Production of Cysteine: Approaches, Challenges and Potential Solution

Nur Izzah Ismail[1], Yumi Zuhanis Has-Yun Hashim[1,3,*], Parveen Jamal[1,3], Rashidi Othman[2,3] and Hamzah Mohd Salleh[1,3]

[1]*Bioprocess and Molecular Engineering Research Unit (BPMERU), Department of Biotechnology Engineering, Kulliyyah of Engineering, International Islamic University Malaysia, P.O. BOX 10, Kuala Lumpur, 50728, Malaysia*

[2]*Department of Landscape Architecture, Kulliyyah of Architecture and Environmental Design, International Islamic University Malaysia, P.O. BOX 10, Kuala Lumpur, 50728, Malaysia*

[3]*Institute for Halal Research and Training (INHART), E52-2 Level 2 Block E5, Kulliyyah of Engineering, International Islamic University Malaysia, P.O. Box 10, 50728, Kuala Lumpur, Malaysia*

Abstract: Cysteine has a wide application in pharmaceutical, foods and cosmetic industries. In the biological system, through its unique properties of sulfur and thiol, cysteine also plays important roles in stability, structure, catalytic activity, and regulation of numerous proteins. In nature, cysteine can be found in animal proteins, fruits, vegetables, legumes and cereal. Due to its wide application, the production of cysteine in large scale is in favour. At present, cysteine is produced from keratin of animal sources as well as through microbial bioconversion and fermentation. Each production method poses its own challenges and limitation; which includes low yield, high-cost and poor consumer acceptance. As such, alternative source for large-scale cysteine production is of interest. Plants are seen to be an attractive substrate for the extraction of cysteine.

Keywords: Amino acid biosynthesis, cysteine production, cysteine purification, sulfur amino acid.

1. INTRODUCTION

Amino acids are compounds of considerable industrial importance, which serve as feed and food additives, taste and aroma enhancers, pharmaceuticals or building blocks for drugs, dietary supplements, nutraceuticals and ingredients in cosmetics. Amino acids are found either in the free-state or as linear chains in peptides and proteins. There are 20 commonly occurring amino acids in proteins which can be classified into essential and non-essential amino acids. Apart from proteins, free amino acids are also being found in biological materials such as plasma and urine and in fruit juice and wine [1]. Cysteine (Cys; C;CAS Number: 52-90-4; chemical formula HO_2CCHCH_2SH), the focus of the study, is one of the non-essential amino acids. Others include alanine, arginine, asparagine, aspartic acid, glutamic acid, glutamine, glycine, proline, serine, and tyrosine. Cysteine is a polar uncharged amino acid that contains thiols (also called mercaptan) which are compounds with sulfhydryl functional group [2]. The low molecular-mass thiol and their disulfides are critical cellular components that play numerous important roles in metabolism and in the antioxidant defense network [3]. As such, cysteine is essential for the entire biological kingdom because of their prominent tasks in primary and secondary metabolism [4].

Although cysteine is categorized as non-essential amino acids, which can be synthesized in the human body, it can be deprived in certain conditions for instance in infants, the elderly, and individuals with certain metabolic diseases. As cysteine can be found in whole foods like nuts, grains, meats, fruits and vegetables, it can be added to the body through intake of these foods or supplements if there is a deficiency.

It was estimated in 2004 that the total amino acid market was $US 4.5billion with annual growth rate of 5-7 % [5]. In line with the increased demand for cysteine, this review is aimed to provide a summary of approaches used to produce cysteine at industrial scale, the challenges and potential solutions.

2. FUNCTIONS AND APPLICATIONS OF CYSTEINE

The high demand for cysteine is due to its multiple roles in metabolism and the myriad of applications in various fields. Figure **1** features the summary of roles and applications of cysteine.

