Preface

Arginine is a conditionally nonessential amino acid, meaning most of the time it can be manufactured by the human body, and does not need to be obtained directly through the diet. Arginine plays an important role in cell division, the healing of wounds, removing ammonia from the body, immune function, and the release of hormones. This book presents topical research in the study of arginine amino acids, including the techniques and methodologies used for the measurement of arginine in different matrixes; alternative metabolic pathways of arginine and their pathophysiological roles; the biosynthesis of arginine in the mammalian liver and kidney; the relationship between the structure and toxicity evaluated by in vitro methods of a series of arginine-based surfactants; the physico-chemical properties of arginine; and the central functions of L-arginine and its metabolites for stress behavior.

Chapter 1 - L-Arginine is one of the most versatile amino acids from the metabolic and physiological point of view. Known funtions for arginine include: substrate for protein and biologically active peptide synthesis, intermediate in ammonia detoxification, hormone liberation, and poliamine and creatine biosynthesis. All these funtions have been recently increased with the discovery of its role as the substrate for the synthesis of nitric oxide, a multifuntional effector involved in vasodilation, neurotransmission, and inmune responses. From the nutritional point of view, L-arginine must be considered as a "semiessential" or "conditionally essential" amino acid in mammals, depending on the developmental stage and health status of the individual. Therefore, the endogenous synthesis and dietary supply both contribute to satisfy arginine needs. In man, biosynthesis seems to be high enough to fulfil normal physiological arginine demand consumption of the amino acid. Such a situation suggests a potential therapeutic use for dietary arginine administration that is just starting to be analyzed. Hence, there is a need for the accurate and reliable quantification of arginine in food and various biological fluids. This current chapter gives a comprehensive overview of the techniques and methodologies currently available for the measurement of arginine in different matrixes. Arginine, as well as the rest of the amino acids, usually needs to be derivatized to make it more detectable. Several derivatizing reagents have been employed for the determination of arginine and each has its advantages and disadvantages.

Chapter 2 - Arginine is not only a protein constituent but it is metabolized through various alternative pathways in mammalian cells. The author review is focused on the two most important alternative pathways: the nitric oxide (NO) synthesis and the arginase reaction. In cells where both pathways are active, their regulation is generally reciprocal due

to the common substrate. This reciprocal regulation was described at the level of both enzyme activities and expressions. Cross inhibition between arginase and NO synthase metabolites can be observed and different cytokines act differently on the expression of both enzymes as well. Arginase and NO synthase isoenzymes are involved in various important physiological processes such as urea cycle, vasodilation, immune defense against certain invaders and neurotransmission. However, the overexpression of these enzymes and overproduction of NO may contribute to the onset of various pathophysiological processes. NO overproduction may be responsible for neurotoxicity, septic shock, type 1 diabetes mellitus or various inflammatory diseases in numerous organs. In addition, inflammatory responses involving the inducible NO synthase may also contribute to the etiology of metabolic syndrome, while impaired NO production by the endothelial NO synthase may be in the background of cardiovascular disorders or preeclampsia. On the other part, the involvement of high arginase expression was observed in cardiovascular disorders and several pulmonary diseases, as pulmonary hypertension, silicosis and asthma. In conclusion, alternative arginine metabolic pathways and their regulation are important factors both in the maintenance of healthy state and in the pathogenesis of various diseases.

Chapter 3 - Amino acids constitute a class of biologically active compounds found either in the free form or as linear chains in peptides and proteins. In addition to their primary function as protein components, they have several biological roles, being important as neurotransmitters, hormones, precursors of complex nitrogen containing molecules and as metabolic intermediates.

Amino acids are molecules containing an amine group, a carboxylic acid group and a side chain that varies between different amino acids. There are 20 amino acids commonly found in proteins, which are classified depending on the polarity of the side chain. According to this criterion they can be non-polar and neutral, polar and neutral, acidic or basic. In plants they are also involved in secondary metabolism, namely in the biosynthesis of phenolic compounds, glucosinolates, cyanogenic heterosides and alkaloids.

The amino acids composition is a reliable indicator of the nutritional value of matrices used for human consumption, but also a useful tool in natural products authenticity. In this work the authors provide an overview on the application of HPLC-UV-vis and GC-FID analysis on the determination of the amino acids profile of several natural matrices: wild edible mushroom species, Brassica oleracea var. costata, Catharanthus roseus, Cydonia oblonga and red wine inoculated with different Dekkera bruxellensis strains. The influence of different factors, such as the collection date, geographical origin and vegetal tissue, in the amino acids composition of the samples are also discussed.

