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INFLUENCE OF THE MUSCLE FIBRE TYPE COMPOSITION AND THE PROPORTION OF CONNECTIVE TISSUE ON THE SENSORY ACCEPTANCE OF BEEFSTEAK TARTARE

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The investigation was carried out on 5 different muscles of 5 fattened bullocks of the Croatian Simmenthal breed aged 15 months and weighing about 400 kg and beefsteak tartare type products made of these muscles. Comparing the structure of the muscles used in the production of the beefsteak tartare, one may conclude that m. psoas major and m. longissimus dorsi are formed by dominantly white dynamic FG muscular fibres representing more than a half of all muscular fibres. In comparison with other muscles, the afore-mentioned muscles contain the least quantity of connective tissue. The investigations showed some statistically irrelevant differences ($P>0.05$) concerning the fibre diameters and volume density of connective tissue in m. psoas major and m. longissimus dorsi (L_2). Some statistically irrelevant differences were also observed in the evaluation of the beefsteak tartare type products made from these muscles. The products prepared from m. semimembranaceus and m. triceps brachii were qualified worse than those made from m. psoas major and m. longissimus dorsi, which were statistically significant ($P<0.05$). As m. semimembranaceus and m. triceps brachii contain less white fibres (41.0% and 43.1%) than red and intermediate ones (59.0% and 56.9%) and also more connective tissue (17.0% and 13.0%) than m. psoas major (6%) and m. longissimus dorsi (9% and 7%), these factors probably contributed to the lower ranking of the products made from m. semimembranaceus and m. triceps brachii.

Keywords: beef muscles, histochemical characteristics, beefsteak tartare quality

From the physiologic point of view, skeleton muscles are active body motors, and from the economic point of view they are the most important part of meat because of their nutritional value by being the richest in protein. The skeleton muscles are built from muscular, connective and fat tissue with nerves and blood vessels grown in.

Striated muscular fibres in muscular tissue constitute a contractile part of the muscles. Red slow contracting oxidative SO fibres, white fast contracting glycolytic FG fibres and intermediary oxidative-glycolytic FOG fibres may be distinguished according to the contraction speed, the quantity of sarcoplasm, the density of myofibrils, the quantity of mitochondria and of myoglobin as well as due to the activity of oxidative enzymes within the fibres (PETER et al., 1972). As to the classification of the muscular fibres, besides the quoted nomenclature, there are also other nomenclatures (BROOKE &

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KAISER, 1970; ASHMORE & DOERR, 1971; BARNARD et al., 1971; GUTH & YELLIN, 1971; ASHMORE et al., 1972).

Most of the skeleton muscles are built heterogeneously, i.e. from all three types of muscular fibres. Muscle fibre composition influences the working capacity and metabolism associated with the quality and gastronomic value of meat.

Besides the muscular tissue, the quality of meat is also influenced by the amount of connective tissue, which participates in the function of the muscle. The connective tissue surrounds each muscle fibre (endomysium), bundles of muscle fibres (perimycium), and forms an external envelope (epimysium), which surrounds the whole muscle.

Beefsteak tartare is a product made from the economically and gastronomically most valuable parts of beef (beefsteak). In the culinary terminology this product is classified as a meal for "cold buffet" (KARAPANDŽA, 1983), which is normally prepared from m. psoas major.

The purpose of the study was to determine to what extent the proportion of muscular and connective tissue within the employed muscles might influence the quality of the beefsteak tartare. This product was made not only from m. psoas major but also from other muscles, which were not considered as a typical raw material assigned to such a purpose. Owing to the similarity of their construction and of their metabolic properties, such muscular fibres are much alike to those found in m. psoas major, m. longissimus dorsi or rather different than those appearing in m. semimembranaceus and m. triceps brachii.

1. Material and methods

The investigations were carried out in "Promes" meat processing plant in Karlovac. Samples of muscles for histomorphological and histochemical investigations were taken immediately after the slaughtering of five fattened bulls of the Croatian Simmenthal breed, kept in intensive breeding and fed with the same food, aged 15 months and weighing about 400 kg. The samples of m. triceps brachii, m. longissimus dorsi, m. psoas major and m. semimembranaceus were taken from both left and right bovine sides. Samples were taken from the same region: m. triceps brachii from the middle of caput longum; m. longissimus dorsi – L_1 at the level of the 10th thoracic vertebra; m. longissimus dorsi – L_2 at the level of the 3rd lumbar vertebra; m. psoas major at the level of the 3rd lumbar vertebra; m. semimembranaceus at the level of the hip joint. Fresh muscular samples, about 1 ml, were frozen just after the sampling in liquid nitrogen and cut into 10 μm thick slices that were stained with hematoxylin and eosin (ROMEIS, 1968) for the appearance of muscle structure or they were submitted to the procedures for the activity of succinic acid dehydrogenase (SDH) (PADYKULA, 1952), of lactic acid dehydrogenase (LDH) (HESS et al., 1958), myosin acid stable (preinkubation in 0.2 M acetate buffer (pH 4.3), 10 min room temperature), alkaline stable (preincubation in 0.1 M sodium barbiturate (10 ml), 0.18 M calcium chloride

