BIOLOGICAL ROLE OF D-α-AMINO ACIDS AND THEIR OCCURENCE IN FOODSTUFFS

— review —

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Abstract: in this paper we review the current understanding of biological and physiological role of D-α-amino acids and the significance of their presence in foodstuffs. The importance of the 19 L-α-amino acids used as building blocks of proteins it is well-known, but the biological role of their D-enantiomers in the body has to be further adequately clarified. Today it is well established the presence of D-α-amino acids in microorganisms, plants, lower animals, mammalian and humans. In food products, D-α-amino acids are generated from L-α-amino acids *via* racemization depending on the processing procedures or the use/presence of microorganisms when fermentation occurs.

Keywords: D-α-amino acids, D-α-amino acid oxidase, racemization, food processing

INTRODUCTION

About 500 α-amino acids (AAs) exist in nature, but only 20 are proteogenic, *i.e.* constitute the building blocks of proteins. With the only exception of Gly they are chiral molecules being their α-carbon atom a tetrahedral stereocentre. Therefore, they can exist as one of two possible stereoisomers. The two configurations, L and D (or *S* and *R*, respectively, in the Cahn-

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Ingold-Prelog nomenclature, with the exception of Cys), are illustrated in figure 1.

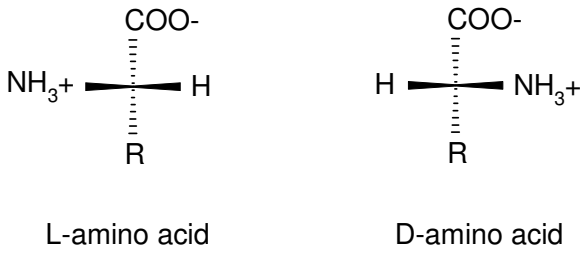

Figure 1. Structural formula of L- and D-α-amino acids

It is worth mentioning that for Ile and Thr, bearing a second stereogenic centre at their β-carbon atom, four stereoisomers are possible. The isomers found in proteins are L-Ile and L-Thr with (2*S*,3*S*) and (2*S*,3*R*) absolute configurations, respectively. The (2*S*,3*R*) stereoisomer of Ile is called L-*allo*-Ile, whereas L-*allo*-Thr indicates the (2*S*,3*S*) stereoisomer of Thr.
Proteins/peptides from living organisms contain predominantly L-AAs (Kvenvolden, 1975).
Both the D- and the L-configurated AAs interconvert one to the other over a period of time, approaching the equilibrium by the racemization process. This process is the result of either a random thermal energy or of a catalyzed chemical mechanism. It was proposed to use the AA D/L ratios as a dating method, as it is known that L-AAs diminish while the D-forms accumulate in dead organisms until equilibrium conditions are reached (Brown, 1985) (Robins et al., 2001). In the case of AAs containing a single stereogenic centre (at the α-carbon atom) the equilibrium ratio is 1/1 (racemic mixture). After the equilibrium is reached the conversion from D- to L-stereoisomer is just as rapid as the conversion from L- to D-stereoisomer. Racemization rate in dead organisms is influenced by different environmental factors, *e.g.* temperature, AA composition of proteins, water content, pH, ionic strength, interaction with bacteria and fungi, etc. As there is a great variability of D/L ratios depending on the environment the AA dating should be used only as a relative dating technique and must rely on other techniques such as ^{14}C.
D-AAs have been considered unnatural AAs, but the development of improved analytical techniques has shown the presence of free and protein-bound D-AAs in considerable amounts not only in prokaryotes but also in eukaryotes including humans.

In *microorganisms*, free and conjugated D-AAs were found in bacteria, yeasts and moulds. In bacteria, some D-AAs (D-Ala, D-Asp, D-Asn, D-isoAsp, D-Glu, D-Gln, D-isoGln) are part of the structure of cell wall peptidoglycans playing an important role in the bacteria resistance to proteolytic digestion (Tipper et al., 1979). Free D-AAs were also detected in cytoplasm of certain microorganisms (Bhattacharyya et al., 1969). In Gram-positive bacteria, D-Ala of lipoteichoic acids (LTA) plays a key role for the immune response, LTA acting as immunostimulatory molecules (Morath et al., 2005). In *plants*, D-AAs, in particular D-Asp, D-Glu, D-Ala, D-Asn, D-Gln, D-Ser, occur naturally in many species in low concentration (0.2-8%) either in free state as in pea seedlings, barley lings, tobacco leaves or hops blossoms or in conjugated forms (peptides) as in pasture grass, wild rice, pea, lentil, tobacco leaves (Brückner et al., 1994). D-Val and D-Leu were detected only in coconut milk (Brückner et al., 2003). Plants can use, metabolize and biosynthesize D-AAs and peptides containing D-isomers.
In *animals*, studies over the last decades have shown the presence of D-AAs, in particular D-Ser and D-Asp in different mammalian tissues and body fluids of insects and humans (Hamase et al., 2002) (Nagata et al., 2007). Part of these D-isomers may originate from the oral and intestinal floras and rumen microorganisms. D-AAs are found also in marine invertebrates, in some bivalves exceeding 1% of the wet weight of organism (Felbeck et al., 1987). In these organisms the D-AAs may originate from the seawater. The presence of D-AAs in tissues and fluids of marine invertebrates can constitute a reservoir for producing L-isomers in extreme conditions (malnutrition, stress). In mammals, D-AAs are considered physiologically active compounds and markers of diseases and aging *via in vivo* racemization of the corresponding L-isomers (Hamase, 2007). In particular D-Ser and D-Asp were found in significant amounts in mammalian tissues being produced by the action of endogenous serin racemase and aspartate racemase (Homma, 2007) (Yoshimura et al., 2003). It has been shown that endogenous D-Ser from cerebral cortex plays an important role in both physiological and pathological processes (Wolosker et al., 1999). Endogenous D-Asp is involved in endocrine system (Long et al., 1989). In different human proteins D-Asp is formed during aging of certain tissues including teeth (Helfman et al., 1975) (Helfman et al., 1976), bones (Ohtani et al., 1998) and eye cataracts (Fujii et al., 2007).

