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Enzymatic Breakdown of Added Tripolyphosphate and Diphosphate in Meat

SUMMARY

The influence of factors of practical importance on the enzymatic breakdown of added tripolyphosphate (TP) and diphosphate (DP) in minced, salted (2 % NaCl) beef muscle was studied using a TLC method. A great variation in the activities of tripolyphosphatase (TPase) and diphosphatase (DPase) of the longissimus muscle between animals was observed. The TPase activity is much higher than the DPase activity. Complete hydrolysis of 0.5 % added polyphosphate in meat at 20°C needs 8 to 20 min for TP and 2 to 15 hours for DP. The TPase activity increases whereas the DPase activity decreases during the first two days post mortem. These effects might be due mainly to the drop in pH post mortem. The pH optimum of the activity lies at pH 5.7 for TPase and at pH 7 for DPase. The dependence of polyphosphatase activities on NaCl concentration shows a maximum at 4 % NaCl for TPase, but a continuous decrease of DPase activity with increasing NaCl concentration.

At 0°C and at freezing temperatures TPase and DPase are entirely inactive. The activities increase with rising temperature, until the enzymes are inactivated by heat denaturation. DPase is more sensitive against heating than TPase. Freezing of tissue has an influence on the enzyme activities.

The increase in water-holding capacity (WHC) of meat, caused by addition of DP, is not reduced even after complete hydrolysis of DP. The effect of TP on WHC increases during the fast breakdown of TP to DP. Only DP seems to be effective on the myofibrillar proteins; TP seems to be effective only after its enzymatic hydrolysis to DP.

ZUSAMMENFASSUNG

Enzymatischer Abbau von Tripolyphosphat und Diphosphat in zerkleinertem Fleisch

Der Einfluß verschiedener, für die Praxis wichtiger Faktoren auf den enzymatischen Abbau von zugesetztem Tripolyphosphat (TP) und Diphosphat (DP) in zerkleinertem, gesalzenem (2 % NaCl) Rindermuskel wurde mit Hilfe einer dünnschichtchromatographischen Methode studiert. Es wurde eine beträchtliche Variation von Tier zu Tier in den Tripolyphosphatase(TPase)- und Diphosphatase(DPase)-Aktivitäten des Longissimus-dorsi-Muskels beobachtet. Die Aktivität der TPase war wesentlich höher als die der DPase. Für eine vollständige Hydrolyse von 0,5 % Polyphosphat im Fleisch bei 20°C sind bei TP 8-20 Min., bei DP 2-15 Stdn erforderlich. Innerhalb der ersten 2 Tage post mortem nimmt die TPase-Aktivität zu, die DPase-Aktivi-

tät ab. Diese Effekte dürften hauptsächlich auf dem pH-Abfall post mortem beruhen. Das pH-Optimum der Aktivität liegt für TPase bei pH 5,7, für DPase bei pH 7. Der Einfluß der NaCl-Konzentration auf die Polyphosphatase-Aktivität zeigt für TPase ein Maximum bei 4 % NaCl, während die DPase-Aktivität mit steigender NaCl-Konzentration kontinuierlich abnimmt.

Bei 0°C und bei Gefriertemperaturen sind TPase und DPase völlig inaktiv. Mit steigender Temperatur nehmen die Aktivitäten zu, bis die Enzyme durch Hitzedenaturierung inaktiviert werden. DPase ist gegen Erhitzung empfindlicher als TPase. Gefrieren des Gewebes hat einen gewissen Einfluß auf die Enzymaktivitäten.

Die durch DP-Zusatz hervorgerufene Erhöhung des Wasserbindungsvermögens (WBV) des Fleisches nimmt selbst bei völliger Hydrolyse des DP nicht ab. Die WBV-Erhöhung durch TP nimmt während des raschen Abbaues von TP zu DP zu. Nur DP scheint von Einfluß auf das myofibrilläre Eiweiß zu sein; TP scheint erst nach enzymatischem Abbau zu DP wirksam zu werden.