(2.5 ml) and destilled water (12.5 ml) for 15 min at room temperature) and standard adenosine triphosphatase (AC, AL, S ATP-ase) (BROOKE & KAISER, 1970) for lipids by means of Sudan III (DADDI, 1968), for connective tissue after the Van Gieson method (ROMEIS, 1968) and for glycogen quantity by means of the PAS method (ROMEIS, 1968).

In order to obtain an accurate view of the size of muscular fibres, transversal cuts of the selected muscles were submitted to the procedure for activity. The diameters of muscular fibres were measured after SONG and co-workers (1963), the obtained results being statistically analysed and presented in a table.

In order to have a better view of the functional capacity of the investigated muscles, muscular fibres were classified according to the changes by a 5 μm pace according to the method of their distributional frequency (BEGO, 1994).

The volume rate of muscular and connective tissue in all investigated muscles was obtained by means of a stereologic analysis (KALIŠNIK, 1982) of histologic slices, with 10 fields of sight being analysed in each histologic slice stained after Van Gieson (ROMEIS, 1968). This analysis was made by means of multiple test system after WEIBEL and co-workers (1966).

The half side of the slaughtered animals, from which muscular samples were taken, were stored in a refrigerated room, the interior temperature being maintained at +4 °C. For sake of its maturation the meat was kept there for 48 h. Even quantities (0.1 kg) of the investigated muscles were taken from each half from the same regions. The samples for histomorphological and histochemical analysis had been excised previously from the same location. The muscle samples, each one separately and during an even time, were minced in electric cutter (AEG Finesse+Electronic). The beefsteak tartare was prepared after a recipe (KARAPANDŽA, 1983), an even quantity of necessary condiments being added to each specific part of the muscle.

For sensorial evaluation the steaks were labelled according to the muscle used for such product: 1 (m. psoas major), 2 (m. semimembranaceus), 3 (m. longissimus dorsi – L_2), 4 (m. triceps brachii), 5 (m. longissimus dorsi – L_1). Each sample contained the same quantity of meat and a corresponding quantity of condiments. Labelled products were served after a one hour storage in refrigerator. The samples of these products were tasted by ten laymen. Each of them had to rate all samples so that the best qualified sample was awarded with 1st position and the worst one occupied 5th position. A statistical analysis of the obtained results was done by analysis of variance and multiple comparison test (SRIĆA-UHLE, 1967). The steak tartare was also tasted by a jury of three experts for flavour and colour.

2. Results and discussion

Table 1 and Fig. 1 present the diameters of muscle fibres within the investigated muscles taken from both flanks of the animal body. The table and the graph show that the muscle fibre diameter within the samples were the following: m. triceps brachii

(20–95 μm), m. semimembranaceus (20–105 μm), m. longissimus dorsi – L_1 (25–100 μm), m. longissimus dorsi – L_2 (25–105 μm), m. psoas major (25–115 μm). The average muscular fibre diameter was: m. triceps brachii (50.33 μm), m. longissimus dorsi – L_1 (53.35 μm), m. longissimus dorsi – L_2 (55.20 μm), m. psoas major (56.51 μm), m. semimembranaceus (51.74 μm).

Table 1. Diameters of muscular fibres within m. triceps brachii (T), m. longissimus dorsi (L_1), m. longissimus dorsi (L_2), m. psoas major (PM), m. semimembranaceus (S)

Statistical evidence	T	L_1	L_2	PM	S
n	1 000	1 000	1 000	1 000	1 000
$\bar{x}$ (μm)	50.33	53.35	55.20	56.51	51.74
$\bar{x}_{min} - \bar{x}_{max}$ (μm)	20–95	25–100	25–105	25–115	20–105
S	14.14	12.50	14.00	13.45	13.97
S_x	0.45	0.40	0.44	0.43	0.40
V	28.09	23.43	25.36	23.80	27.00