BIOLOGICAL SIGNIFICANCE OF D-α-AMINO ACIDS

The presence of D-AAs in human tissues is well accepted today. The excretion of the D-stereoisomers of AAs in physiological fluids is influenced by age, diet, physiological state and antibiotic therapies. Some D-AAs, in particular D-Asp, D-Ser and D-Ala are abundant in urine and blood sera. D-AAs have beneficial activities and applications in biomedical sciences but some of them increases during human pathologies or diseases.
Known biological functions (beneficial and harmful effects) of D-AA are summarized in table 1.

Table 1: Beneficial and adverse effects of D-α-amino acids in humans

D-α-amino acid	Beneficial role	Reference	Adverse effect	Reference
D-Ala	-Important for the immunosuppressive cyclosporine A biosynthesis -Novel cancer gene therapy paradigm	(Hoffmann et al., 1994) (Stegman et al., 1998)	-Pathophysiology of Alzheimer's disease (high D-Ala and low D-Asp) -Renal damages -Increased free radicals production -Associated with cow mastitis	(Csapó et al., 1995) (Hamase et al., 2002) (Gonzalez-Hernández et al., 2003)
D-Val	Inhibition of hepatic tumor growth	(Sasamura et al., 1998)	-Associated with cow mastitis -Autogenesis and maintenance of tumors	(Csapó et al., 1995)
D-Leu	Therapeutic properties as analgesic	(Man et al., 1987)	-Associated with cow mastitis -Maintenance of tumors	(Csapó et al., 1995) (Fisher, 1998)
D-Met			Maintenance of tumors	(Sasamura et al., 1998)
D-Phe	-Treatment of depression and Parkinson's disease -Therapeutic properties as analgesic	(Iumatov et al., 1991) (Spatz et al., 1975) (Walsh et al., 1986)		

	-Stress protective action			
D-Tyr	Antiinflammatory activity	(Hansford et al., 2003)	Increase of production of free radicals	(Gonzalez-Hernández et al., 2003)
D-Trp	Niacin activity	(Shibata, 1999) (Shibata et al., 2000)		
D-Asp	-Antinociceptive effect -Neuroendocrine role -Role in reproduction	(D'Aniello, 2007) (D'Aniello et al., 2005) (Onat et al., 1995)		
D-Arg	-Cancer treatment -Dilator action	(Calver et al., 1991) (Szende et al., 2001)	Light toxicity (DL50 2800 mg/kg in mice)	(Navarro et al., 2005)
D-Ser	-Endogenous modulator of the retina -Neuronal activity -Modulator of glutamatergic neurotransmission	(Bauer et al., 2005) (Estevens et al., 2003) (Miller, 2004) (Wolosker et al., 2002)	-Inductor of nephrotoxicity -Pathophysiology of Alzheimer's disease -Renal damages	(Hamase et al., 2002) (Ganote et al., 1974) (Man et al., 1987)
D-Thr			Affects the neurotransmitter balance in the brain	(Boehm et al., 1998)
D-Cys	-Beneficial effects in acute alcohol intoxication -Protection in cancer therapeutics	(Roberts, 1995) (Tsukamoto, 1990)	-no mutagenicity	(Glatt et al., 1985)
D-Pro	Antitumor effect in association with PEG-DAO (D-amino acid oxidase-polyethylene glycol)	(Fang et al., 2002)		

It is known that natural and synthetic D-AAs containing peptides are used as antibiotics: gramicidin, bacitracin, polymixins, tirocidins, actinomycin,

valinomycin. These antibiotics act by disrupting bacterial cell membranes through ion channels formation.

