Introduction
1.1. Introduction

Acrylamide (AA) \(\text{CH}_2=\text{CHCONH}_2\), is a water soluble vinyl monomer that is formed in many common foods at elevated temperatures. Over many years the polymer has been used in the laboratory to separate proteins by electrophoresis (Friedman, 2003). AA is also a component of tobacco smoke (Bergmark, 1997; European Commission, 2000). AA is readily polymerizable to polyacrylamide which has multiple applications in chemical and manufacturing industries-for example, as a flocculant for clarifying drinking-water, as a sealant for construction of dams and tunnels, as a binder in the paper and pulp industry and in dye synthesis. The risk of AA to health was shown in 1997 when a large water leakage happened during the building of a tunnel in Sweden and large numbers of dead fish and paralyzed cattle were found near the construction site (Keramat et al., 2011). A high level of AA was found in blood of tunnel workers. The ubiquity of AA-haemoglobin adducts led to a hypothesis that AA might be ingested through diet (Reynolds, 2002). Then in early 2002, high concentrations of AA were reported in certain surface fried, baked, and deep-fried foods (Swedish National Food Administration, 2002). Germany and Sweden laboratories also confirmed these results (Gertz and Klostermann, 2002; Rosen and Hellenas, 2002). This discovery dramatically increased the interest in nonindustrial sources of AA exposure to the general public. Research in many European countries and the United States determined that AA is formed primarily in carbohydrate-rich foods prepared or cooked at high temperatures (i.e., >120 ºC) (Tareke et al., 2002). Further studies showed that AA is formed during the Maillard browning reaction from a heat-induced reaction between the amino acid asparagine and the carbonyl group of glucose (Mottram et al., 2002; Stadler et al., 2002). Another pathway for AA formation was suggested to originate from wheat gluten (Claus et al., 2006a). 5- Hydroxymethylfurfural (HMF), an intermediate in the Maillard reaction (MR) was shown to be bioactivated \textit{in vitro} to 5-sulfoxymethylfurfural (SMF) through sulfonation of its allylic hydroxyl group, catalyzed by sulfotransferases which represented a potential health risk due to its cytotoxic and mutagenic activity (Ulbricht
et al., 1984; Abraham et al., 2011). It has also been suggested that AA can be formed from lipid-rich foods by a reaction between ammonia and acrolein (Yasuhara et al., 2003). Some data indicated that the type of oil used for deep frying can influence the formation of AA (Becalski et al., 2003). Potential of AA formation was also related to the sugar content such as glucose and fructose (Biedermann et al., 2002).

Tareke et al. (2000) reported a link of AA to form specific haemoglobin adducts in rat fed with fried food. Nervous system in humans and animals was damaged due to AA exposure (Lopachin and Lehning, 1994; Tilson, 1981), and AA reproductive toxicity was reported by Costa et al. (1992). AA is also considered with mutagenic and carcinogenic properties in experimental rodents in vitro and in vivo (Dearfield et al., 1995). These findings have attracted considerable interest worldwide, because AA has been classified as “probably carcinogenic to humans” by the International Agency for Research on Cancer (IARC, 1994).

