Production, Purification and Applications of L-asparaginases
Sikander Ali*, Ayesha Akram, Arfa Jabbar, Rabia Riaz and Unaiza Sarwar
Institute of Industrial Biotechnology, Government College University, Lahore, Pakistan

ABSTRACT

L-Asparaginase is found in many living organisms including animals, plants and microorganisms. Owing to the difficult extraction procedures, microorganisms are preferred for its industrial scale production. Commonly L-asparaginase is present in three dimensional tetrameric structure but other forms also exist depending on its different sources. Its biochemical properties are also dependent on the organism from which it is obtained. L-asparaginase acts on amino acid L-asparagine in two main steps, first an intermediate is formed and then it is attacked by a water molecule and is hydrolyzed into ammonia and aspartic acid. Deficiency of L-asparagine causes inhibition of nucleic acid synthesis in malignant cells which results in cell death. L-Asparaginase can be produced by both solid state and submerged fermentation, but the SSF is more economical and eco-friendly. Enzyme purification is done either by protein precipitation, dialysis, and chromatography or liquid-liquid extraction procedures. L-Asparaginase is involved in treating acute lymphoblastic leukemia (ALL). It is also used essentially in food industry, biosensing and amino acid metabolism.

Keywords: ALL, SSF, SmF, Biosensors, Antineoplastic

I. INTRODUCTION

L-asparaginases (E.C. 3.5.1.1) are present in a variety of living organisms like plants, animals (mammals, birds), microorganisms (bacteria, fungi, algae, yeast, actinomycetes) and some rodents, but not in humans. It is an amido-hydrolase which hydrolyzes amide group of asparagine to ammonia and aspartate. In 1904, Lang first found this enzyme and in 1922 Clementi first observed it in the blood serum of guinea pigs (Clementi, 1922). The discovery of L-asparaginase as an antitumor began in 1953 by Kidd, when lymphomas of rat and mice were treated with serum of guinea pigs (Kidd, 1953). Later in 1961, Broome showed that guinea pigs serum actually contained L-asparaginase enzyme of comparative substrate specificity which caused deterioration of tumor cells (Broome, 1963). Normal body cells synthesize L-asparagines by asparagines synthetase, but the lymphoma cells need its huge amount from the external source for their quick malignant growth. L-asparaginase causes its starvation, blocks protein synthesis and inhibit DNA and RNA, hence; the death of cancerous cells results. At industrial level, L-asparaginase is being produced from microbial species both by submerged and solid state fermentation, and purified by crystallization, dialysis, ion exchange and gel filtration methods. SSF is preferred over SmF due to its cost effectiveness and high yield for industrial scale enzyme production. Some of the commercially available asparaginase include Colapase, Crasnitin, Pasum, Elspar and Pegasparagmasum. There are many challenges in the production of such enzyme which may have fewer side effects, because prokaryotic enzyme often shows hypersensitivity, anaphylaxis, hyperglycemia, hypo-albuminemia, coagulation abnormalities, azotemia and immune inactivation problems (Haskell et al., 1969).

L-asparaginase plays a significant role in pharmaceuticals when used in combination chemotherapy drug for treating lymphoblastic leukemia, acute myelomonocytic leukemia, reticulosarcoma, malignant diseases, melanomas and Hodgkin’s lymphomas (Appel et al., 2007). In food industry, it prevents acrylamide formation, a neurotoxin, during high temperature (at 120°C) processing of starch containing foods. It also plays an essential role in the biosynthesis of amino acids (e.g., Aspartic amino acids) such as lysine, therionine and methionine (Kumar et al., 2013).
II. STRUCTURE OF L-ASPARAGINASE

L-asparaginase usually exists in a tetrameric form, but hexamers, dimmers and monomers are also found to occur in some species. Most bacterial, mainly E. coli and Erwinia species, enzymes are present in similar three dimensional quaternary and tertiary structures (Lubkowski et al., 2002). E. carotovoara enzyme is made up of two tetramers, it further consist of four monomers having 14 α-strands, 8 β-strands, a big N-terminal, and a small C-terminal domain. The active site is present between the two monomers; this site is formed according the channel of amino acids. Amino acids like Thr15 and Thr95 are accountable for the catalytic activity of L-asparaginases. The molecular weight of L-asparaginase formed by E.coli is 130 kDa, whereas Erwinia asparaginase is between 130kDa and 138kDa (Kumar and Sobha, 2012).