D-α-AMINO ACIDS IN FOODSTUFFS. MECHANISM OF FORMATION.

Usually, food proteins contain L-isomers of AAs, but some D-stereoisomers occur in foods either naturally originated or processing-induced under specific conditions, *e.g.* high temperatures, strong acid and alkali, fermentation processes or in case of adulteration/fortification of non-fermented foods (Chiavaro et al., 1998) (Friedman et al., 1984) (Hayase et al., 1975). Free D-AAs are not significant in diet (except some marine foods) but are component of proteins which are susceptible to racemization by processing. Regarding the taste, usually D-AAs taste sweet as compared to their L-isomers which generally have bitter flavor. The hydrophobic groups of the side chains of D-Val, D-Leu, D-Trp, D-Phe are involved in the intensity of sweet taste, which is greater than that of D-Ala. In some cases, the sweetening power of D-AAs with hydrophobic groups is higher than that of sucrose (Linden et al., 1999).
The main D-stereoisomers of AAs that occur in different food products are given in Table 2.

Table 2: D-α-Amino acids in different foodstuffs

Type of D-α-amino acid	Food products	Reference
D-Ala, D-Asp, D-Glu, D-Val, D-Leu, D-Ile, D-Ser	yoghurt, kefir, curdled milk, cheese (Gouda, Cheddar, Emmentaler, Parmesan), ewe's milk	(Brückner et al., 1992)
D-Ala, D-Asp, D-Asn, D-Glu, D-Gln, D-Leu, D-Met	Lactic fermented juice of carrots	(Brückner et al., 1989)
D-Ala	Cabbage juice	(Brückner et al., 1989)
D-Ala, D-Gln	Sourdough before baking	(Gobetti et al., 1994)
D-Asp, D-Glu, D-Phe	Roasted coffee	(Palla et al., 1989)
D-Pro, D-Ala, D-Glu, D-Asp	Beer, wine, vinegar	(Calabrese et al., 1995)
D-Leu, D-Pro, D-Phe	Honey	(Pawlowska et al., 1994)
D-Asp, D-Ala, D-Phe, D-Leu, D-Val	Heated almonds	(Man et al., 1987)
D-Asp, D-Ala, D-Phe, D-Leu, D-Val	Heated bacon	(Fuse et al., 1984)
D-Asp, D-Ala	Processed chicken muscle	(Liardon et al., 1983)
D-Asp, D-Ala, D-Phe, D-Leu, D-Val, D-Met	White bread toast	(Bunjapamai et al., 1982)

D-AAs are quite common in dairy products and fermented beverages (beer, vinegar, wine) resulting from bacterial activity of different microorganisms. It is considered that fermented milk products contain higher amounts of D-AAs (D-Ala, D-Glu, D-Asp) than pasteurized or UHT milk/milk products (Csapó et al., 2007). Variable amounts of D-AAs were found in sourdough as a result of acidic bacteria and yeast used in fermentation and also in roasted coffee, processed fish meals, honey, hamburger meat, liquid spices and eggs. In fruits and fruit juices D-AAs could originate from different sources: plant, soil, microorganisms or heat treatments (pasteurization) of juices.

The presence of D-AAs in foods can be used as an important biomarker of thermal treatment, microbial quality and adulteration or fortification of the products. In milk, it is considered that a concentration above 4% of D-Ala represents an indicative of microbial milk contamination. In honey, the enantiomeric ratio of leucine and proline represents a molecular marker of age, processing and storage of product. The presence of D-Pro in wines and vinegar is used as an indicator for age dating (Chiavaro et al., 1998).

The findings of D-AAs in commercial foods has stimulated extensive studies on the racemization mechanisms that take place in processed foods: base-catalyzed or acid-catalyzed, catalyzed by microbial enzymes or by the Maillard reaction. One of the fastest protein-bound L-AA which converts *via* racemization to the corresponding D-form is D-Glu.

The most important mechanism of formation of D-AAs is racemization under alkali conditions. *The base-catalyzed racemization* of AAs is governed by acidic proton abstraction from the chiral center of the molecule by bases due to resonance stabilization of the planar generated carbanion (Bodanszky, 1984), as shown in figure 2.

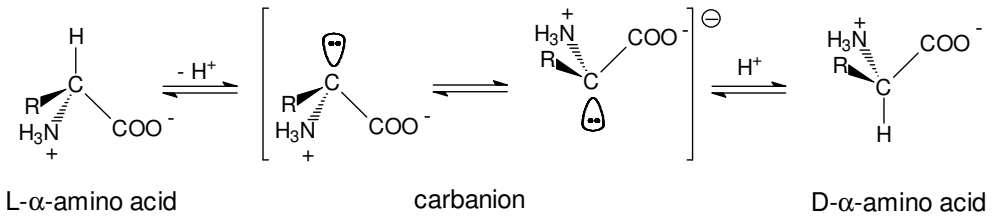

Figure 2. Mechanism of base-catalyzed racemization

Racemization follows the rate expression:

$$\ln\left\{\frac{1+D/L}{1-D/L}\right\} = 2k_i t$$

The rate of racemization $2k_i$ depends on whether the AAs are in free state or linked into peptides/proteins. Racemization of some AAs (Ser, Cys, Thr) generates not only the D-stereoisomer but also xenobiotic AAs which are very reactive and can have toxic effects (dehydroalanine, lysinoalanine) (Hayashi, 1982).

