DER XXI EUROPAISCHE KONGRES
DER MITARBEITER WFI FUR FLEISCHINDUSTRIE

Der gesamten Union Wissenschsftliches Forchungsinstitut
für Fleischindustrie Ud SSR

DAS STUDIUM DER STABILITAT LYSOSOMALISCHEN FERMENTEN BEIM AUTOLYSEPROZES DES RINDFLEISCHES

P.E.Pavlovsky,E.I.Simbireva

A N N O T A T I O N

Das Studium der Niveauänderung der freicn Aktivität der lysosomalischen Fermenten bei der Fleischaufbewahrung ist vom praktischen Interesse für die Bewertung der Vorgänge der fermentativen autolytischen Verwandlungen der Hauptkomponenten wie Z.B. der Eiweissestoffen,Kohlenhydraten,Lipiden u.a.

Die Lysosomen Wurden mit Hilfe der differentialen zentrifugung.Man studierte auch die Dynamik der Anderung der allgemeinen,freien und mit zusammen hängenden Aktivität den cathepsinen A und C, α-glucosidase, α-lipase,phospholipase C, collagenase beim Autolyse (2^{o}C).

Die Untersuchungen haben gezeigt das der Niveau der freien und mit Lisosomen zusammenhängenden Aktivität der hauptlysosomalen Hydrolasen nicht gleich ist.Es wurde verschiedene Stabilität dieser Fermenten geklart.

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L'ETUDE DE LA STABILISATION DU FERMENTS LYSOSOMALES PENDANT L'AUTOLYSE DE LA VIANDE DE BOEUF

P.E.Pavlovski,E.I.Simbireva

A N N O T A T O N

L'etude du changement des niveaux de l'activité libre des ferments lysosomales durant le stockage de la viande présente un intérêt pratique pour apprécier le caractère des transformations fermentaires autolyŷiques des composant principaux:des protéines,des hydrates de carbone,des lipides et des autres.

On dégagesit les lysosomes par le centrifugage differentielle.On a étudié a l'autolyse (2^{o}C) la dynamique du changement de l'activité générale libre et liée avee des lysosomes des cathepsines A et C; α-glucosidase; α-lipase; phospholipase C; collagenase.

Les e'tudes out montré que le niveau de l'activité des hydrolases principaux libre et liée avee des lysosomes h'est pas égal.Une stabilité différente de ces ferments a été révèlêẽ.

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THE STUDY OF LYSOSOMAL ENZYMES STABILITY DURING THE AUTOLYSIS PROCESS OF BEEF

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A B S T R A C T

The study of the changing levels of enzymes free activities during meat storage is of particular concern for those interested in discovering the character of enzymatic,autolytic conversion of the main meat components as proteins,carbonhydrides,lipids and ets.

Lysosomes were removed by differential centrifugation. The conversion dynamic of a common,free and lysosomal bound activity of cathepsins A and C, α-glucosidase, α-lipase,phospholipase C,collagenase was observed during the autolysis process.

The observation showed that the level of a free and lysosomal bound activity of the main lysosomal hydrolases was not identical.It was discovered that those enzymes had various stabilities.

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ИЗУЧЕНИЕ СТАБИЛЬНОСТИ ЛИЗОСОМАЛЬНЫХ ФЕРМЕНТОВ В ПРОЦЕССЕ АВТОЛИЗА ГОВЯЖЬЕГО МЯСА

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А Н Н О Т А Ц И Я

Изучение изменения уровней свободной активности лизосомальных ферментов при хранении мяса представляет практический интерес для суждения о характере ферментативных,автолитических превращений основных компонентов: белков,углеводов,липидов и других.

Лизосомы выделяли дифференциальным центрифугированием.Изучали динамику изменения общей,свободной и связанной с лизосомами активности катепсинов А и С, α-глюкозидазы, α-липазы, фосфолипазы "С",коллагеназы при автолизе /2^{o}С/.

Исследования показали,что уровень свободной и связанной с лизосомами активности основных лизосомальных гидролаз неодинаков.Выявлена различная стабильность этих ферментов.

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THE STUDY OF LYSOSOMAL ENZYMES STABILITY DURING THE AUTOLYSIS PROCESS OF BEEF

P.E.Pavlovsky, E.I.Simbireva

Enzymatic conversion of proteins, carbonhydrates, lipides, phospholipids, which are the results of the autolysis process, is of great importance for such meat properties as delicasy, flavour and taste. In connection with the above mentioned the studing of the releasing of enzymes from the lysosomes, enzyme activity changing and stability of lysosomal enzymes is of great interest. This paper deals with the studing of cathepsins A, C, collagenase, α-glucosidase, α-lipase and phospholipase C stability during the autolysis process.

