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Essentiality of dietary amino acids for antioxidative defense

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The amino acids are not only building blocks for proteins synthesis. They also participate in many other biochemical processes and functions: cell signaling pathways, antioxidative reactions, acid-base balance, gene expression, regulation of immune function, and many others. The aim of this study was to present scientific data concerning the essentiality of dietary non-essential amino acids (NEAA) for antioxidative defense in the body, and to present data about their composition and quantity in Bulgarian foods. Information is provided on the content of amino acids with antioxidant activity (Cys,Glu, Gly, Met and total content of S-amino acids) in 27 Bulgarian food products.The data were obtained by the classical method for analysis of amino acids – ion exchange chromatography with post-column derivatization. The importance of NEAA for the antioxidant defense of the body through their participation in glutathione, taurine and hydrogen sulfide synthesis is clarified. Their role in activating the antioxidant capacity of human serum albumin – an important endogenous antioxidant is outlined. Examples are listed showing effective implementation of antioxidant amino acids at thermal processing of dietary fats substituting the synthetic additives.The current data clearly showed that non-essential amino acids, especially those containing extra thiol, thioether, or extra amine group, have strong antioxidant activities. On the basis of the essentiality of dietary non-essential amino acids for the antioxidative defense of the human body, their broad participation in preventive and healthy diets is strongly recommended.

Keywords: Amino acids, Antioxidation, Glutathione, Taurine, Human serum albumin, Additives

INTRODUCTION

The concept identifying amino acids (AA) as essential (EAA) and non-essential (NEAA) has been implemented by the nutritional science for more than a century. One of the basic topics of the scientific research in this period was dedicated to the assessment of dietary protein quality that cumulated a great amount of research energy. That was exactly the aspect by which the proteinogenic amino acids were classified in two major groups – essential (Ile, Leu, Val, Lys, Met, Thr, Phe, Trp,His) and non-essential (Ala, Arg, Asn, Asp, Cys, Glu,Gln, Gly, Pro, Ser, Tyr) and the ratio between their content was adopted as a marker of dietary protein quality[1-3].

Essential amino acids are not synthesized in human organisms thus determining their importance as essential ones that have to be supplied through the diet in order to realize the specific protein synthesis.All research was focused on those amino acids which availability determines the sufficiency of food protein. Studies on the role of replaceable amino acids also started during the last decades of the past century. The replaceable amino acids with their variable chemical composition and structure outline a broad spectrum of biochemical reactions specific for their particular involvement. The following more important processes could be marked among this variety: regulation of cell signalling pathways (Arg, Gln, Gly), antioxidant defense of the organism (Glu,Cys,Gly),maintaining of the acid-alkaline balance (Gln),involvement in immune function processes(Arg, Gln, Pro), and others[4, 5].

The research efforts to clarify the "diet-health" relationship established the "Antioxidant hypothesis", determining the important role and significance of dietary components with antioxidant activity, in the leading place. The introduction of the antioxidant hypothesis explaining the effects of food on human health triggered comprehensive studies searching for compounds substantiating the antioxidant power of the food. The variety of food compositions showed presence of nutrients and of a number of bioactive compounds with antioxidant activity, determining the importance of food for strengthening the antioxidant defense of the organism and for delaying the aging processes [6, 7].

The current studies on the antioxidant activity of non-essential amino acids are concentrated on two aspects. The first one is associated with the role of essential amino acids against oxidative stress in the organism, supplied through the diet [8, 9]. The second one surveys the options to use those amino acids in food technologies as antioxidant additives [5, 10, 11]. This broad spectrum of NEAA implementation in oxidation processes requires comprehensive knowledge on the amino acid

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composition of foods and on their activity in various biological media as well.

The aim of the current study was to present scientific data concerning the essentiality of dietary non-essential amino acids (NEAA) for the antioxidative defense in the body, and to present data about their composition and quantity in foods, typical for the Bulgarian diet.

MATERIAL AND METHODS

An analysis and assessment of recent scientific information from the last decade was conducted, clarifying in a new light the role and importance of NEAA.The survey covered a total of 27 food products typical for the Bulgarian diet. The results for the content of amino acids associated with the organism antioxidant defense were taken from our data base for the amino acid composition of Bulgarian food products that did not contain data about fruits and vegetables because of their poor protein content. The amino acid composition was determined after hydrochloric acid hydrolysis with 6M HCl at 110°C for 22 hours by the classic method of Speckman, Stein and Moor using ion exchange chromatography with post-column derivatization with ninhydrin and UV detection at 570nm (CLA-5, Hitachi). The total protein content in the samples was determined by Kjeldahl method where the content of studied amino acids was expressed on the basis of 100 g protein. The amino acids glutamine, cysteine, glycine, and the essential amino acid methionine as cysteine precursor and sulfur-containing amino acid were in the focus of the present study.Cystine and cysteine were determined as a total complying with the routine analytical recommendations after their oxidation with performic acid to cysteic acid [12].