In metabolism, cysteine constitutes the almost exclusive metabolic entrance for reduced sulfur into cell

*Address correspondence to this author at the Bioprocess and Molecular Engineering Research Unit (BPMERU), Department of Biotechnology Engineering, Kulliyyah of Engineering, International Islamic University Malaysia, P.O. BOX 10, Kuala Lumpur, 50728, Malaysia; Tel: +603 6196 5767; Fax: +603 6196 4442; E-mail: yumi@iium.edu.my

ISSN: 1927-3037/14

metabolism where it is required for biosynthesis of essential compounds including methionine, thiamine, biotin, coenzyme A, and Fe/S clusters [6, 7]. Cysteine also plays crucial roles in protein folding, assembly, and stability through disulfide-bond formation [4, 7] as well as in cellular processes such as redox cycles, detoxification of heavy metals and xenobiotics, and metabolism of secondary products [8]. For instance, cysteine was found to have antioxidant properties in fruits and vegetables [2].

In pharmaceutical field, cysteine is used as antidote [9] and it is best known for its ability to counter acetaminophen toxicity. It has been used successfully to treat GSH deficiency in a wide range of infections, genetic defects and metabolic disorders, including HIV infection and chronic obstructive pulmonary disease (COPD) [10, 11]. In addition, cysteine can be used to treat oral cavity infection such as glossitis and gingivitis [12].

In cosmetics area, cysteine is used as a substitute for thioglycolic acid in permanent hair wave preparation due to its ability to break disulfide bonds in keratin. Thioglycolic was previously used to prepare hair for perms but it has unpleasant odor and is an allergenic [13]. Further, cystine (two cysteine molecules formed cysteine) is used in nail care as it promotes proper fingernail growth, hardness and functionality [14]. The acetylated cysteine (*N*-acetylcysteine) is being used in the formulation of safe and effective products for anti-aging and anti-atrophy skin care products [15].

In food industry, especially in bakery application, cysteine is used as flour additives to break up the gluten in flour, thus reducing its stickiness and facilitating the kneading of the dough [13]. Theoretically, protein-protein interactions through covalent disulphide bond formation can be disrupted by adding cysteine [16] which would increase elasticity of the formed dough, helping it to rise during baking [17].

As animal feed, cystine is considered efficacious in partially meeting the requirements of sulphur-containing amino acids in all animal species [18]. L-cysteine has also been shown to have prophylactic potential against nitrate poisoning in ruminant species [19].

3. SOURCES OF CYSTEINE

Cysteine builds disulfide bridges which contribute to the stability of proteins thus enabling the formation of strong fiber strands such as hair, wool and feathers, as well as horns, hooves and nails as they contain large amount of cysteine [13]. As such, these are the common sources of cysteine. However, cysteine can also be found in vegetables and fruits. Study done by Demirkol *et al*. [2] revealed the various levels of cysteine concentration detected in certain fruits and vegetables by HPLC. In vegetables, the cysteine concentrations are ranged between 4 – 349 nM/g wet weight. The lowest concentration is obtained from avocado and the highest is red pepper while no cysteine was detected in carrot, potato and broccoli. Furthermore, the levels of cysteine present in fruits were less compared in vegetables. The highest level of cysteine was 58 nM/g wet weight in papaya and the lowest level was 7nM/g wet weight in lemon.

In dietary proteins, the level of cysteine is relatively low and does not exceed 5% of total amino acids. Among plant proteins, cereal protein contains 3 to 5% of cysteine, while legume protein contains about 2 to 3.5% of cysteine. Generally, animal proteins contain higher level of cysteine compared to vegetable sources [20].

