MYOGLOBIN CONTENT AND ß-HYDROXYACYL-CoA-DEHYDROGENASE ACTIVITY OF MUSCLES AS A TOOL TO DIFFERENTIATE BETWEEN FRESH AND THAWED BEEF, SKELETAL MUSCLES AND OFFALS OF SLAUGHTER OF BEEF AND VEAL

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SUMMARY:
The activity of the mitochondrial enzyme ß-hydroxyacyl-CoA-dehydrogenase (HADH) in the press juice of intact muscle tissue allows to differentiate between fresh and thawed meat. The product of the myoglobin content (M) and the HADH-activity (H) of muscles, the so called MH-value, provides a possibility to distinguish between skeletal muscles and offals of slaughter. The latter aspect is very important for minced meat.

Byproducts of cattle slaughtering as heart, tongue, cheek meat, diaphragm and tail as well as shoulder, top round, loin and diaphragm of calf were examined with regard to their myoglobin content, the HADH-activity and the resulting MH-values. Heart, tongue and cheek meat of cattle show like diaphragm higher MH-values than "normal" beef. Muscles of tail behave like "normal" beef muscles. With the exception of tail it is possible to distinguish between "normal" skeletal muscles and "offals" of slaughter from cattle.

There are no essential differences in the MH-values of various cross striated muscle types of grown up cattle and calf. Muscles of cattle show a slightly higher myoglobin content, whereas its HADH-activity is lower than in veal. Diaphragm of calf can be clearly differentiated from "normal" veal.

INTRODUCTION:
For consumer protection it is necessary to provide methods, which allow to differentiate between fresh and thawed meat, "normal" skeletal muscles and "offals" of slaughter. The latter aspect is very important in the case of minced meat, which is normally imported as a salted and frozen product and used in the production of sausages. In the European Community the import duty for "offals" of slaughter is lower than for "normal" skeletal muscles. Therefore this agreement can seduce to declare "normal" beef as eatable "offals" in order to pay only a lowered tax for import and to sell it as "normal" processing beef with the regular profit. As there exist no reliable histological procedures, an enzymatic test was developed, which represents in combination with the myoglobin content of the muscles a possibility for the differentiation between skeletal muscles and "offals" of slaughter.

Because of its properties, the enzyme ß-hydroxyacyl-CoA-dehydrogenase (HADH) proved to be convenient for the test. This enzyme is involved in the oxidation of fatty acids in the cells and is tightly bound to the membranes of muscle cell mitochondria. An increase of the enzyme activity in the press juice of meat is obtained after freezing and thawing of the meat, as HADH is released from the membrane to the sarcoplasm by this procedure (Gottesmann and Hamm, 1984; Gottesmann und Hamm, 1987). Neither storage above freezing temperatures of meat before comminution nor comminution and salting nor frozen storage reduce its total HADH-activity by destruction of the enzyme (Gottesmann und Hamm, 1986). Gottesmann and Hamm (1987) developed a qualitative HADH test, which indicates thawed meat by a colour change in the mixture. Without this indication method the total activity of the enzyme in the meat can be determined by means of an UV-spectrophotometer. The quantified activity of this enzyme in the press juice of meat is sufficient to differentiate between fresh and thawed frozen meat.

Gottesmann and Hamm (1986) used another test combination to distinguish between diaphragm and "normal" skeletal muscles from cattle. The product of the HADH-activity (H) and the content of myoglobin (M), the so called MH-value, served as an indicator for the identification of these two muscle-types. Generally a very close correlation between the myoglobin content and the HADH-activity of different muscle types was found (Gottesmann and Hamm, 1986; El-Badawi and Hamm, 1970, 1972; Hamm and Gottesmann, 1984, 1985).

In the following investigations the described method was applied for the differentiation between "normal" cross striated muscle-types of cattle and byproducts of cattle slaughtering as diaphragm, heart, tongue, tail and cheek meat. The MH-values of diaphragm and various skeletal muscles of calf were also determined with regard to possible differences in grown up cattle and calf.

