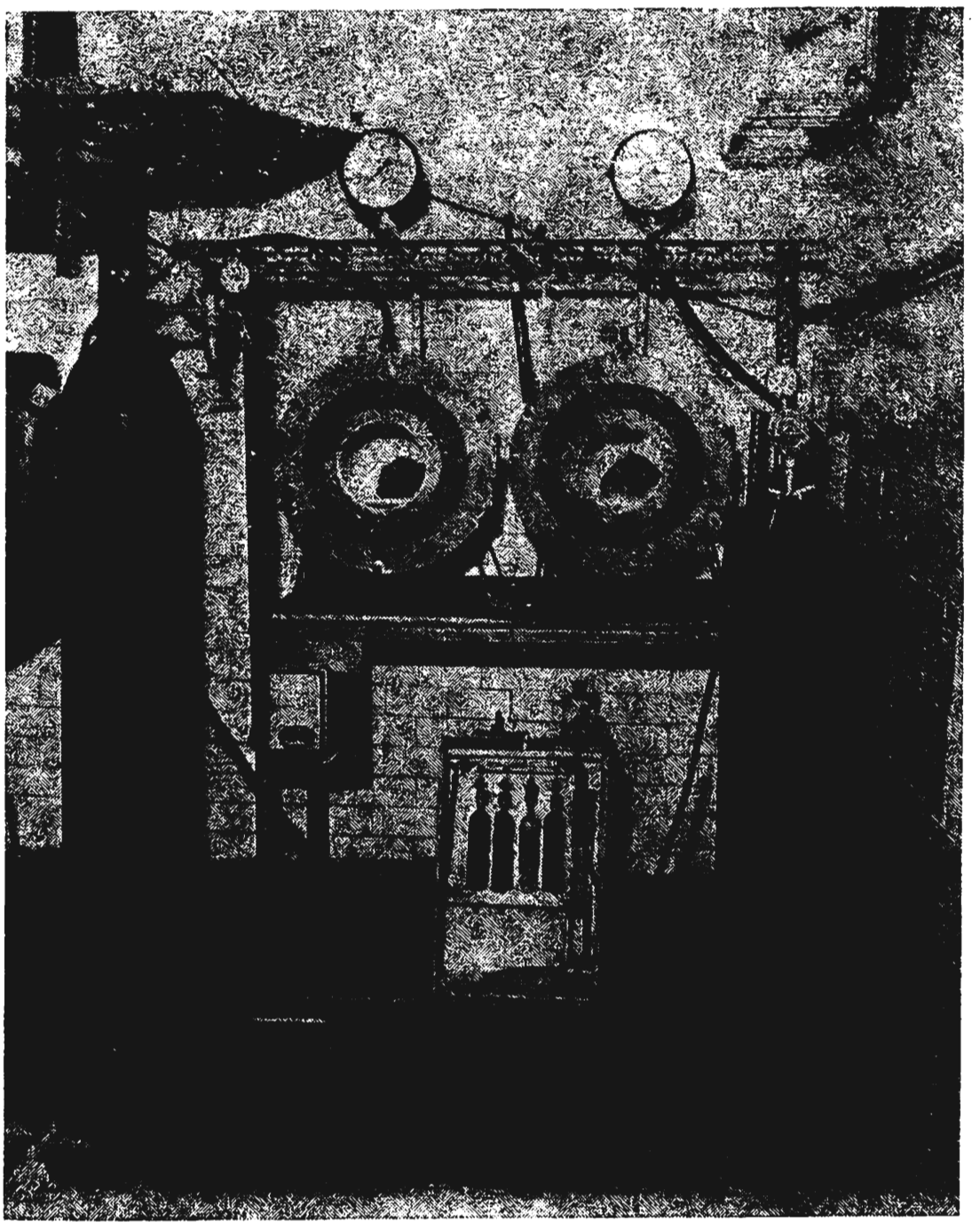

Fig. 1. Set of gas-tight containers

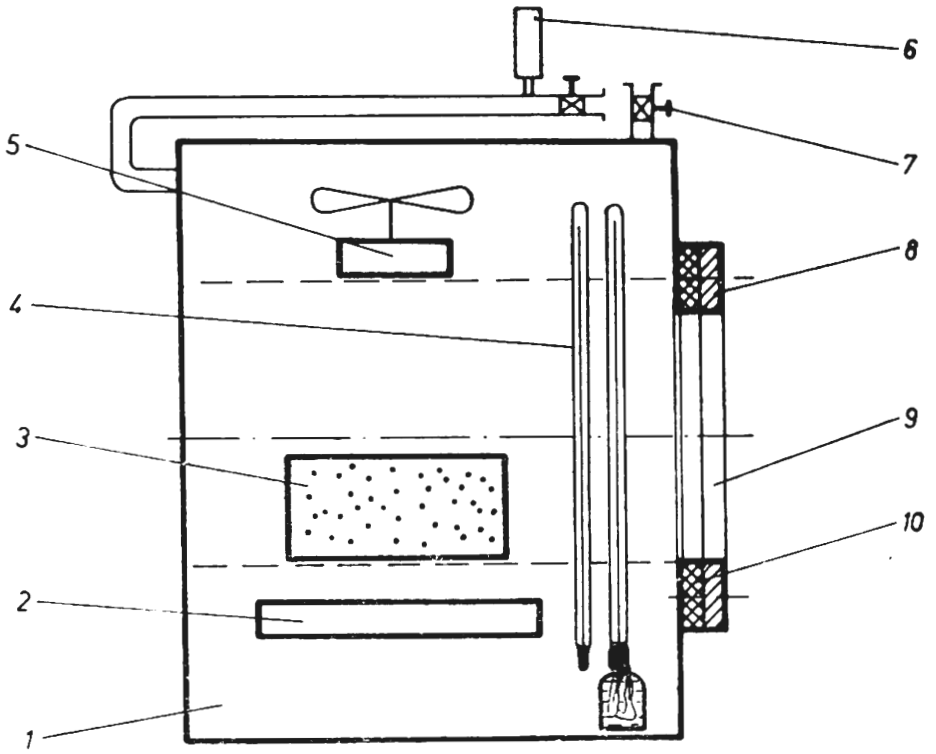

Fig. 2. Cross-section of gas-tight container: 1 — container, 2 — tray, 3 — meat sample, 4 — psychrometer, 5 — fan, 6 — compound manometer, 7 — gas value, 8 — cover, 9 — sight-glass, 10 — seal

the required composition of the gas mixture was obtained in the container.

The second method of filling the containers with a gas mixture provides, first, for an evacuation of air up to a pressure of 10 kPa. Next, the containers were supplied with nitrogen and carbon dioxide in amounts necessary to obtain the required composition of the gas mixture. For example to achieve mixture B, nitrogen in an amount corresponding to a pressure of 80 kPa was fed into the container. Then, pressure in the container was levelled out to atmospheric pressure by an addition of CO_2. After mixing the gases in the container, the preset composition of the gas mixture was obtained. The particular pressure values were determined experimentally. By filling the containers using evacuation of the air in order the obtain the required composition of the gas mixture, 53% less N_2 and 15% less CO_2 were used than when evacuating air from the containers by means of nitrogen.

