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Production of high-lysine-content biscuit and examination of the absorption of lysine in humans

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Abstract. In the Medical and Health Centre of the University of Debrecen, we examined the changes in the free amino acid content of the blood serum of control and experimental individuals after consumption of 2,000 mg of lysine-laden biscuits. We baked the biscuits at 130 °C, during which the greater part (70–75%) of the lysine was not converted into Maillard reaction products. After 30–60 minutes of consumption of the biscuits, the free lysine content of the blood serum increased significantly in the experimental and control group with 41–46%, and even

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after three hours of consumption the level was 20% higher than in the initial concentration. The free arginine content of the blood serum did not change after the consumption of control and lysine biscuits neither in the control nor in the experimental group. Therefore, the free lysine/free arginine ratio of the individuals consuming lysine increased significantly compared to the initial and the control group's value. The antioxidant level of the blood serum in the control group remained unchanged after the consumption of the control biscuit, while in the case of the experimental individuals who consumed lysine-fortified biscuits it increased by 40–45% compared to the initial level. Summing up: After consumption of the biscuits with 2,000 mg of free lysine, the concentration of free lysine in the blood serum, its free lysine/free arginine ratio and antioxidant level increased significantly. Our researches have clearly demonstrated that the active substances of the biscuit got into the blood serum, so the investigation of the active substance and the evaluation of the physiological effects are definitely recommended in the long run.

1 Introduction

Lysine (also known as 2,6-diaminohexanoic acid, or α, ε-diamino caproic acid) is one of the twenty protein-building amino acids, perhaps the most important essential amino acid, which also plays the role of limiting amino acid in most plant foods (except for leguminous plants), since compared to human needs it can be found in the smallest proportion. It is essential for humans and all farmed animals; the daily human need is 1.0–1.5 g, which can be satisfied only with the combined consumption of appropriate animal and plant proteins. Cereals regularly consumed by humans (wheat, rice, or maize) as well as foodstuffs made from them are deficient in lysine; among frequently consumed plant-based proteins, soy protein has a relatively high lysine content (*Csapó & Csapóné*, 2004). The estimated lysine needs – as according to WHO recommendations – of infants, young and school children, and adults are outlined in *Table 1.*

Table 1: Estimated lysine needs (mg/BWT/day) based on WHO recommendations

Amino acid	Infants (3–4 months)	Young children (2 years)	School children (10–12 years)	Adults
Lysine	103	64	44–60	12.0

BWT = body weight (kg)

In many countries of the developing world, lysine deficiency is a prevailing factor due to the predominance of low-protein plant-based diet. Lysine supplementation of wheat proteins was first practised in the mid-50s, though with little success due to the legal regulations of that period (*Flodin*, 1997). Lysine supplementation of animal feeds has been a daily practice in Hungary since the late 70s, made necessary by the general use of intensive livestock farming. The annual worldwide use of synthetic lysine in animal nutrition is reported to be of 800,000 tons, nowadays regulations also providing for food supplementation (*Toride*, 2007).

Besides its many biological functions, we must highlight that lysine takes an active share in treating the symptoms of the herpes simplex virus (HSV). To make use of this functionality, our intention was to introduce a significant amount of lysine into the human organism to monitor through our experiment how it becomes effective and gets absorbed, whether it has therapeutic benefits, and if so, then to have a look at its efficiency in preventing the development of herpes as well as in the treatment of the already developed disease.

2 Literature review

2.1 Absorption and therapeutic effects of lysine in combating HSV

Experiments in human nutrition have demonstrated that absorption from the digestive system of synthetic lysine and that of lysine with protein intake is practically the same. In 5–7 hours following consumption, lysine rapidly finds its way to the muscle tissues, where both its intra- and extracellular concentration becomes higher than in any other tissue. Lysine is an antagonist of arginine which is absolutely necessary for HSV reproduction (*Griffith et al.*, 1981). Lysine inhibits arginine absorption from the small intestines, its reabsorption in the kidney, and its transport through the plasma membranes, whereas in *in vivo* experiments it has been shown to inhibit arginine's HSV-growth-stimulating effect. The increase of the lysine/arginine ratio is crucial in combating HSV.