MATERIALS AND METHODS:
From five beef carcasses about 150 g of heart, tongue, diaphragm, cheek muscle and a mixture of tail muscles were dissected, packaged in polypropylene bags and frozen at -30°C. The adequate amounts of M. longissimus dorsi (loin), M. adductor (top round), M. supraspinam (shoulder) and diaphragm of calf were treated under the same conditions. After 3 weeks of frozen storage the samples of beef and veal were thawed during the night at 0°C.

Determination of the HADH-activity: The following investigations were performed according to Gottesmann and Hamm (1986): About 50 g of muscle tissue were ground and exactly 5 g homogenized in 50 mM potassium phosphate buffer (pH 7,6) in the presence of Triton X 100, centrifuged and afterwards filtered. The activity of the enzyme HADH was determined as described by Gottesmann and Hamm (1986). Data are presented in international units per gramm of raw material (U/g).

Determination of the myoglobin content: The content of hemoglobin in muscles is in comparison to the myoglobin content very low. Therefore the total content of hemin was determined according to the method of Hornsey (1956,1957). The myoglobin content was calculated in consideration of the weight of raw material in (mg/g).

RESULTS AND DISCUSSION:
Beef: The content of myoglobin, the HADH-activity and the calculated product of both, the so called MH-value of heart, tongue, outer cheek muscle, diaphragm and a mixture of tail muscles of cattle are shown in table 1. Gottesmann and Hamm (1986) found, that refrigerated storage at +2°C, frozen storage at different temperatures as well as comminution and salting are not influen-

cing the activity of HADH and the content of myoglobin. The determined MH-value of diaphragm corresponds apart from a slight deviation with the data of Gottesmann and Hamm (1986). Outer cheek muscle and heart of cattle showed higher MH-values than diaphragm from the same species. Heart with a MH-value of 433 can unequivocally be distinguished from cheek (208) and diaphragm (168). With regard to a myoglobin content of about 9 mg/g heart and diaphragm are comparable. MH-values of more than 400, as determined in the case of heart, are exclusivly due to very high HADH-activities. The possible biochemical reasons for the increased activity of the mitochondrial enzyme HADH in different muscle species can be an increased concentration of the enzyme respectively a variation in the isoenzyme composition.

Table 1: Myoglobin content, ß-hydroxyacyl-CoA-dehydrogenase activity and MH-value of different beef muscles, which have been stored at -30°C for 3 weeks. Number of used animals = 5. The MH-value of each muscle is the mean value of the product of myoglobin content and HADH-activity of the 5 animals. Data for skeletal muscles (Gottesmann and Hamm, 1986[a]) are shown for comparison.

muscle species	myoglobin [mg/g] x ± s	HADH-activity [U/g] x ± s	MH-value x ± s
heart	9,3 ± 0,2	46,6 ± 15,9	433 ± 147,6
tongue	6,7 ± 0,1	19,2 ± 5,6	129 ± 38,2
outer cheek mus.	8,3 ± 0,3	26,3 ± 6,2	208 ± 48,9
diaphragm	9,0 ± 0,1	19,0 ± 3,3	168 ± 26,6
tail muscles	5,8 ± 0,3	9,8 ± 3,0	58 ± 19,2
loin[a)]	4,7	6,5	26
top round[a)]	5,0	8,8	44
shoulder[a)]	5,7	9,6	55

The myoglobin content of tongue is lower than in heart, outer cheek muscle and diaphragm, whereas its HADH-activity is comparable with that of diaphragm. As shown in table 1 the MH-values of diaphragm (168) and outer cheek muscle (208) are significantly higher than in tongue (129).

In contrast to these results the determined value for a mixture of tail muscles is essentially lower. Beef tail showed a myoglobin content of 5,8 mg/g, a HADH-activity of 9,8 U/g and a calculated MH-value of 58. In consideration of these values there is no fundamental difference to "normal" cross-striated skeletal muscles of cattle (Gottesmann and Hamm, 1986).