INTRODUCTION

Addition of polyphosphates as diphosphate or tripolyphosphate to sausage emulsions or cured meat products improve the quality of the product by increasing of water-holding capacity and resulting in a better distribution of fat. With respect to this practical application of polyphosphates it is of interest that already several years ago an enzymatic breakdown of TP*) and DP by muscle proteins was observed (1,6,8,9). However, results of experiments with isolated enzymes (6,8,9) do not reflect the conditions which exist in meat and meat products. Little is known about the influence of pH, added sodium chloride, temperature, time post mortem etc. on the enzymatic hydrolysis of polyphosphates in meat (1). The knowledge of these influences would be important for legal consequences resulting from the determination of added polyphosphates in meat products, for the best application of phosphates in meat processing and also for judging the physiological effect of polyphosphates in meat products.

Therefore, the effect of different factors on the enzymatic breakdown of TP and DP in minced muscle tissue was studied. After completion of this work a brief paper on hydrolysis of DP and TP in meat was published by MIHALYI-KENGYEL and KÖRMENDY (5). The results of their few experiments are in good agreement with our findings.

MATERIAL AND METHODS

Beef muscle (M. longissimus dorsi) was ground at different times post mortem. 1 % solutions of $Na_5P_2O_7$ or $Na_4P_2O_7$ were mixed with the ground tissue. Usually, a total amount of 50 %1) water was added. NaCl was added

*) Abbreviations used: DP = diphosphate; TP = tripolyphosphate; DPase = diphosphatase; TPase = tripolyphosphatase; P_i = inorganic monophosphate; WHC = water-holding capacity.

1) The percentage of added water, NaCl and polyphosphates is related to the meat without additives.

and the pH or temperature were adjusted to the desired experimental conditions. At different times after addition of the polyphosphate, 10 or 15 g of the sample were homogenized with the same amount of 10 % TCA for 30 sec. In the TCA extracts DP or TP and DP were determined by the TLC-method of NERAAL and HAMM (7). The P_i-content was determined according to the method of KENNEDY and WEETMAN (4). For the determination of WHC the procedure of GRAU and HAMM (2) was used.

RESULTS AND DISCUSSION

The activities of TPase and DPase were measured by determining the rate of breakdown of TP and DP in the meat sample. Additional information was obtained by following the increase of the P_i content in the sample. The enzyme activities are expressed as µmoles polyphosphate broken down per minute by 1 g muscle tissue.

If not explicitly indicated, 0.5 % polyphosphate, 2 % NaCl and 50 % water were added to the post-rigor muscle (about 7 days post mortem). The presence of NaCl was necessary because in meat processing polyphosphates are mostly used in combination with NaCl.

Polyphosphatase activity of beef muscle

The enzymatic breakdown of TP in muscle follows the schema shown in Fig. 1. TP is hydrolyzed to DP and P_i. After reaching a maximum, the DP concentration decreases because of the DPase activity of tissue which splits DP into two moles of P_i.

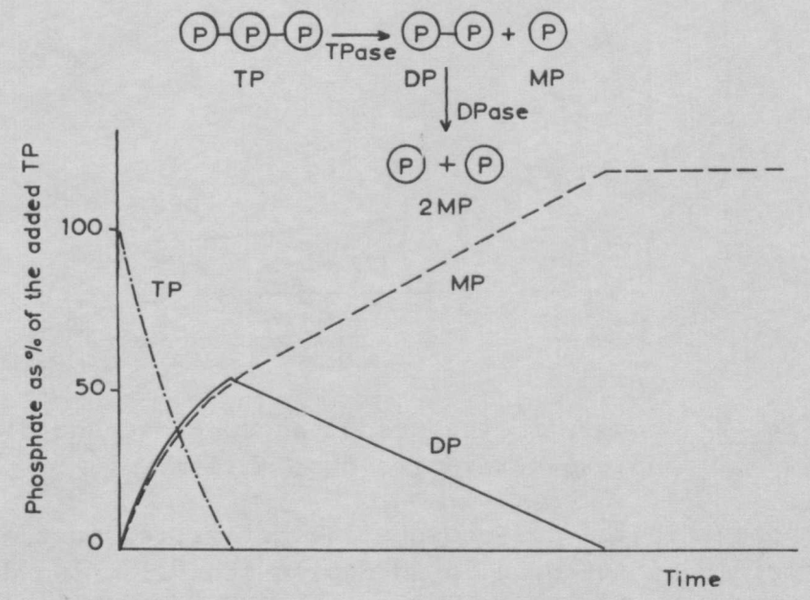

Fig.1 Schema of the enzymatic breakdown of tripolyphosphate in muscle tissue

A great variation of the TPase activity within the longissimus muscle of different animals was observed. In the post-rigor muscles of 8 animals, which had about the same pH value, the TPase activity varied between 0.7 and 1.7 µmoles/min/g tissue at 20°C. This means that, at that temperature, 0.5 % added TP is completely broken down within only 8 to 20 min.