L-lysine has been proven in several experiments as effective in treating symptomatic herpes simplex virus, while both in *in vivo* and *in vitro* experiments it has exhibited herpes-fighting properties. *Kagan* published his results as early as in 1974, pointing out that the administration of L-lysine suppresses the viral replication of herpes simplex, allowing of an effective treatment of herpes. *Thein & Hurt* (1984) carried out an experiment with 26 participants

predisposed to develop HSV. The experimental subjects were consuming 1,000 mg of L-lysine throughout a year, causing the free lysine level of the serum to increase to 165 nmol/ml – whenever this level was maintained or increased due to the continuous administration of lysine, the incidence rate of herpes would significantly decrease in comparison to control.

Based on a survey questionnaire, *Ferroli et al.* (1996) established that more than half of the patients suffering from herpes would have a significantly improved quality of life following the regular consumption of lysine-containing products. *Richardson & Pearson* (2003) patented products in the form of tablets, creams, and solutions, containing L-lysine, zinc, selenium, copper, and cysteine, all of them found suitable for treating symptomatic HSV.

Due to space limitations, we have touched upon the major factors alone regarding the role of lysine in treating herpes, but the following studies also confirm its anti-herpes effects: *Algert et al.* (1987), *Armstrong & Elenbaas*, (1983), *Ayala & Krikorian*, (1989), *Benmohamed et al.* (2005), *Boutell et al.* (2003), *Brandimarti & Roizman*, (1997), *Digiovanna & Blank*, (1984), *Digiovanna et al.* (1985), *Ferroli et al.* (1996), *Griffith et al.* (1978, 1981, 1987), *Ishihara et al.* (1989), *Kagan* (1974, 1983), *Luo & Aurelian*, (1992), *Masterson* (1986), *McCune et al.* (1984), *Milman et al.* (1980, 1987), *Park et al.* (1982), *Ruyechan & Olson*, (1992), *Simon et al.* (1985), *Smirnova et al.* (1999), *Thein & Hurt*, (1984), *Tomblin & Lucas* (2001), and *Walsh et al.* (1983).

2.2 Is overconsumption of lysine possible?

The question may arise as to whether the overconsumption of lysine could cause any problems in the human organism. Researches have demonstrated that there is no threat of overconsumption either under normal nutritional conditions or in cases of lysine supplementation. Researches suggest that the prevention of HSV requires a daily consumption of 500–3,000 mg of lysine, but for maintaining a steady level 500–1,000 mg seem to be sufficient, while 3,000 mg should be applied in severe cases exclusively and for short periods of time (*Meredith et al.*, 1986; *Duncan et al.*, 1996). If, however, an amount above the necessary level would enter the human organism, it would be used up as energy. The degradation of lysine is a highly complex process since four of its six carbon atoms take part in acetoacetyl-CoA formation (ketogenic amino acid), while the other two are converted into carbon dioxide as a consequence of decarboxylation. Unlike in the case of other amino acids, removal of the ε-amino group is particularly problematic here as there is no suitable enzyme to

perform this task. There are two known routes for the removal of the ε-amino group: one of them involves the formation of cyclic intermediates (piperidine carboxylic acids, pipecolate) and the other one the formation of saccharopine in the liver by condensation of the ε-amino group and α-ketoglutarate. At the intersection of these two routes, lysine is converted at first into aminoadipate-semialdehyde and then into acetoacetyl-CoA (*Csapó & Csapóné*, 2007).

In summary, we can say that L-lysine intake, in any form, improves the biological value of lysine-deficient proteins, contributes to the optimal development of the young organism, and – besides its many other therapeutic effects – can be efficiently used in combating the herpes virus.

3 Research objectives

The abovementioned facts and explanations are very convincing in that lysine supplementation can be extremely useful in the prevention of many diseases as well as in their treatment in case they have already developed. The question arises, then, as to in what form lysine should be introduced into the organism. Intake in the form of medicines or medicinal preparations is problematic and is subject to special licenses, whereas the marketing of functional foods with medicinal properties has no limitations. Setting out from the above, we have decided to deliver lysine into the organism in the form of biscuit and examine how lysine is absorbed in the human body and how it increases the free lysine content of the blood serum. Our secondary aim was to look into how the duration and temperature of baking influences the utilization of lysine in the body.

4 Materials and methods

4.1 Research location

We conducted our examinations in the Medical and Health Centre of the University of Debrecen, in compliance with the laws and regulations applicable in Hungary and in possession of the licences for experimentation on humans. The Institution has at its disposal all resources in staff and equipment necessary for carrying out a Phase II.a. human experiment. Examination of the free lysine content of the biscuit and blood serum was performed at the Department of Food Science, Faculty of Miercurea Ciuc, Sapientia Hungarian University of Transylvania.