Because of the analytical data for the myoglobin content and the HADH-activity byproducts of cattle slaughtering as heart and outer cheek muscle show similar or even higher MH-values than diaphragm and are not mutually to distinguish in a mixture with diaphragm. Tongue shows a MH-value, which is between diaphragm and skeletal muscles. The mixture of tail muscles behaves like skeletal muscles.

Taking into account the data for beef, which were already published (Gottesmann and Hamm, 1986; Hamm and Gottesmann, 1984, 1985), an upper limit for MH-values of "normal" beef can be fixed at 110, whereas byproducts of slaughtering show MH-values above 125 (table 1). Based on these results there exists a possibility to scrutinize imported processing meat, which is normally purchased in a ground, salted and frozen condition, with regard to the customs declaration and the paid custom duties.

<u>Veal:</u> The MH-value of diaphragm from calf (129 ± 30,8) is significantly lower than in the same muscle of grown up cattle (table 1; table 2). This is also expressed in the values of the myoglobin content (8,0 ± 0,5 mg/g) and the activity of HADH (16,2 ± 4,4 U/g), which are lower than in beef diaphragm.

Table 2 also includes the myoglobin content, the HADH-activity and the calculated MH-values for loin, top round and shoulder of calf (35 - 55).

Table 2: Myoglobin content, ß-hydroxyacyl-CoA-dehydrogenase activity and MH-value of different veal muscles, which have been stored for 3 weeks at -30°C. Number of the used animals = 5. The MH-values were calculated as described in table 1.

muscle species	myoglobin [mg/g] x ± s	HADH-activity [U/g] x ± s	MH-value x ± s
loin	3,7 ± 0,3	9,4 ± 2,1	35 ± 9,3
top round	3,8 ± 0,3	9,0 ± 1,3	35 ± 5,0
shoulder	5,2 ± 1,1	10,6 ± 2,3	55 ± 14,2
diaphragm	8,0 ± 0,5	16,2 ± 4,4	129 ± 30,8

A comparison with the data for the same beef muscles (MH: 26 - 55; Gottesmann and Hamm, 1986) does not reveal essential differences. For beef loin a MH-value of 26 results, whereas in the case of the muscle originating from calf a value of 35 is calculated. The HADH-activity is decisive for this difference, because the determined activity in the muscle of calf (9,4 U/g) is higher than in beef (6,5 U/g). The myoglobin content of the latter muscle in veal is lower than in beef (Gottesmann and Hamm, 1986).

Gottesmann and Hamm (1986) determined in beef top round a MH-value of 44, which was in the muscle from calf only 35. With regard to the myoglobin content the same behaviour as demonstrated in the previous example was found [5,0 mg/g (beef); 3,8 mg/g (veal)]. In diaphragm of calf the myoglobin content (8,0 mg/g) is again lower than in the case of the same muscle from grown up cattle (9,0 mg/g). Nevertheless it is also in this case possible to differentiate clearly between "normal" beef, "normal veal" and diaphragm from calf (MH = 129).

CONCLUSIONS:
In publications of Gottesmann and Hamm (1986) and Hamm and Gottesmann (1984, 1985) the correlation between the myoglobin content and the HADH-activity is always pointed out. This correlation is demonstrated for cattle and calf in figure 1. There is a linear course in the dependency between the myoglobin content and the HADH-activity for skeletal muscles inclusive diaphragm with a more positive slope in the case of calf than in the case of cattle. This results from higher HADH-activities at lower myoglobin contents for calf in comparison with cattle. There exists also a nearly linear course between byproducts of slaughter and skeletal muscles from cattle with a less steep slope excluding diaphragm.

Figure 1 expresses clearly, that it is not possible to differentiate between veal- and beef-skeletal muscles using the presented method. But diaphragm of both animals can unequivocally be distinguished from skeletal muscles.