The quality meat stored was assessed at 8÷10 days interval. Paralelly, an evaluation was made of the quality of control samples stored in air. The determinations were repeated three times.

ANALYTICAL METHODS

On the basis of a number of published studies [1, 2, 12, 13, 14, 15, 25, 26, 32] the following indices were selected for an assessment of the changes in the standard of the material tested: colour of the surface, pH, water holding capacity, thermal drip, weight losses, total and non-

protein nitrogen, content of soluble and amine nitrogen and a microbiological analysis. The colour of the meat surface was assessed on the basis of changes in metmioglobin (MMb) concentrations determined according to the method of Ledward [14] and Stewart [31].

When calculating the result expressed in percent MMb, use was made of Judd's tables [7]. pH was measured in a water meathomogenate using a pH-meter type N 512, by the meat water ratio as 1:1 [3]. The water holding capacity was determined by the method of Grau and Hamm as modified by Pohja and Niinivaara [27] and by the centrifuge method [6]. In the case of the last method, the meat samples were submitted to the action of the centrifugal force corresponding to a value of relative acceleration of 680 g, at a centrifugation time of 30 min and temperature 1÷4°C. The thermal drip was determined after the cooking of meat samples in 0.6% Na Cl solution at 94÷96°C [33]. Weight losses were calculated from the difference of the weight of meat samples before and after the storage, taking into account the drip collected on trays. Total nitrogen was determined by the Kjeldahl's method using a sample of approx. 0.5 g, and nonprotein nitrogen by Helander's method [10]. When determining the content of soluble protein, extraction was carried out in conditions described by Dyer [8]. Soluble protein in the extract obtained was determined by the burette method acc. to Snow [29]. The content of free amine nitrogen was determined by the colorimetric method after reaction with copper ions. The microbiological evaluation of meat was conducted in conditions applied by the Polish sanitary-epidemiological stations [5].

CHARACTERISTIC OF THE MATERIAL

The meat used for the tests has the following characteristic: temperature 4°C; MMb concentration 23,4÷29.5%; pH 5.42÷5.88; water holding ability 11.7÷19.0 cm^3/100 g of comminuted meat; thermal drip 27.1÷37%; total nitrogen 31.37÷35.22 mgN/1 g tissue; non protein nitrogen 4.65÷÷5.44 mg N/1 g tissue; soluble nitrogen 9.46÷16.69 mg N/1 g of meat tissue; aminic nitrogen 1.07÷1.26 μg/1 g; total amount of microorganisms on the surface of meat $(6.58 \div 7.60) \cdot 10^4$/1 cm^2.

DISCUSSION OF THE RESULTS

The results of the assessment of the quality of meat stored are presented on Figs. 3÷12 and in Table. Each dot on the diagrams is an arithmetic value obtained from 3 repetitions. In order to evaluate the amount of

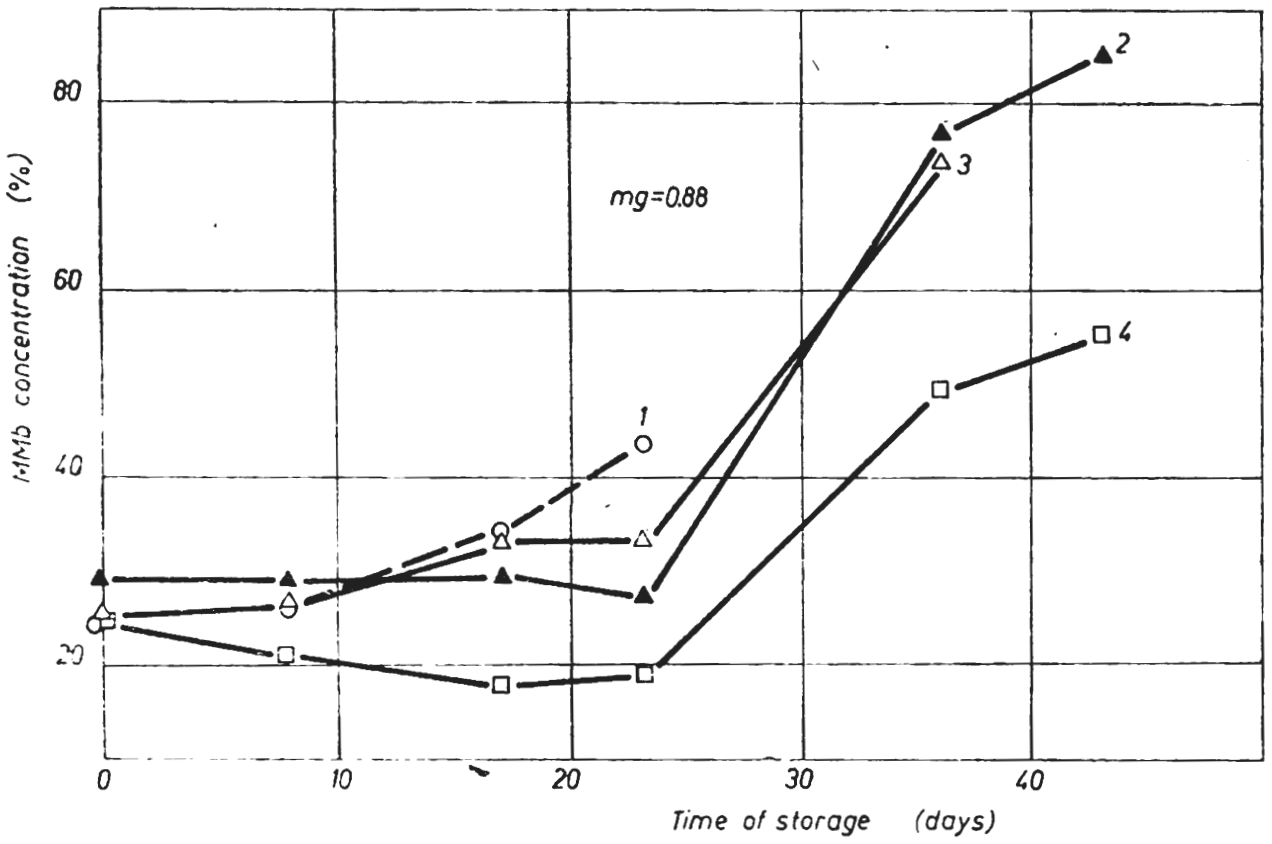

Fig. 3. Changes in MMb concentration on the meat surface depending on the gas mixture composition; 1 — control conditions, 2 — 30% CO_2, 69% N_2, 1% O_2, 3 — 10% CO_2, 89% N_2, 1% O_2, 4 — 20% CO_2, 79% N_2, 1% O_2

