IMPROVED DEVICE AND PROCEDURE FOR THE DETERMINATION OF PROTEIN DIGESTION "IN VITRO"

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The device described in this paper is an improved version of a classical device of Pokrovsky-Ertanov (Pokrovsky-Ertanov, 1965). As distinct from the prototype, containing three operational cells, this device of make PLFGB-10 for proteins digestion "in vitro" contains 10 cells fixed in foam plastic sleeves, positioned in lower and upper parallel organic glass panels, attached firmly to each other (a ten-cell device of Lipatov N.N. for enzymic hydrolysis of proteins). Each outer thermostatting cup of these cells has one coaxially positioned cup, made of organic glass, the bottom of the latter being a semi-permeable membrane made of pre-boiled-off cellophane hermetically connected with the cup by two crimping rubber rings. The inner cups by means of special adapters are fixed in an operational cell in such a way, that the bottoms of all the cups are in the same plane.

Above the upper panel, fixing the operational cells by means of six racks, limiting the movement down and horizontally, a removable panel with two handles is mounted, on which are positioned a driving motor and bearings of the axles of vertical mixers, coaxially immersed in the inner cups of the cells. The vertical mixers have two working elements with crossing geometric axles, perpendicular to each other, and a geometric axle of rotation. The drive of the mixers is done by one electric motor by means of a clamp, consecutively bending around diametrically opposite segments of pulleys, fastened at upper parts of their axles. The rate of mixers rotation, equal to 1.0 s^{-1}, is close to the frequency of peristaltic movements. The thermostatting of the outer and therefore the inner cups is accomplished owing to intensive circulation of waterglycerol mix through the jackets of the former ones, the temperature of this mix is maintained by ultrathermostat at the level of 37.0±0.5°C.

In carrying out the base procedure of Pokrovsky- Ertanov on the device PLFGB-10, a specimen of the product is taken, in which a fraction of total mass of protein had been already determined by the device "Kel-Foss-Automatic" . The mass of this specimen is recalculated to have 150 mg of protein. The prepared specimen is thorougly pounded with a pestle in a porcelain mortar in the presence of 5-7 ml of glycine buffer with ph 2.2, taken from the total volume of 15 ml, that will be used later. Then the panel with mixers is removed from the device, and from the operational cells the inner cups are taken out, to which from the mortar a homogenized specimen is quantitatively transferred and then poured by the left amount of glycine buffer. Then the inner cup is placed into the operational cell, and through the hole in the adaptor, 65 ml of similar glycine buffer is poured into the space between the inner and outer cups; in so doing the equal hydrostatic levels in the inner cups are provided.

15-20 min. after the temperature of buffer solution in the inner cup reached 37.0±0.5°C which is controlled by a liquid thermometer, it is added with 15 mg of crystalline pepsin of standard activity, and the panel with mixers is placed on the device. After 3 hours of digestion, proceeding at constant thermostatting and mixing of the content of the inner cup, the mixers are turned off and the panel is removed from the device. The inner cups are carefully taken from the operational cells, and 1 ml of hydrolyzate is taken from the outer cup. Then the neutralization of the content of the inner cups by the NaOH solution is carried out, after completion of which each cup is added with 15 ml of alkaline buffer with ph 8.4. The content of the outer cups is removed by means of a rubber bulb, the cups are rinsed with water, which then removed in a similar way, then the inner cups are placed into them, and through the hole in the adapter an intercup space is poured over by a buffer of pH 8.4, in so doing the equality of hydrostatic levels in inner and outer cups being provided. 15-20 min. after the temperature of the inner cup reached 37±0.5°C, 15 ml of crystalline trypsin of standard activity is introduced in it, and a panel with mixers is placed upon the device. 3 hours later, similarly as after

the first 3 hours, the hydrolyzate is taken from the inner cups. The efficiency of the protein hydrolyzate of the studied object is judged by the quantity of tyrosine passed through a cellophane membrane from the inner to the outer cup during 3 hours of digest by pepsin, trypsin and by the sum, corresponding to 6 hours. The amount of tyrosine present in dialyzate is determined according to Lawrie (Pokrovsky, Ertanov, 1965; Zhuravskaya, 1973) with the help of the spectrophotometer "Specol-11" at a wavelength of $\lambda = 750$ nm.

The up-dating of the base procedure of Pokrovsky-Ertanov as proposed by the authors of this paper and its distinction from the known modifications is in the essence of presentation of the final result. The thing is, that the expression of results of studying of protein digestion in vitro in such units as μg tyrosine/ml dialyzate, mg tyrosine/g of dry matter and even mg tyrosine/g protein as used in meat industry and special literature, does not objectively reflect the efficiency of proteins hydrolysis by pepsin and trypsin in vitro and in many instances can be and is the reason of wrong conclusions. The authors of the paper have come to such a conclusion as a result of long-standing work, connected with studying and analysis of amino acid composition and digestability in vitro of the raw materials and products containing protein, that are produced and have good perspectives for production in future by meat and dairy industry, paying attention to the fact that the proteins of the most objects differ from each other by the fraction of total mass of tyrosine by 1.5-2.0 fold and more. Thus, the beef muscle and thermally treated gel from sodium caseinate, subjected to pre-fermentation in brine, have the following indices of pepsin-trypsin digestibility: for beef muscle - 19.4 mg tyrosine/1 g protein, for caseinate gel - 26.6 mg tyrosine/1 g protein. Without taking into account the initial fraction of total mass of tyrosine in the protein of the compared objects, one can come to the conclusion (Pokrovsky, Ertanov, 1965) which seems not contradicting to common concepts, that the efficiency of pepsin-trypsin digestibility in vitro of sodium caseinate surpasses the similar index, corresponding to the protein of the raw fermented muscle tissue. However, if one takes into account, that the fraction of total mass of tyrosine in protein of beef muscle, subjected to fermentation when curing, is on average 3.41 g/100 g of protein, and that of the sodium caseinate - 6.31 g/100 g of protein, then by the formula (Lipatov, 1988):

$$\rho = \frac{10\,\pi}{T} \qquad (1)$$

one can obtain the results, indicating that the efficiency of pepsin-trypsin digestion of the protein of thermally treated caseinate gel ($\rho = 42.16\%$) is 1.35 less than the efficiency of pepsin-trypsin digestibility ($\rho = 56.89\%$) of fermented not thermally treated beef tissue. In the formula (1) the following designations are accepted:

ρ - digestibility of protein of the investigated object, expressed in % to the initial fraction of the total mass of tyrosine in it;
π - protein digestibility of the investigated object expressed in mg of tyrosine/1 g protein;
T - a fraction of total mass of tyrosine in the protein of the investigated object, g/100 g of protein;
10 - coefficient of proportionality,

$$\frac{\text{g of protein} \cdot \text{g \%}}{\text{mg} \cdot \text{100 g of protein}}$$

References

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Fig. 1. A ten-cell device of Lipatov N.N. for enzymic hydrolysis of proteins