# Investigation of the Nutritive Value of Dry Sausage

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In the evaluation of the nutritive value of foods, beside net protein content, the protein quality indices, which can characterize protein quality by numerical values, play a gradually increasing role.

As the direct estimation of protein quality by means of biological assays is an expensive and time consuming process, the importance of in vitro chemical methods, which can give as much information concerning the quality of protein as the biological assays at highly reduced expenses, is more and more acknowledged.

Of the chemical indeces based upon gross amino acid content data ( Morup-Olesen Index, FAO/WHO Index, Chemical Score, Korpáczy Index ) only some are able to predict the quality and the biological value of mixtures of proteins, thus are applicable to meat products.

The present work is concerned with the applicability of the above mentioned indeces for the characterisation of protein quality, demonstrated on the example of one of our best known export products, of dry sausage.

The results of our investigations have proved the biological quality of dry sausage is one of the highest among meat products.

# Untersuchung des biologischen Wertes der Dauerwurst

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In der Beurteilung der Qualität der Lebensmittel, neben Proteingehalt, kommen Kennziffer, die die Proteinqualität numerisch angeben können, zu immer grösserer Rolle. Wegen der grossen Zeit- und Kostenforderung der biologischen Methoden für die direkte Bestimmung der Proteinqualität kommen in vitro chemische Methoden in den Vordergrund, die über Proteinqualität die gleiche Informationen geben können, mit wesentlich kleineren Aufwänden.

Aus den auf die Aminosäurezusammensetzung der Produkte beruhenden chemischen Indexen ( Morup-Olesen Index, FAO/WHO Index, Chemical Score, Korpáczy Index ) sind manche fähig, den biologischen Wert auch im Falle von Proteinmischungen z.B. in Fleischprodukten zu bestimmen.

In der vorliegender Arbeit wurde die verwendbarkeit der verschiedenen chemischen Indexe zur Charakterisierung der Proteinqualität der der Produkte untersucht, als Beispiel diente die Dauerwurst.

Aufgrund unserer Ergebnisse kann man feststellen, dass der biologische Wert ( Biological Value ) der Dauerwurst einer der höchsten unter den Fleischwaren ist.

5.15

Examen de la valeur biologique du saucisson sec

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Dans l'estimation de la valeur nutritive des aliments à côté de la teneur en protéine nette les indices de la qualité des protéines, qui caractérisent numeriquement la qualité des protéines, jouent un rôle croissant.

Parceque l'estimation directe de la qualité des protéines avec des expériments biologiques est très exigeante et absorbante de temps l'importance des méthods chimiques qui donnent une information aussi suffisante concernant la qualité des protéines pour un prix très reduit, est plus en plus reconnue.

Parmi les indices chimiques basés sur la composition aminoacidique ( indices: MORUP-OLESEN, FAO/WHO, KORPÁCZI, Chemical Score ) seulment une part est capable de prédire la qualité et la valeur biologique des mélanges des protéines.

Dans la cadre de ce travail de recherche l'applicabilité des ces indices pour la caractérisation de la qualité des protéines est démontré par l'examen d'un des produits les plus connus de la Hongrie: le saucisson sec. Les résultats du travail démontrent que la valeur biologique du saucisson sec est une des plus grandes parmi les produits de viande.

Исследования биологической ценности специальной высококачественной сырокопченой кольбасы

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При определении качества пищвых продуктов, наряду с количественными показателями содержания белков, всё большую роль играют и численные показатели качества белков. Так как непосредственное определение качества белков методом " биоассайс" требует длительного времени и больших затрат, разработанные химические методы " ин витро" которые с наименьшими затратами оьеспечивают такую же точность проверки.
Некоторые из индексов,применяемые на основе определения данных общего аминокислотного состава /Morup-Olesen Index FAO-WHO, Chemical Score, Korpáczy Index/ пригодны и для определения биологических ценностей смеси белков ,а также мясных продуктов.
В данной работе, на примере самого известного мясного экспортного продукта -специальной высококачественной сырокопченой венгерской кольбасы, показана возможность применения химических индксов, пригодных для анализа качеста белков.
На основе результатов исследований установлено, что по биологической ценности, венгерская специальная сырокопченая кольбаса является самой ценной среди продуктов мясной промышленности.

