Lysine is an essential amino acid, which is limited in foods of plant origin, especially in cereals. The heat-treatment of products containing proteins and reducing sugars results in formation of Maillard reactions during which the cross-linkages among epsilon amino groups (ε-NH₂) and reducing sugars are created. Thus the protein-carbohydrate complex is formed. This complex contains an unreactive (unavailable) lysine, which is bound to reducing sugars and is not available in body. Hereby, the nutritive value of feeds and foods decreases. When a standard analytical method for analyses of amino acids is used, in products containing protein-carbohydrate complexes, it is not possible to analyze the content of reactive (available) and unreactive (unavailable) lysine, but only the content of total lysine. Therefore, when the standard amino acid analysis is used, the content of lysine in heat-treated feeds and foods is overestimated. In order to avoid this, some methods for determination of reactive lysine were developed. Among the best known, the homoarginine and furosine methods are included. Using these methods, in evaluation of nutritive value of feeds and foods, is of great importance because they allow to determine the extent of proteins, which were damaged during the heat treatment and thus we obtain information on objective nutritional protein quality of the product.

Keywords: Feed, food, Maillard reaction, protein, reactive lysine

INTRODUCTION

The knowledge about the nutrient composition of foods and feeds is important, because based on this information we are able to find whether the full-value products for nutrition are used and whether a required content of nutrients is provided for organism. The amino acids are the basic organism building units. The foods of plant origin, general cereals, are limited in some essential amino acids, especially lysine. It is essential, both for humans and for animals. During the heat-treatment, in the raw materials and foods which contain proteins and reducing sugars, the functional (Singh, 1991) and nutritional (Friedman, 1999a; 1999b; 1999c) properties of final products are changed as a result of changes in the structure of proteins. The attractive and low attractive odors and colors are formed and the nutritive value of products is reduced (Ajandouz et al., 2008). When the high temperatures are used, during the heat-treatment of raw materials, Maillard reactions are formed (Borrelli et al., 2003). It is chain complex of chemical reactions in which proteins, amino acids and reducing sugars are involved. The products of these reactions are responsible for the brown color and changes in organoleptic properties of feeds. Moreover, the nutritive value in the heat-treated feeds is decreased mainly as a result of decline the available lysine content (Bjork et al., 1984). During the Maillard reaction the crosslinking among ε-amine groups and reducing sugars are formed to give the unreactive (regenerated) lysine which is not digestible in the body (Fastinger and Mahan, 2006). When the content of lysine is analyzed using a standard amino acid analysis the overestimation of lysine occurs, because in this way the content of total lysine, both reactive and unreactive, is determined.

Chemical changes in heat treated foods

Besides the changes in organoleptic properties, during the heat treatment, in raw materials either plant or animal origin containing proteins and reducing sugars (glucose, lactose), a decline in nutritive value occurs as a result of formation undigestible protein-carbohydrate complexes (Bjorck et al., 1984). Irrespective of conditions which are used during the processing not all the amino acids are involved in formation of these linkages. A presence of ε-amine group in lysine is responsible for a low availability of proteins because this amino group is interactive with other compounds in feeds and thus makes them unavailable. During these interactions following reactions are formed: 1) reaction of ε-NH₂ group of lysine with carbonyl groups of sugars, are further transformed to Maillard browning products (Adrian, 1974; Bjarnason and Carpenter, 1970), 2) formation of unnatural amide (isopeptide) cross-link products between ε-NH₂ groups of lysine and carbonyl and amide groups of aspartic and glutamic acid or asparagine and glutamine residues (Bjarnason and Carpenter, 1970; Hurrell and Carpenter, 1977; Waibel and Carpenter, 1972), 3) formation of cross-linked amino acids such as lysinoalaine (Friedman, 1978; Stemberg et al., 1975), 4) interaction of ε-NH₂ with tannins and quinones (Bjarnason and Carpenter, 1970; Dale et al., 1980; Muindi and Thomke, 1981), 5) in vivo and in vitro methylation of ε-NH₂ groups to form mono-, di-, and trimethyl lysine side chains (Friedman and Gumbmann, 1981; 1979) and 6) steric blocking by adjacent disulfide bridges (Matthes, 1968).