Figure 1: 3-D Tetrameric structure of L-asparaginase II in E. coli

III. MECHANISM OF ACTION

L-asparagine is synthesized in humans by asparagine synthetase and the tumor cells need non-essential amino acid in larger quantity for growth. For treatment of acute lymphoblastic leukemia, this enzyme is injected in the body where it hydrolyzes L-asparagine, making it deficient for the cancerous cells (Broome, 1993). Figure 2, shows the difference between asparagines processing in normal and timorous cells.

Figure 2: L-asparaginase action in tumor and normal cells

Hydrolysis of L-asparaginase is a two step process. In step one, a beta-acyl enzyme intermediate is formed by nucleophilic attack and in step two, the intermediate formed is attacked by a water molecule that generates aspartic acid and ammonia (Michalska and Jaskolski, 2006). Figure 4, is depicting a two step process of asparagines conversion to aspartate.

Figure 3: Hydrolysis of L-asparagines

It also has capacity to catalyze other reactions such as enzyme produced by Serratia marescens can hydrolyze 5% L-glutamine and similarly when produced by E.coli and Erwinia chrysenthemi. Some microbial species like Pseudomonas and Acinetobacter sp. produce L-asparaginase with the same glutaminase and asparaginase activity and L-asparaginase hydrolyze L-glutamine right after L-asparaginase hydrolysis. Their hydrolysis is similar due to their structural similarities but L-glutamine is a competitive inhibitor of L-asparagine (Van Den, 2011).

IV. SOURCES OF L-ASPARAGINASES

Although L-asparaginase is found in diverse groups of plants and animals but owing to its difficult extraction procedures, only the microbial sources are explored. Microorganisms help in large scale production for its use in pharmaceuticals as a chemotherapeutic agent, food industry and as a biosensor. Some of the microbial sources are as follows:
A. Bacterial and Fungal Sources

L-asparaginase is produced by Gram positive as well as Gram negative bacteria found both in terrestrial and marine environments, but Gram negative species has got more importance. There are its two types produced by Gram negative bacteria. Type I includes cytosolic enzymes are quantitatively expressed and show activity on L-asparagine and L-glutamine amino acids. Type II is specific for L-asparagine under anaerobic conditions (Sanches et al., 2001). E. coli, Erwinia chrysanthemi, E. carotovora and many other bacterial species are in clinical use, over years, as antitumor agents in the treatment of ALL (Narayana et al., 2008). Fungi are potential source of L-asparaginas along with bacteria. Fungi, being eukaryotic closely resemble humans, therefore; the chances of immunological reactions are lesser. The main reason of their importance is that they produce enzyme extracellularly, so it is easy to extract and purify it (Shrivastava et al., 2012).

B. Actinomycetes, Yeast and Algal Sources

Actinomycetes are present throughout in nature, soil and water but those which are present in living organisms are known to be good for their enzymatic activity. They are also considered a better source as compared to fungi and bacteria. L-asparaginas are also produced from yeasts such as Pichia sp., Saccharomyces sp., Aspergillus sp., Rhodotorula., Spobolomyces etc., exhibiting antitumor activities (Nigarethinam et al., 1995). Chlamydomonas species and a yellow green alga, Vaucheria uncinata are known to produce L-asparaginase under specific conditions of temperature, pH and other environmental factors (Usha et al., 2011).