# Investigation of the Nutritive Value of Hungarian Dry Sausages

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## Introduction:

A considerable amount of human protein requirement is provided by animal proteins, especially by meat and meat products. In order to inform the consumers about the product they buy several characteristics have to be stated by the manufacturer ( and controlled by regulatory agencies ) such as net calory content, chemical composition, net protein content and (or protein nutritional quality. In the USA e.g. government regulations require that certain food items meet a minimum biological value and that the Protein. Efficiency Ratio of the product be evaluated and used in food labelling. The protein nutritive value depends essentially on two factors: the amount of protein present and the biological value of the protein. While the amount of protein can be measured very easily, a reliable test, which is reasonably rapid, accurate and reproducible, inexpensive and simple, and is applicable to a wide range of complex and ) or processed food products is still lacking for assessing the biological value.

The possibility of determining the biological value of food and feed proteins by in vitro chemical methods has been intensively studied in our laboratory in the past few years. The aim of this paper is to try to demonstrate the applicability of chemical indices for the characterization of protein nutritional quality of meat products.

A certain kind of raw sausage - Hungarian salami - was chosen as an example, for raw sausage type products are manufactured and exported in great quantities.

## Theoretical aspects

Protein quality is the capacity of a protein to meet the amino acid and nitrogen requirements of an organism, it relates to the efficiency with which various food proteins are used for synthesis and maintenance of tissue protein.

Protein quality can be measured either directly or indirectly. The direct methods are biological assays of which there are two general types: ones based upon body weight gain ( e.g. Protein Efficiency Ratio ) (PER) and ones based on nitrogen balance techniques ( e.g. Biological Value ) (BV). The concept of characterizing the nutritive value of a protein by the PER technique is beeing heavily criticized (1,2) as the true protein quality is measured the latter bioassays. They also offer the advantage that they can be used not only for the characterization of the end product but also at various stages of manufacture as well as for the control of raw materials. Moreover they can serve as a basis for optimalization of technology parameters, food and feed formulations and they can be used in the quality evaluation of food products containing additives or substitutes.

Although there is a constantly growing need for objective protein quality testing for the above mentioned purposes, the use of the direct biological assays is greatly hindered by the fact that they are extremely time comsuming and expensive. These shortcomings are overcome by the indirect methods, which are less expensive, more rapid and easier to perform. The information concerning the nutritive quality of a protein given by these methods is in certain cases equivalent to that resulting from bioassays (3).

At present, work is progressing on assays of the biological value of proteins by microbiological, enzymatic and chemical methods and by the combinations of these three methods, although there are a number of difficulties facing succesful application of short methodologies. Adventages, disadvantages and limitations of these methods have been excellently reviewed recently (3, 4).

Use of chemical indices for protein quality assessment

The procedure of assessing protein quality from amino acid data is overwhelmingly simple to all other methods: only amino acid data and a calculating machine are necessary for an estimate to be made. The biological value of proteins is predicted by chemical indices, which describe the relationship between amino acid data and biological value by means of matehematical functions. Generally:

$$\text{Eq. 1} \quad (BV)^x = f\left[\left(\frac{x_{i1}}{x_{r1}}, \quad \frac{x_{i2}}{x_{r2}}, \ldots \quad \frac{x_{ij}}{x_{rj}}\right)\right]$$

where $(BV)^x$ = the predicted biological value

$x_{ij}$ = the concentration of the j-th amino acid in the sample

$x_{rj}$ = the concentration of the j-th amino acid in the reference protein the amino acid composition of which is considered to be ideal

j = running index for amino acids which are considered to be indispensable ( essential )

n = total number of essential amino acids

A great number of chemical indices have been suggested and used for protein quality evaluation, which differ from one another in the following assumption:

- which amino acids are considered to be essential
- what is the amino acid composition of the reference protein i.e. which amino acid pattern is considered to meet the requirements ideally
- what type of mathematical formula is used for calculation of the index.

Nowadays there is a general agreement that the following amino acids are essential for humans: threonine, valine, isoleucine, leucine, tyrosine and phenyl-alanine (aromatic amino acids) cystine and methionine (sulphur amino acids), lysine, tryptophane.

Since egg had been demonstrated as a protein of high biological value, it was suggested as a reference standard for scoring purposes (5), later it was replaced by a hypothetical reference protein (6), the composition of which was repeatedly revised (7,8) MORUP and OLESEN (9) suggested to use as a reference the amino acid composition of a mixture (egg-potatoe) which had been hitherto found experimentally to have the highest biological value (1o,11).

As far as the calculation of the chemical indices from amino acid data is concerned, the usual procedure is to compare the given amino acid in the sample and in the reference protein, the fraction obtained is used for further calculations. A method frequently used is the calculation of the geometrical mean of the $\frac{x_i}{x_r}$ fractions. ( Essential Amino Acid Index, Modified Essential Amino Acid Index ).

Historically, the first approach to protein quality assessment by means of chemical indices was the Chemical Score (5) based on the theory of limiting amino acids. The calculation of the Chemical Score is very simple: the least of the above mentioned fractions is taken and expressed as percentage.