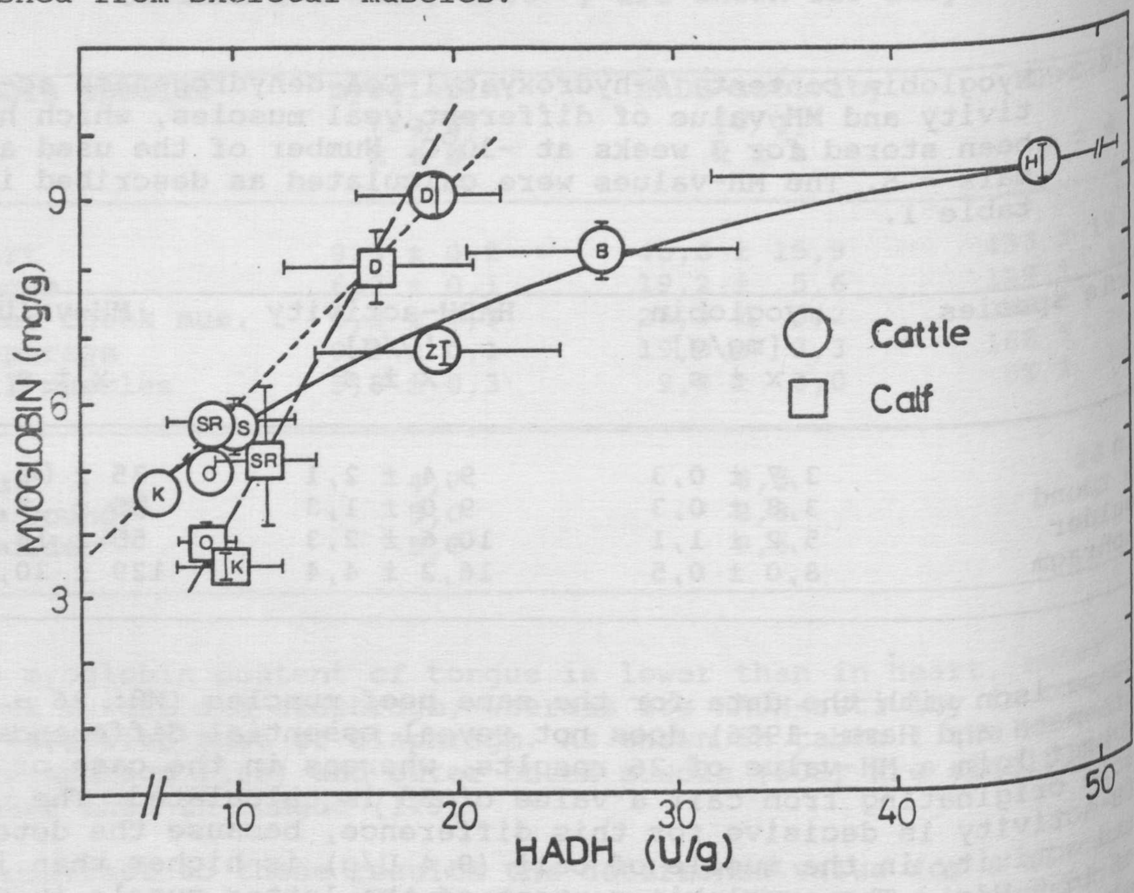

Figure 1: Correlation between myoglobin content and HADH-activity for different muscles from cattle ◯ and calf □ . There is a linear course in the dependency of myoglobin content upon HADH-activity for beef (-----)- and veal (——-) skeletal muscles and diaphragm. An almost linear course for this dependency is shown for tongue, outer cheek muscles, heart and skeletal muscles of cattle (not broken line).
D = diaphragm; Z = tongue; B = outer cheek muscle; H = heart; K = loin; O = top round; S = tail muscles; SR = shoulder.

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