The DPase activity is much lower than the TPase activity. The activities measured varied between 0.023 and 0.15 µmoles/min/g tissue at 20°C. Therefore, the time needed for a complete hydrolysis of 0.5 % added DP at this temperature varied between 2 and 15 hours.

Influence of time post mortem

There are considerable changes in polyphosphatase activities during storage of meat after slaughter at 4°C. Either 0.5 % TP or DP was added at different times post mortem, and the polyphosphatase activities were measured at 20°C. As Fig.2 shows, the TPase activity increases within the first 2 or 3 days post mortem. However, the DPase activity decreases during this period. There are no remarkable changes in the polyphosphatase activities during further storage of beef.

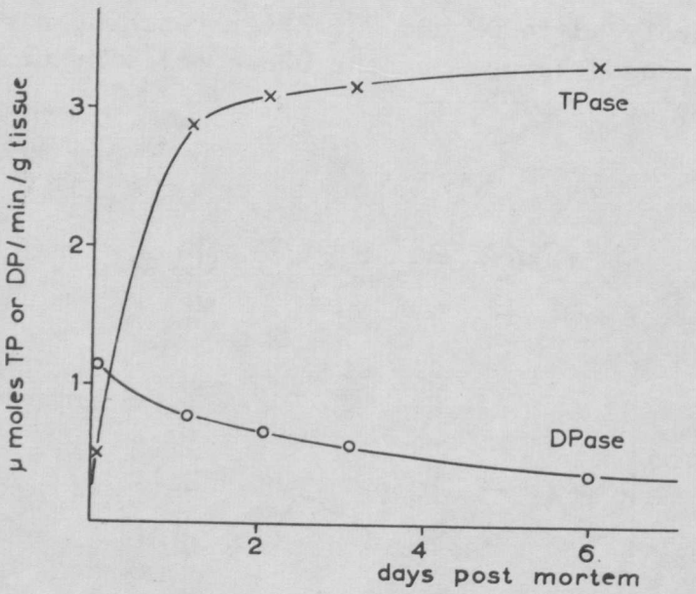

Fig. 2 Changes in the tripolyphosphatase and diphosphatase activities post mortem

These changes in the polyphosphatase activities post mortem can be explained mainly by the drop in pH during the first two days after slaughter caused by the glycolytic formation of lactic acid (see the next pragraph). Also changes in the interaction of actin and myosin and in the concentration of free inorganic ions may have had some influence.

Influence of pH

The TPase activity shows a pronounced pH optimum at pH 5.7 (Fig.3). Therefore, the shift in pH post mortem from 7 to 5.5 explains the increase in the TPase activity during the first two days after slaughter. The pH optimum of the DPase activity lies around pH 7 (Fig. 4). This may be the main reason for the post mortem decrease in DPase activity.