Although the Chemical Score is now superseded, the FAO/WHO Index (8) returns to this method of calculation, with the difference, that instead of the amount of the individual amino acids, their ratios to the total essential amino acids are used in forming the fraction. (Eq.2.)

It was of primary importance to realize that amino acid ratios higher than 1 are unbeneficial for the optimal ratio. If the sample contains more of the amino acid in question than the reference protein, i.e. either of the above mentioned fractions is greater than 1, the usual procedure is to use its reciprocal (Eq.3.).

Eq. 2.

$$\text{FAO/WHO Index} = \min \left[ \frac{\dfrac{x_{ji}}{\sum_{j=1}^{n} x_{ji}}}{\dfrac{x_{jr}}{\sum_{j=1}^{n} x_{jr}}} \right] \cdot 100$$

Eq. 3.

$$BV^x = f\left(\frac{x_i}{x_r}\right) \qquad \text{if} \qquad x_i \leq x_r$$

$$BV^x = f\left(\frac{x_r}{x_i}\right) \qquad \text{if} \qquad x_r < x_i$$

It was suggested by KORPÁCZY et al. (12) to take in consideration the amount of the non-essential amino acids in expressing protein quality as their role is not negligable in maintaining nitrogen balance:

KORPÁCZY Index =

Eq. 4.

$$75 \sqrt[8]{\prod_{i=1}^{8} \frac{x_i}{x_{i\,egg}}} + 25 \cdot \frac{\sum_{j=1}^{11} c_{j\,egg} - \sum_{j=1}^{11} c_j}{\sum_{j=1}^{11} c_{j\,egg}}$$

( $c_j$ = the concentration of the j-th non-essential amino acid in the sample, $c_{j,egg}$ - in the egg )

All the above mentioned calculations yield indices which are more or less able to characterize the biological value of a protein, their use, however, is limited because of the following drawbacks:

- Although they show a certain correlation with the biological value they are not capable of giving an absolute index number representing protein quality, they can only be used for ranking between proteins.
- As a consequence of this, if these indices are used for the calculation of the biological value of a protein mixture, the results are absolutely unreal and do not agree with those of the biological assays.

While the above mentioned chemical indices are more or less ba[sed u]pon theoretical assumptions, the approach of MORUP and OLESEN (9) is essentially different, as the authors determined the correlation between the biological value and amino acid data by regression analysis which yielded the following function:

Eq. 5.

$$(BV)^x = \prod_{j=1}^{8} \left[ \frac{\dfrac{x_{ji}}{\sum_{j=1}^{8} x_{ji}}}{\dfrac{x_{jr}}{\sum_{j=1}^{8} x_{jr}}} \right]^{\alpha_j}$$

Where $\alpha_j$ is the regression coefficient (exponential) for each essential amino acid.

The fraction in paranthesis is substituted by its reciprocal if its value is greater than as defined in Eq. 3.

The MORUP-OLESEN index measures the biological value of proteins on an absolute scale, the correlation coefficient for actual and predicted biological value was found to be o,99. This index can be readily used for assessment of the biological value of protein mixtures as well, giving essentially the same results as the feeding trials.

The authors have also used the MORUP OLESEN index successfully in characterizing food and fe[e]d formulations (13).

## Materials and Methods

### Samples

Five dry sausage - Hungarian salami - samples were used for the investigations, originating from various factories. They were manufactured at beginning of 1979, at various points of time.

### Preparation of the Samples

The samples were homogenized in a meat chopper. Samples for amino acid analysis and for digestibility determination were defatted by fivefold extraction with pethroleum ether at room temperature. The nitrogen content of the samples was determined by the Kjeldahl procedure as specified by Hungarian Standard (14).

A factor of 6,25 was used for the calculation of raw protein. The digestibility of the defatted samples was determined by prolonged digestion (48 hours) with pepsin in acidic medea ( pH~1 ) as specified by Hungarian Standard (14).

### Amino acid analysis

Samples (~ 7o mg) were hydrolized with 6 N HCl for 24 hours at 11o°C in sealed tubes under nitrogen. The hydrolyzates were neutralized according to SPITZ (15) and filled up to a volume of 25 ml, a 1 ml aliquot was used for the analysis. Two parallel determinations were performed per samples.

Amino acids were determined by an automatic amino acid analyzer type AAA 881 (Microtechna, Prague) using Ostion LG-KS o8o3 resin. Amino acids were eluted by a constant molarity buffer system ( o,2 M $Na^+$) with citrate buffers of increasing pH values (3,25, 4,25, 7,o, 9,5) Continous ninhydrine reaction was used for detection.