C. Biochemical Sources

L-asparaginase occurs in different types of animals, plants and also in microorganisms. Mostly L-asparaginas also have activity of glutaminases, only about 3-9% of its total activity. The metabolism of both D-glutamine and D-asparagine occurs at the same active site of enzyme by hydrolysis of ester and amide bonds (Nakamura et al., 1971). The weight of L-asparaginase of E.coli is about 1,38,000-1,41,000 Da. The enzyme obtained from Erwinia has different iso-electric points and hence; exhibits average immunological activity. The Michaelis constant in Michaelis-Menten Kinetics of asparaginase is 100 times lower than glutamine. However, high asparaginase concentration does not deplete glutamine’s serum (Whelan and Wriston, 1979). The PEG modified enzyme is utilized in hypersensitivity reactions to natural form of asparaginase. As the PEG add up 5000 Da to the molecular weight so the molecular weight of natural enzyme is slightly lower than the modified. The activity of enzyme depends upon the biological sources and conditions of the reaction (Yap et al., 1981).

V. PHARMACODYNAMICS

The asparagine is hydrolyzed and converted in ammonia and aspartate, serum asparagines are also exhausted with the administration of asparaginase. Malignant cells are incapable to supply their own asparaginase supply because of their lowered asparaginase synthase activity (Miller et al., 1969). The deficiency of asparaginase results in the disfunctioning of protein synthesis and also causes the delay in the inhibition of synthesis of nucleic acids. It results in the cell death. Asparaginase metabolizes the glutamine and also shows relative substrate specificity (Yoshimoto, 1986).

VI. PHARMACOKINETICS

L-aspariginase is intra-muscularly and intra-venously introduced in malignant treatment disease. According to the researches, the concentration time curve shows plasma half-lives of 4 to 15 h, which is against the first order pharmacokinetics. Researchers also propose that after the enzyme uptake, redistribution into plasma takes place. There is another plasma activity of asparaginases that supports the first order pharmacokinetics (Ohnuma et al., 1970). A scientist named Broome found that there is low activity of serum and spleen of mice with high hepatic activity followed by the treatment by L-aspariginase. It was suggested that the large amount of aspariginase is eliminated through the recirculatory system (Broome, 1968). A scientist named Hall concluded after observing plasma activity of the asparaginases (E.coli and Erwinia asparaginase) that elimination actually takes place by reticulo-endothelial system. Brueck et al. supported this hypothesis with his research on plasma and urine samples taken after administration of asparaginase. The plasma activities were half with intramuscular administration than that.
with intravenous administration (Hall, 1970). It was reported that with dose of about 5000 U/kg per day there is low asparaginase activity in CSF. Investigations performed on asparaginase treatment in monkey and patient concluded that asparaginase activity of 2,100 U/L results in depletion of Cerebrospinal fluid and the serum (Riccardi et al., 1981).

A. Intramuscular and Intravenous administration

Intravenous and intramuscular routes were compared to observe therapeutic efficiency and side effect. It was observed that there is no significant difference but some anaphylactic reactions were observed. They might have occurred because of some protein contamination. The severe reactions were considered to be the relative difference between two modes of administrations (Loos and Borsos, 1972).

B. Drug Interactions

The interaction between drugs and asparaginase depends upon the enzyme induced deficiencies. The administration of those substances that inhibit the synthesis of protein, anti-metabolites and the alkylating agents have shown the capacity to damage the toxicity of these agents (Capizzi., 1974). It was experimentally found that both time interval and sequence between doses of anti-metabolite, methotrexate and asparaginase are effective in therapeutic terms (Capizzi and Cheng, 1981). The optimal time interval between asparaginase and MTX is 9-10 days when asparaginase is given before the MTX (Capizzi and Castro, 1974). Some experiments on animals have shown that late administration of asparaginase is linked with the defensive effect of healthy cells in body. This effect can be compared with rescue function of decreasing tetrahydrofolic acid (Capizzi, 1975). Some animal base experiments have confirmed the direct relationship between MTX and asparaginase sensitivity.