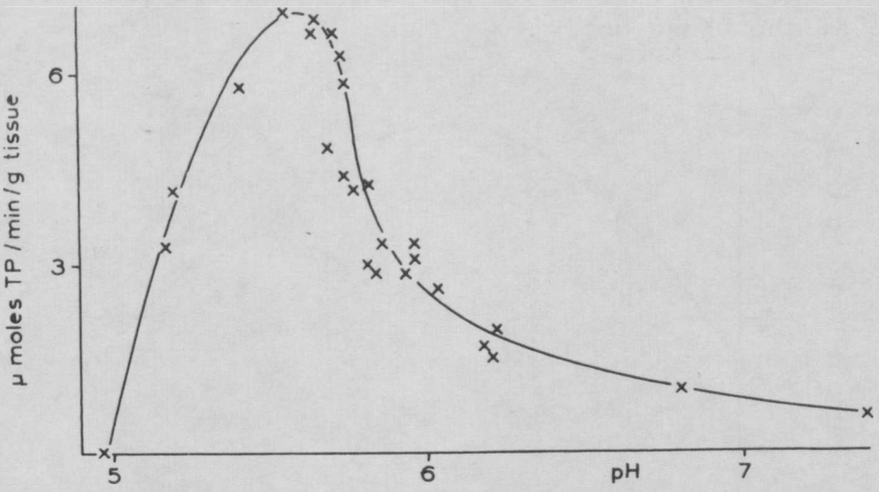

Fig. 3 Influence of pH on the tripolyphosphatase activity of muscle tissue

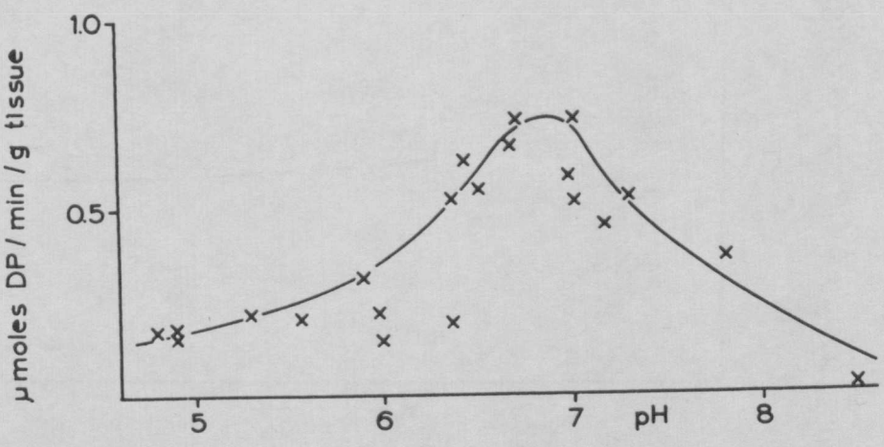

Fig. 4 Influence of pH on the diphosphatase activity of muscle tissue

Influence of sodium chloride

The activities of muscle polyphosphatases are influenced by added NaCl. In the range between 0 % and 4 % NaCl the activity of TPase increases with increasing NaCl concentration (Fig. 5). A further increase in NaCl concentration results in a decrease of TPase activity. A similar inhibition by high salt concentrations is also seen with many other enzymes.

The dependence of DPase activity on the NaCl concentration does not show a maximum (Fig.6). Increasing the NaCl concentration results in a decreasing of the DPase activity.

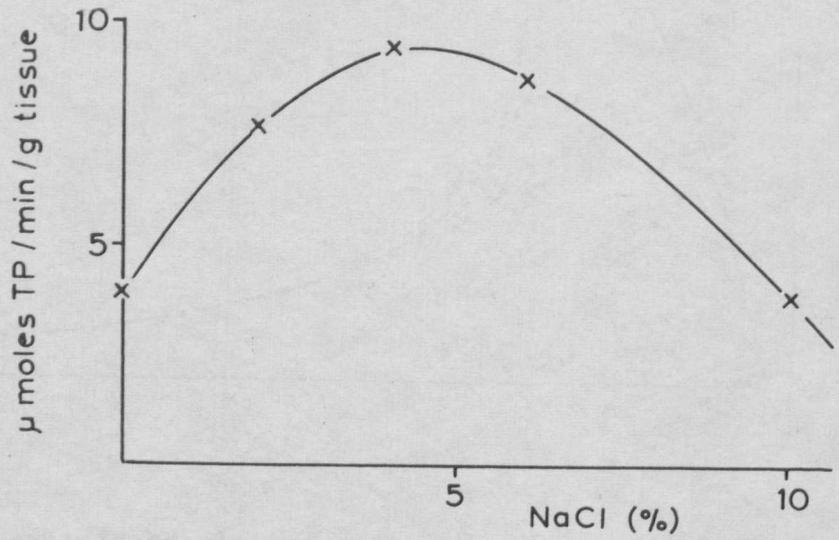

Fig. 5 Influence of added sodium chloride on the tripolyphosphatase activity of muscle tissue