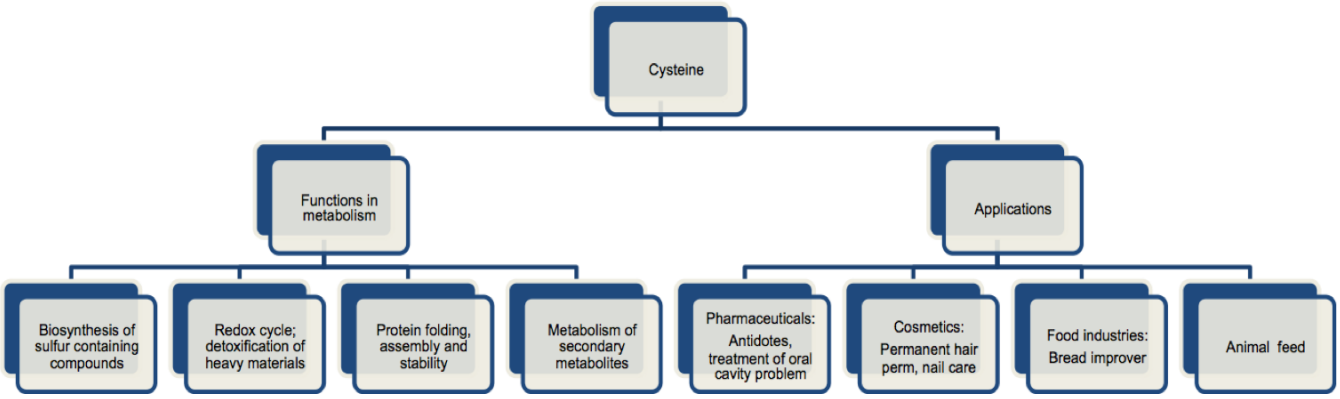

Figure 1: Functions and applications of cysteine.

3.1. Biosynthesis of Cysteine in Microbes and Plants

The synthesis of cysteine in bacteria and plants can be subdivided into three steps as the following: (i) the assimilatory sulfate reduction which provides reduced sulfur in terms of sulfide, (ii) the synthesis of the carbon (C) and nitrogen (N) containing the backbone for cysteine, and (iii) the incorporation of reduced sulfur into the organic backbone [4].

In bacteria, the synthesis of C/N-backbone of cysteine is catalyzed by serine acetyltransferase (SAT, EC 2.3.1.30), which transfers an acetyl-moiety from acetyl-Coenzyme A (acetyl-CoA) to serine, leading to *O*-acetylserine (OAS) formation. Then, *O*-acetylserine (thiol) lyase (OAS-TL, EC 4.2.99.8) is responsible for the synthesis of cysteine in the presence of free sulfide [6, 7, 21].

In contrast, released cysteine represents the first form of reduced organic sulfur in plant cells. The sulfide production and synthesis of most amino acids in higher plants occurs in plastids, cytosol and mitochondria [22-26] because both SAT and OAS-TL are localized in these compartments [27-29]. Synthesis pathway in plants and bacteria is shown in the Eq. 1:

$$\begin{aligned}&\text{L-serine + O-acetyltransferase (SAT) +}\\&\quad\text{acetyl-CoA} \rightarrow \text{O-acetylserine (OAS) + CoA}\\&\text{O-acetylserine (thiol) lyase (OAS-TL) +}\\&\quad H_2S \rightarrow \text{L-cysteine} + H_2O + \text{acetate}\end{aligned} \qquad \text{(Eq. 1)}$$

Production of cysteine from microbes like *E. coli*, *Salmonella typhimurium*, *Corynebacterium glutamicum*, *Lactococcus lactis*, *Pseudomonas putida*, *Saccharomyces cerevisiae*, *Bacillus sphaericus* and Streptomyces sp. has been reported. SAT represents the rate limiting component and its activity is exclusively found in association with OAS-TL in the cysteine synthase complex. OAS-TL is present in large excess due to the activity of SAT-free homodimers [30].

In most bacteria, the precursor for cysteine synthesis is serine. For instance, enteric bacteria like *Salmonella typhimurium* and *E. coli* synthesize cysteine from serine. In addition, the coryneform glutamic acid producing bacteria like *C. glutamicum, Brevibacterium lactofermentum,* also synthesize the cysteine from serine, although they have the methionine biosynthetic pathways. However, some microorganisms like *Saccharomyces cerevisiae, Lactococcus lactis* and *Pseudomonas putida* do synthesize cysteine from methionine.

As compared to *E.coli,* the production of cysteine from *Corynebacterium glutamicum* is relatively low, which is 290 mg/L [31]. In another study, the addition of 3-chloro-L-alanine to the medium enhanced the formation of OAS-TL and it can be substituted for OAS as a substrate for OAS-TL [19]. By this method, the high yield of cysteine was obtained (70g/100 ml). However, large-scale production of cysteine in microorganisms is mainly hampered by intrinsic regulatory mechanism of the cys-regulon as well as cysteine toxicity at elevated concentrations [32].

