ENZYMATIC STUDY OF MEAT QUALITY

ZENG Shi-yuan et al

Food Science Department
Hangzhou Institute of Commerce,P.R.China

INTRODUCTION

Meat quality is a composite manisfestation of various factors, which depend upon the metabolic states of the muscle tissue, the environmental conditions of animal before slaughter and also the treatments after slaughter. The biochemical events which occur in muscle tissues both in living animal and post slaughter stage may give a clue to understanding the quality attributes of meat. Choosing enzyme--the unique, important bioactive molecule as key substance, we attempted to uncover the intrinsic correlations between the biochemical characteristics of muscle tissue and meat quality.

In the early sixties, Dahl (1) comfirmed Hunter's finding (2) that the creatine content in lean meat was constant. Dahl also used creatine as an index of meat product quality. Zdenek Dvorak (3) after a series determinations of creatine contents of fresh pork, beef, mutton etc. verified that there was a good linear relationship between creatine content and net muscle protein. He suggested that creatine content may be used as an index of fresh meat quality.

As the interconversion of creatine and phosphocreatine--the energy reservoir for muscle contraction, is under the action of creatine phosphate kinase (CPK), it is reasonable to deduce that there may be a relationship between the creatine content and CPK activity. After comparing three different assay methods for CPK, we determined the CPK activities of different kinds of muscles and at the meantime, the creatine or total creatinine content.

Inspired by some preliminary results, we further broadened the enzyme spectrum of muscle to those enzymes such as phosphohexose isomerase (PHI), which genetic locus has linkage with the HAL genetic locus (4) of the commonly recognized, in recent years, the stress-sensitive gene of pig, and lactate dehydrogenase (LDH) which is an important enzyme in muscle glycolysis, in order to disclose the relationship between enzymes and meat quality and moreover to search for the biochemical mechanism of meat quality changes.

MATERIALS AND METHODS

(I) Samples

(1) Muscles M.longissimi dorsi, M.(I), M.psoas major, M.(II), M.supraspinatus, M.(III), M.lumbar eye, M.(IV), the lumbar part of M.(I), M.biceps femoris, M.(V). Others: Muscles from front and hind legs, from neck and back.

(2) Sera from the same animals

Diferent batches of samples were taken instantly after slaughter at Hangzhou Meat Factory, or supplied by Animal Husbandry Institute of Zhejiang Academy of Agricultural Sciences.

(II) Preparation of test solutions

(1) For CPK assay To each muscle sample (10g. minced), a sufficient amount of 0.01 M. KCL solution was added. Homogenized, filtered and centrifuged. The supernatant was diluted adequately and used for assay.

(2) For PHI assay Use 0.02 N. KOH solution, others as above.

(3) For LDH assay Same as for CPK for muscles, sera were diluted before use.

(4) For creatine and total creatinine To 5g. of minced muscle sample, 100 ml. of 3% CCL_3COOH solution was added and homogenized. The mixture was filtered and the filtrate was used for creatine and total creatinine determination.

(III) Methods

(1) Assay for enzyme activity

(a) CPK (EC 2.7.3.2.) Inorganic phosphate (IP) method (5), diacetyl (DA) method (6), pH method (7) with modifications.

(b) PHI or GPI (EC 5.3.1.9.) Fructose colorimetric method (8)

(c) LDH (EC 1.1.1.27.) Pyruvate colorimetric method (9)

(2) Determination of creatine and total creatinine

(a) Alkaline picrate method (10) For creatine and total creatinine

(b) Diacetyl method Referred to (6), with modifications.

(3) Protein content Biuret method

(IV) Definition of enzyme activity unit

CPK:

IP method: 1μg of phosphorus produced at 37 ℃, 30 min.

DA method: 1 μmole of creatine produced at 37 ℃, 1 hr.
pH-method: 1 microequivalent of $[H^+]$ released after 1 min. at 25 ℃.
PHI: 1 μg. of fructose produced at 30 ℃. for 10 min.
LDH: 1 μmole of pyruvic acid formed at 37 ℃. for 15 min.

(V) Instruments and reagents

DS-1 high speed homogenator, pH S-2 pH meter, TGL-16 high speed benchtop centrifuge, 721 spectrophotometer, 751 UV/VIS spectrophotometer. Reagents: biochemical grade or A.R. grade

RESULTS

(I) Activity of CPK was correlated to the creatine or total creatinine content

(1) IP method

CPK activity (units/g.meat)	159.77±15.60
creatine content (mg./g.meat)	2.76±0.21

n=9, 95% confidence, r=o.88
Sample: Mixture of muscles from neck and back, front and hind leg and M.(I).