$P>0.05$ – L_2 : PM
$P<0.05$ - in all other muscles
n: number of samples; $\bar{x}$: medium value; $\xi\, \bar{x}_{min} - \bar{x}_{max}$: minimal – maximal value;
S: standard deviation; S_x: medium error of standard deviation; V: variance

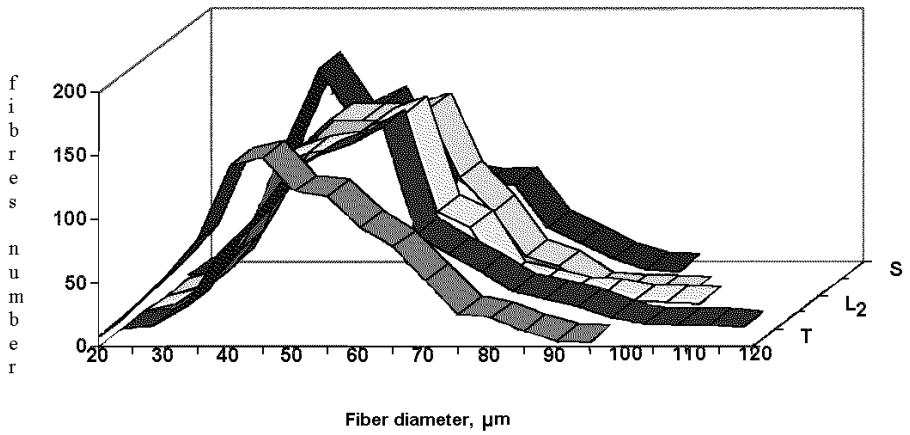

Fig. 1. Frequency distribution of muscle fibres within m. triceps brachii (T), m. longissimus dorsi-level of 10th thoracic vertebra (L_1), m. longissimus dorsi-level of 3rd lumbar vertebra (L_2), m. psoas major (PM), m. semimembranaceus (S) by 5 μm segments. ▩: T; ■: PM; ▨: L_2; □: L_1;▩: S

Red SO fibres, white FG fibres and intermediary FOG fibres were found in all investigated muscles. Table 2 shows the correlation between muscle fibre types and their average diameter. With their quantity and diameters white fibres dominate in all muscular samples m. triceps brachii (41.0%), m. semimembranaceus (43.1%), m. longissimus dorsi (51.2% and 55.0%), m. psoas major (57.8%), the average white fibre diameter in the same muscles being 64.15 μm, 65.09 μm, 63.42 μm and 65.15 μm, 66.08 μm, respectively. Red fibres are of smaller diameter and they are less numerous within each muscular sample. In two muscles these fibres are more numerous, and in other three fibres they are less numerous than in the intermediary ones. According to their average diameter, they are smaller than the intermediary fibres. Red fibres appear in m. triceps brachii (30.60%), m. semimembranaceus (30.00%), m. longissimus dorsi (21.40% and 19.50%), m. psoas major (16.60%), the average red fibre diameter in the same muscles being 33.65 μm, 36.38 μm and 36.75 μm, 37.10 μm, 36.84 μm, respectively. Intermediary fibres are of bigger diameter than the red ones. In the total quantity of fibres, the percentage of intermediary fibres is 28.40%, 26.90%, 27.40% and 25.50%, 25.60%, with their average diameters being 49.52 μm, 47.47 μm, 47.50 μm and 47.55 μm, 47.66 μm, respectively.

Table 2. Rate (%) and average diameter (μm) of white fibres (FG), red fibres (SO) and intermediary fibres (FOG) within the investigated muscles

Fibre type	T	L_1	L_2	PM	S
FG	41.00 %	51.20 %	55. 00 %	57.80 %	43.10 %
	64.15 μm	63.42 μm	65.15 μm	66.08 μm	65.09 μm
SO	30.60 %	21.40 %	19.50 %	16.60 %	30.00 %
	33.65 μm	36.75 μm	37.10 μm	36.84 μm	36.38 μm
FOG	28.40 %	27.40 %	25.50 %	25.60 %	26.90 %
	49.52 μm	47.50 μm	47.55 μm	47.66 μm	47.47 μm

T: m. triceps brachii; L_1: m. longissimus dorsi at the level of the tenth pectoral vertebrum; L_2: m. longissimus dorsi at the level of the third lumbal vertebrum; PM: m. psoas major; S: m. semimembranaceus

Statistical analysis of the data as well as the comparison of the investigated muscles show that only the differences between the fibre diameter of m. longissimus dorsi (L_2) and m. psoas major is not statistically relevant ($P>0.05$), such differences are statistically significant in all other muscles ($P<0.05$).