Cysteine biosynthesis in plants is the most important pathway in the sulfur cycle in nature because the fixation of inorganic sulfur into cysteine. SAT and OAS-TL are the major regulatory factors in the biosynthesis of cysteine in plants. It has been suggested that the availability of OAS is one of the major limiting factor for cysteine biosynthesis in plants [33] and it also acts as the control metabolite for the pathway itself [34]. The SAT and OAS-TL enzymes are strongly evolutionary conserved, both structurally and functionally [35].

Manipulations of plants have been done in aiming for overexpression of these enzymes [34-36].Products of these genes are targeted into three cellular components which are cytoplasm, mitochondria and chloroplast. Overexpression of SAT and OAS-TL in transgenic plants led to increase of plant's potential sulfate assimilation into cysteine, and subsequently into glutathione [34, 35]. Increased level of cysteine and glutathione contents further implies that the accumulation of OAS might induce sulfate intake and reduction which are necessary for cysteine synthesis.

Production of cysteine in plants is aimed on one hand on agronomical traits such as nutritional quality; on the other hand on production of biopharmaceutical and bioactive substances for plant defense [38]. From biotechnology point of view, transgenic plants with high cysteine are developed to improve its agricultural properties like increase the defense systems towards various form of stress; xenobiotics, heavy metals, drought and heat which benefit the plant itself [34]. In addition, they can be potentially applied for the phytoremediation of soil and water or might have an environmental advantages in some conditions, for instance, they can be more tolerant to stress [35]. To the best of our knowledge, there is no large scale production of cysteine from plants nor there is attempt to study the extraction of cysteine from plant for commercial application.

4. PRODUCTION METHODS OF CYSTEINE

Several approaches have been used to produce cysteine at industrial level. This includes extraction of cysteine from keratin hydrolysate, enzymatic bioconversion and fermentation.

4.1. Keratin Hydrolysate

Traditionally, cysteine is produced from keratins through protein hydrolysate. The main sources of keratin are from animal and human materials such as feathers, hair, bristles and hooves. In Asia, China is the largest producer of cysteine. Cysteine is extracted using activated charcoal and concentrated hydrochloric acid prior to electrolysis. One ton of hair yields 100 kg of cysteine and it requires 27 kg of hydrochloric acid to extract 1 kg of hair [13]. However, feathers are the material of choice in China for cysteine production. Although this is the easiest way to produce cysteine but it suffers from a low yield, unpleasant odor and problem of waste treatment [39].

4.2. Enzymatic Bioconversion

Cysteine is being produced through enzymatic process by conversion of DL-2-amino-Δ^2-thiazoline-4-carboxylic acid (DL-ATC) to cysteine using *Pseudomonas* sp. [7, 9, 39]. The authors described this method as having potential in producing high yield with low energy requirement during production. Using this approach, DL-ATC is chemically synthesized and the conversion of DL-ATC to cysteine involved three enzymes; ATC racemase, L-ATC hydrolase and S-carbamyl-L-cysteine hydrolase [39] with the following successive steps: (i) enzymatic reclaimation of D-ATC to L-ATC; (ii) a ring-opening reaction of L-ATC to *N*-carbamyl-L-cysteine (L-NCC) as intermediate; and (iii) hydrolysis of L-NCC to L-cysteine [7, 39].

Enzymatic production of cysteine can also be achieved using tryptophan synthase of *Escherichia coli* which catalyzed the synthesis of cysteine from various β-substituted-L-alanines and sulfides with L-serine as the best substrate. In order to make the approach cost effective, glycine is used to produce L-serine [9].

4.3. Fermentation

High production of cysteine in small scale fermentation can be achieved using cysteine-producing bacterial strains cultured in different media composition. This approach has been reported to minimize the cost and has potential commercial exploitation [40].