Tryptophane was determined by ion exchange chromatography, following hydrolysis with 4 N NaOH according to DÉVÉNYI (16), using an automatic amino acid analyzer type LYZ - 75 (CHINOIN, Budapest). Chromatograms were evaluated manually.

### Computation

Chemical indices were calculated by the BIOLERT program, developed by the authors (17,18) using an ODRA 12o4 computer of the Technical University Budapest. The program calculates the following chemical indices: Chemical Score, Modified Essential Amino Acid Index, Korpáczy Index, FAO/WHO index and Morup-Olesen Index.

## Results and Discussion

Net protein content and amino acid data of the samples are shown in Table 1. Only the amounts of essential amino acids are detailed, sulphur- and aromatic amino acids are shown togather. The data presented are averages of two determinations. For the sake of comparison the chemical composition of a German salami is also presented as well as the amino acid composition of fat half-swine.

The digestibility of the samples was found to be very high, close to 1. The digestibility was considered to be the same for every essential amino acid for the calculation of the chemical indices.

The chemical indices calculated are presented in Table 2. As it seen in the table, all chemical indices rank the dry sausages very high, and there is a proper agreement among the various indices. There is only one exception, in case of sample C, which was ranked by Morup-Olesen index to a low value. However, this fact is readily explained by the fact, that the amount of threonine was found in case of this sample slightly higher, than in the other samples. The Morup-Olesen Index is very sensitive to threonine, as the fraction containing this amino acid has the highest exponent in the product of multiplication. We remind that the analytical errors in amino acid determination strongly effect the chemical indices, as recently demonstrated by the authors (19).

The data presented show, that there is no significant difference from the point of view of the biological value of sausages manufactured at various factories by the same technology. Neither was a difference observed between Hungarian and German salami samples, by comparison to literature data.

The biological value of raw sausage is comparable to that of its raw material: fat pork. This is very important because during processing technologies the biological value of the product is usually deteriorated as compared to the starting material because of protein denaturation, crosslinking reactions, loss of the availability of certain amino acids etc. As contrasted with this, the digestibility of dry sausages significantly improves during ripening of the product, and - as proved by amino acid analyses - the amino acid composition of the product is essentially the same as that of the raw material. This is in concordance with literature data as the improvement in digestibility of raw sausage type meat products during ripening has been demonstrated recently and was explained by limited protein degradation of meat the oligo- and small polypeptides.

To sum up the results: we have demonstrated that Hungarian raw sausages have a good predicted biological value, as calculated by any of the chemical indices. The biological value of raw meat, and the digestibility is improved.

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able 1

| | | Hungarian Salami amples | | | | | German Salami (2o) | Fat half swine (2o) |
|---|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | | |
| Net Protein % | | 18,2 | 17,6 | 17,2 | 18.1 | 18,8 | 16,9 | 9.8 |
| Essential Amino Acids g/1oog protein | THR | 4,84 | 4,96 | 5,69 | 4,66 | 5,12 | 4,1o | 4,69 |
| | VAL | 5,o1 | 4,61 | 4,17 | 4,48 | 4,91 | 5,oo | 5,2o |
| | ILE | 4,33 | 4,54 | 3,78 | 4,84 | 3,95 | 4,83 | 5,1o |
| | LEU | 7,9o | 7,64 | 6,85 | 8,34 | 9,13 | 7,19 | 7,35 |
| | LYS | 8.75 | 6,84 | 5,78 | 7,85 | 7,98 | 8,o3 | 8,16 |
| | TRP | 1,13 | 1,15 | 1,53 | 1,56 | 1,69 | o,84 | 1,33 |
| | CYS+MET | 3,64 | 4,26 | 3,36 | 3,51 | 3,72 | 3,47 | 3,67 |
| | TYR+PHE | 6,67 | 6,55 | 5,85 | 7,64 | 6,21 | 6,91 | 7,56 |
| Digestibility | | o,91 | o,94 | o,92 | o,93 | o,95 | - | - |

Table 2

| Chemical Indices | Hungarian Salami Samples | | | | | German Salami | Fat half swine |
|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | | |
| M E A I | 7o,2 | 72,4 | 65,2 | 74,3 | 74,3 | 72,4 | 64,9 |
| KORPÁCZY | 62,8 | 63,3 | 57,8 | 65,2 | 65,6 | 74,9 | 73,5 |
| FAO/WHO | 85,3 | 81,8 | 81,1 | 75,2 | 82,7 | 83,4 | 76,7 |
| MORUP-OLESEN | 67,8 | 58,4 | 36,4 | 82,3 | 58,8 | 69,6 | 81,o |