Chapter 4 - This review summarizes the impressive work performed by several teams of research to discover the biosynthesis of arginine in mammalian liver and kidney and the different steps which led to identify the metabolites involved in this reaction. Successive purification steps allowed the isolation and characterization of two enzymatic proteins, namely argininosuccinate synthetase and argininosuccinate lyase. New approaches including molecular biology gave insights into the molecular characteristics of ASS and ASL genes, mRNAs, and proteins. Furthermore, new techniques such as Northern blot, Western blot, and immunocytology became excellent tools to analyze the expression on ASS and ASL genes in the different organs of several mammalian species. ASS and ASL are homotetramers with subunits of 46 and 51 kDa, respectively. ASS and ASL are generally co-localized in the same cell type and are widely distributed in various organs.

Chapter 5 - The enzymes argininosuccinate synthetase (ASS) and argininosuccinate lyase (ASL) convert L-citrulline into L-arginine. In mammalian kidneys, L-arginine is essentially produced in the proximal convoluted tubules (PCT). Almost all the studies performed on this renal metabolic pathway were restricted to male animals. The authors experiments were first conducted to determine whether female rat kidneys express ASS and ASL genes and the results were compared to those of the males. The expression of ASS and ASL was analyzed by Western blot analyses in the different zones dissected from rat kidneys. ASS protein was localized by immunofluorescence. Finally, the authors tested whether sex influences the renal level of ASS and ASL proteins, as determined by Western blot analyses. The author results reveal that high levels of ASS and ASL proteins were detected in female as well as in male rat kidneys. The relative abundance of ASS and ASL proteins was higher in the cortex (superficial > deep) than in the outer stripe of the outer medulla. Immunolocalization studies clearly showed that ASS expression was restricted to the proximal tubules. PCT exhibited the highest level of ASS compared with the proximal straight tubules (PST). The levels of ASS and ASL proteins were similar in female and male rat kidneys. In conclusion, female rat kidneys express the two enzymes involved in the production of the conditionally essential amino acid L-arginine. No sexual dimorphism in ASS and ASL expression was found in the rat kidney.

Chapter 6 - Surfactants are one of the most representative chemical products which are consumed in large quantities every day on a worldwide scale. The use of surfactants in everyday life is almost unavoidable. The development of less irritant, less toxic, consumer-friendly surfactants or surfactant systems is, therefore, of general interest. During the last 20 years, the author group has been developing new biocompatible surfactants derived from amino acids. Among them, arginine derivative surfactants constitute a novel class that can be regarded as an alternative to conventional cationic surfactants due to their multifuncional properties and the renewable source of raw materials used during the synthesis process. These characteristics make them candidates of choice as additives in pharmaceutical, food and cosmetic formulations. Evaluation of the irritant potential in vivo, of new products or ingredients, for pharmaceutical use, is required by law in most EU countries, prior to human exposure. However, due to increasing concern over animal use and in lights of its potential ban in the near future, alongside with the obvious ethical implications of using directly human subjects, in vitro alternative methods should now be encouraged. This review reports on the relationship between the structure and toxicity evaluated by in vitro methods of a series of arginine-based surfactants including surfactants with one single chain, gemini surfactants, and surfactants with glycerolipid-like structure.

Chapter 7 - L-Arginine (2-amino-5-guanidinpentanoic acid) is a product of great importance in medicine, food and pharmaceutical industry. L-Arginine is a basic, genetically coded α-amino acid, one of the twenty most common natural amino acids. It is essential for human ("semi-essential") [1-3]. Arginine plays a significant role in cell division, healing of wounds, removing ammonia from a body, immune function, and release of hormones [2,4-5].

Chapter 8 - L-Arginine is an essential amino acid for birds, carnivores and young mammals and a conditionally essential amino acid for adults. L-Arginine can be catabolized by four sets of enzymes in mammalian cells, resulting in the production of urea, L-ornithine, L-proline, L-glutamate, polyamines, nitric oxide, creatine, agmatine, etc.. Unlike mammals, birds lack carbamyl phosphate synthetase, one of the urea cycle enzymes necessary for the synthesis of L-citrulline from L-ornithine in the liver and kidney. Therefore, it is impossible

to synthesize L-arginine in birds, and L-arginine is classified as an essential amino acid for birds. In this chapter, the authors introduce recent studies about central functions of L-arginine and its metabolites for stress behavior. In particular, the functions in avian species are focused upon. In neonatal chicks, centrally injected L-arginine induces sedative and hypnotic effects under social separation stress. Among L-arginine metabolites, L-ornithine, L-proline and L-glutamate would especially contribute to these effects.