In red muscular fibres the activity of LDH and SDH as well as of AC ATP-ase is strong, with the activity of S and AL ATP-ase being weak. These fibres contain quite a lot of lipids and a smaller quantity of glycogen. White muscular fibres show a weak activity of LDH and SDH and of AC ATP-ase, a strong activity of S and AL ATP-ase, few fats and a rather big quantity of glycogen. Intermediary fibres show a rather strong activity of S and AL ATP-ase, a weak activity of AC ATP-ase, a moderate activity of LDH and SDH, with the quantity of lipids and glycogen varying in these fibres.

The results obtained by measurement of the rate of muscular and connective tissue within the investigated muscles show a dominating position of muscular tissue. The volume density of muscular tissues is: m. triceps brachii (0.83 mm°– 83%), m. longissimus dorsi – L_1 (0.91 mm° – 91%), m. longissimus dorsi – L_2 (0.93 mm° – 93%), m. psoas major (0.94 mm° – 94%), m. semimembranaceus (0.87 mm° – 87%), but the volume densities of connective tissue are 0.17 mm – 17%, 0.09 mm° – 9%, 0.07 mm°–7%, 0.06 mm°–6% and 0.13 mm°–13%, respectively. Statistical analysis of the data as well as the comparison of the investigated muscles show that only the differences between volume density of connective tissue of m. longissimus dorsi (L_1 and L_2) and m. psoas major, m. longissimus dorsi (L_1) and m. longissimus dorsi (L_2) is not statistically relevant ($P>0.05$), such differences are statistically significant in all other muscles ($P<0.05$).

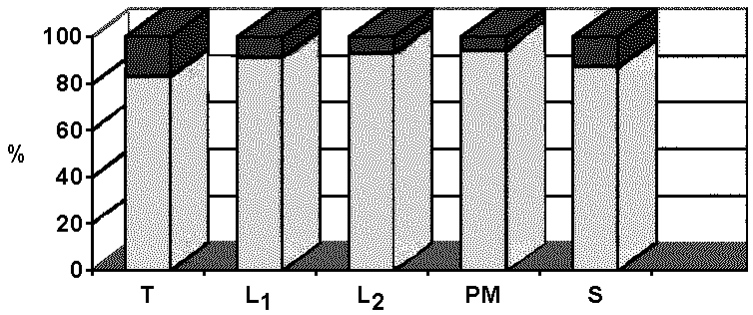

Fig. 2. Percentage proportion rate of muscular and connective tissue within m. triceps brachii (T), m. longissimus dorsi-level of 10th thoracic vertebra (L_1), m. longissimus dorsi-level of 3rd lumbar vertebra (L_2), m. psoas major (PM), m. semimembranaceus (S). □: Muscular tissue; ■: connective tissue

Table 3. Volume density of connective tissue within m. triceps brachii (T), m. longissimus dorsi (L_1), m. longissimus dorsi (L_2), m. psoas major (PM), m. semimembranaceus (S)

Statistical evidence	T	L_1	L_2	PM	S
n	100	100	100	100	100
$\bar{x}$ (mm°)	0.17	0.09	0.07	0.06	0.13
S	0.05	0.03	0.02	0.02	0.04
V	29.41	33.33	28.57	33.33	30.77

$P>0.05$ – L_1 : PM; L_2 : PM; L_1 : L_2
$P<0.05$ – in all other muscles
n: Number of samples; $\bar{x}$: medium value; S: standard deviation; V: variance

The results of sensorial evaluation are shown in Table 4. One may state that product (L_1) 5 obtained minimum points, which means that it was qualified as the best one. The maximum points was given to product 4 (T), meaning it was qualified as the worst one.

The results of variance analysis by multiple comparison test (SRIĆA-UHLE, 1967) show that the sum of points, ranging 20–40 points, is not statistically relevant ($P>0.05$). There was no relevant difference concerning the qualifications of products No. 1, 3 and 5 (PM, L_2, L_1), the products No. 2 and 4 (S, T) obtaining statistically relevant low ranging ($P<0.05$).

Preparing the beefsteak tartare type products, the panel of three experts noticed that these products were of different colouring, although their flavour was the same: the beefsteak tartare made from m. psoas major and m. longissimus dorsi (L_2) was ochre-reddish, the one made from m. longissimus dorsi (L_1) was ochre-brown, and another made from m. semimembranaceus and m. triceps brachii was darkish red-brown.