4.2 The product under study

For our study, we made use of the *Detki* biscuit, previously prepared with lysine supplementation, having the following major properties: lemon-flavoured calcium source, increased lysine content, and tested antioxidant activity – in the production of the *Detki Keksz Édesipari Kft.* The product contained the following ingredients: 63.4% wheat flour, vegetable fats, sugar, isosugar, whey powder, 3.8% L-lysine hydrochloride, gluten, 0.8% calcium-carbonate, aromas, raising agents (ammonium-hydrogen-carbonate, sodium-hydrogen-carbonate), antioxidants, tartaric acid, soya lecithin as emulsifier, salt, and it may contain traces of nuts, peanuts, and egg-powder. Biscuit ingredients per 100 g of product were: caloric value: 1,955 kJ, protein: 8.9 g, carbohydrate: 67.6 g – containing sugar: 21.1 g, fat: 17.5 g – containing saturated fatty acids: 8.2 g, dietary fibre: 0.2 g, sodium: 17 mg, calcium: 400 mg, L-lysine: 3,000 mg – containing free L-lysine: 2,300 mg, antioxidant activity: 76.5 mg vitamin C equivalent determined with FRAP method.

4.3 Baking time and temperature combinations in the production of biscuit

Since the baking process of biscuit takes up 30 minutes on average, we did not change the baking time, only the temperature in the production of the biscuit. Baking was performed at 120, 130, 140, 150, 160, 170, and 180 °C, each time followed by an examination of the biscuit composition – more specifically its free amino acid and total amino acid content, paying particular attention to free lysine – and its antioxidant activity, determined with FRAP method and expressed in vitamin C units (mg/kg).

4.4 The study protocol of lysine absorption

Volunteers selected for the clinical experiments had to undergo medical examinations prior to participation in the study. During these examinations, their state of health and physical condition was measured, their dietary habits were assessed via questionnaire-based interviews, and certain necessary blood tests were also carried out. Afterwards, six participants consumed six 100 g biscuits per head, each portion containing 333 mg of lysine hydrochloride; thus, the total lysine consumption amounted to 2,000 mg at the beginning of the experiment. At the same time, two further participants consumed six biscuits likewise, but without lysine supplementation – they formed the control group. Blood was drawn and the composition of these blood samples was determined

in the members of both groups immediately before consuming the biscuits and then after 15, 30, 60, 120, 180, and 240 minutes. Following centrifugation, blood plasma was divided into three parts, and its antioxidant, lysine, and calcium content was determined.

4.5 The employed analytical methods

To determine the antioxidant level of the blood plasma, we used the FRAP (Ferric Reducing Ability of Plasma) method, during which we added 500 µl of FRAP reagent to 100 µl of blood serum and measured light absorption at 593 nm, from which we calculated the vitamin C antioxidant equivalent.

Concentration of free amino acids in the blood plasma was performed according to *Csapó et al.* (2008). In doing so, we centrifuged the blood samples, precipitated the proteins with trichloroacetic acid, and determined the free amino acids of the protein-free solution with INGOS AAA-400 amino acid analyser, using post-column ninhydrin derivatization. Derivative absorbance was measured at 440 nm (proline and hydroxyproline) and at 570 nm (all other amino acids) and, besides lysine, all other amino acids were also determined by comparison with the standard chromatogram. In determining the calcium content of the blood plasma, we took 200 µl of it and digested it with a mixture of 1 ml concentrated HNO_3 and 0.5 ml concentrated H_2O_2 for 30 minutes at 80 °C. The final digest was diluted to 10 ml with deionized water, and the concentration of the solution was determined with Thermo Iris Intrepid II inductively coupled plasma optical spectrometer.

5 Results and discussion

5.1 Total and free amino acid content of the high-lysine-content biscuit

The total amino acid content is characteristic of wheat and of the components added to wheat flour during biscuit production, except that the added lysine has slightly changed the proportions. We measured a 2.98% lysine content in the high-lysine-content biscuit, which amounted to 24.5% in terms of protein. Apart from lysine, owing to the ratio of the components, glutamic acid (26.3%) and proline (8.0%) were also highly prevalent in the protein. Part of the 2.98% lysine is the initial lysine content of the wheat, and the other part comes from the added lysine. We measured a 0.37% arginine content in the high-lysine-content biscuit, which adds up to 3.0% in the protein, indicating that the

high-lysine-content biscuit has a lysine content almost 9 times higher than its arginine content.