Base-catalyzed racemization occurs in alkali-treated proteins (casein, lactalbumin, soy protein, corn protein, wheat proteins) at a temperature greater than 50°C (Master et al., 1979). The racemization is produced during different types of food processing, such as extraction of proteins from vegetal sources or bones from meat carcasses, inactivation of mycotoxins, protein inhibitors, elimination of nucleic acids from a single-cell biomass. The process of β-elimination increases with pH, heat and time of treatment. Casein heated at 230°C for 20 minutes contains 31% of D-Asp (Hayase et al., 1975). Soybean protein treatment under conditions of 0.1 N NaOH and 75°C leads to racemization half-life of essential Cys in less than 30 minutes (Friedman et al., 1985). It is considered that racemization of AAs in proteins is significantly higher than racemization of free AAs. However, the combination of heat and alkali produces higher rates of racemization of essential AAs than only heat treatment of foods.

Acid-catalyzed racemization occurs at a slower rate than base-catalyzed racemization and consists in protonation of carboxyl group of an L-AA which facilitates the elimination of α-CH proton with the consequent generation of asymmetric dehydroalanine, which regenerates in an equimolar mixture of D- and L-isomers, as shown in figure 3.

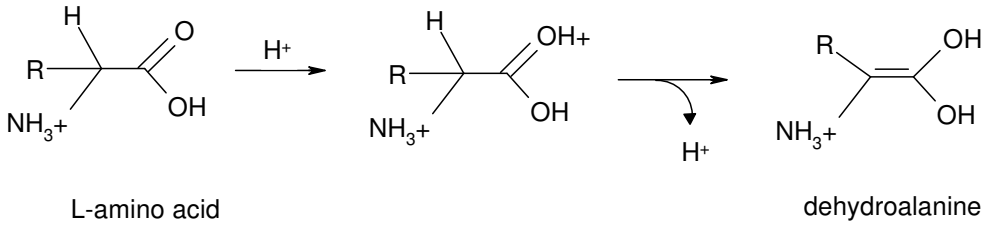

Figure 3. Mechanism of acid-catalyzed racemization

In fermented foods, an *enzymatic racemization* is involved by the action of racemases and epimerases from bacteria (Brückner et al., 1992). The mechanisms involve either the isomeric inversion catalyzed by pyridoxal-phosphate coenzyme of the microbial racemase or the conversion of L-Lys in D-Lys by microbial and plant enzymes *via* pipecolic acid (Fangmeier et al., 1981).

Literature data report the formation of D-AAs in many vegetal and animal food products as a result of the *Maillard reaction*, which is a non-enzymatic browning reaction in food products (Brückner et al., 2001). The reaction takes place between sugars and amino groups. Recently it has been shown that the mechanism of action involves the formation of relatively stable Amadori compounds (fructose-AAs) and carbanions (Pätzold et al., 2006).

Despite of their beneficial role in some diseases, D-AAs in diet could have negative effects, primary affecting the digestibility of food proteins and their nutritional value. Enzymatic hydrolysis of proteins containing D-AAs at peptide bonds with L-AAs is slower than that of native proteins, generating nonmetabolizable and nutritionally antagonist products. D-stereoisomers of essential AAs are poorly utilized, the efficiency of their use in diet strongly depending on the activity of D-AA oxidase (DAAO). Mammals absorb and metabolize D-AAs *via* enzymatic (DAAO) oxidation in mitochondria to the corresponding α-keto acids which further undergo stereospecific transamination yielding the L-enantiomer normally metabolized. Literature data report inhibition of DAAO activity by some synthetic preservatives used in food and apple juices, like potassium sorbate (Oguri et al., 2007). Biochemically, intake of high amounts of D-AAs from processed foods can lead to an increase of the DAAO system. Another pathway of D-AAs metabolism involves the epimerases and racemases. Potential toxic effects of some D-AAs can be induced by oxidative damage of their metabolic products as H_2O_2 (Ercal et al., 1996). Certain D-AAs can increase free radicals inducing lipoperoxidation in liver and kidney under specific pathological conditions (Cortes-Rojo et al., 2007).

