Pineapple is the second most popular tropical fruit next to bananas. Pineapple (*Ananas comosus* L.) originates from tropical South America. The varieties of pineapple differ greatly in both shape and taste. Most of the varieties of pineapple are free of seeds and are self sterile. The pineapple is a xerophyte can survive in dry conditions for a long time. Mist, dew and rainwater are collected by the leaves and stored.

The global production of pineapple is 24785762 MT and India is sixth largest producer of pineapple, contributing 1571000 MT which is approximately 6.35% of the total production (FAO, 2013). The cultivation of pineapple is followed in various countries like Thailand, Philippines, China, Brazil, India, Nigeria, Costa Rica, Mexico, Indonesia and Kenya. Today, it is a commercially important fruit crops in India. It is being cultivated in hilly areas of North-Eastern India and humid coastal regions of peninsular India. It is grown in Meghalaya, Assam, Sikkim, West Bengal, Tripura, Mizoram, Karnataka, Goa and Kerala on a large scale, whereas in Maharashtra, Gujarat, Andhra Pradesh, Orissa, Bihar, Uttar Pradesh and Tamil Nadu on a small scale. There is a good demand of pineapple in the processing industry and in the internal markets as well. Indian pineapple is exported to U.K., Nepal, U.A.E. and Spain. The main products of export are juice and canned slices.

The pineapple fruit is a good source of vitamins, enzymes, fiber and minerals which provide balanced nutrition and helps in maintaining ideal weight. It is good for the digestive system. It contains vitamin A, B & C and minerals like magnesium,
calcium, iron and potassium. Hundred grams of pineapple contains 82.79g water, 12.30g carbohydrates, 1.12g protein, 0.94g fiber and 0.33g ash (Fasoyiro et al., 2005). The raw fiber content of the pineapple pulp, core and shell is 24, 62 and 65% respectively (Pardo et al., 2014). Alcohol insoluble solids (AIS) of the pineapple fruit mash contained 21.73% of pectin, 38.4% of hemicellulose and 40.20% of cellulose (Mohammad et al., 2010). Investigations on pineapple juice concentration and on other processed products of the pineapple juice have been carried out on worldwide bases largely because of its flavor and taste (Dev et al., 1982). To improve consumers appeal, particular concern is the recovery of the best quality juice from pineapple. The pineapple juice extraction on large scale is carried out by a two step pressing of comminuted solids of pineapple. The residual pulp remaining after juice extraction still contains some extractable material such as flavor, particulate, soluble solids, etc. Presently, the Indian pineapple industry disposes the residue, which is mainly used for animal feed (Dev et al., 1982; Joseph and Mahadevaia, 1988). It is possible to extract valuable juice components from pulp by adding cell wall liquefying enzymes such as pectinases, cellulases and hemicellulases.

At present, the enzymes are commonly used in many industrial applications, and the demand for more stable, highly active and specific enzymes is growing rapidly. The global market for industrial enzymes was worth nearly $4.5 billion in 2012 and nearly $4.8 billion in 2013 (http://www.bccresearch.com). Use of cellulases, hemicellulases and pectinases began in early 1980s, first in animal feed followed by food applications. Subsequently, these enzymes were used in the laundry, textile as well as in the paper and pulp industries. During the last two decades, the use of cellulases, hemicellulases and pectinases has increased considerably, especially in textile, food, brewery and wine as well as in pulp and paper industries.
Pectinases, cellulases and hemicellulases have a wide range of applications in food biotechnology. These enzymes are used in juice extraction and clarification in fruits and vegetables. The fruit and vegetable juices are good for human health and their production is commercially important. The availability of nutritious components from fruits and vegetables is thus facilitated throughout the year by selling of their juices to a large number of consumers.

The production of vegetable and fruit juices requires methods for juice extraction, clarification and stabilization. During the early 1930s, when fruit industries began to produce juice, the yield was low, and many difficulties were encountered in filtering the juice to an acceptable clarity (Uhlig, 1998). Currently, a combination of pectinases (endo and exo-polygalacturonases, pectin lyase, pectin acetyesterase, pectin methylesterase, endo- and exo-arabinases and rhamnogalacturonase), cellulases (cellobiases, endoglucanases and exoglucanases) and hemicellulases (endo- and exo-xylanases, xyloglucanases, galactanases and mannanases) collectively called macerating enzymes are used in the extraction and clarification of fruit and vegetable juices (Galante et al., 1998a; Grassin and Fauquembergue, 1996). During the production of juice from fruits such as apples, pineapples and pears, the whole fruits are crushed to pulp mash, which, after mechanical processing (pressing, centrifuging and filtering), yields a clear fruit juice and a solid phase called pomace (Galante et al., 1998b). Macerating enzymes are generally used in two steps: (1) in the maceration of the fruit pulp for its liquefaction, which increases the juice yield and also reduces the processing time. The maceration also improves the quality of the juice by the extraction of valuable fruit components, and (2) clarification of the juice by lowering the viscosity and thus improvement of the filtration properties and stability of the final product. The pectinases are generally used for this purpose. Thus, these enzymes play a key role in
the extraction of juice from fruits and vegetables and their demand is expected to increase.

The enzymatic juice extraction process has a number of advantages over mechanical processing of several fruit pulps. In particular, the use of cellulases and pectinases has been an integral part of modern fruit processing technology involving treatment of fruit mashes as they not only facilitate easy pressing and increase in juice recovery but also ensure the highest possible quality of end products (Kilara, 1982; Roumbouts et al., 1978). These enzymes not only help in softening the plant tissue but also lead to release of cell contents that may be recovered with high yield (Chesson, 1980). The pectinases and cellulases are enzymes, which act upon the pectin and cellulose polysaccharides respectively.