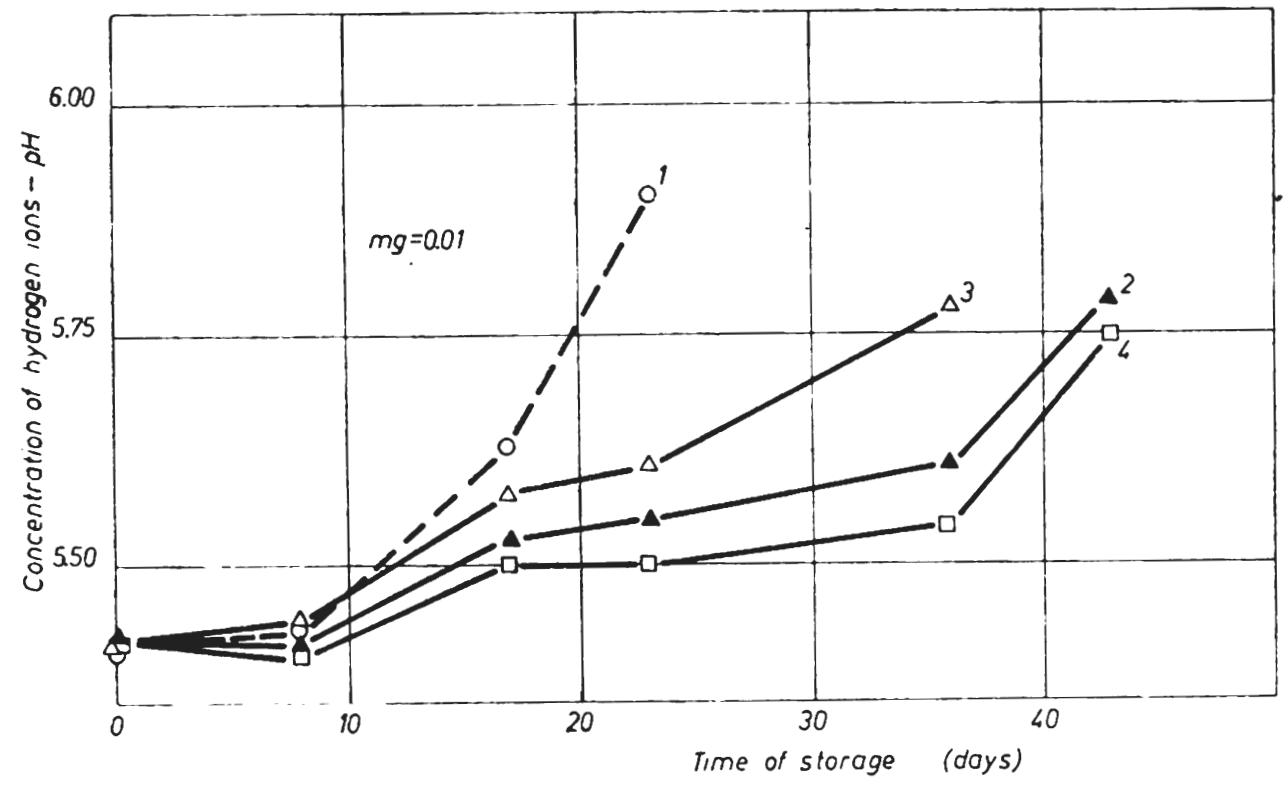

Fig. 4. Changes of pH in meat depending on the gas mixture composition; explanations — see Fig. 3

Table. Microbiological assessment of meat stored in gas mixtures

Group of microorganisms	Number of colonies per 1 cm² of surface or count				
	initial state			after 36 days storage	
	mesophilic bacteria	psychrophilic bacteria	gas mixture	mesophilic bacteria	psychrophilic bacteria
Total number	$7.6 \cdot 10^4$	$6.58 \cdot 10^4$	control conditions*)	$2.8 \cdot 10^6$	$2.3 \cdot 10^6$
			A	$1.5 \cdot 10^7$	$1.2 \cdot 10^7$
			B	$7.0 \cdot 10^5$	$6.1 \cdot 10^6$
			C	$2.9 \cdot 10^5$	$2.5 \cdot 10^6$
Enterobacteriaceae	$1.45 \cdot 10^4$	$2.88 \cdot 10^4$	control conditions	$6.8 \cdot 10^6$	$6.3 \cdot 10^5$
			A	$2.0 \cdot 10^6$	$5.8 \cdot 10^6$
			B	$1.2 \cdot 10^5$	$4.2 \cdot 10^5$
			C	$8.0 \cdot 10^4$	$5.3 \cdot 10^4$
Coccidians and streptococci	$1.8 \cdot 10^2$	$1.5 \cdot 10^2$	control conditions	$1.5 \cdot 10^4$	$9.0 \cdot 10^3$
			A	$8.0 \cdot 10^5$	$5.6 \cdot 10^5$
			B	$2.8 \cdot 10^4$	$1.3 \cdot 10^5$
			C	$1.0 \cdot 10^3$	$4.5 \cdot 10^3$
Anaerobes count	absent in 0.1 g	absent in 0.1 g	control conditions	10^{-3}	absent in 0.1 g
			A	10^{-3}	10^{-3}
			B	10^{-3}	10^{-3}
			C	10^{-2}	10^{-3}
Enterococci count	absent in 0.1 g	absent in 0.1 g	control conditions	10^{-2}	absent in 0.1 g
			A	10^{-3}	absent in 0.1 g
			B	10^{-2}	absent in 0.1 g
			C	absent in 0.1 g	absent in 0.1 g

*) After 16 days storage

error, guaranteed error was calculated using the parametric test of Student.

$$mg = t\alpha, \delta \cdot \sum_{i}^{n} \frac{(x_i - \bar{x}^2)}{(n-1)\, n}$$

The average guaranteed error for each analytical discriminant is given in the Figs.

Changes of colour expressed as changes of methioglobin concentration on the surface of the meat samples tested are shown in Fig. 3. As seen from the curves in the diagram, a distinct increase of MMb concentration

for all mixtures took place afted 23 days storage. The lowest increase of MMb was observed in mixture B. After 43 days storage in this mixture, MMb concentration at the surface of meat samples amounted to 55% and in mixture C — 85%. In mixture A the concentration of MMb after 36 days was equally high and amounted to 77%. This was also a time when meat began to deteriorate. Comparing the results shown on the diagram it is possible to say that mixture B was the best especially as in the initial period of storage (up to the 23rd day) the content of MMb on the surface of meat did even decrease. Meat stored in control conditions had the worst colour after 23 days and signs of deterioration appeared already after 17 days.

pH of meat (Fig. 4) displayed the most favourable changes in samples kept in mixtures B and C, the speed of pH growth in meat stored in mixture B was shower than in mixture C. After 43 days pH amounted to 5.75 which proved the high standard of meat. The growth of pH in case of mixture A was very distinct, especially after 23 days. In control samples after 17 days of storage, pH increased dramatically which was linked with rapidly progressing unfavourable changes of the meat tissue.