In large scale production, advance technology is used to ferment the improved strain-producing cysteine that will produce high yields. Wacker Fine Chemical is one of the industrial producers of cysteine through fermentation. The company uses a new approach by optimizing the existing metabolism of the bacterium's native metabolism. This enable large quantity of cysteine to be released by the bacterium into the fermentation media, as 90 percent of pure cysteine ends up in the final product which fulfills the quality standards for foods and pharmaceutical industries [17].

Generally, for effective production of cysteine through fermentation, the improvement of precursor supply and weakening of product reuptakes are very important. L-serine is the precursor for L-cysteine; however it is very difficult to produce L-serine by direct fermentation. Thus, it requires metabolic engineering approach to produce L-cysteine by combining the overexpression of genes encoding for serine-biosynthetic enzymes and deletion of gene encoding for serine-degrading enzyme [41]. In addition to feedback inhibition-insensitive SAT, low activity of cysteine degradation and high activity of cysteine-export in the host cells are very crucial for efficient cysteine fermentation.

5. ISOLATION AND PURIFICATION OF CYSTEINE

Direct and cost effective process for isolation and purification of cysteine from complex substance mixture are still considered unsolved problems especially in large scale productions [42]. Currently, there are several processes to purify cysteine from complex mixture (keratin hydrolysate) or fermenter broth. The processes to purify cysteine are summarized in Table **2**.

6. CONCLUSION

In conclusion, cysteine has a great versatile role in our life. The production of cysteine in large scale is in favour. Concerning about the safety and the acceptance of the society, production of cysteine should meet the requirement of the legislation especially in food and pharmaceutical industries. Conventionally, cysteine is produced from keratin of animal sources which poses the halal/haram issues as well as not being accepted by certain communities like the vegetarians. Meanwhile, the production of cysteine

Table 1: Comparison of Cysteine Production by Various Methods

Method of production	Raw material	Details procedure	Challenges	Remarks	References
Extraction from protein hydrolysates	Human hair and animal feathers	Extraction from acid hydrolysate of the keratinous protein in human hairs and animal feathers	Low yield, unpleasant odors, problem of waste treatment	Impose HALAL issues	[9,17]
Bioconversion process by *Pseudomonas*	Asymmetrical hydrolysis of DL-2-amino-Δ^2-thiazoline-4carboxylic acid (chemically synthesis)	*Pseudomonas* exhibit activities involved in asymmetrical hydrolysis of DL-2-amino-Δ^2-thiazoline-4carboxylic acid (D-ATC) to L-cysteine. Steps involved: Enzymatic racemization of D-ATC to L-ATC A ring-opening reaction of L-ATC to N-carbomonyl-L-cysteine (L-NCC) as intermediate Hydrolysis of L-NCC to L-cysteine	Unstable L-cysteine producing enzyme that is formed in *Pseudomonas*, limited production of L-cysteine due to product inhibition	Industrially used by Ajinomoto Co.	[40]
Biosynthesis	*Corynebacterium glutamicum*	Three-step pathway: Basically, L-cysteine is synthesized through the same pathway as *E.coli*, although *C.glutamicum* has two methionine biosynthetic pathways (direct sulfuration and transsulfuration pathways unlike *E. coli.* Both SAT and OAS of *C. glutamicum* have been partially purified and characterized.	It has 2 methionine pathways but it cannot synthesized L-cysteine from L-methionine	Production relatively low compared to *E.coli* (290 mg/L)	[41]
	Tryptophan synthase of *E. coli*	Bacterial strain, *E. coli* was cultured in minimal medium supplemented with acid hydrolyzed casein and L-tryptophan Tryptophan synthase of *E. coli* catalyzed the synthesis of L-cysteine from various β-substitute-L-alanines (e.g: L-serine) and sulfides	L-serine was the best substrate for cysteine production, but it is relatively expensive	Under optimum condition, 114 g/l of cysteine was formed in a molar yield of 94% based in L-serine added	[9]
Enzymatic	*Bacillus sphaericus*	Bacteria strain and cultivation Preparation of resting cells Resting cell reaction of l-cysteine production Analytical method of l-cysteine: Gaitone's acid ninhydrin Amino acid analyzer	Only L-isomer of 3-chloroalanine was converted to L-cysteine, which is a major limitation for its commercial application	Enabled the highest production of L-cysteine (7g/100ml) and with 80-85% conversion of the added 3-chloro-L-alanine	[21]
Microbial fermentation	Direct fermentation from glucose	There have been two approaches to obtaining an SAT that is less sensitive to feedback inhibition: The engineering of SAT from *E. coli* through site directed or random The use of the natural SAT, which is desentisized from higher plants	Cysteine degradation in media	SAT that is less sensitive to feedback inhibition is used in this study	[9]