Several analytical methods are used to detect D-AAs in tissues and food products. These methods are based on enantiomeric separation techniques, either enzimatically or chemically (reaction with an optically pure reagent or uses of chiral stationary phases): HPLC analysis consisting of pre-column derivatization with DAAO and chemical modification with *o*-phenylendiamine (Oguri et al., 2005), gas-chromatography (Casal et al., 2000), gas-chromatography coupled to mass spectrometry (Brückner et al., 2001), capillary electrophoresis (Vandenabeele-Trambouze et al., 2000),

immunocytochemical methods (Schell et al., 1995), enzymatic methods using D-AA oxidase (Nagata et al., 1987).

CONCLUSIONS AND FUTURE ASPECTS

In the present review we have considered the presence of D-AAs in microorganisms, plants and animals, their adverse and health-promoting effects, occurrence in foodstuffs in particular in processed food products. We presented also a brief summary of the principal analytical methods used for D-AAs determination. Foods are complex mixtures in which not only L-AAs from proteins play an important role but also other naturally occurring AAs and D-AAs.

Detection of D-AAs in foodstuffs is used as biomarker of microbial quality, thermal treatment and adulteration/fortification of the products. The main negative effect of the presence of D-AAs in foods is the decreasing of protein digestibility. Evidence showed that in some cases low digestibility of proteins can be successfully used in weight control diets or in chronic pain control. Complete significance of D-AAs in foods is not entirely known and there is still a great demand to thoroughly investigate their role.

REFERENCES

1. Bauer, D., Hamacher, K., Bröer, S., Pauleit, D., Palm, C., Zilles, K., Coenen, H., Langen, K.-J., Preferred stereoselective brain uptake of D-serine-a modulator of glutamatergic neurotransmission, *Nuclear Medicine and Biology,* 2005, 32(8), p. 793-797
2. Bhattacharyya, S., Banerjee, A., D-amino acids in yeast cell pool, *Can. J. Microbiol.,* 1969, 15, p. 1107-1109
3. Bodanszky, M., in *"Principles of peptide synthesis"*, Akademie-Verlag, Berlin, 1984
4. Boehm, G., Cervantes, H., Georgi, G., Jelinek, J., Sawatzki, G., Wermuth, B., Colombo, J.P., Effect of increasing dietary threonine intakes on amino acid metabolism of the central nervous system and peripheral tissues in growing rats, *Pediatr. Res.,* 1998, 44, p. 900–906
5. Brown, R.H., Amino Acid Dating, *Origins*, 1985, 12(1), p. 8-25
6. Brückner, H., Hausch, M., Detection of free amino acids in food by chiral phase capillary chromatography, *J. High Resol. Chromatogr.,* 1989, 12, p. 680-684
7. Brückner, H., Jaek, P., Langer, M., Godel, H., Liquid chromatographic determination of D-amino acids in cheese and cow milk. Implication of starter

cultures, amino acid racemases and rumen microorganisms and nutritional considerations, *Amino Acids,* 1992, 2, p. 271-284
8. Brückner, H., Westhauser, T., Chromatographic determination of D-amino acids as native constituents of vegetables and fruits, *Chromatographia,* 1994, 39, p. 419-426
9. Brückner, H., Schieber, A., Determination of amino-acid enantiomers in human urine and blood serum by gas chromatography-mass spectrometry, *Biomed. Chromatogr.*, 2001, 15, p. 166–172
10. Brückner, H., Justus, J., Kirschbaum, J., Saccharide induced racemization of amino acids in the course of the Maillard reaction, *Amino acids,* 2001, 21, p. 429-433
11. Brückner, H., Westhauser, T., Chromatographic determination of of D- and L-amino acids in plants, *Amino Acids*, 2003, 24, p. 43-55
12. Bunjapamai, S., Mahoney, R.R., Fagerson, I.S., Determination of D-amino acids in some processed foods and effect of racemization on *in vitro* digestibility of casein, *J. Food Sci.,* 1982, 47, 1229-1234
13. Calabrese, M., Stancher, B., Riccobon, P., High-performance liquid chromatography determination of praline isomers in Italian wines, *J. Sci. Food Agric.*, 1995, 69, p. 361-366
14. Calver, A., Collier, J., Vallance, P., Dilator actions of arginine in human peripheral vasculature, *Clin. Sci. (Lond).*, 1991, 81, p. 695-700
15. Casal, S., Oliveira, M.B., Ferreira, M.A., Gas chromatographic quantification of amino acid enantiomers in food matrices by their N(O,S)-ethoxycarbonyl heptafluorobutyl ester derivatives, *J. Chromatogr. A*, 2000, 866, p. 221–230
16. Chiavaro, E., Bonardi, S., Maggi, E., *Ind. aliment.,* 1998, 37(376), p. 1397-1400
17. Chiavaro, E., Caligiani, A., Palla, G., Chiral indicators of aging in balsamic vinegars of Modena, *Ital. J. Food Sci.*, 1998, 10, p. 327-329
18. Cortes-Rojo, C., Clemente-Guerrero, M., Saavedra-Molina, A., Effects of D-amino acids on lipoperoxidation in rat liver and kidney mitochondria, *Amino Acids*, 2007, 32, p. 31-37
19. Csapó, J., Csapó-Kiss, Z., Stefler, J., Martín, T.G., Némethy, S., Influence of mastitis on D-amino acid content of milk, *J. Dairy Sci.,* 1995, 78, p. 2375–2381
20. Csapó, J., Varga-Visi, E., Lóki, K., Albert, Cs., The influence of manufacture on the free D-amino acid content of Cheddar cheese, *Amino Acids,* 2007, 32, p. 39-43
21. D'Aniello, A., D-Aspartic acid: an endogenous amino acid with an important neuroendocrine role, *Brain Research Reviews* 2007, 53(2), p. 215-234
22. D'Aniello, G., Ronsini, S., Guida, F., Spinelli, P., D'Aniello, A., Occurrence of D-aspartic acid in human seminal plasma and spermatozoa: Possible role in reproduction, *Fertility and Sterility,* 2005, 84(5), p. 1444-1449