Pectinases or Pectinolytic enzymes are a heterogeneous group of related enzymes that hydrolyze the pectic substances, present mostly in plants. Pectinolytic enzymes are widely distributed in higher plants and microorganisms (Whitaker, 1990). They are of prime importance for plants as they help in cell wall extension (Ward and Moo-Young, 1989) and softening of some plant tissues during maturation and storage (Aguilar et al., 1990). They also aid in maintaining ecological balance by causing decomposition and recycling of waste plant materials. Plant pathogenicity and spoilage of fruits and vegetables by rotting are some other major manifestations of pectinolytic enzymes (Perombelon and Kelman, 1980; Fraissinet et al., 1996; Collmer et al., 1986). It has been reported that microbial pectinases account for 25% of the global food enzymes sales. Almost all the commercial preparations of pectinases are produced from fungal sources. *Aspergillus niger* is the most commonly used fungal species for industrial production of pectinolytic enzymes (Kotzekidov, 1991; Barnby et al., 1990).
The largest industrial application of pectinases are in fruit juice extraction and clarification. Pectins contribute to fruit juice viscosity and turbidity. A mixture of pectinases and amylases is used to clarify fruit juices and is reported to decrease filtration time up to 50% (Blanco et al., 1999). Pectinases in combination with other enzymes, viz., cellulases, arabinases and xylanases, have been used to increase the pressing efficiency of the fruits for juice extraction (Gailing et al., 2000). Cellulase refers to a group of hydrolytic enzymes (cellulases) capable of hydrolyzing cellulose to glucose. Cellulolytic enzymes are produced by a large number of microorganisms which include fungi and bacteria (Enari, 1983). In microorganisms, the enzymes are either cell-bound or extra cellular. The ability to produce extra cellular cellulolytic enzymes is widespread in fungi and these enzyme systems have been most extensively studied (Enari, 1983; Wood, 1985). However, cellulases are also produced by plants and animals. Several kinds of cellulases are known, which differ structurally and mechanically.

These enzymes are used to perform a multitude of functions including removal of cell walls or crude fiber to release valuable components (enzymes, flavors, polysaccharides and other proteins) from plants cells to improve nutritional value of foods or to prepare plant protoplasts for genetic research (Mandels, 1985). There are at least three major types of cellulolytic enzymes produced by fungi: endoglucanases, cellobiohydrolases and cellobiases.

The juice from pineapple may be extracted from the juicy part and from the residual part. The pineapple pulp for the juice extraction is produced from the juicy part of the fruit and the remaining core, trimmings and skin part is the left out (residual) product. The juice extracted from the core, trimmings and skin of the pineapple is referred as mill juice (Ben-gera and Kramar, 1969; Tran, 2015). The core and the
trimmings can be used to extract the low brix juice. The pulp suspension, viscosity, foaming, and filtration properties of pineapple juice have been attributed to the presence of a natural gum (Dull, 1958; Bates, 1964; Chenchin et al., 1978). The pulp suspension and viscosity due to the presence of these natural gums may be undesirable properties if low-viscosity products are desired and thus a suitable method for its removal may be needed. The pineapple gum is neutral polysaccharide composed predominately of galactomannans. The gums can be hydrolyzed by enzymes such as pectinases, cellulases and hemicellulases (Chenchin et al., 1978).

**JUSTIFICATION**

The pectinases and cellulases have wide range of applications. There have been several studies concerning the pectinase or cellulase production individually from different cheaper raw materials under submerged and solid state fermentation. However, the production of both pectinase and cellulase by same organism under same fermentation conditions can be more economical and time saving practice and hence required to be investigated.

India is the sixth largest producer of pineapple and contributes more than 6% of the world’s production. Pineapple is commercially considered as an important fruit but the potential of the fruit is not fully tapped. The area requires wider research in terms of utilization of residue, enhanced juice yield with optimum overall acceptability. The application of the commercial enzymes can improve the juice yield and quality. The crude cellulase and pectinase may also be used for the improvement of juice yield, quality as it is more economical and eco-friendly, provided it is produced from GRAS fermentation.

A low brix mill juice produced from the core and trimmings of the pineapple can be concentrated to increase its brix and pooled to the pineapple juice. The pulp
suspension and viscosity due to the presence of these natural gums may be undesirable properties if low-viscosity products are desired and thus a suitable method for its removal is needed. The degumming of this concentrated mill juice can be done using crude and commercial enzymes to improve its quality and filtration properties. The economy of the process thus adopted can be evaluated by calculating the expenditure and the outcome while using commercial and crude enzymes. The present study was therefore, undertaken to use the crude and commercial enzymes (purified enzymes) for the improvement of the pineapple juice yield and quality with the following objectives:

- To select pure cultures, based on the production of pectinases and cellulases.
- To standardize the fermentation procedures for maximum production of pectinases and cellulases from these microorganisms and to characterize the pectinases and cellulases in terms of activity and kinetic parameters.
- To study the effect of crude and purified enzymes (commercial enzymes) in juice extraction to improve the yield of pineapple juice from pulp with respect to the optimum quality.
- To optimize the treatment parameters of crude and purified enzymes (commercial enzymes) for the maximum juice recovery with optimum quality by using Response Surface Methodology.
- Enzymatic degumming of pineapple mill juice using crude and purified enzymes (commercial enzymes) and then the optimization of the processing conditions under the different range of time and temperature.
- To evaluate the economy of the process.