Changes of the amount of forced drip as external reflection of the changes in water holding capacity determined by the method of Grau and Hamm are presented on curves in Fig. 5. After eight days of storage a larger amount of drip was found in all conditions tested compared with the initial value. In case of mixture B this difference was the smallest. In a later period of storage the amount of drip was smaller

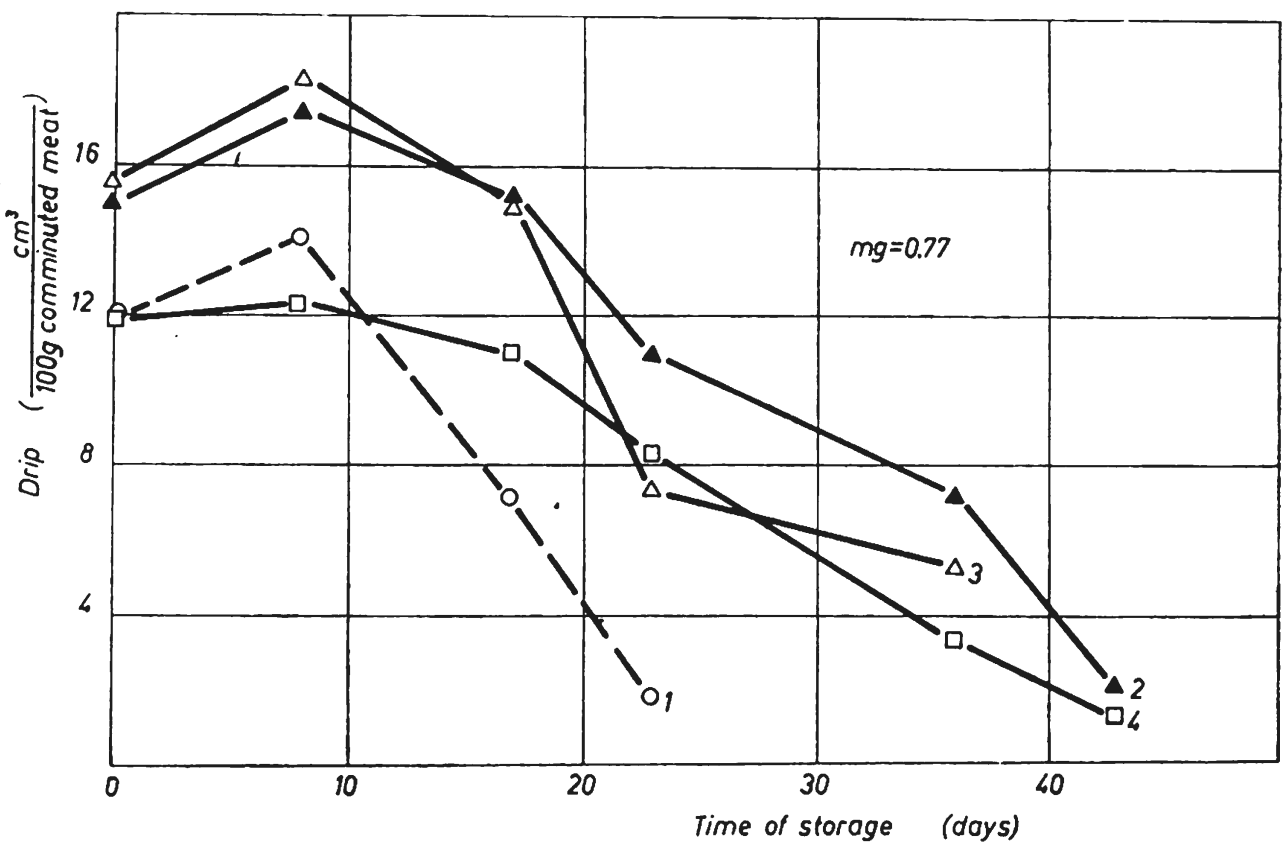

Fig. 5. Changes in the water holding capacity depending on the gas mixture composition. Grau and Hamm method; explanations — see Fig. 3

which points to a gradual improvement of the water holding capacity. Similar changes were observed also in the case of the water holding capacity determined by the centrifuge method (Fig. 6).

A comparison of the changes in the water holding capacity with changes of hydrogen ions concentration gives a basis for a determination of their mutual dependency. The growth of pH which took place after 8 days of storage was accompanied by a reduction of the amount of pressed juice both in the Grau and Hamm method and the centrifuge method. In the former case, changes in water holding capacity corresponded more closely to changes in hydrogen ion concentration than in the latter case. In order to thoroughly determine this dependence an attempt was made to establish the multiple correlation coefficients and