23. Ercal, N., Luo, X., Matthews, R.H., Armstrong, D.W., *In vitro* study of the metabolic effects of D-amino acids, *Chirality,* 1996, 8, p. 24-29
24. Estevens, E.R., Esguerra, M., Kim, P.M., Newman, E.A., Snyder, S.H., Zahs, K.R., Miller, R.F., D-Serine and serine-racemase are present in the vertebrate retina and contribute to the physiological activation of NMDA receptors, *Proc. Nat. Acad. Sci. USA,* 2003, 100, p. 6789–6794
25. Fang, J., Sawa, T., Akaike, T., Maeda, H., Tumor-targeted delivery of polyethylene glycol-conjugated D-amino acid oxidase for antitumor therapy *via* enzymatic generation of hydrogen peroxide, *Cancer Research,* 2002, 62, p. 3138-3143
26. Fangmeier, N., Leistner, E., Conversion of D-lysine into L-lysine *via* L-pipecolic acid in *Nicotiana glauca* L. plants and cells suspension cultures, *J. Chem. Soc. Perkin Trans.,* 1981, 1, p. 1769-1772
27. Felbeck, H., Wiley, S., Free D-amino acids in the tissues of marine bivalves, *Biol. Bull.*, 1987, 173, p. 252–259
28. Fisher, G.H., Appearance of D-amino acids during aging: D-amino acids in tumor proteins, *EXS*, 1998, 85, p. 109–118
29. Friedman, M., Gumbmann, M.R., Masters, P.M., Protein-alkali reactions: chemistry, toxicology and nutritional consequences, in *"Nutritional and toxicological aspects of food safety"*, edited by Friedman M., Plenum, New York, 1984
30. Friedman, M., Liardon, R., Racemization kinetics of amino acid residues in alkali-treated soybean proteins, *J. Agric. Food Chem.*, 1985, 33, p. 666-672
31. Fujii, N., Shimmoyo, Y., Sakai, M., Sadakane, Y., Nakamura, T., Morimoto, Y., Kinouchi, T., Goto, Y., Lampi, K., Age-related changes of alpha-crystallin aggregate in human lens, *Amino Acids,* 2007, 32, p. 87-94
32. Fuse, M., Hayase, F., Kato, H., Digestibility of proteins and racemization of amino acid residues in roasted foods, *J. Jpn. Soc. Nutr. Food Sci.,* 1984, 37, p. 349-354
33. Ganote, C.E., Peterson, D.R., Carone, F.A., The nature of D-serine-induced nephrotoxicity, *Am. J. Pathol.,* 1974, 77, p. 269–276
34. Glatt, H., Oesch, F., Mutagenicity of cysteine and penicillamine and its enantiomeric selectivity, *Biochem Pharmacol*, 1985, 34(20), p. 3725-3728
35. Gobetti, M., Simonetti, M.S., Rossi, J., Cossigni, L., Corsetti, A., Damiani, P., Free D- and L-amino acids evolving during sourdaough fermentation and baking, *J. Food Sci.,* 1994, 59, p. 881-884
36. Gonzalez-Hernández, J.C., Aguilera-Aguirre, L., Pérez-Vazquez, V., Ramírez, J., Clemente-Guerrero, M., Cortés-Rojo, C., Saavedra-Molina, A., Effect of the amino acids on some mitochondrial functions in rat liver, *Amino Acids,* 2003, 24, p. 163–169