The examination of the free amino acid content concluded that the 3,000 mg added lysine has dropped to 2,300 mg – 700 mg have decomposed or transformed during the baking process. Lysine is a component of the Maillard reaction, during which various coloured products (brown pigments) and aroma compounds are created by the reaction of protein and carbohydrates. Reaction stages are realized through complex Schiff-base formation, Amadori and Heyns rearrangements, dehydration and deamination steps, and Strecker degradation. The end-products of the reaction series are the coloured melanoidins and the hydroxymethyl-furfurol (HMF) capable of further reactions, while antioxidant compounds are also created in small quantities. Although these transformations reduce the available amount of lysine, they are useful for causing the formation of colour, flavour, aroma substances, and antioxidants as well.

According to our studies, 60–80% of L-lysine remained unchanged in the biscuit during the 30-minute baking process at 130 °C, resulting a lysine/arginine ratio in the end-product that is suitable for therapeutic purposes. The converted lysine created antioxidants, which enhanced the therapeutic benefit and the palatability traits of the product. We have established that the mono- and disaccharides significantly affect the amount of the resulting antioxidants, on the one hand, and that using fructose and dextrose, besides sucrose, is beneficial for this purpose, on the other hand.

The amino acid composition of the high-lysine-content biscuit as functional food shows a significant difference from that of the control biscuit. As an effect of lysine supplementation, the free amino acid content of the biscuit increased to 2.19% as compared to the control's 0.0034%, the total lysine content increased from 0.13% to 2.82%, while in the protein from 1.6% to 25.8%. The lysine/arginine ratio increased from 0.36 to 55.58 in the free amino acid fraction, whereas in terms of the total lysine content from 0.30 to 4.78. These findings are extremely important since a lysine/arginine ratio below 1 is a favourable condition for the viral replication of herpes, whereas values above 2–4 will have a therapeutic effect. The approx. 100 mg free lysine content of the 5-g biscuits can secure the 1–3 g/day lysine intake necessary to achieve the desired effect, this implying the consumption of 10–30 biscuits. The anti-herpes effect of the obtained products was verified with double-blind clinical trials.

We have established that baking temperature and time have a significant influence both on the amount of free lysine left in the product and on the

amount of the resulting antioxidants. 30 minutes of baking time at 120 °C left 95% of the lysine in free form, while this proportion dropped below 20% at 180 °C. Antioxidant activity varies inversely to this, as measured in vitamin C equivalent this value barely exceeds 0 at 120 °C, whereas at 180 °C it can reach as much as 600–700; however, in the control biscuit, this value range was 0–150. Extending baking time from 15 to 60 minutes increased antioxidant activity from 20–25 to approx. 120–140 in the 1%-lysine-content biscuit, while this value ranged between 15 and 50 in the control biscuit. In view of the available measurement data, we have optimized the technical parameters (baking time and temperature) for the conversion of high antioxidant and low lysine content.

5.2 Changes in the lysine content of blood serum samples

In the two control individuals, the free lysine content of the blood plasma increased from 2.80–3.00 mg/100 ml to 2.95–3.13 mg/100 ml in a period of half an hour, and then decreased to the average value of 2.55–2.88 mg/100 ml. The free lysine content of the experimental subjects' blood serum ranged between 2.77 and 3.98 mg/100 ml before the consumption of the high-lysine-content biscuit – these values increased to 3.62–5.11 after half an hour and to 3.84–6.05 after an hour, followed by a significant drop in all individuals by the end of the fourth hour. Values measured after half an hour and after an hour respectively have increased one and a half times as compared to the initial measurements, while control individuals showed virtually no such changes at all.

Table 2: Average amount of free lysine concentration in the blood serum of experimental and control subjects in function of the period after consuming the biscuits as compared to initial values

Free lysine in blood	Time lapsed after consuming the biscuits (hour)				
plasma/initial value (1.00)	0.5	1	2	3	4
Experimental subjects	1.41	1.46	1.36	1.19	1.08
Control subjects	1.05	0.94	0.89	0.91	0.96

We used one-way analysis of variance to demonstrate that the consumers of Liziner had a significant increase in their free lysine level in the blood serum, while we could not detect any significant change in the case of the control group.