The longissimus dorsi muscle of bovine was the subject of the research. The muscle was isolated just after the slaughter from the old animals (3-4 years) Grade one. That muscle was stored at 2°C.

Lysosomes were isolated from muscle tissue homogenate by differential centrifugation at 0-2°C /1/.

Both a common and a lysosomal bound activity were determined in the muscle tissue homogenate and in an isolated lysosomal fraction, in which lysosomal membranes were destoyed by detergent - triton X-100. Enzymatic free activity was established in the supernatant layer, which had been received after the precipitation of the lysosomes.

Enzymatic activities were established on the specific substrats. The cathepsin A activity was determined by Iodice /2/; cathepsin C - by Caldwell and Groijean /3/; α-glucosidase - by Beck and Tappel /4/; α-lipase - by Shligin /5/; phospholipase C - by Lehmann /6/. The collagenase activity in muscle and intermuscle connective tissue was determined by Sory and Zaharia /7/; the level of protein - by Lowry.

The changes of a common, free and bound enzymatic activity are shown on fig.1-3.

Our observatios showed that the releasing of cathepsin A from lysosomes is of an unusual nature. After 3 hours of autolysis 11% of cathepsin is released from lysosomes (fig.2, slope 1). However, in the following twenty four hours the degree of the releasing enzyme from lysosomes was not large. We can received a maximum activity (44%) of enzyme on the fourth day (in 96 hours) only. After that the cathepsin A in a free form is inactivated.

The degradation of cathepsin A bound activity runs gradually during all the period of autplysis (fig.3, slope 1). On the 12-th day we can receive the largest inactivation of bound enzyme.

As it was observed, the cathepsin A has a relatively high stability. Its common activity does not change during the first four days (fig.1, slope 1). A further autolysis process causes a visible cathepsin degradation. After 12 days of meat storage the enzyme still has a certain common activity.

The cathepsin C is notable for its higher degree of releasing from lysosomes. In the first hours of muscle autolysis free cathepsin activity, which is 20% of that of the common activity is determined (fig.2, slope 2) and after four days of an autolysis this value is 95%. Then the degradation of free enzymatic activity begins.

The cathepsin C is a high stabile enzyme. Its first common activity does not change after two days of muscle storage. On the 3-d - 4-d days it changes slowly and only after 4 days the degradation of its activity begins. But on the 10-th day the cathepsin C still has a certain degree of a high activity.

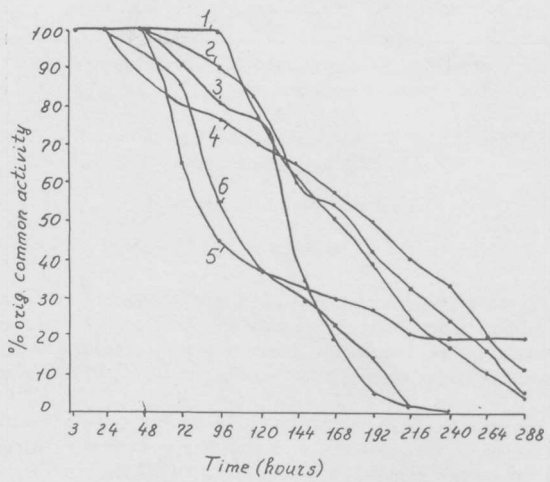

Fig.1
The change of a common activity:
cathepsin A-1; cathepsin C-2; collagenase-3;
α-glucosidase-4; α-lipase-5; phospholipase C-6
during beef muscle autolysis (2°C).

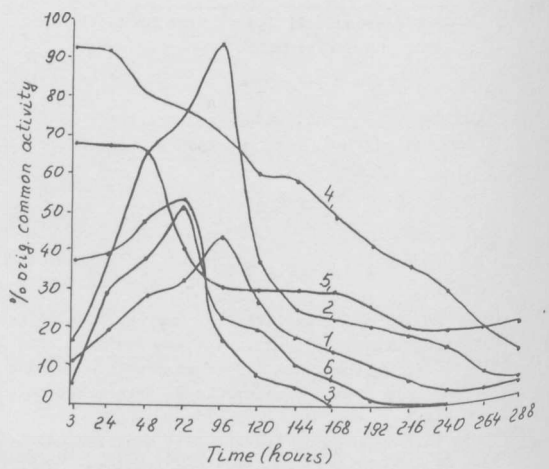

Fig.2
The change of a free activity:
cathepsin A-1; cathepsin C-2; collagenase-3;
α-glucosidase-4; α-lipase-5; phospholipase C
during beef muscle autolysis (2°C).