For this reason, food authorities in many countries have requested food manufacturers to take measures to limit AA formation in their products. Considerable attempts have been made to mitigate the formation of the potential hazardous, Maillard reaction products (MRPs) in thermally processed foods with special attention to AA either by selection of suitable raw material (Claus et al., 2006b; De-Wilde et al., 2006), changes in the formulation, or by optimization of the process technology. The main mechanism of AA formation in foods is the reaction between reducing sugars and free asparagine i.e., the Maillard reaction. Therefore, AA formation was thought to be reduced by using L-asparaginase (EC.3.5.1.1; L-asparagine amidohydrolase) that catalyses the deamination of L-asparagine into L-aspartic acid and ammonia, making L-asparagine unavailable for Maillard reaction (Kornbrust et al., 2010). This enzyme is also used for the treatment of selected types of haematopoietic diseases such as acute lymphoblastic leukaemia and non-Hodgkin lymphomas (Muller and Boos, 1998). The use in anti-cancer therapy is based on ability of L-asparaginase to cleave L-asparagine, an amino acid essential for lymphoblast’s growth, to ammonia and L-aspartic acid in serum and cerebrospinal fluid. Lymphoblast’s have an unusually high requirement for
L-asparagine and cannot synthesize sufficient endogenous L-asparagine due to very low levels of L-asparagine synthetase and are dependent on serum levels of asparagine for their proliferation and survival. Due to inability of these cells to increase L-asparagine synthetase production, L-asparaginase administration leading to starvation for this amino acid resulted in death of the cells (Ohnuma et al., 1970; Kiriyama et al., 1989; Kotzia and Labrou, 2007). L-asparaginase received increased attention in recent years as food processing aid to reduce the formation of AA in starch-based foods that were baked, roasted or fried (Tareke et al., 2002). During baking of bread dough, a complex cascade of nonenzymatic reactions, so-called Maillard reaction, is chiefly responsible for the development of attractive aroma, colour and flavour (Lindenmeier and Hofmann, 2010). The MRPs in foods have certain nutritive consequences and biological effects that must be considered, since both benefits and possible risks have been envisaged. As positive biological effects, improved palatability and their proven antioxidant activity (Seiquer et al., 2008) together with some chemopreventive effects have been cited (Somoza et al., 2005). Moreover, some MRPs are considered to be toxic contaminants in foods as AA is derived from the Maillard reaction of L-asparagine with carbonyls (IARC, 1994). The enzyme L-asparaginase has also been studied for application in L-asparagine biosensor for leukemia (Verma et al., 2007a).

The production of L-asparaginase from a reliable process in large scale is required to meet the increasing demand of enzyme in food industry to reduce AA levels in fried foods and for developing therapeutic agents to treat cancer. L-Asparaginase is present in a wide range of organisms including animals, microbes, plants, and in the serum of certain rodents but not in human beings (Bessoumy et al., 2004). Although L-asparaginase has been found in various plant and animal species, due to the difficulty in extraction procedure of this enzyme, other potential sources like microorganisms were screened. Microorganisms like fungi and bacteria have proven to be very efficient and inexpensive sources of this enzyme. L-asparaginase is produced by few microorganisms. (Kotzia and Labrou, 2007; Laan et al., 2008; Matsui et al., 2008). Although
production and purification techniques have been developed for L-asparaginase recovery from the bacteria, enzyme yields have been low (Kenari et al., 2011). Even though the enzyme is produced by various microorganisms, *Escherichia coli* and *Erwinia chrysanthemi* L-asparaginases possess antineoplastic activity and have been used successfully in the treatment of leukemia (Fu and Sakamoto, 2007; Maloney, 2010; Savitri et al., 2003). Unfortunately, a therapeutic response by patients rarely occurs without some evidence of toxicity (Narta et al., 2007). The long-term usage of these agents has lead to allergic reactions and new L-asparaginase with new immunological characteristics is needed. The side effects are due to allergic responses, and the use of L-asparaginases from different organisms can alleviate this problem. L-asparaginase from bacterial sources has been shown to have adverse side effects in human trials (Verma et al., 2007b). Therefore, there is a need for L-asparaginase from alternate sources. It has been observed that eukaryotic microorganisms like yeast (Jones and Mortimer, 1973) and filamentous fungi (*Aspergillus*, *Penicillium* and *Fusarium*) produce L-asparaginase with less adverse effects (Sarquis et al., 2004).

The present work was carried out with the main objective to isolate, optimise the medium composition, operating conditions for the enhanced production of L-asparaginase from fungal sources using cheap agricultural by-products. Studies on purification, characterization and modification of L-asparaginase were carried out. Attempts were made to use the purified L-asparaginase in reducing AA formation in fried and baked foods.

### 1.2. Bibliography


Bessoumy AA, Sarhan M & Mansour J. Production, isolation, and purification of L-asparaginase from *Pseudomonas aeruginosa*


Maloney KW. *Erwinia* asparaginase: coming closer to an understanding of its use in pediatric acute lymphoblastic leukemia?. *Pediatric Blood Cancer*, 2010; 54;189-190.


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