5.3 Changes in the arginine content and free lysine/free arginine ratio of blood serum samples

We have observed 2.51–3.88 mg/100 ml and 1.88–4.39 mg/100 ml of free arginine content in the control and in the experimental individuals respectively, suggesting considerable differences between the subjects. In function of the time lapsed after consuming the biscuits, the free amino acid content did not change in a statistically verifiable manner – thus, practically it remained constant throughout the experiment. We can make similar statements in terms of all other amino acids, whose concentration showed changes to varying degrees during the experiment, but we could not demonstrate significant differences between the experimental and the control group.

Then, we studied the blood serum's free lysine/free arginine ratio in the blood plasma, and established that this ratio was practically the same in the experimental (0.91–1.29) and control subjects (0.85–1.15). While the ratio remained unchanged in control subjects, experimental individuals showed increasing tendencies as follows: 1.38-fold increase after half an hour, 1.35-fold after an hour, 1.29-fold after two hours; 1.19-fold after three hours, and 1.14-fold after four hours (*Table 3*).

Table 3: Average ratio of free lysine/free arginine content in the blood serum of experimental and control subjects in function of the period after consuming the biscuits as compared to initial values

Free lysine/free arginine ratio as compared to initial value (1.00)	Time lapsed after consuming the biscuits (hour)				
	0.5	1	2	3	4
Experimental subjects	1.37	1.35	1.29	1.19	1.14
Control subjects	0.98	0.97	0.87	1.03	0.99

We used variance analysis to find out that the free lysine/free arginine ratio in the blood serum of those consuming Liziner has significantly increased compared to both the initial value and the control group. The control group did not show any significant change regarding this ratio in the function of time.

6 Changes in the antioxidant level of blood serum samples

The antioxidant level of the control individuals' blood serum ranged between 31.2 and 37.7 mg vitamin C equivalent/1000 ml in one case and between 59.5 and 68.3 in the other case. Relevant differences could be observed between

experimental subjects as well in terms of antioxidant level (36.0–117.7), wherefore we could not detect significant differences in this respect between the two groups. However, when we did not take antioxidant level per se but studied it in its relation to the initial value, we could state that this level had significantly increased in the case of high-lysine-content biscuit consumers (*Table 4*).

Table 4: Average antioxidant level – determined with FRAP method – in the blood serum of experimental and control subjects in function of the time lapsed after consuming the biscuits

Antioxidant activity (mg vitamin C/1000 ml) as compared to initial value (1.00)	Time lapsed after consuming the biscuits (hour)				
	0.5	1	2	3	4
Experimental subjects	1.36	1.41	1.35	1.45	1.40
Control subjects	0.96	1.00	1.11	1.04	1.07

7 Conclusions

In the Medical and Health Centre of the University of Debrecen, we examined the changes in the free amino acid content of the blood serum of control and experimental individuals after consumption of high-lysine-content biscuits. We opted for a baking temperature of the biscuit (130 °C) with a view to leaving the greater part of lysine in free form and converting only a smaller part (20–25%) through the Maillard reaction. By changing baking temperature between 120 and 180 °C, we were able to change the concentration and ratio of free lysine and of the Maillard reaction products, but since our aim was to increase the lysine level in the blood serum we kept to a temperature of 130 °C. We set the free lysine content of the biscuit to 3% so that the 5-g biscuit would contain 100 mg of lysine: this way, we could work out that a daily consumption of 10–30 biscuits would secure the necessary amount of lysine intake in order to achieve the desired therapeutic effect. 30–60 minutes after the consumption of 2,000 mg of free lysine, the free lysine content of the experimental group's blood serum showed a significant increase of 41–46% in comparison with the initial value, yielding a 20% higher concentration in relation to the initial measurement even after three hours have passed following consumption. After the consumption of control biscuits, the free lysine concentration increased in the control subjects' serum, but this was not a significant change.

The free arginine content of the blood serum did not change either in control or in experimental subjects following the consumption of the control and the

high-lysine-content biscuit respectively. As a consequence, the free lysine/free arginine ratio in the blood serum of the individuals consuming lysine increased significantly compared to the initial value and to the control group alike.

The antioxidant level of the control subjects' blood serum has remained virtually unaffected by the consumption of control biscuits, whereas in the case of experimental individuals consuming high-lysine-content biscuits this level has increased by 40–45% in relation to the initial value.

In summary, we may conclude that after the consumption of biscuits with 2,000 mg of free lysine the concentration of free lysine in the blood serum, its free lysine/free arginine ratio and antioxidant level increased significantly. Our researches have clearly demonstrated that the active substances of the biscuit got into the blood serum, so the investigation of the active substance and the evaluation of the physiological effects are definitely recommended in the long run.

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