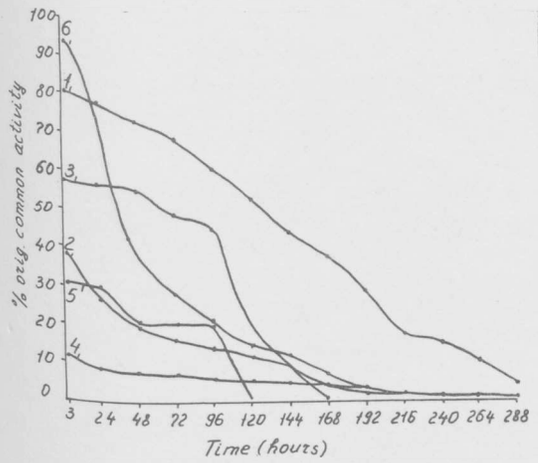

Fig.3
The change of a bound activity:
cathepsin A-1; cathepsin C-2; collagenase-3;
α -glucosidase-4; α-lipase-5; phospholipase C-6
during beef muscle autolysis (2°C).

The results of the expirements showed that there was no collagenase activity in muscle tissue.But the lysosomal fraction of the intermuscle connective tissue has a higher collagenase activity.

In the very first hours of autolysis a large amount of enzyme is isolated from lysosomes.A free collagenase activity is 40% of that of the common activity after 3 hours of an autolysis (fig.2,slope 3).We receive a maximum activity of hydrolase after 72 hours of muscle storage.

The way,the collagenase common (fig.1,slope 3) and lysosomal bound activities (fig.3,slope 3) change is identical.Collagenase is a relatively stabile enzyme.After two days of autolysis for the first time we can observe enzyme inactivation,the following degradation of the common and bound activity runs slowly and a common inactivation is reached on the 10-th day of meat storage.

The observation showed that the lysosomal activity of α -glucosidase is 10% of that of the common activity (fig.3, slope 4),but the extralysosomal activity is 90% of that of the common activity (fig.2,slope 4). A low level of α-glucosidase in lysosomes can probably be explained by the presence of numerous glucosidases in the cytoplasme and other subcellular fractions.

An activity of α-glucosidase bound with lysosomes changes slowly during the first 4-5 days of autolysis and on the 9-10-th day a full enzyme releasing is observed.

The activity of the extralysosomal α-glucosidase does not change during the first four hours,but later on it slowly degradates.After 120-h of autolysis the activity of this glucosidase consist of 60% of the original activity.After this the degradation of the extralysosomal α-glucosidase becomes more intensive and in 12 days of autplysis it falls to its lowest level.

The slow degradation of the common (fig.1,slope 4) activity and the free α-glucosidase activity can be explained by the high stability of the extralysosomal α-glucosidases.

The results showed that the lysosomal α-lipase activity was 30% of that the common activity (fig.3,slope 5) but in supernatant its activity is about 70% (fig.2,slope 5).The low content of α-lipase in lysosomes seems to be connected with the distribution of the enzyme in other subcellular fractions. The lysosomal α-lipase is a labile enzyme.The bound lysosomal activity of α-lipase had a minimum in 5 days of autolysis.

Extralysosomal α-lipase is characterized by its high stability,after 12 days of muscle storage the free activity of α -lipase is 20% of that of the common activity.

Phospholipase C differs from other lysosomal hydrolases by its high degree of releasing from lysosomes.After the first day of autolysis its free activity (fig.2,slope 6) is 30% of that of the common activity.The maximum activity of the free enzyme being received in 72 hours of autolysis .This is confirmed by the changes connected with yhe lysosomal activity of phospholipase C (fig.3,slope 6).

Phospholipase C is a relatively labile enzyme.During the first 24 hours of autplysis its common activity practically does not change (fig.1,slope 5) only.Further autolysis causes a sharp degradation of the common activity.Complete enzyme inactivation is received after 10 days of muscle tissue storage.

SUMMARY

1.The development of the autolysis process causes the appearence enzyme releasing from lysosomes and the increase of the free activity of enzymes.Later one a degradation of all types of activities is visible.

2.Different levels of enzyme stability were established during the process of autolysis of beef muscles (2°C).

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