(2) DA method

Sample	M.(I) (N=10)	M.(II) (N=9)
CPK activity (units/ml. solution)	94.84±0.99	90.93±3.48
(1) total creatinine	3.53±0.385	3.29±0.299
(2) creatine	3.89±0.443	3.56±0.346
correlation coefficient	(1) 0.95 (2) 0.95	(1) 0.80 (2) 0.77

(II) Activities of CPK were different among different muscles

(1) Comparison of CPK activity between two or three kinds of muscles

(a) DA method

Sample	M.(I) (N=9)	M.(II) (N=9)
CPK activity (units/ml. solution)	94.84±0.99	90.39±3.48
	p<0.01	
soluble protein (mg./ml. solution)	10.29±0.25	9.96±0.53
relative activity	9.23±0.20	9.15±0.76

(b) pH method

Sample	M.(I) (N=9)	M.(II) (n=9)
CPK activity (microeq.[H] /g.meat)	1128.6±248.73	895.7±203.75
	p<0.05	

(c) Simultaneous method (DA method for both items)

Sample	CPK activity (x10 units/ g.meat)	p value <
M.(I)	8.64+1.21	0.05
M.(II)	7.83+0.94	0.001
M.(III)	6.70+0.83	0.001

Sample	creatine content umole/g.meat	p value <
M.(I)	3.29+1.40	0.05
M.(II)	2.80+1.08	0.01
M.(III)	2.55+1.28	0.05

N=10, 95% confidence

Correlation coefficient

Sample	CPK activity	creatine content	
M.(I)	0.88	0.98	
M.(II)	0.87	0.96	(n=10)
M.(III)	0.89	0.99	

(III) Activity of CPK was correlated with that of PHI

Sample	M.(IV)	M.(V)
CPK activity	930.80±20.57	640.64±65.45
	p<0.001	
PHI activity	2486±13.9	2378±448.5
	p< 0.05	
correlation coeff.	0.85	0.72

n=5, 95% confidence

(IV) LDH activities were also different between two different kinds of muscles

Sample	M.(IV)	M.(V)	Serum
LDH activity	1004.4 ±111.11	785.3 ±166.03	6.56 ±1.14

DISCUSSION

By comparing three different methods for assaying CPK activity, we found that IP method was redundant, pH-method showed its merit in time-saving but regretfully was found liable to fluctuation. After repeated tests with modifications, it was accepted along with DA method.

Individual divergences were found among different animals both in CPK activity and in creatine content. But there were correlations among different muscles i.e if the CPK activity(or creatine content) of one kind of muscle of animal A is higher than that of animal B, then the activity (or content) of the second kind of muscle of A is also higher than that of B, and so forth. The correlation coefficient between creatine contents is higher than that between CPK activity. This can well explain why the correlation coefficients between CPK activity and creatine content were different for different muscles. (as 0.95 for M.(I), 0.77 for M.(II)). And the difference in creatine content were not in disconformity with the work or finding of Dahl and Hunter, who did not differentiate the different muscles but determined muscles in whole.

So far as the variation coefficient is concerned, the value of CPK is close to that of PHI, But that of LDH is larger. This situation occurred in both muscles. And no matter which enzyme, the variation cofficient of M.(V) is greater than M.(IV). When comparing the LDH activities of pig muscles with the serum LDH activity, we got the following relationship: if LDH activity in muscle is high, the activity in serum will be low, and vice versa. There were 11 pairs with this ralationship. This phenomenon is in conformity with that found in human beings and perhaps can manifest the different metabolic state of muscles of different individuals.

The last and the most important aspect is the correlationship between PHI and CPK. As in recent years, the halothane test used widely in testing PSS and PSE low quality meats, our finding of the correlationship between PHI and CPK is exciting. Since PHI is a promising enzyme in predicting meat quality at least in the aspect of edible protein, while the link of genetic locus of PHI with HAL^n locus of porcine stress sensitive gene is well established, (4),(11), the amino acid sequence data of PHI can be used to design an oligonucleotide which would hybridize to the corresponding DNA sequence. The cloned GPI gene would be used as a probe to look for RFLPs (12) linked to the GPI locus and hence to the HAL^n locus. Thus as a tool to resolve the problem of meat quality, more works can be done with PHI as well as CPK, the important enzymes in muscle.

CONCLUSION

I) Activity of CPK was positively correlated to the creatine or total creatinine contents.

II) Activities of CPK were different among different kinds of muscles by three different assay methods and the activities were correlated with the contents of soluble protein.

III) Activity of CPK was correlated with that of PHI.

IV) Activities of both PHI and LDH were different among different muscles just the same as that of CPK.

V) CPK, as an index of meat quality, has been proved unequivocally. PHI, not only is valuable in predicting the normal meat quality and in differentiating and id

entifying the normal from the low quality meat, but also in elucidating some theoretical problems.

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