Undigestible lysine-sugar complex – unreactive lysine

The best known reactions between lysine and reducing sugars are the Maillard reactions during which protein-saccharides complexes, not available in organism, are formed (Figure 1). During this reaction, ε-amine group of lysine binds to molecules of reducing sugars and thus the ideal digestibility and availability of lysine is decreased (Stein et al., 2006). Only lysine which is not linked to the reducing sugars (reactive lysine) is available in organism, while lysine linked to sugars is unavailable (Finot and Magennat, 1981).
New formed lysine-sugar complex, which is resistant to enzymatic digestion in the small intestine, is cleaved during the acid hydrolysis which is one of the steps when amino acids are analyzed (Finot and Magnenet, 1981). During this step the amadori products are hydrolyzed to three amino acids: regenerated - unreactive lysine, furosine and pyridosine (Finot et al., 1968), whereas reactive lysine remains as lysine. Amadori products are the intermediate formed in the formation of advanced glycation end products. These constituents are one of the factors which are responsible either for development or for deterioration of degenerative diseases such as diabetes or arteriosclerosis. During the glycation, the linkages among molecules of protein and sugars are formed resulting in damage and dysfunctional structure of proteins. Hereby the biological structure of protein is changed and biological value is reduced. Regenerated lysine, which is formed during the decomposition of amadori products, is structurally identical to the reactive lysine (Finot et al., 1968). Lysine that appears in chromatogram, when standard amino acid analysis is used, is composed of reactive and unreactive lysine. Therefore it is not possible to distinguish between reactive and unreactive lysine. This leads to the overestimation of lysine concentration in heat treated products. Therefore it is necessary to find, especially in heat-treated products containing proteins and reducing sugars, the content of available lysine.

For this purpose some methods for analysis of chemically and nutritionally available lysine were developed (Bodwell, 1976; Concon, 1975; Ostrowski, 1978). Among the best known, homoaarginine and furosine methods belong.

### Furosine method

Using furosine method the content of reactive lysine is analyzed based on the furosine concentration in heat-treated products. Furosine is amino acid which arises after acid hydrolysis of Amadori products, which are formed within the Maillard reaction by interacting ε - amino groups of lysine with glucose, lactose or maltose (Nursten, 1981), during acid hydrolysis of amino acid analysis (Finot et al., 1968).

Thus furosine is considered as useful indicator of the degree of damage proteins during the Maillard reactions in many plant foods such as soy, barley, malt (Molnar-Perl et al., 1986), potato, rice (Resmini and Pellegrino, 1991), marmalades and fruit foods for newborns (Rada-Mendoza et al., 2004). The amadori products represent 32 % of furosine, 40 % of regenerated lysine, 28 % of pyridosine (Bujard and Finot, 1978). Since the proportion of these products is constant, concentration of regenerated lysine is calculated after analysis of furosine concentration. To determine the peak of furosine in chromatogram at 570nm, c-N-2-furomethyl-lysine is used (Palm et al., 2008).

The content of Amadori products (AP) is calculated as follows: AP (%) = furosine (%)/32(100)

Regenerated lysine which represents 40 % of Amadori products is calculated: Regenerated lysine (%) = amadori product (%) x (40/100)

The peak of lysine which is appeared in chromatogram is identical with content of total lysine – reactive and regenerated (unreactive). The amount of regenerated lysine, calculated based on concentration of furosine, is subtracted from the total amount of lysine and thus reactive lysine is calculated: Reactive Lys (%) = total Lys - regenerated Lys

### Homoarginine method

The homoarginine method, during which a conversion of reactive lysine to homoaarginine occurs, is possible to use for determination of reactive lysine (Kimmel, 1967). Within the guanidination reaction, lysine which is not linked to sugars is converted to the homoaarginine before the sample is subjected to acid hydrolysis of protein (Kimmel, 1967). When homoarginine method is used the content of reactive lysine is determined based on the analyzed amount of homoaarginine which is formed during the guanidination reaction. The O-methylisourea reacts in an alkaline medium with ε-amino group to form a homoaarginine (Figure 2).

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1. **Homoaarginine method**
2. **Furosine method**
3. **Homoarginine method**
4. **Guanidination reaction**

The amount of Lys recovered after guanidination is quantified using the following equation:

Lys recovery (%) = 100 x [(mmol of homoaarginine / (mmol homoaarginine + mmol lysine)]

The use of this technique is important in terms of nutritional quality evaluation of feeds and foods. Torbatinejad et al. (2005) found, in selected cereals, the differences in content of total and reactive lysine when this method was used. The content of total lysine was higher than reactive. The difference between reactive and total lysine ranged from 20 - 54 %. The large differences between total and reactive lysine suggest a considerable decrease of available lysine in the breakfast cereals that have been tested. Some works, reported 10% destruction and 20% inactivation of lysine in breakfast cereals (Erbersdobler and Hupe, 1991). Also Ruthefurd et al. (2006) reported that conventional analysis of digestible total lysine content significantly overestimated available lysine in breakfast cereals, on average 37%.

### CONCLUSION

During the heat-treatment of products due to cross-linking between lysine and reducing sugars the damage of proteins occurs. Protein-sugar complexes are unavailable in an organism and therefore the nutritional quality of final products is reduced. When the standard amino acid analysis is used, in heat treated foods and feeds, it is not possible to determine the content of available and unavailable lysine and therefore the content of lysine is overestimated. To avoid this, it is necessary to use methods which provide the reactive lysine content. Among the most suitable methods homoaarginine and furosine method belong.

Acknowledgments: This article was written during realization of the project “ZDRAVIE no. 26220220176” supported by the Operational Programme
Research and Development funded from the European Regional Development Fund.

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