VII. L-ASPARAGINASE PRODUCTION FROM MICROORGANISMS

Microorganisms can be easily modifies for the production of desired substances. For the production of L-asparaginase, different organisms like yeast, actinomycetes and some marine organisms can be used. These sources are used to avoid immunological reactions by bacterial L-asparaginase (Savitri et al., 2003).

A. Bacterial L-asparaginase Fermentation

Different bacterial strains specially Erwinia aroideae and one Hydrogenomonas eutropha are very efficient in producing higher amount of L-asparaginase (Peterson and Ciegler., 1972). Bacterial L-asparaginase (strains of E. chrysanthemi and E. coli) are used for treating Acute Lymphoblastic Leukemia. Different bacterial L-asparaginases possess different affinity, stability and pH. E. coli and E.chrysanthemi are known as main microbial production source at industrial scale. Bacterial asparaginase differs in their pharmacokinetics, biochemical and serological nature (Kumar and Sobha, 2012). Different factors may affect its production rate i.e pH, concentration of C and N sources, temperature, aeration, time and microbial agent (Zia et al., 2013). Among bacteria, the Enterobacteriaceae members are the best for asparaginase production. The activity of asparaginase is increased to 21% by adding 6% n-dodecane with 80 % increase in E.coli concentration via 12.7%. The asparaginase production is stimulated through adding Asn, yeast or tryptone and 10g/L lactose as carbon source for Erwinia aroideae batch cultivation (Alegre and Minim., 1993). The rod shape, gram negative bacteria Enterobacter cloacae can utilize Asn as C & N source and produce asparaginase intracellularly (Nawaz et al., 1998). Enterobacter aerogenes is also known to produce asparaginase with different C sources like glycerol, lactose, glucose and mannitol (Geckil et al., 2005). However, it has lower activity of about 0.60 U/mL. Recently, solid-state fermentation is good alternative of submerged fermentation.

VIII. L-ASPARAGINASE PRODUCTION BY FERMENTATION

L-asparaginase production involves different parameters i.e., temperature, potential of hydrogen, concentration of oxygen and carbon dioxide (Bascomb et al., 1975). The commercial production includes the techniques of fermentation and strain selection. Production of L-asparaginase includes two techniques: Submerged fermentation (SmF) and Solid-state fermentation (SSF).
A. L-Asparaginase Production by Submerged Fermentation (SmF)

Submerged fermentation requires optimized liquid medium for the growth of microorganisms. This process includes a closed reactor containing oxygen concentration and a liquid broth medium. It uses existing microorganisms. Bacteria are used because high moisture content is required for their growth. Fungal sources include *Aspergillus niger*, *Aspergillus terreus*, *Fusarium* and *Penicillium* (Patro and Gupta, 2012). Synthesis of L-asparaginase includes different factors such as temperature, pH, composition of nutrients and the time of fermentation (Kumar and Sobha, 2012). Production of L-asparaginase by using fungal species exhibits the highest activity of about 58IU/ml. the fungal species includes *A. terreus*. The agricultural waste corn steep is used as a source for its production. *Saccharomyces cerevisiae* ASP3 gene was cloned in *Pichia Pastoris* and its production was about 800U/g per cell mass that reduces the immunological reactions (Ferrara et al., 2006). Table 1 shows the L-asparaginase production by submerged fermentation using different microorganisms.

### TABLE I. L-ASPARAGINASE PRODUCTION BY SmF

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Medium</th>
<th>Factors</th>
<th>Production</th>
<th>Citation</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. terreus</em></td>
<td>Praline medium 2</td>
<td>Temp. 30°C, pH 6.2</td>
<td>388 IU/L</td>
<td>Squia et al., 2004</td>
</tr>
<tr>
<td><em>T. thermophila</em></td>
<td>Tryptone, yeast extract, glucose</td>
<td>Temp. 30°C, pH 7.0</td>
<td>1400 IU/g protein</td>
<td>Pits and kysa-Kidits, 2001</td>
</tr>
<tr>
<td><em>S. griseus</em></td>
<td>Aspartase, dextrose salt medium</td>
<td>Temp. 35°C, pH 8.0</td>
<td>38.7 IU/mL</td>
<td>Meena et al., 2015</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>Peptone 4.0</td>
<td>pH 8.5 batch</td>
<td>117 IU/L</td>
<td>Doj Jeong, 1972</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>Tryptone, glucose, yeast</td>
<td>Temp. 28°C, pH 7.0</td>
<td>1520 IU/g dry weight of soil</td>
<td>Peterson and Ciegel, 1969</td>
</tr>
</tbody>
</table>