Table 4. Sensorial evaluation of the beefsteak tartare type products

Tester number	Product 1	Product 2*	Product 3	Product 4*	Product 5
1	2	5	1	4	3
2	4	2	5	3	1
3	3	5	1	4	2
4	4	3	1	5	2
5	2	3	4	5	1
6	1	5	2	4	3
7	2	4	1	5	3
8	1	5	4	3	2
9	2	4	3	5	1
10	1	5	2	4	3
Total points	22	41	24	42	21

*$P<0.05$

1: m. psoas major; 2: m. semimembranaceus; 3: m. longissimus dorsi (L_2); 4: m. triceps brachii; 5: m. longissimus dorsi (L_1)

The fact that white fibres are considerably bigger than red ones and that the red fibre type can metabolize and deposit more lipids than the white fibres resulted in a generally accepted opinion that red and white meat differ as to their quality, as well as that the factors able to speed or slow such processes could greatly influence the meat quantity and quality.

Due to the dominant breeding orientation onto meat production, a limited animal muscular activity may influence the proportion of fibre types as well as their volume and their metabolic process, thereby the quality of meat products, the real purpose of such animals breeding.

Differences in chemical composition existing between white and red meat, i.e. considering the proportion of muscular fibre types as well as the quantity of connective and fat tissue, may have some effects on its sensorial properties, principally on its softness and succulence (SCHEPER, 1962; LEE, 1984; AUGUSTINI & TEMISAN, 1986).

Within the muscular system each muscle is adapted to different functions it has. Many muscles contain white, red and intermediary fibres (WANDER et al., 1990). Therefore, one may state that the muscles presenting a uniform construction are rather infrequent, such as m. pectoralis in broilers of the "Jata" line (KAUFMAN et al., 1997; JELIĆ et al., 1998). It means that the majority of muscles is of a heterogeneous composition.

Muscular fibres do not contract with the same power and frequency. Some muscular fibres are fast contracting and others are slow contracting, i.e. their contraction power is more or less strong. So some muscle exert their function more or less frequently. There are some differences not only between several muscles and several muscular groups but also between identical muscles in different specimens as well as between several parts of the same muscle.

The results of our histomorphological and histochemical investigations of muscles confirm that within all investigated muscles three types of muscular fibres were found, i.e. red SO fibres, white FG fibres and intermediary FOG fibres.

By measurements of diameters in some fibre types within the investigated muscles it was stated that the red high oxidative fibres are of smaller diameter than white and intermediary fibres. Because of their small diameters, red fibres do not have a capacity to store big quantity of energy necessary for functioning. In these fibres it is compensated by a better vascularization, i.e. by more capillaries that enable the conducting of adequate quantities of the energetically important matters for a corresponding metabolic intensity (GAUTHIER, 1970). The S and AL ATP-ase activity within red fibres is generally weak, with the AC ATP-ase activity being stronger, which corresponds to the findings by BROOKE and KAISER (1970). For contraction energy they use the oxidative processes of fatty acids breakdown by means of the Krebs cycle, which is proved by a stronger activity of SDH and LDH considered as typical indicators of this cycle. These fibres are slow contracting, they have less contractive power and a bigger resistance to the strain, being able of longer functioning.

In all investigated muscles, white fibres have bigger diameters than red and intermediary fibres and they have greater contracting power. The blood supply of these fibres does not match the intensity of their contraction processes. They contain a bigger quantity of glycogen and few lipids, as well as a have weaker SDH and LDH activity, a stronger S and AL and a weaker AC ATP-ase activity. They are dominantly fast contracting. Within the sarcoplasm the acidity augments and the strain appears because of relatively poor vascularization. The appearance of strain causes the reduction of contractive capacity of fibres and these fibres are capable of only a short besting and fast functioning. In our investigations the intermediary fibres are mainly of medium diameters. As the source of energy for contraction they equally use oxidative and glycolytic metabolic ways. In our investigations the intermediary fibres show a

moderate SDH and LDH activity as well as a stronger S and AL ATP-ase activity and a weak AC ATP-ase activity. The quantity of glycogens and lipids within these fibres varies, in some fibres being bigger than in other ones.

Considering the former statements it can be concluded that the diameter of muscle fibres are closely connected with metabolic or contractive capacities of these fibres. The proportion of several types of muscular fibres are indicative as their working capacity is concerned. Muscular fibres of bigger diameters are stronger, the quality of the muscle itself depending of such fibres. A stronger S and AL ATP-ase activity is a sign of a faster contraction, a bigger activity of oxidative enzymes being symptomatic for greater muscular endurance.