Table 2: Isolation and Purification Process of Cysteine. Adapted from Boehm [42]

Process/Method	Detail procedures	Drawback/ Weakness
Isolation of soluble cystine in purified form	Cysteine and other derivatives are converted to cystine. Cystine is reduced subsequently to give cysteine	Complicated and cysteine must be prepared in two- stage process *via* intermediate cystine
Direct isolation of cysteine from a solution containing cysteine, cystine, serine and inorganic salt	Cystine and inorganic salt are crystallized at 20°C by adding hydrogen chloride and removed by filtration. Remaining solution contains cysteine,	Limited to the solution that contained serine, cysteine, cystine and inorganic salt. Low yield and low purity
Combination of ion exchange, crystallization and other known methods	No detail information on specific procedures	-
Removal of solids by centrifugation or membrane filtration with subsequent isolation and purify of amino acid by means of ion exchange, concentration and crystallization	No detail information on specific procedures	-
Ion exchange chromatography	Fermenter broth comprises of an oxidizing agent, cysteine binding to an ion exchanger and form ion exchange as an eluent	Simple, cost effective and applicable for industrial application

from bacteria through bioconversion and fermentation is hampered by its high start-up cost and inherent inhibition feedback pathway resulting in low yield.As there is at present, no production or extraction of cysteine from plant source in large scale, it is very timely to explore this area.

ACKNOWLEDGEMENT

The authors have no conflict of interest regarding this paper. This study was supported by IIUM Research Grant.

REFERENCES

[1] Margaret IT. Amino acid analysis: an overview. In Cooper C, Packer N, Williams, K. eds., Amino acid analysis protocols. Humana Press: New Jersey 2001, pp. 1-7.

[2] Demirkol O, Adams C, Ercal N. Biologically important thiols in various vegetables and fruits. J Agric Food Chem 2004; 52: 8151-4. http://dx.doi.org/10.1021/jf040266f

[3] Kusmierek K, Bald E. Reduced and total glutathione and cysteine profile of citrus fruit juices using liquid chromatography. Food Chem 2008; 106: 340-4. http://dx.doi.org/10.1016/j.foodchem.2007.05.043

[4] Wirtz M, Droux M. Synthesis of the sulfur amino acids: cysteine and methionine. Photosynth Res 2005; 86: 345-62. http://dx.doi.org/10.1007/s11120-005-8810-9

[5] Leuchtenberger W, Huthmacher K, Drauz K. Biotechnological production of amino acidsand derivatives: current status and prospects. ApplMicrobiolBiotechnol 2005; 69: 1–8. http://dx.doi.org/10.1007/s00253-005-0155-y

[6] Wirtz M, Droux M, Hell R. O-acetylserine (thiol) lyase: an enigmatic enzyme of plant cysteine biosynthesis revisited in *Arabidopsis thaliana.* J Exp Bot 2004; 55: 1785-98. http://dx.doi.org/10.1093/jxb/erh201

[7] Wada M, Takagi H. Metabolic pathways and biotechnology of L-cysteine. Appl Microbiol Biotechnol 2006; 73: 48-54. http://dx.doi.org/10.1007/s00253-006-0587-z

[8] Saito K. Regulation of sulfate transport and synthesis of sulfur-containing amino acids. Curr Opin Plant Biol2000; 3: 188-95.

[9] Ishiwata K, Nakamura T, Shimada M, Makiguchi N. Enzymatic production of L-cysteine with tryptophan synthase of *Escherichia coli*. J Fermentation and Bioeng1989; 67: 169-72.