B. L-asparaginase Production By Solid-state Fermentation

Solid-state fermentation requires optimized solid substrate with minimal content of moisture for the growth of microorganisms. Solid substrate is used as a source. SSF is used in different processes like bioremediation, bio detoxification, production of enzymes and secondary metabolites. It uses waste and residues as a cheap source for microorganisms (Watanabe et al., 2002). L-asparaginase produced by actinomycetes shows high activity by this technique (Basha et al., 2009). Substrate used in this technique includes wheat bran, sesame oil, soy bean, meal and sugar cane. *Aspergillus niger* use glycine as a solid substrate and produced high amount of L-asparaginase. Environmental and nutritional factors are very important in its production. *Cladosporium* species use wheat bran for the production of L-Asparaginase and involve different factors such as pH, Temperature, concentration of oxygen and moisture show enzyme activity of 3.74U. Production of L-asparaginase by using sesame oil includes temperature and aeration rate etc. and shows enzyme activity of 310U/g. L-Asparaginase was also produced by *Pseudomonas aeruginosa* and purification was done by ammonium sulphate fractionation (El Bessoumy et al., 2004). Solid state fermentation has disadvantage because scaling of bioreactor is difficult due to the mass and heat transfer in the system. Table 2 indicates the L-asparaginase production by solid state fermentation using microorganisms and their substrates.

### TABLE II L-ASPARAGINASE PRODUCTION BY SSF

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Substrate</th>
<th>Factors</th>
<th>Enzyme activity</th>
<th>Citation</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. aeruginosa</em> 50071</td>
<td>Soy bean meal</td>
<td>Temp. 37°C, pH 7.4, 40% w/v</td>
<td>142.18U</td>
<td>Abdel Fattah and Otama, 2002</td>
</tr>
<tr>
<td><em>A. Niger</em></td>
<td>Glycine max</td>
<td>Temp. 30°C, pH 6.5</td>
<td>40.9±3.35U of dry substrate</td>
<td></td>
</tr>
<tr>
<td><em>Cladosporium</em></td>
<td>Wheat bran</td>
<td>Temp. 30°C, pH 5.8</td>
<td>3.74U</td>
<td>Kumar et al., 2013</td>
</tr>
<tr>
<td><em>A. terreus</em> MTCC 1782</td>
<td>Oil cake</td>
<td>Temp. 30°C, pH 6.2</td>
<td>30.3IU/mL</td>
<td>Basker and Ren-ganathan, 2009</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>Soy bean meal</td>
<td>Moisture content 70%</td>
<td>8.5IU</td>
<td>Hosamani and Kaliwal, 2011</td>
</tr>
</tbody>
</table>

### IX. PURIFICATION OF L-ASPARAGINASE

L-asparaginase purification includes chromatography, alkaline lysis, ion exchange etc. the purification was done through cation-exchange chromatography followed by affinity chromatography, finally through dialysis (Lee et al., 1986). L-asparaginase free of glutaminase was purified by ammonium sulphate fractionation to
avoid the effects of glutaminase. The process of ammonium sulphate fractionation was followed by DEAE column chromatography. The value of Vmax is 4.45U⁄µg and for Km is 0.657mM (Kumar et al., 2011). L-asparaginase normally shows activity at pH 7-9 but enzyme produced from *T. thermophilus* shows activity at 9.2.