The skeleton muscles have an elastic structure adapted to the function they perform in the body as it concerns their metabolic and elastic supporting component. Therefore, in our investigations we tried to determine the connective tissue muscular proportion within the mentioned groups of muscles.

The results show that in all investigated muscles the volume of muscular tissue was bigger than the one corresponding to connective tissue. It was found that m. psoas major contained the least quantity of connective tissue. Few connective tissue was also found within m. longissimus dorsi, this being particularly manifest in the region of the third lumbar vertebra. More connective tissue was detected within m. semimembranaceus and m. triceps brachii than in the afore-mentioned muscles. It permits a conclusion that the muscles such as m. psoas major and m. longissimus dorsi, which have a little function in moderate movements and a bigger one in forced movements, contain relatively less connective tissue, with white fibres representing more than 50% of all fibres.

As to the evaluation of the quality of some products, the man is the best "measuring instrument" (RISTIĆ, 1987), because the most important part of such testing is performed by human organs of senses (sight, smell, taste, touch). The grading expressed by qualifications or ranking is based on sensory impressions. A degustation tester, converted into a "measuring instrument", receives sensorial stimuli transferred to his own organs of senses (BUČAR, 1983).

Statistically no significant differences could be found by means of the sensorial evaluation of the beef steak tartare type products in our investigations of the products made from m. psoas major and m. longissimus dorsi (L_1 and L_2). The products made from m. semimembranaceus and m. triceps brachii were ranged low, which was statistically significant ($P<0.05$).

Concerning the flavour, the beefsteak tartare products did not vary essentially, although there were some differences in their colouring. The products made from m. psoas major and m. longissimus dorsi (L_2) were of an ochre-reddish colour, those made from m. longissimus dorsi (L_1) were ochre-brown, the products made from m. triceps brachii and m. semimembranaceus being of a darkish red-brown colour.

Comparing the structure of the investigated muscles used in production of the beef steak tartare, one may conclude that m. psoas major and m. longissimus dorsi are formed by dominantly white dynamic muscular fibres representing more than a half of

all muscular fibres. In comparison with other muscles, the afore-mentioned muscles contain the least quantity of connective tissue. The investigations showed some statistically irrelevant differences concerning the fibre diameters in m. psoas major and m. longissimus dorsi (L_2). Some statistically irrelevant differences were also observed in the evaluation of the beef steak tartare type products made from these muscles. The products made from m. semimembranaceus and m. triceps brachii were qualified worse than those made from m. psoas major and m. longissimus dorsi, which were statistically relevant ($P<0.05$). As m. semimembranaceus and m. triceps brachii contain less white fibres (41.0% and 43.1%) than red and intermediate ones (59.0% and 56.9%) and also more connective tissue (17.0% and 13.0%) than m. psoas major (6%) and m. longissimus dorsi (9% and 7%), this factor probably influenced lower ranking of the products made from m. semimembranaceus and m. triceps brachii. Although the products made from m. semimembranaceus and m. triceps brachii were ranked lower than the ones made from other muscles, they were no less savoury. Therefore, it may be concluded that, besides m. psoas major, some other more or less valuable muscles such as m. longissimus dorsi can be also used to make a beefsteak tartare, which is indeed a common household practice also confirmed by sensorial evaluation.

3. Conclusions

Having in mind the meat quality and quantity, besides the animals species, breed and age, from the point of view of its economic and gastronomic (culinary) utility a dominant type of muscular fibres as well as a proportion of connective and fat tissue may directly influence the taste of the product itself. Muscles owing to their topographic and anatomic position are less charged having a bigger muscular mass and containing more white than red and intermediary fibres, with few connective tissue, are gastronomically more propitious for some special dishes like beefsteak tartare than the muscles usually exposed to more strains. The classification of meat for sale gives an accurate idea of its culinary utility, although such insight is not a complete one.

Our studies of steak tartare quality prepared from the muscles of various types, function and load, from the same animals species, breed, sex and age, kept and fed under the same conditions, slaughtered and processed in the same way have shown that the finished product of less loaded muscles comprising more white fibres and less connective and intramuscular fat tissue, is of better taste than the product containing less white fibres and more connective tissue. In addition to m. psoas major, equally good beefsteak tartare can be prepared from m. longissimus dorsi. A good beefsteak tartare can be made of other muscle too, but on the account of quality grade.

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