37. Hamase, K., Morikawa, A., Zaitsu, K., D-Amino acids in mammals and their diagnostic value, *J. chromatogr., B, Biomed. Sci. Appl.*, 2002, 781(1-2), p. 73-91
38. Hamase, K., Morikawa, A., Zaitsu, K., D-amino acids in mammals and their diagnostic value, *J. Chromatogr. B,* 2002, 781, p. 73–91
39. Hamase, K., Sensitive two-dimensional determination of small amounts of D-amino acids in mammals and the study on their functions, *Chem. Pharmaceut. Bull.,* 2007, 55(4), p. 503-510
40. Hansford, K.A., Reid, R.C., Clark, C.I., Tyndall, J.D.A., Whitehouse, M.W., Guthrie, T., McGeary, R.P., Schafer, K., Martin, J.L., Fairlie, D.P., D-Tyrosine as a chiral precursor to potent inhibitors of human nonpancreatic secretory phospholipase A2 (IIa) with anti-inflammatory activity, *Chembiochem.,* 2003, 4, p. 181–185
41. Hayashi, R., Lysinoalanine as a metal chelator: an implication for toxicity, *J. Biol. Chem.,* 1982, 257, p. 13896-13898
42. Hayase, F., Kato, H., Fujimaki, M., Racemization of amino acid residues in proteins and poly(L-amino)acids during roasting, *J. Agric. Food Chem.,* 1975, 23, p. 491-494
43. Helfman, P.M., Bada, J.L., Aspartic acid racemization in tooth enamel from living humans, *Proc. Natl. Acad. Sci. USA,* 1975, 72, p. 2891-2894
44. Helfman, P.M., Bada, J.L., Aspartic acid racemization in dentine as a measure of aging, *Nature* 1976, 262, p. 279-281
45. Hoffmann, K, Schneider-Scherzer, E, Kleinkauf, H, Zocher, R., Purification and characterization of eucaryotic alanine racemase acting as key enzyme in cyclosporin biosynthesis, *J. Biol. Chem.,* 1994, 269, p. 12710–12714
46. Homma, H., *Amino Acids,* 2007, 32, p. 3-11
47. Iumatov, E.A., Sarychev, E.I., Kozlovskii, I.I., Mineeva, M.F., Demidov, V.M., Morozov, I.S., Kozlovskaia, M.M., Enhanced resistance to emotional stress through the use of D-phenylalanine, *Zh. Vyssh. Nerv. Deiat. Im. I. P. Pavlova,* 1991, 41(1), p. 156-61
48. Kvenvolden, K.A., Advances in the geochemistry of amino acids, in "Annual Review of Earth and Planetary Science", edited by Donath F.A., 1975, 3, p. 183-212
49. Liardon, R., Hurrell, R.F., Amino acid racemization in heated and alkali-treated proteins, *J. Agric. Food Chem.,* 1983, 31, p. 432-437
50. Linden, G., Lorient, D., Amino acids and peptides, in "New ingredients in food Processing: biochemistry and agriculture", Woodhead Publishing, Cambridge, 1999

51. Long, Z., Homma, H., Lee, J.A., Fukushima, T., Santa, T., Iwatsubo, T., Yamada, R., Imai, K., Biosynthesis of D-aspartate in mammalian cells, *FEBS Lett.,* 1989, 43, 231-235
52. Man, E.H., Bada, J.L., Dietary D-amino acids, *Annu. Rev. Nutr.,* 1987, 7, p. 209–225
53. Master, P.M., Friedman, M., Racemization of amino acids in alkali-treated food proteins, *J. Agric. Food Chem.,* 1979, 27, p. 507-511
54. Miller, R.F., D-Serine as a glial modulator of nerve cells, *Glia.,* 2004, 47, p. 275–283
55. Morath, S., von Aulock, S., Hartung, T., Structure/function relationships of lipoteichoic acids, *Journal of Endotoxin Research*, 2005, 11(6), p. 348-356
56. Nagata, Y., Akino, T., Ohno, K., Kataoka, Y., Ueda, T., Sakurai, S., Shiroshita, K., Yasuda, T., Free D-amino acids in human plasma in relation to senescence and renal disease, *Clin. Sci.,* 1987, 73, p. 105-108
57. Nagata, Y., Sato, T., Enomoto, N., Ishii, Y., Sasaki, K., Yamada, T., *Amino Acids,* 2007, 32, p. 137-140
58. Navarro, E, Alonso, S.J., Martin, F.A., Castellano, M.A., Toxicological and pharmacological effects of D-arginine, *Basic Clin. Pharmacol. Toxicol.,* 2005, 97(3), p. 149-154
59. Oguri, S., Nomura, M., Fujita, Y., A new strategy for the selective determination of D-amino acids: enzymatic and chemical modification for pre-column derivatization, *J. Chromatogr. A,* 2005, 1078, p. 51-58
60. Oguri, S., Watanabe, K., Nozu, A., Kamiya, A., Screening of D-amino acid oxidase inhibitor by a new multi-assay method, *Food Chem.*, 2007, 100 (2), p. 616-622
61. Ohtani, S., Yamamoto, T., Masushima, Y., Kobayashi, Y., Changes in the amount of D-aspartic acid in the femur with age, *Growth Dev. Aging,* 1998, 62, p. 141-148
62. Onat, F., Toker, F., Aslan, N., Oktay, S., Berkman, K., Antinociceptive effect of D-aspartic acid in mice, *Pharmacology Biochemistry and Behavior* 1995, 51(4), p. 715-719
63. Palla, G., Marchelli, R., Decennia, A., Casnati, G., Occurence of D-amino acids in foods, *J. Chromatogr.,* 1989, 33, p. 577-582
64. Pawlowska, M., Armstrong, D.W., Evaluation of enantiomeric purity of selected amino acids in honey, *Chirality,* 1994, 2, p. 270-276
65. Pätzold, R., Brückner, H., Gas chromatographic determination and mechanism of formation of D-amino acids occurring in fermented and roasted cocoa beans, cocoa powder, chocolate and cocoa shell, *Amino Acids,* 2006, 31, p. 63-72
66. Roberts, J.C., *Amino acids* 1995, 8(2), p. 113-124