**X. IMMOBILIZATION OF L-ASPARAGINASE**

Inhibitory effect of L-asparaginase involves two major factors, half-life of enzyme and its substrate affinity (Chang, 2013). Polyethylene glycol and L-asparaginase conjugate does not bind to the active site but asparaginase attaches there to avoid allergic reactions. As a result, production of antibodies against asparaginase reduces and half-life of enzyme increases (Zhang et al., 2004).

**A. Downstream Processing**

Downstream processes contribute to about 50-80% of the total yield of proteins. This can be improved by combination with other techniques of purification (Dutra-molino et al., 2014).

**B. Protein Precipitation**

Precipitation is one of the oldest methods to purify the molecules. It causes disruption of the protein structure and forms precipitates. This process has advantages of low cost and simple equipment. Precipitant can also be recycled. Asparaginase from different sources required precipitation. The process start with addition of (NH4)2SO4 to the enzyme extract. 35-100% of the salt concentration was added to the solution. Precipitation increased by about 26.9 fold with centrifugation. This process was followed by dialysis (Amena et al., 2010). Ethanol can also be used as a precipitation agent.

**C. Liquid-liquid Extraction**

This process was achieved by aqueous two-phase systems (ATPS). It is defined as the removal of solute from the liquid phase during contact with the mixture of liquid. Affinity ligands are used for partition. ATPS is used for Asparaginase purification (Qin and Zhao, 2003). Cells are treated with 9.4%w/v K2HPO4 and 15% triton X-100. Cell disruption is combined with homogenization for purification of this enzyme. This process increases the yield 52-73% and the enzyme activity by 78.60-94.80U⁄mg (Jian-Hang et al., 2007).

**D. Chromatography**

High level of purification is achieved by this technique. About 68.9 fold asparaginase, from *Bacillus* sp., is purified by ammonium sulphate fractionation and column chromatography (Singh et al., 2013). Asparaginase by *Cladosporium* was purified by cellulose ion exchange and enzyme activity was 83.3U⁄mg.

**XI. APPLICATION OF L-ASPARAGINASE**

**Pharmaceutical Industry; Antineoplastic Action**

L-asparagine hydrolyzes into ammonia and aspartate was observed in bovine’s tissues. Later on L-asparaginase hydrolase was also confirmed and detected in horse and pig organs (Furth and Friedmann, 1910). Further L-asparaginase detection was carried out in guinea pig serum (Clementi, 1922). The antitumor activity of the enzyme was identified later on. L-asparaginase of *E. coli* had inhibitory capacity on tumors in rats (Mashburn and Wriston, 1964). L-asparaginase is also a chief chemotherapeutic substance for treating lympho-proliferative and lymphoma diseases. Figure 4, shows processing its processing in normal and cancerous cells.

**Figure 4: Antineoplastic action in blood**

**A. Anticancer Drugs**

Use of L-Asparaginase as anticancer drugs is one of its most widely used applications. It is used in combination with vincristine and glucocortiocoid to treat ALL. It is a
very important antitumor drug. It is used widely in treatment of malignancies such as Hodgkin’s diseases, ALL (Schrappe et al., 2000), Myelomonocytic leukemia, Melanomas, Reticulosarcomas, Myelocytic leukemia, and Lymphosarcomas (Kidd, 1953; Broome, 1968). L-Asparagine is involved in protein synthesis and tumor cell growth. L-asparaginase has capacity to change L-Asparagine to aspartic acid. Due to the existence of L-Asparaginase, malignant cells become deficient in important growth factors, the depletion of asparagine, die off the cells. Although L-Asparaginase is present in many living organisms like animals, microorganisms and plants but asparaginase used in treatment of ALL, is taken only from E. coli species. Asparaginase is used for curing acute leukemias and non-Hodgkin lymphomas in 226 children aged between 6.1 and 14.0 years who suffered from stage III and IV lymphoblastic non-Hodgkin lymphoma. An analysis was carried out on the relationship between outcome and a variety of factors involving gender, disease stage, degree of WBC rise, Platelet count, CNS involvement, bone marrow involvement, primary tumor site, degree of LDH increase and before treatment allocation. On the basis of origination from the lymphatic cell system various analysis on patients with acute myelogenous leukaemia were reported who experienced protocol treatment. Another treatment was given to adult patients with myelomonocytic leukaemia. When they were treated with asparaginase monotherapy, some patients get a complete decrease, some achieved good partial reduction and duration reduced to only 1–3 months. L-asparaginase also performs an essential role in the biosynthesis of aspartate amino acids family. Amino acids that are synthesized by it are lysine, threonine, methionine. Kreb’s cycle is used in its synthesis, but on the other hand it can also be produced by the activity of L-asparaginase enzyme (Sinha et al., 2013).