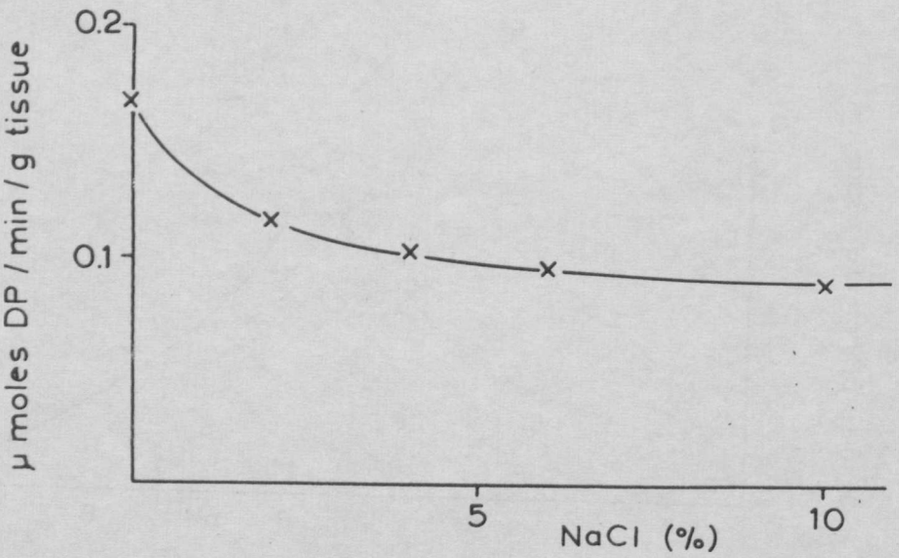

Fig. 6 Influence of added sodium chloride on the diphosphatase activity of muscle tissue

Influence of temperature

The rate of the enzymatic breakdown of TP and DP in muscle tissue depends strongly on the temperature. Between +5° and +40°C an increase in temperature of 10°C causes a doubling of the DPase activity. At 4°C the hydrolysis of 0.5 % DP needs several days; at 0°C no DP was broken down. Therefore, the amount of added polyphosphates determined in meat products depends strongly on the time and temperature of storage of sausage emulsion or cured products.

The activity of DPase and TPase increases with rising temperature until the enzyme is inactivated by heat denaturation. In meat, which was kept at 42°C for 30 min, a 80 % inactivation of the TPase activity was found; heating at 50°C for the same time caused a 98 % inactivation. DPase is more resistant against heating. Heating at 43°C for 30 min did not influence the activity, but heating at 54°C for the same time reduced the DPase activity 85 %.

During smoking and cooking the polyphosphatases are completely inactivated, but such treatments can cause nonenzymatic hydrolysis of polyphosphates. Because of the possibilities of enzymatic and nonenzymatic hydrolysis of polyphosphates during processing it is hardly possible to estimate the amount of added polyphosphates by determination of these compounds in the finished product.

Influence of freezing

At freezing temperatures the polyphosphatases of muscle tissue are entirely inactive. After freezing of post-rigor beef at -18°C, storage at this temperature for 11 days and thawing, the same TPase activity was found as before freezing. Pre-rigor frozen and thawed beef showed the same TPase activity as the nonfrozen post-rigor beef. The DPase activity was reduced by freezing and thawing.

Enzymatic breakdown of polyphosphates and water-holding capacity

For the practical use of polyphosphates the question of interest is whether the enzymatic hydrolysis of added TP and DP lowers the favourable effect of these additives on WHC of beef. As Fig. 7 shows, the increase in WHC caused by the addition of DP is not reduced even after complete hydrolysis of DP. This is analogous to the fact that the WHC of pre-rigor salted meat does not decrease even after a complete breakdown of ATP (3).