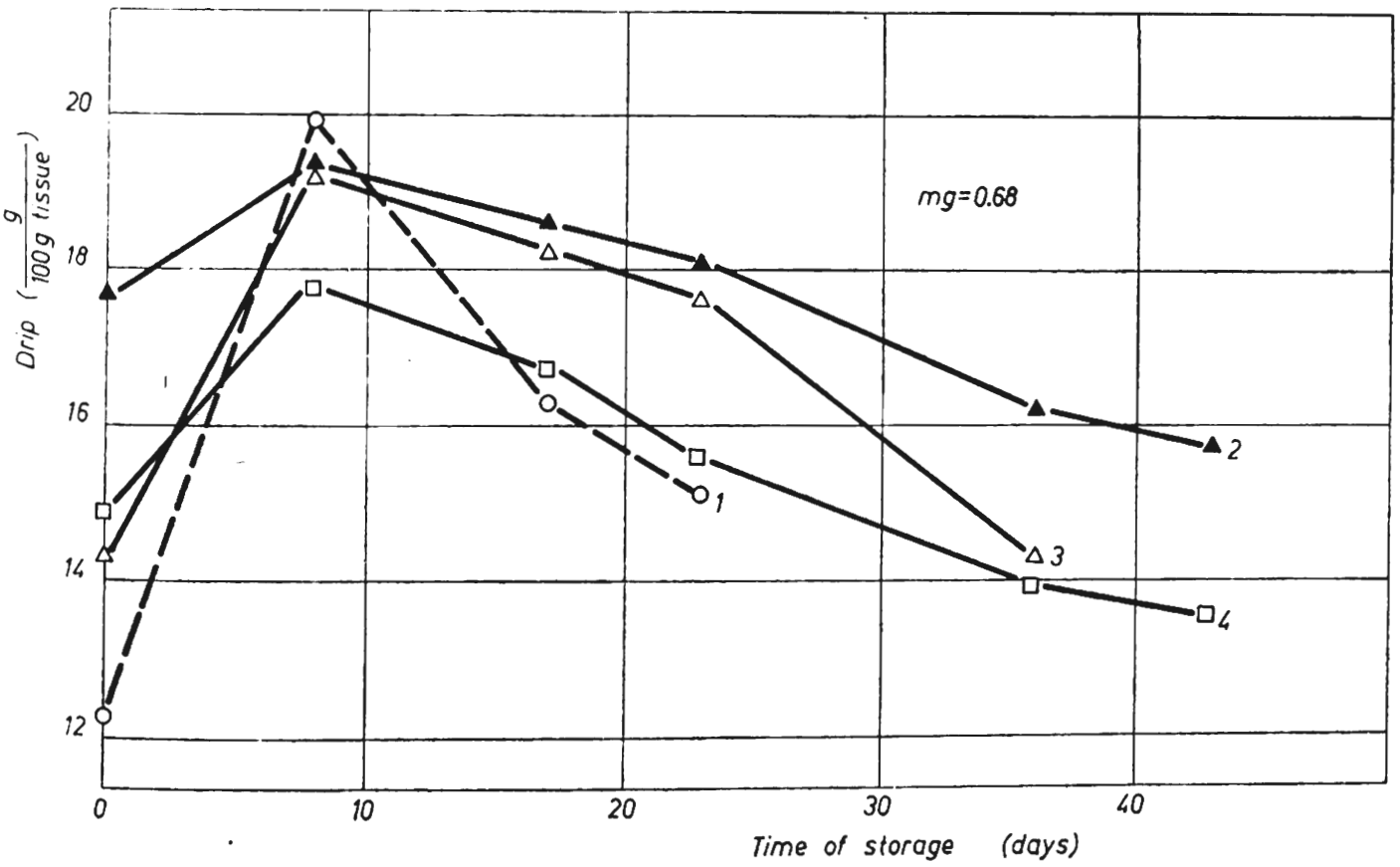

Fig. 6. Changes of the water holding capacity depending on the gas mixture composition. Centrifuge method; explanations — see Fig. 3

the rectilinear regression coefficients. Calculations were made on an Odra 1204 computer. Changes in pH (X) and of the water holding capacity (Y) determined by the method of Grau and Hamm (Fig. 7) may be described by the following rectilinear equation:

$$y = -30.05 \cdot x + 177.04 \text{ cm}^3/100 \text{ g force meat}$$

At a significance level $\alpha = 0.05$, the confidence interval for the directivity index is comprized between — 38.33 and -22.76. The correlation coefficient is high and amounts to $r = -0.821$. As much as 90.5% of experimental points are comprized within 95% of the confidence interval of water holding capacity changes.

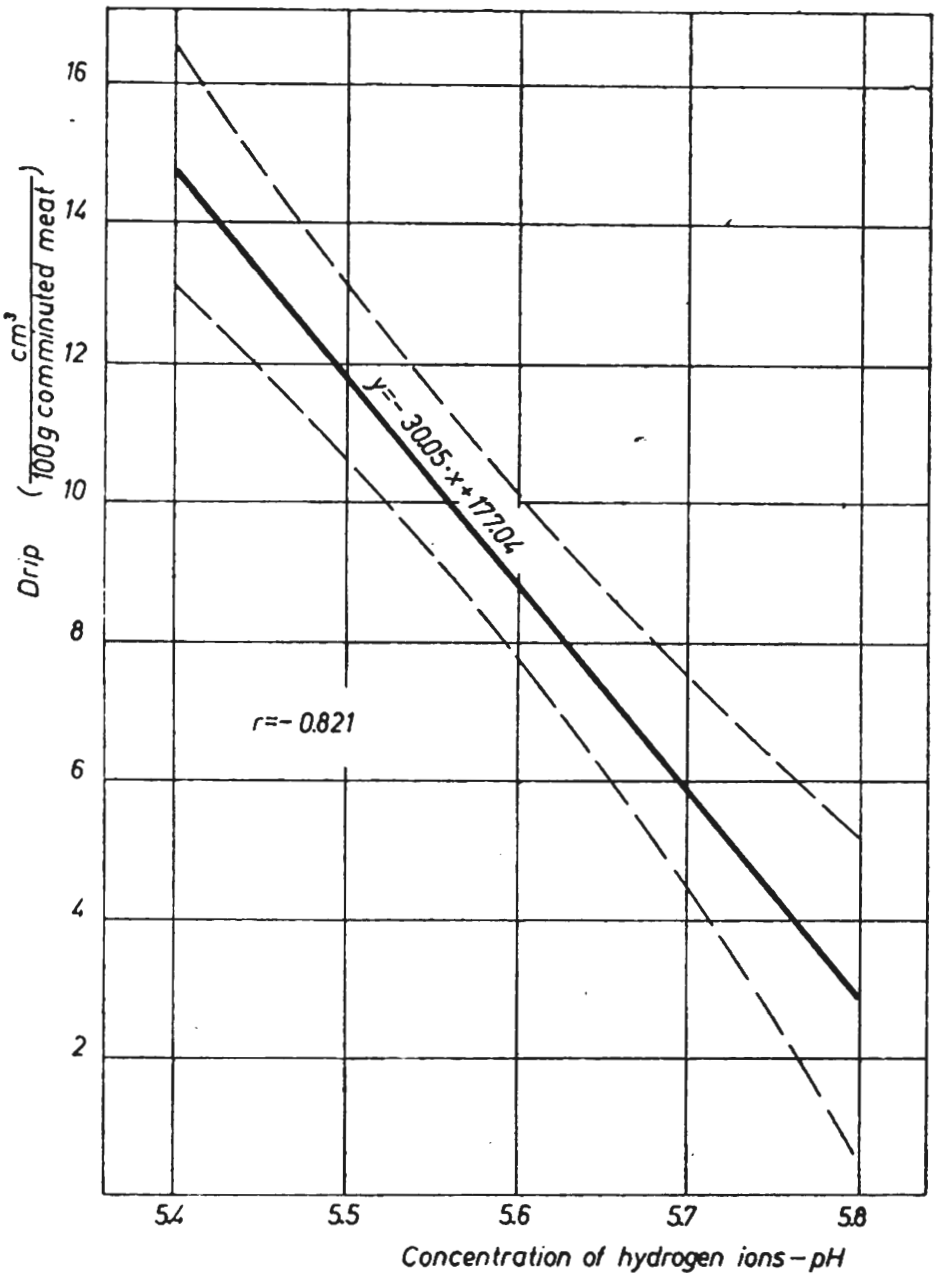

Fig. 7. Dependency between the water holding capacity determined by Grau and Hamm method and the pH value of meat; — — — — — — — confidence interval of mean value y

Changes of the amount of thermal drip from meat stored in all the conditions tested (Fig. 8) were comprized within the limits characteristic for tender meat [32].

The content of total nitrogen in particular meat samples amounted to 30-34 mg N/1 g tissue and was subject to insignificant changes only, irrespecively of the conditions of storage.