XII. L-asparaginase Role In Biosensor

In order to analyze the levels of asparagine in leukemia or food industry, L-Asparaginase is used for expansion of a biosensor. Although there are various techniques for analysis of L-asparagine such as XPS, SEM, TEM and XRD, but use of biosensor is most effective one. Biosensor technology has many advantages over spectroscopy techniques, as it is more reliable and cheap while the spectroscopy techniques are high cost and less favorable. Mode of action of biosensor is based on the activity of asparaginase. Hydrolysis of asparagines results into the production of ammonium ions and thus causes change in potential of hydrogen. This results into change in color (Kumar et al., 2013).

XIII. L-asparaginase Role In Food Industry; Acrylamide Formation

It is also used in food industry. Acrylamide is neurotoxin (Gokmen and Plazoglu, 2008), known by various other names like 2-propenamide, ethylene carboxamid and propenamide. Its molar mass is 71.08 g/mol (Friedman, 2003). It is a colorless odorless, crystalline solid and carcinogenic to human. It is used as a food processing agent. The development in food industry have shown to acrylamide is synthesized by Millard reaction (Tareke et al., 2000). L-asparaginase has the ability to convert L-asparagine to L-aspartate. It is commonly used to decrease the number of precursors for Millard reaction. Some fungal asparaginases utilised in food industries now a day’s involve L-asparaginase by different sources such as Aspergillus niger and Aspergillus oryzae (Morales et al., 2008). Acrylamide is produced in fried and baked foods when reducing sugars are condensed with a carbonyl source. L-asparagine is the main amino acid that is involved in this phenomenon. Acrylamide is mainly obtained by heat induced reactions. Reaction is carried out between the α-Amino group and Carbonyl groups of reducing sugars (Zyzak et al., 2003). The amide chain present in the acrylamide structure is provided by L-asparagine. Reduction and removal of L-asparagine is an important approach for decreasing acrylamide amount in foods (Pedreschi et al., 2008). Several different ways are involved in reduction of L-asparagine, selection of vegetal species with lower level of L-asparagine, deletion of enzymes involved in biosynthesis of L-asparagine by suppression of specific genes, acid hydrolysis of L-asparaginases and acetylation process of L-asparagine to form N-acetyl-L-asparagine, preventing creation of acrylamide (Friedman, 2003).

XIV. CONCLUSION

L-Asparaginase is an industrially important enzyme, produced by animals, plants, bacteria, fungi, algae and actinomycetes. It has a three dimensional tetrameric
structure consisting of four monomers of different molecular weight. In timorous cells it is produced by asparagines synthetase. It acts by hydrolyzing the amino acid L-asparagine to aspartic acid and ammonia. Mostly bacterial species are used for its extraction due to the easy extraction procedures. It can be produced by fermentation, submerged or solid state, and purified by cation exchange chromatography, filtration, precipitation and dialysis. It is used to treat leukemia and other malignant diseases. In food industry, it prevents acrylamide formation and carries out amino acid metabolism.

**XV. REFERENCES**


[20]. Geckil, H., B. Ates, and S. Gencer. 2005. Membrane permeabilization of Gram-negative bacteria with potassium phosphate/hexane aqueous phase system for the release of L-


[44]. Sanches, M., K. Krauchenco and I. Polikarpov. 2001. Structure substrate complexation and