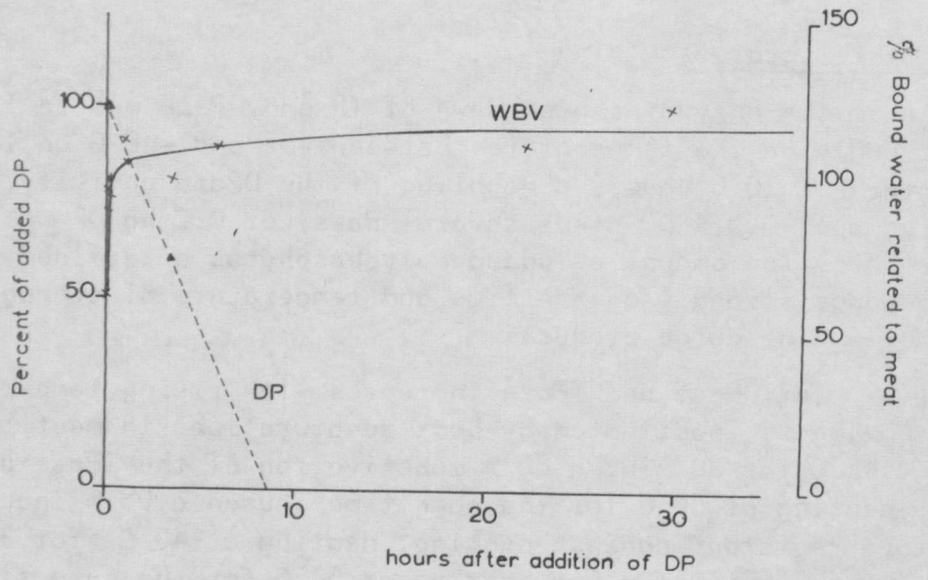

Fig. 7 Hydrolysis of diphosphate and change in water-holding capacity (WBV) in muscle tissue. 100 % added water

As to the effect of TP, the WHC measured immediately after addition of the polyphosphate does not decrease but even increased during the fast breakdown of TP to DP (Fig. 8). This result seems to support the suggestion of YASUI et.al.(9) that only DP is effective on actomyosin and that the TP is effective only after it is first hydrolyzed to DP by the myosin-TPase.

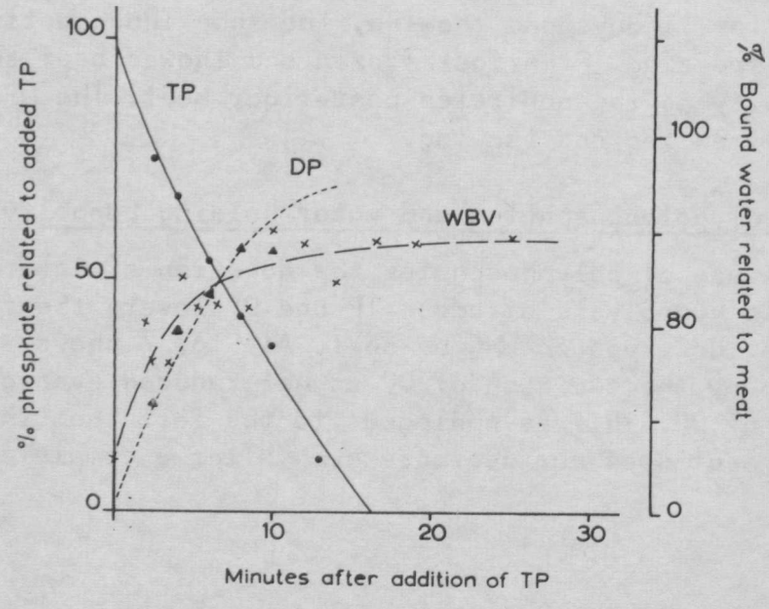

Fig. 8 Breakdown of triphosphate and change in water-holding capacity (WBV) in muscle tissue

Further investigations

Extended studies about the kinetics of TPase and DPase in the complex system of muscle tissue were carried out. The effect of Ca^{++}, Mg^{++}, EDTA, and of the concentration of added adenosine triphosphate and adenosine diphosphate as well as the subcellular distribution of the polyphosphatases were studied. The results of these experiments will not be discussed here but published later on.

REFERENCES

(1) AWAD, K.K.:M.Sci.thesis, University of Alberta,Edmonton(Canada); ref.: DeMan, J.M.in:"Phosphates in Food Processing"(Ed.J.M. DeMan and P.M.Melnychyn),p.38-48.AVI Publ.Company Inc. Westport,Conn. 1971.

(2) GRAU, R. and R.HAMM:Zeitsch.Lebensmittel-Untersuch. u. -Forsch. 105, 446 (1957).

(3) HAMM,R.: "Kolloidchemie des Fleisches".Verlag Paul Parey.Berlin, Hamburg. 1972.

(4) KENNEDY,J.F. and D.A.WEETMAN: Analyt.Chim.Acta 55, 448 (1971).

(5) MIHALYI-KENGYEL,V. and L.KÖRMENDY: Acta Alimentaria 2,No.1,60(1973).

(6) NAKAMURA,S., M.YAMAGUCHI,J.MORITA and T.YASUI: Bull.Meat, Meat Prods. 5, 19 (1969).