RESULTS AND DISCUSSION

The results of this survey are presented in two parts. The first one includes analysis of current scientific data about the antioxidant properties of essential amino acids engaged in the antioxidant defense of the organism and in their implementation as antioxidant additives in food technologies. The second part presents the content of those amino acids in Bulgarian food products.

Engagement of NEAA in the antioxidant defense of the organism and their implementation as antioxidant additives

The engagement of essential amino acids in the antioxidant defense is performed through various biochemical pathways. Three of them were in the focus of the present survey: glutathione synthesis, taurine synthesis and involvement in the activation of the antioxidant capacity of human serum albumin (HSA).

Glutathione is an important endogenous antioxidant, part of a precisely coordinated antioxidant enzyme system of peroxidases, synthases and reductases. Glutathione synthesis (tripeptide – gamma-glutamyl-cysteinyl-glycine) requires the presence of the amino acids glycine, cysteine and glutamine (Gly, Cys, Glu). There are several proposed and described mechanisms for explanation of the antioxidative activity of those amino acids. They can take part in antioxidative defenses also through antioxidative enzymes (glutathione peroxidase, superoxide dismutase, and H_2O_2- peroxidase).NEAA have a role in disposal of ammonia, oxidants, and xenobiotics, and in anti-inflammation and regulation of cell's apoptosis.

Glutathione is one of the earliest compounds established as a redox-system involved in biological oxidation.In1889 it was stated that reduced glutathione (GSH) is the body's own master antioxidant. About 30 years ago the scientists began to understand its functions and discovered how to raise its levels. GSH is a small protein molecule produced in every cell of the human body performing many biological roles [13].

Glutathione in its oxidized form contains a disulfide bridge (linking two glutathione molecules) and in its reduced form – sulfhydryl groups. Being a good reductant together with NADPH it participates in the disposal of free radicals. It plays the role of a co-factor of glutathione peroxidase participating in the disposal of hydrogen peroxide and the free hydroxyl radical [14,15].Based on the data of Choe and Min [7]amino acids could be accepted as radical scavengers quenching singlet oxygen.The scientific publications have revealed many different data on synergistic interactions with tocopherols and other primary antioxidants, as well as evidence for antioxidant activity of secondary compounds, produced by reaction between AA and oxidized lipids [5]. Some studies supplied results about chelating (to metals) properties of amino acids and peptides that catalyze the breakdown of hydroperoxides into free radicals [16]. The antioxidative activity of GSH is based specifically on the presence of sulfur atom - crucial for scavenging peroxides (H_2O_2), and other free radicals. The master antioxidant role of GSH is the recycling of vitamin C, vitamin E,lipoic acid, and the regulation of caspases, controlling apoptotic cell death, by redox reactions through glutathione and thioredoxin. The thiol status is an important factor in the therapeutic control of different diseases [13, 17].

L-cysteine plays an important role in glutathione synthesis. It is a semi-essential amino acid which, besides being a glutathione precursor together with glutamate and glycine, participates also in protein synthesis.Besides from the diet, this amino acid could be supplied by trans-sulfuration pathway from methionine and through decomposition of proteins. Various studies using diet supplementation with amino acids building glutathione have shown elevated level of synthesized glutathione in the liver of test animals[18]. Other studies have presented even quantitative evaluations in a sense that moderate amino acid supplementation definitely brought about elevated glutathione level but exceeding the intake dose caused reduced glutathione concentration in the liver tissue – a fact that has not been fully explained yet. There are multiple studies on diet supplementation with cysteine at different diseases and almost all of them confirmed elevated glutathione levels and enhanced antioxidant capacity[19-22]. Certain studies on *per oral* glutathione import have not revealed changes in its serum and tissue level in the organism, thus suggesting and emphasizing the role of the amino acids, building it.Those data clearly showed the necessity of knowledge on the content of above mentioned amino acids in the foods constituting the diet in order to compile precise healthy dietary regimes providing the necessary glutathione synthesis in the organism.

The antioxidant activity of the amino acid cysteine is supported by the evidence for its involvement in production of taurine and hydrogen sulfide which possess antioxidant, anti-inflammatory and neuroprotective activity, thus enhancing the esteem of this amino acid as participant in the antioxidant defense of the organism[13]. Stipanuk's results[23, 24] proving that a diet rich in sulfur-containing amino acids caused the activation of the enzyme L-cysteine dioxygenase involved in taurine-formation process support this concept.