Changes in the content of non protein nitrogen (Fig. 9) varied according to the composition of the gas mixtures. The content of non protein nitrogen in meat samples stored in mixtures B and C over a period of 36 days stayed, practically, at an unchanged level and amounted to 5 mg N/1 g of the tissue. After the period, this index grew to 6 mg N/1 g of the tissue. In samples stored in mixture A the growth of non protein nitrogen took place after 23 days, and in control conditions even earlier, i.e. already after 17 days of storage. This growth is connected with a deterioration of meat quality.

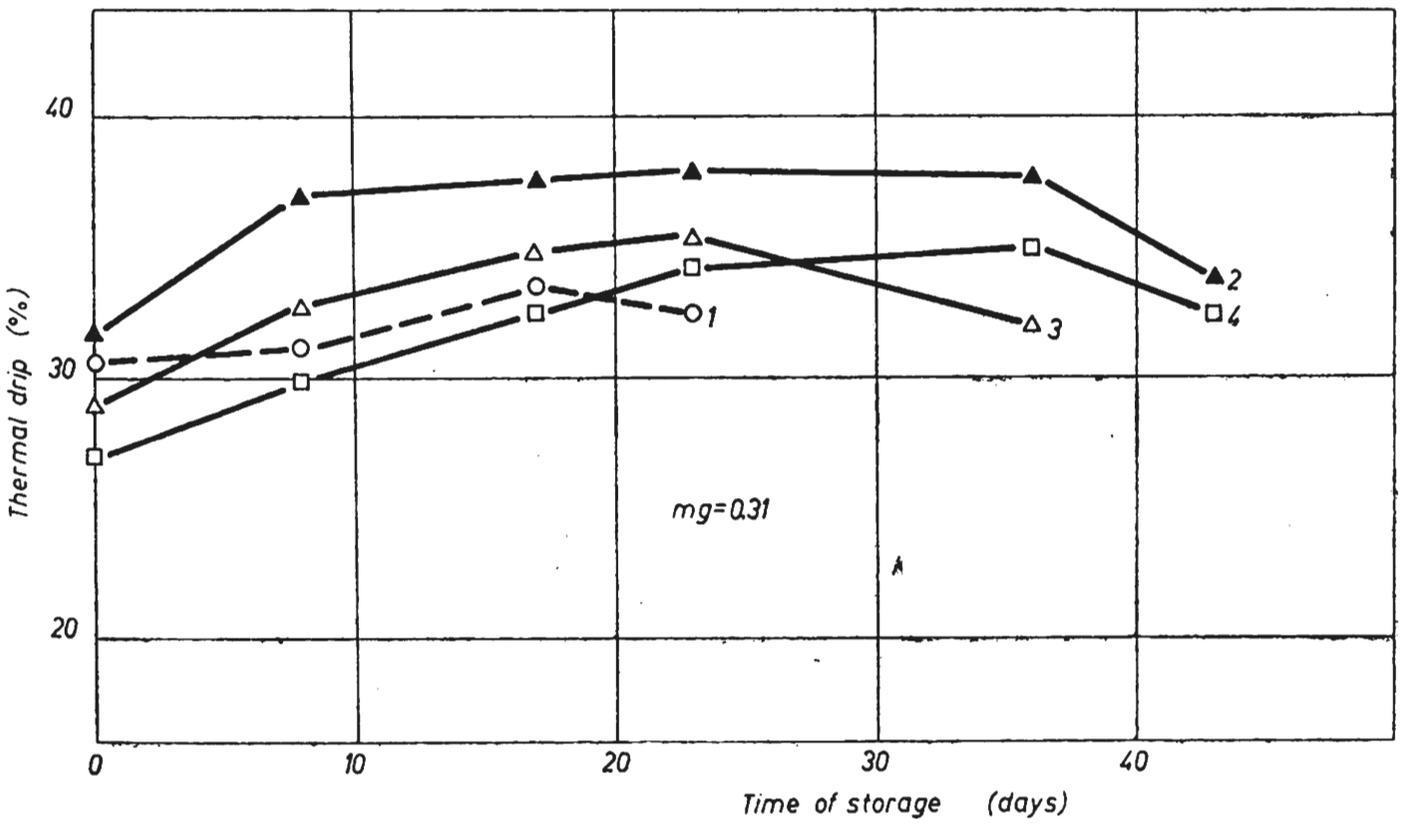

Fig. 8. Changes in the thermal meat drip depending on the gas mixture composition; explanations — see Fig. 3

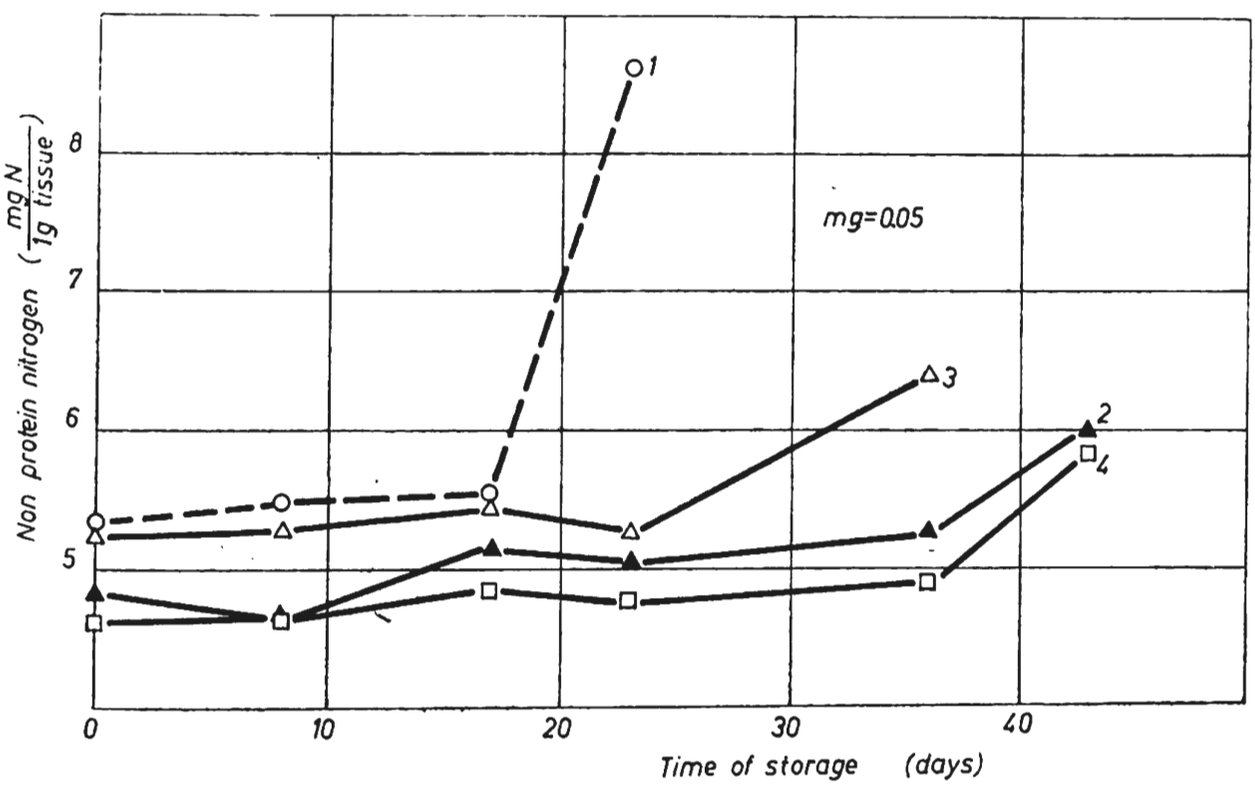

Fig. 9. Changes of non protein nitrogen content depending on the gas mixture composition; explanations — see Fig. 3