[10] Atruki KR, Mantovani JJ, Herzenberg LA, Herzenberg LA. N-acetylcysteine: a safe antidote for cysteine/GSH deficiency. Curr Opin Pharmacol 2007; 7: 1-5. http://dx.doi.org/ 10.1016/j.coph.2007.04.005

[11] Millea PJ. N-actylcysteine: ultiple clinical applications. Am Fam Physician 2009; 80: 265-69.

[12] Ziggioti A, Lualdi P. Mouth-soluble pharmaceutical compositions containing acetyl-cysteine. United State Patent US 4,970,236.1990.

[13] Renneberg R. High grade cysteine no longer has to be extraxted from hair. In Demain, AL, eds. Biotechnology for beginners. Academic Press: Amsterdam 2008, pp.106.

[14] Iorizzo M, Piraccini BM, Tosti A. Nail cosmetics in nail disorders. J Cosmet Dermatol 2007; 6: 53-8. http://dx.doi.org/10.1111/j.1473-2165.2007.00290.x

[15] Hillebrand G, Bush RD. Use of N-acatyl-L-cysteine and derivatives for regulating skin wrinkles and/or skin atrophy. Great Britain Patent EP 0 734 718 A2. 1992.

[16] Lambert IA, Kokini JL. Effect of L-cysteine on the rheological properties of wheat flour. Cereal Chem 2001; 78: 226-30.

[17] Wacker Fermentation-Grade. Discovery a new dimension of pureness: vegetarian cysteine. Germany: Wacker Chemie AG. 2010

[18] European Food Safety Authority (EFSA). Scientific Opinion on the safety and efficacy of L-cystine for all animal species. EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), Parma, Italy EFSA Journal 2013; 11(4): 3173

[19] Takahashi, J, Young, BA. Prophylactic effect of L-cysteine on nitrate-induced alterations in respiratory exchange and metabolic rate in sheep. Anim Feed Sci and Tech 1991; 35: 105–13. http://dx.doi.org/10.1016/0377-8401(91)90103-Y

[20] Bos C, Huneau J, Gaudichon C. Sulfur amino acids contents of dietary proteins: daily intakes and requirement. In Masella R, Mazza G, eds. Glutathione and sulfur amino acids in human and health and disease. John Wiley & Sons Inc: New Jersey 2009, pp. 21-33.

[21] Dhillon GS, Nagasawa T, Yamada H. Microbial process for L-cysteine production. Enzyme Microb Technol 1987; 9: 277-80. http://dx.doi.org/10.1016/0141-0229(87)90003-2

[22] Droux M. Plant serine acetyltransferase: new insights for regulation of sulphur metabolism in plant cells. Plant Physiol Biochem 2003; 41: 619-27. http://dx.doi.org/10.1016/S0981-9428(03)00083-4

[23] Lunn JE, Droux M, Martin J, Dounce R. Localization of ATP-sulfurylase and O-acetylserine(thiol)lyase in spinach leaves. Plant Physiol 1990; 94: 1345-52. http://dx/doi/10.1104/pp.94.3.1345

[24] Rolland N, Droux M, Dounce R. Subcellular distribution of O-acetylserine (thiol) lyase in cauliflower (*Brassica oleracae* L.) in fluorescence. Plant Physiol 1992; 98: 927-35. http://dx.doi.org/10.1104/pp.98.3.927

[25] Ruffet M-L, Droux M, Dounce R. Subcellular distribution of serine acetytransferase from *Pusimsativum* and characterization of an *Arabidopsis thaliana* putative cytosolic isoform. European J Biochem 1995; 227: 500-9.