The major taurine precursor is cysteine-sulfinate. The enzymes cysteine dioxygenase(CDO), cysteinesulfinate decarboxylase(CSD), and cysteaminedioxygenase pathways [25] participate in taurine synthesis.There is evidence that low cysteine levels block CDO activity while its elevated concentration causes quick recovery and enhancement of the enzyme activity in the adipocytes and liver[24].This sensitivity of the enzyme to cysteine level is an important factor for taurine formation as it can be a regulating factor in its synthesis. Studies on the role of cysteine sulfinate have shown that 66% of it participated in taurine production and the remaining part – in sulfite production(34%)[24].Taurine, besides its antioxidant activity has also cytoprotective effects, participates in the regulation of intracellular Ca-concentration, in the movement of ions and neurotransmitters, etc.It is accepted as a protective factor against the effect of various environmental contaminants and variety of drug forms[26, 27].The increased production of H_2S is also a preventive mechanism against oxidative stress caused mainly in the brain by the elevated presence of glutamate and its accumulation into asynaptic cleft. Dysfunction of glutamate transporters is associated with various neurodegenerative diseases[28,29].Lu *et al*.demonstrated that H_2S has a potential therapeutic value for oxidative stress-induced brain damage[30].There is abundant information on food protein hydrolysates, amino acids residues and free amino acids with antioxidant activities, based on scavenging of free radicals or reactive species, but mechanisms of action have not been studied in details[30-32]. In this aspect many studies focus on the antioxidant properties of human serum albumin (HSA)[8, 33-35]. NEAA have particular relationship with the antioxidant activity of HSA that acts as antioxidant in biological systems to oxidative stress in aging and inflammation processes.Various studies have proven a relationship between the redox change in HSA and the oxidation of several amino acid residues. The antioxidant activity of HSA is strongly dependent on Cys34, and could serve as a bio marker for evaluation of systemic redox states, important for the efficacy of the therapy and disease progression[8, 34]. Quantitative studies on the role of cysteine 34 and methionine residue in the antioxidant activity of serum albumin have shown respectively 61% and 29% *versus* singlet oxygen; 68% and 61% *versus* hydrogen peroxide, thus clearly defining the more important role of cysteine [33].The analysis of the information explicitly identifies the role of amino acids in the antioxidant defense enhancing in this way the spectrum of compounds with antioxidant properties activating the antioxidant potential of the organism.

From practical point of view the implementation of amino acids as antioxidant additives in fats and oils undergoing thermal processing provokes certain interest. The data from scientific publications show that amino acids act as primary antioxidants but also as antioxidants synergic to alfa-tocopherol. Amino acids containing a thiol group or extra amine group, such as arginine and cysteine have high antioxidant activity while those with functional groups as amide, carboxyl acid

imidazole or phenol have relatively lower antioxidant activity. A study on the antioxidant activity of 20 amino acids added to soy oil undergoing thermal processing at 180°C showed significantly higher antioxidant activity of the amino acids compared to the synthetic antioxidant *tert*-butylhydroquinone (TBHQ) routinely implemented in technological processes [5].

Filipenko and Gribova [10] investigated the effect of amino acids on the processes of sunflower oil oxidation. The higher levels of antioxidant activity of certain amino acids is associated with the greater number of nitrogen atoms in their molecule. The authors have proven that both amino acids and their salts had antioxidant activity.Amino acids, as natural compounds, are recommendable for implementation instead of synthetic antioxidants that now have a long history of implementation in food technologies.

Content of AA with antioxidant potential in Bulgarian foods

Table 1 presents data about the content of amino acids with antioxidant potential in 27 Bulgarian foods. It lists data about glutathione-building NEAA – Cys, Glu, Gly and the essential amino acid Met as sulfur-containing and engaged in cysteine synthesis. Cysteine exists mainly in the form of cystine because of rapid oxidation that is why the table lists the total content of the two amino acids Data are provided about the total protein content of foods, and the content of each amino acid was calculated *versus* it.