The content of amino nitrogen increased with the time of storage (Fig. 10). For mixture A, after 23 days this growth was much more rapid than for mixtures B and C. Meat stored in air featured a distinct growth of this index over the whole period of the tests.

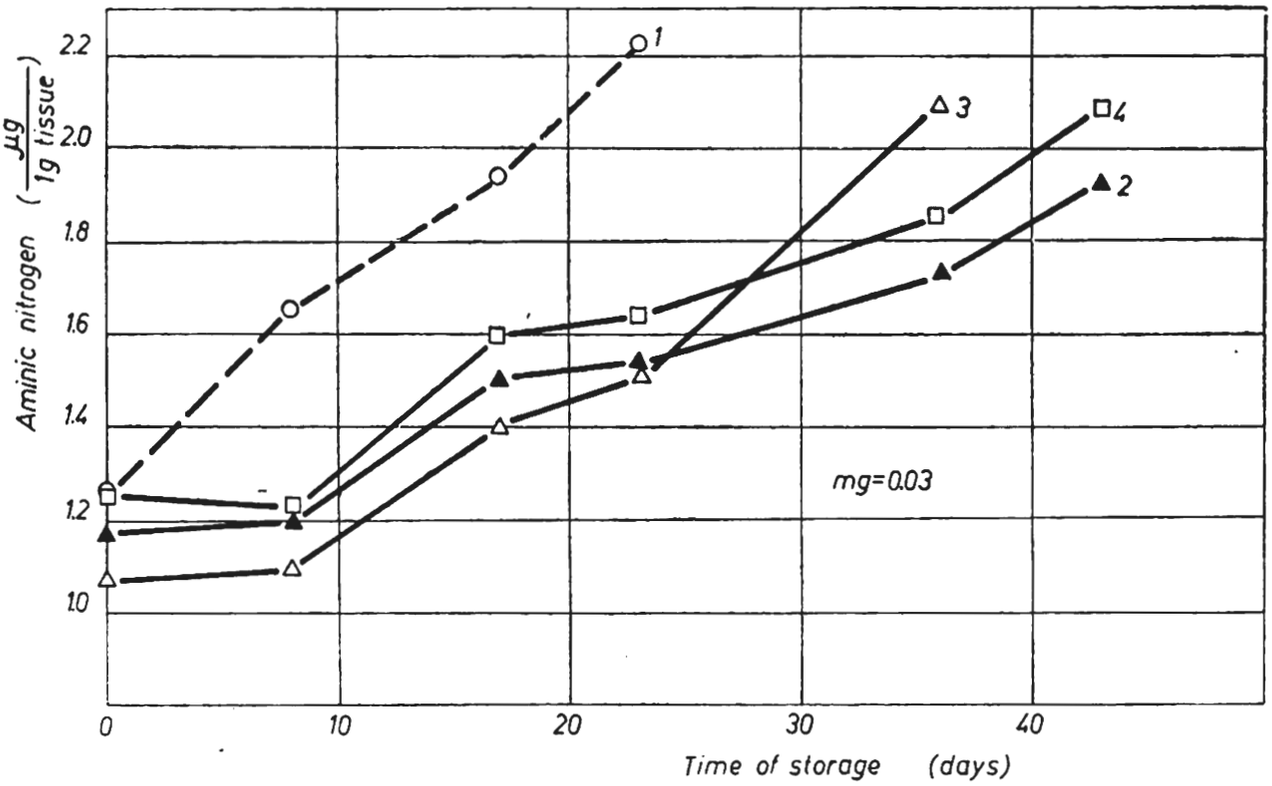

Fig. 10. Changes of the amino nitrogen content depending on the gas mixture composition; explanations — see Fig. 3