67. Robins, J., Jones, M., Matisoo-Smith, E., Amino acid racemization dating in New Zealand: An overview and Bibliography, Auckland University, Auckland, New Zealand, March 20, 2001
68. Sasamura, T., Matsuda, A., Kokuba, Y., Tumor growth inhibition and nutritional effect of D-amino acid solution in AH109A hepatoma-bearing rats, *J. Nutr. Sci. Vitaminol.* (Tokyo), 1998, 44(1), p. 79-87
69. Schell, M.J., Molliver, M.E., Snyder, S.H., D-serine, an endogenous synaptic modulator: localization to astrocytes and glutamate-stimulated release, *Proc. Natl. Acad. Sci. USA*, 1995, 92(9), p. 3948–3952
70. Shibata, K., Niacin Activity of D-Tryptophan in Rats, *Reports of the Research Committee of Essential Amino Acids*, Japan, 1999, 154, p. 43-48
71. Shibata, K., Sawabe, M., Fukuwatari, T., Sugimoto, E., Efficiency of D-Tryptophan as Niacin in Rats, *Biosci. Biotechnol. Biochem.,* 2000, 64(1), p. 206-209
72. Spatz, H., Heller, B., Nachón, M., Fischer, E., Effects of D-phenylalanine on clinical picture and phenethylaminuria in depression, *J. Biol. Psychiat.* 1975, 10, p. 235
73. Stegman, L.D., Zheng, H., Neal, E.R., Ben-Yoseph, O., Pollegioni, L., Pilone, M.S., Ross, B.D, Induction of cytotoxic oxidative stress by D-alanine in brain tumor cells expressing *Rhodotorula gracilis* D-amino acid oxidase: A cancer gene therapy strategy, *Hum. Gene Ther.,* 1998, 9, p. 185–193
74. Szende, B., Tyihak, E., Trezl, L., Role of arginine and its methylated derivatives in cancer biology and treatment, *Cancer Cell. Int.,* 2001, 1, p. 3-8
75. Tipper, D.J., Wright, A., The structure and biosynthesis of bacteria cell walls, in "*The bacteria*", edited by Sokatch J.R., Ornston L.N., Academic Press, New York, 1979
76. Tsukamoto, S., Kanegae, T., Nagoya, T., Shimamura, M., Mieda, Y., Nomura, M., Hojo, K., Okubo, H., Effects of amino acids on acute alcohol intoxication in mice-concentrations of ethanol, acetaldehyde, acetate and acetone in blood and tissues, *Arukoru Kenkyuto Yakubutsu Ison (JAPAN),* 1990, 25(5), p. 429-40
77. Vandenabeele-Trambouze, O., Albert, M., Boyle, C., Couderc, F., Commeyras, A., Despois, D., Dobrijevic, M., Grenier-Lousta-lot, M.F., Chiral determination of amino acids by capillary electrophoresis and laser-induced fluorescence at picomolar concentrations, *J. Chromatogr. A*, 2000, 894, p. 259–266
78. Walsh, N.E., Ramamurthy, S., Schoenfeld, L., Hoffman, J., Analgesic effectiveness of D-phenylalanine in chronic pain patients, *Arch. Phys. Med. Rehabil.*, 1986, 67, p. 436–439
79. Wolosker, H., Sheth, K.N., Takahashi, M., Mother, J.P., Brady, R.O.Jr., Firris, C.R., Snyder, S.H., Purification of serine-racemase: biosynthesis of the neuromodulator D-Ser, *Proc. Natl. Acad. Sci. USA*, 1999, 96, p. 721-725

80. Wolosker, H., Panizzutti, R., De Miranda, J., Neurobiology through the looking-glass: D-serine a new glial-derived transmitter, *Neurochem. Int.,* 2002, 41, p. 327–332
81. Yoshimura, T., Esak, N., Amino acid racemases: Functions and mechanisms, J. Biosci. Bioeng. 2003, 96(2), p. 103-109