[26] Wirtz M, Hell R. Production of cysteine for bacterial and plant biotechnology: Application of cysteine feedback-insensitive isoforms of serine acetyltranferase. Amino Acids 2003; 24: 195-203. http://dx.doi.org/10.1007/s00726-002-0313-9

[27] Brunold C, Rennenberg H. Regulation of sulfur metabolism in plants: first molecular approaches. Prog Bot 1997; 58: 164-86. http://dx.doi.org/10.1007/978-3-642-60458-4_7

[28] Hell R. Molecular physiology of plant sulfur metabolism. Planta 1997; 202: 138-48. http://dx.doi.org/10.1007/s004250050112

[29] Hesse M, Mayer U, Jurgens G. Cytokinesis in flowering plants: cellular process and development integration. Curr Opin Plant Biol 1998; 1: 486-91. http://dx.doi.org/10.1016/S1369-5266(98)80040-X

[30] Droux M, Ruffet M-L, Douce R, Job D. Interactions between serine acetyltransferase and O-acetylserine(thiol) lyase in higher plants. Structural and kinetic properties of the free and bound enzymes. Eur J Biochem 1998; 255: 235-45. http://dx.doi.org/10.1046/j.1432-1327.1998.2550235.x

[31] Wada M, Awano N, Haisa K, Takagi H, Nakamori S. Purification, characterization and identification of cysteine desulfhydrase of *Corynebacteriumglutamicum*, and its relationship to cysteine production. FEMS Microbiol Lett 2002; 217: 103-7.

[32] Soerensen MA, Pedersen S. Cysteine, even in low concentrations, induces transient amino acid starvation in *Escherichia coli*. J Bacteriol 1991; 173: 5244-6.

[33] Saito K, Yokoyama H, Noji M, Murakoshi I. Molecular cloning and characterization of plant serine acetyltransferase playing a regulatory role in cysteine biosynthesis from watermelon. J Biol Chem 1995; 270: 16321-6. http://dx.doi.org/10.1074/jbc.270.27.1632

[34] Harms K, von Ballmoos P, Brunold C, Hofgen R, Hesse H. Expression of a bacterial serine acetyltransferase in transgenic potato plants leads to increased level of cysteine and glutathione. Plant J 2000; 22: 335-43. http://dx.doi.org/10.1046/j.1365-313x.2000.00743.x

[35] Sirko A, Blaszczyk A, Liszewska F. Overproduction of SAT and/or OASTL in transgenic plants: a survey of effects. J Exp Bot 2004; 55(44): 1881-8. http://dx.doi/10.1093/jxb/erh151

[36] Urano Y, Tomofumi M, Noji M, Saito K. Molecular cloning and functional characterization of cDNAs encoding cysteine synthase and serine acetyltransferase that may be responsible for high cellular cysteine content in *Allium tubersom*. Gene 2000; 257: 267-77. http://dx.doi/10.1016/S0378-1119(00)00399-1

[37] Saito K, Kurosawa M, Tatsuguchi K, Takagi Y, Murakoshi I. Modulation of cysteine biosynthesis in chloroplast of transgenic tobacco overexpressing cysteine synthase [O-Acetylserine(thiol)-lyase]. Plant Physiol 1994; 106: 887-95. http://dx.doi.org/10.1104/pp.106.3.887

[38] Hoefgen R, Kreft O, Willmitzer L, Hesse H. Manipulation of plant thiol contents in plants. Amino Acids 2001; 20: 291-9.

[39] Ryu OH, Ju JY, Shin CS. Continuous L-cysteine production using immobilized cell reactors and product extractors. Process Biochem 1997; 32: 201-9. http://dx.doi.org/10.1016/S0032-9592(96)00061-1

[40] Ali NM, Shakoori FR, Shakoori, AR. Improvement in cysteine production by local bacterial isolates. Pak J Zool 2011; 43: 805-808.

[41] Peter-Wendisch P, Stolz M, Etterich H, Kennerknecht N, Sahm H, Eggeling L. Metabolic engineering of *Corynebacteriumglutamicum* for L-serine production. Appl Environ Microbiol 2005; 71: 7139-7144. http://dx.doi.org/10.1128/AEM.71.11.7139-7144.2005

[42] Boehm A. Process for purifying L-cysteine. United States patent US 20080190854A1. 2008.

Received on 23-06-2014 Accepted on 26-08-2014 Published on 15-10-2014

DOI: http://dx.doi.org/10.6000/1927-3037.2014.03.03.3