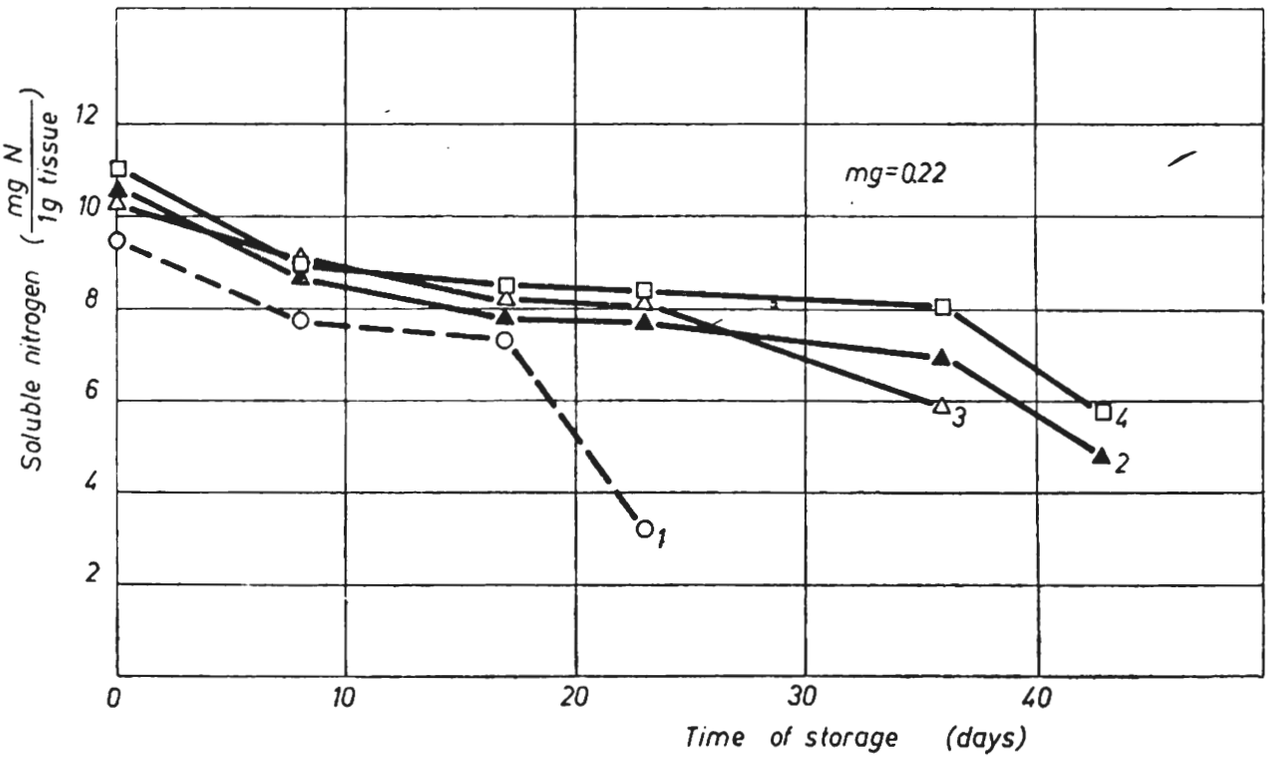

Fig. 11. Changes of soluble nitrogen content depending on the gas mixture composition; explanations — see Fig. 3

The amount of protein soluble nitrogen (Fig. 11) calculated from the difference between the content of total soluble nitrogen and non protein nitrogen, changed in a similar way in mixtures B and C. In mixture A, after 23 days storage a rapid drop of this index was observed which may point to a deterioration of the meat standard in this period of time. In the case of control samples the course of changes in the content of protein nitrogen was the less advantageous. A definite deterioration of the meat standard took place as soon as after 17 days of storage.

On the basis of physico-chemical assessment of the quality of meat tested discussed above, it is possible to say that mixture B is the best suited for long prolongated storage of meat. In this connection, additional determinations were made of meat weight losses in this mixture (drip and drying). The determinations were made taking into consideration the 30-day period of storage (Fig. 12). The amount of drip was 0.27% and drying losses — 0.89%. Weight losses amounted to a total of 1.16% and did not exceed the lower limit of the amount of natural losses observed by Baranowski during one-stage cooling meat [2]. In control conditions, the amount of drip and of drying losses were many times higher in the same time and amounted to 2.86% and 2.04% respectively, i.e. a total of 4.9%. The comparatively high losses of weight result from the large surface of cuts and from the lack of natural protection which is constituted in the case of whole carcasses by their external layer. Weight losses will be much smaller in industrial conditions. A reduction of weight losses in meat stored in a gas mixture as compared to losses in meat stored in air yields essential economic effects when this method is applied in industrial practice.

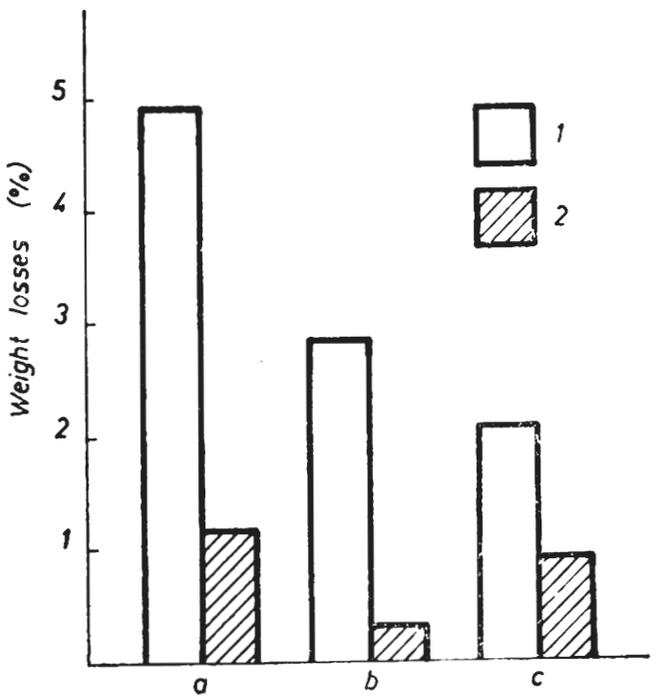

Fig. 12. Weight losses of meat samples stored in gas mixture comprising 20% CO_2, 79% N_2, 1% O_2 and in control conditions; 1 — control conditions, 2 — gas mixture; a — total weight losses, b — drip, c — drying loss

The microbiological assessment (Table) showed that meat stored in air deteriorated after 16 days, and in a gas mixture after 36 days. Symptoms of a lovering standard of samples kept in mixtures B and C took place after 43 days only. The gas mixtures used are the strongest inhibitors of microorganisms of the Enterobacteriacae genus, followed by coccidians and staphylococci. The higher the concentration of CO_2 in the mixture, the slower the growth of microorganisms. Mention is due

to the fact that the total initial number of microorganisms per 1 cm^2 of the surface of meat used for the tests was relatively high and amounted to $6.58 \div 7.60) \cdot 10^4$.

It is possible to further prolongate the period of storage provided that the initial number of microorganisms on the surface of meat will be lower. This fact is confirmed both by the authors' own studies and data obtained by other authors [23].

The final conclusion of the studies made is that taking into account all the physicochemical and microbiological determinations, a mixture of gases: 20% CO_2 + 79% N_2 + 1% O_2 should be considered the most suitable for a prolongation of the period of storage of beef, at a temperature close the cryoscopic. In these conditions, the time of meat storage amounts to 43 days and it is three-times longer than storage in air.

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Authors address: 90-537 Łódź, Gdańska 166

Z. Niedzielski, L. Krala

WPŁYW SKŁADU MIESZANINY GAZOWEJ NA CZAS SKŁADOWANIA WOŁOWINY

Instytut Chemicznej Technologii Żywności, Politechnika, Łódź

Streszczenie

Przebadano wpływ trzech mieszanin gazowych na przedłużenie okresu składowania wołowiny w temperaturze zbliżonej do krioskopowej. Stosowano następujące mieszaniny:
A — 10% CO_2, 89% N_2 1% O_2
B — 20% CO_2, 79% N_2 1% O_2
C — 30% CO_2, 69% N_2 1% O_2

W tym samym czasie próbki mięsa były składowane w atmosferze powietrza (warunki kontrolne). Opracowano dwie metody napełnienia zbiorników mieszaniną gazów. Jakość mięsa w okresie składowania oceniano na podstawie następujących wskaźników: zmiany barwy, pH, zdolności utrzymywania wody, wycieku cieplnego, ubytku masy, azotu ogólnego, niebiałkowego, rozpuszczalnego i aminowego oraz analizy mikrobiologicznej. W celu ustalenia korelacji pomiędzy wartością pH i zdolnością utrzymywania wody obliczono współczynniki regresji i korelacji.

Na podstawie otrzymanych wyników można stwierdzić, że najbardziej sprzyjające warunki daje mieszanina gazowa zawierająca 20% CO_2. W tych warunkach można składować mięso przez 43 dni, tj. 3 razy dłużej niż w warunkach kontrolnych.