(7) NERAAL,R. and R.HAMM: Fleischwirtschaft 52, 1171 (1972).

(8) YASUI,T.,M.SAKANISHI and Y.HASHIMOTO: J.Agric.Food Chem.12,392(1964).

(9) YASUI,T.,T.FUKAZAWA,K.TAKAHASHI,M.SAKANISHI and Y.HASHIMOTO: J.Agric. Food Chem. 12, 399 (1964).

R. NERAAL et R. HAMM

Dégradation enzymatique du tripolyphosphate et du pyrophosphate (diphosphate) ajoutés dans la viande broyée.

L'influence de divers facteurs de grande importance technologique sur la dégradation enzymatique du tripolyphosphate (TP) et du pyrophosphate (DP) ajoutés est étudiée sur le muscle de boeuf broyé et salé à 2 % de NaCl à l'aide d'une méthode par chromatographie sur couche mince. On a observé d'un animal à l'autre des différences considérables d'activité enzymatique tripolyphosphatase (TPAse) et pyrophosphatase (DPAse) sur le Longissimus dorsi. L'activité TPAse est notablement plus intense que celle de la DPAse. Pour une hydrolyse complète de 0,5 % de polyphosphate dans la viande à 20°C il faut de 8 à 20 minutes pour le TP et de 2 à 15 heures pour le DP. L'activité TPAse augmente, l'activité DPAse baisse pendant les 2 premiers jours post-mortem. Ces deux effets doivent principalement reposer sur la chute du pH post-mortem. L'optimum d'activité se situe à pH 5,7 pour la TPAse et à pH 7 pour la DPAse. Le maximum d'activité polyphosphatase est observé pour une concentration en sel NaCl de 4 %, alors que l'activité DPAse décroit de façon continue à mesure que la concentration en NaCl croit.

La TPAse et la DPAse sont totalement inactives à 0°C et aux températures de congélation. Les activités augmentent avec la température jusqu'à la température d'inhibition thermique des enzymes. La DPAse est plus sensible à la chaleur que la TPAse. La congélation des tissus a une influence certaine sur les activités enzymatiques.

L'augmentation de pouvoir de rétention en eau (PRE) dûe à l'addition de DP ne s'abaisse pas même après hydrolyse complète du DP. L'augmentation de PRE dûe à l'addition de TP augmente pendant la dégradation rapide de TP en DP. Seul DP semble avoir une influence sur les protéines myofibrillaires. TP semble n'etre actif qu'après dégradation enzymatique en DP.

Ферментативное расщепление триполифосфата и дифосфата, добавленных к измельченом мясе

Р. Нераал и Р. Хамм

Влияние факторов, имеющие практическое значение, на ферментативное расщепление триполифосфата (ТФ) и дифосфата (ДФ), добавленных к измельченой и посоленой говядини (2% соли), исследованно методом тонкослойной хроматографии. Замечены большие вариации активности триполифосфатазы (ТФазы) и дифосфатазы (ДФазы) из разных образцов m.long. dorsi . Активность ТФазы была болъше чем ДФазы. Для полного гидролиза 0,5% полифосфата добавленого к мясе при 20° С было нужно 8 - 20 мин. в случае ТФ, а 2 - 15 часов в случае ДФ. В течении двух первых суток после убоя активность ТФазы повышается, а ДФазы понижается. Эти эффекты обусловлены в основном послесмертным снижением pH. Оптимальный pH для ТФазы 5,7, а для ДФазы 7,0. Влияние концентрации повареной соли на активностъ полифосфатаз показнвает максимальное значение при 4% для ТФазы, а активностъ ДФазы понизается с повышением концентрации.

При 0° С и при температурах замораживания ТФаза и ДФаза полностью неактивные. С повышением температуры активность повышается до момента инактивации ферментов тепловой денатурацией. ДФаза больше чувствительная к действию теплоты. Замораживание показивает некоторое влияние на активность ферментов.

Повышение влагопоглащаемости мяса вызванно добавкой дифосфата не понижается даже при полном гидролизе ДФ. Повышение влагопоглощаемости продолжается при быстром расщеплении ТФ в ДФ. Кажется что только ДФ влияет на миофибрилярные белки, а ТФ деятельный лишъ только после ферментативного расщепления до ДФ.