Table 1. Content of AA with antioxidant potential in Bulgarian foods

№	Food Product	Protein (%)	AMINO ACIDS /g / 100 g protein				
			Cys + Cystine	Met	Glu	Gly	Total S-amino acids
GRAINS AND PULSES							
1.	Bread, wheat, white	6.73	1.39	1.50	34.83	3.26	2.89
2.	Bread, wheat, black	7.34	1.62	1.45	33.52	3.35	3.07
3.	Bread, rye	4.65	1.63	1.56	27.73	3.73	3.19
4.	Rice	6.46	1.00	2.25	18.94	3.92	3.25
5.	White beans	19.14	0.66	1.13	17.64	3.78	1.79
6.	Lentils	23.33	0.76	0.77	18.39	3.79	1.53
7.	Green peas	23.50	0.86	0.92	18.62	3.93	1.78
MILK AND DAIRY PRODUCTS							
8.	Fresh cow's milk	3.21	0.77	2.38	22.49	1.57	3.15
9.	Fresh sheep's milk	4.68	0.69	2.49	20.58	1.56	3.18
10	Yogurt - cow	3.24	0.71	2.38	22.10	1.63	3.09
11	White cow's cheese	16.41	0.61	2.36	21.36	1.61	2.97
12	White sheep's cheese	15.59	0.69	2.30	21.33	1.61	2.99
13	Yellow cheese "Vitosha"	24.60	0.73	2.19	22.16	1.48	2.92
14	Yellow cheese "Balkan"	24.02	0.80	2.17	22.13	1.49	2.97
15	"Cheddar" cheese	24.06	0.55	2.58	20.46	1.56	3.13
16	"Roquefort" cheese	21.18	0.69	2.50	20.89	1.60	3.19
MEAT AND MEAT PRODUCTS							
17	Veal	20.51	0.88	2.67	16.59	4.36	3.55
18	Beef	18.97	1.07	2.05	16.28	5.67	3.12
19	Pork	12.49	0.67	2.46	15.92	5.48	3.13
20	Pork filet	20.60	0.75	2.83	15.41	3.99	3.58
2.	Lamb	20.58	0.93	2.38	15.34	6.05	3.31
22	Chicken (white meat)	23.43	0.74	2.74	15.52	3.96	3.48
23	Chicken (red meat)	19.72	0.92	2.73	16.40	4.28	3.65
24	Egg (hen)	12.57	2.03	3.49	13.31	2.95	5.52
FISH							
25	Carp	18.51	0.67	2.92	15.99	4.39	3.59
26	Trout	22.16	0.67	3.34	14.66	4.71	4.01
27	Mackerel	17.37	0.73	3.17	14.63	4.36	4.0

The last column shows the total content of sulfur-containing amino acids based on published data on their role in a number of biochemical processes.

The table does not list data about the content of the investigated amino acids in fruits and vegetables because of the specificity of those foods, characterized by a broad spectrum of bioactive compounds, and very low protein content suggesting yet lower level of the investigated amino acids. Similar values would have greater theoretical importance rather than practical applicability. The data in the table could be used for more comprehensive characteristics of the particular foods and for elaboration of preventive diets.

CONCLUSION

The presented scientific information on the role of NEAA in the antioxidant defense of the organism enables the enhancement of the spectrum of nutrients with antioxidant potential and revision of the amino acids "essential – nonessential" classification framework because there is a lot of new evidence for amino acids which, although non-essential for the protein synthesis in the body are essential for certain biochemical processes.

The application of amino acids as additives is a new option to remove the synthetic antioxidants undesired by the consumers from various foodstuffs.

The presented data for amino acid amount in certain foods will facilitate the establishment of preventive and healthy nutrition of the Bulgarian population and will enrich the technologists' information about new food products.

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ЕСЕНЦИАЛНОСТ НА АМИНОКИСЕЛИНИТЕ В ХРАНАТА ЗА АНТИОКСИДАНТНАТА ЗАЩИТА

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(Резюме)

Аминокиселините са не само изграждащи единици за протеиновия синтез. Те участват също в много други биохимични процеси и функции: клетъчни сигнални пътища, антиоксидантни реакции, киселинно-основен баланс, генна експресия, регулиране на имунната функция и много други. Целта на това изследване е да представи научни данни за значението на неесенциалните аминокиселини (NEAA) в храната за антиоксидантната защита на организма и за техния състав и количество в български храни. Представена е информация за съдържанието на аминокиселини с антиоксидантна активност (Cys, Glu, Gly, Met и общо съдържание на S-аминокиселини) в 27 български хранителни продукти. Данните са получени по класическия метод за определяне на аминокиселини – йонообменна хроматография със следколонна дериватизация. Изяснено е значението на NEAA за антиоксидантната защита на организма чрез участието им в синтеза на глутатион, таурин и водороден сулфид. Подчертана е ролята им за активиране на антиоксидантната способност на човешкия серумен албумин– важен ендогенен антиоксидант.Приведени са примери за ефективно прилагане на антиоксидантни аминокиселини при термичната обработка на мазнини, заместващи синтетичните антиоксиданти. Настоящите данни ясно показват, че неесенциалните аминокиселини, особено тези, съдържащи допълнителна тиолова, тиоетерна или аминогрупа, имат висока антиоксидантна активност. Имайки пред вид значението на неесенциалните аминокиселини в храната за антиоксидантната защита на човешкия организъм се препоръчва използването им в превантивни и здравословни диети.