Chapter 9 - Amino acids (AA) are the most important energetic substrate during fish larval development. Estimation of AA requirements is, therefore, crucial to formulate suitable diets for aquaculture species in order to obtain better larval survival, growth and general fish performance. AA larval requirements can be estimated using the AA profile of fish carcass as it is a good indicator of fish larval requirements. The AA profile can later be corrected with the AA bioavailability and a fair estimation of larval AA requirements is achieved. Once the AA requirements are determined they need to be compared to the AA profile of the fish larval diets. Most fish larval stages are still dependent on live feed, such as rotifers and Artemia, to survive and grow. Therefore, correction of the AA profiles of the live feed must be done enriching or supplementing the live feed diet. The effect of feed supplementation with AA can be tested at long term through zootechnique trials or at short term using the tube-feeding technique. This review intends to evaluate the current knowledge of arginine requirements and the effect of arginine supplementation on the metabolism of this AA in several marine fish larvae such as white seabream, Diplodus sargus, and Senegalese sole, Solea senegalensis. Arginine was chosen because it is an indispensable AA involved in metabolic pathways such as protein synthesis, urea production, metabolism of glutamic acid and proline, and synthesis of creatine and polyamines. It is also considered to be in deficiency in the diet of some fish.

Chapter 10 - Polypeptides composed of arginine-rich domains play a key role in gene regulation, such as nuclear localization signal which can penetrate nuclear membrane. Recent studies indicated that arginine-rich cell-penetrating peptides (CPPs) possess the ability to penetrate plasma membrane without receptors. Length of peptides with arginine residues determines the efficiency of cell internalization. Also, D- or L-form of arginine residues have different effects on internalization efficiency. In the author investigations, arginine-rich CPPs could serve as efficient shuttles to deliver proteins, DNAs, RNAs or nanoparticles (such as quantum dots) into animal or plant cells in a covalent (CPT), noncovalent (NPT), or covalent and noncovalent (CNPT) protein transduction manner. The penetrating mechanism of CPPs was still controversial, but more evidences indicated that these peptides enter cells through multiple internalization pathways. No cytotoxicity and high transduction efficiency highlight great advantages of these CPPs in cargoes or drug delivery. Therefore, studies of arginine-rich CPPs could launch a new page in pharmaceutics, therapeutics or cell biology.

Chapter 11 - Asparagus (Asparagus officinalis L., cv. Welcome) plants inoculated with arbuscular mycorrhizal fungi (AMF) (Glomus intraradices and Gigaspora margarita). Dry weight of shoots and roots were increased in deep-sea-water added asparagus plants with or without presence of AMF, though higher dry weight was observed in deep-sea-water added AMF-inoculated plots. Thus, plant growth promotion via symbiosis appeared in mycorrhizal asparagus plants. As for disease tolerance, incidence and severity of root rot was lower in AMF plants than in non-AMF plants. On the other hand, several free amino acids , such as asparagine, glutamine, aspartic acid, alanine, GABA, tyrosine, ornithine and lysine were increased in shoots and roots of most of the mycorrhizal asparagus plants. From these findings, it is suggested that plant growth promotion through AMF and tolerance to Fusarium

root rot occurred in mycorrhizal asparagus plants. In addition, several free amino acid contents are stimulated by the symbiosis, supposing that the changes might be associated with the disease tolerance.

Chapter 12 - It is known that many regulatory peptides take part in haemostatic reactions of the organism, influence on all phases of blood coagulation and fibrinolysis. At the same time formed in process degradation of regulatory peptides fragments have independent biological activity. For example, many short peptides have fibrinolytic, anticoagulant and antitrombotic effects in blood. By stage-by-stage proteolysis it is possible to define structural conditionality of most regulatory peptides effects and to define the minimum fragment, responsible for its biological activity. Any modification of peptide molecule can cause changes of its biological efficiency. Therefore the study of peptides degradation ways, and also structure and effects of formed fragments, has the great interest and significance. In the present study summarizes the results of own researches about the influence on various links of the haemostasis system of same regulatory peptides fragments and different olygopeptides, containing the arginine amino acid in different positions of peptides chain.

Chapter 13 - Recent studies with chromation immunoprecipitation assay and mutational analysis in binding sites of the regulators demonstrated unexpected biological mechanisms in the transcriptional regulation of ARG1. Two roles of Mcm1p were identified at ARG1: a Gcn4p-mediated positive transcriptional role and a negative role involving Arg80p, Arg81p, and Arg82p. Mcm1p contributed to active transcription at the ARG1 promoter by increasing the binding of the activator Gcn4p and by recruiting the co-activator complex SWI/SNF at ARG1 under Gcn4p-induced conditions. Mutational analysis of the ARG1 promoter also revealed a positive role for the Mcm1p binding site in ARG1 transcription and growth to overcome arginine starvation in the absence of Gcn4p. A transcriptional negative role for Mcm1p was apparent in the recruitment of the whole repressor ArgR/Mcm1p complex, which contributed to dampening the activating function of Gcn4p at ARG1 in arginine-replete cells. Concerning the mechanism of ARG1 transcription, the most interesting finding was that ARG1 transcription could be controlled by different mechanisms with the Mcm1p binding sites through either the presence or absence of complete amino acids under condition of arginine starvation.