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PROCESS STANDARDIZATION FOR THE PRODUCTION OF BAKER'S YEAST FROM MANGO STONES

By

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(2010-12-111)

THESIS

Submitted in partial fulfillment of the requirement for the degree of

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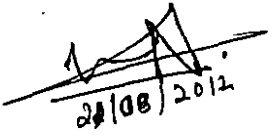
DECLARATION

I hereby declare that the thesis entitled **"Process standardization for the production of baker's yeast from mango stones"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.



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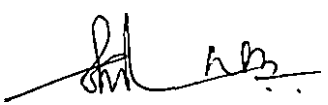
Certified that the thesis entitled **"Process standardization for the production of baker's yeast from mango stones"** is a bonafide record of research work done independently by Mr. NIKHIL V. M under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.



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INTRODUCTION

1. INTRODUCTION

Mango (*Mangifera indica*) which belongs to the family Anacardiaceae, is one of the most cultivated fruits in the world and India contributes major part of the world production. It is considered as the 'King of fruits' owing to its luscious taste, captivating flavour and high nutritive qualities. Presently, it occupies 2309 thousand ha which is 37.8 per cent of total area under fruit crops with a total production of 12750 thousand MT which is 18.6 per cent of total fruit production (NHB, 2011)

Mango is used extensively by the food processing industry for preparation of a wide variety of products. Both ripe and unripe mangoes are utilized for this purpose. Ripe mango is processed into variety of products including pulp, jam, juices, beverages, mango leather, powder, canned slices *etc.* while the unripe mango can be processed into pickle, chutney, brined slices, amchur *etc.* Major components of mango fruit include pulp (45-60%), peel (20-30%) and stone (20-40%). Mango processing industries generate huge quantity of solid waste comprising mainly of peels and stones.

Majority of wastes from fruit and vegetable processing industries are seasonal and they do not decompose rapidly. The major part of this is mostly discarded and it is the main source for increasing the environmental pollution. Utilization of this waste is both a necessity and challenge. Production of value added products from agro-industrial waste is now a focusing area, as it reduces pollution in the environment.

Most of the developing countries of the world have been facing malnutrition problems. The deficiency of protein in human food and animal feed is well recognized due to the rapid growth of population. The global shortage of food and feed protein has prompted researchers to seek protein production from both conventional and non conventional sources. Proteins are the essence of life processes and are important to all living beings. Microbes like yeast, bacteria and fungi are sources of proteins and hence use of single cell protein or microbial protein can solve the global food problems.

Single cell protein (SCP) is dried cells of microorganisms such as algae, bacteria, yeast, moulds and higher fungi that can be used as protein supplement for both human and animal feeds. Microorganisms have high protein content and short growth times, leading to rapid biomass production and is independent from the environmental conditions. The use of fungi, especially yeasts for SCP production is more convenient, as they can be easily propagated using cheap raw materials and easily harvested due to their bigger cell sizes and flocculation densities. Moreover, bioconversion of agricultural and industrial wastes to protein rich food and fodder stocks has an additional benefit of making the final product cheaper. SCP production has the potential for feeding the ever increasing world population at cheaper rates. Production of microbial proteins by fermentation of agricultural waste products is one of the most promising approaches for increasing the availability of protein. In addition, utilization of these waste products provides alternative substrates and helps in solving pollution and waste disposal problems.

Food grade yeasts are used as source of high nutritional value proteins, enzymes and vitamins, with applications in the health food industry as nutritional supplements, as food additives, condiments and flavouring agents. Yeast cell matter consists of 40-60 per cent protein and is particularly rich in most of the B group vitamins.

The most common food grade yeast is *Saccharomyces cerevisiae,* also known as baker's yeast, which is used worldwide for the production of bread and baking products. Yeast SCP is considered as a potential protein source for humans as well as animals. The only species fully acceptable as food for humans is *Saccharomyces cerevisiae* (Baker's and Brewer's yeast).

Mango stones form the main waste generated by the mango processing industry. One of the main problems associated with mango stones is that during processing the pulp attached to the stones is not removed completely. The adhering pulp attracts flies and microorganisms especially fungi and yeast and cause pollution to the environment. The pulp has a TSS as high as 22^0B and low pH (5-5.5) which is

most favourable for growth of yeast and single cell protein production. Hence the project was envisaged to study the feasibility of utilizing mango stones as a substrate for SCP production. To be economically feasible, optimum culture condition for maximum biomass yield and intracellular protein has to be standardized. Hence the present study was undertaken with the following objectives.

1. Standardization of pretreatments of the substrate for baker's yeast production.
2. Optimization of culture conditions and growth supplements for baker's yeast production.
3. Utilization of SCP enriched flour for preparation of biscuit.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Mango *(Mangifera indica),* the King of fruits, is grown throughout several parts of the world, but more than three-quarters of the world's production is in the Asian region and currently India shares about 56 per cent of the total mango production in the world. Ripe mango is processed into a variety of products including pulp, jam, juices, beverages, mango leather, powder, canned slices *etc.* Mango fruit processing industries generate two types of waste, including solid waste (peel and stones) and liquid waste (juice and waste water) and utilization of this waste is both a necessity and challenge (Reddy *et al.*, 2011). During processing the pulp attached to mango stones is not removed completely which attracts microorganisms especially fungi and yeast. Mango fruit pulp is one of the best substrates for yeast growth because of its high TSS and favourable acidity (Splittstoesser, 1994). Nowadays, Baker's yeast, *Saccharomyces cerevisiae* has received much attention as a potential food protein source. Moreover, agricultural waste represents an inexpensive source of substrate for the production of single cell protein (Batt and Sinskey, 1987).

Research findings already reported by various workers in the related fields are reviewed under the following headings.

2.1 Importance of fruit waste utilization

Bhalerao and Mulmuley (1989) reported that during fruit processing, more than 50 per cent of fruit weight goes as waste in the form of peel, fruit stones *etc.*

The inedible portions of fruits, which are discarded by consumers and fruit processing units go as waste. A variety of value added products can be manufactured if wastes are utilized in an organized manner (Bhalerao and Mulmuley, 1989).

Production of value added products from agro-industrial and food processing wastes is now a focusing area, as it reduces pollution in the environment, in addition to value addition. Also, wastes can be efficiently disposed and capacity utilization of processing units can be increased (Srivastava and Sanjeevkumar, 1994; Anand and Maini, 1997).

Fehr *et al.* (2002) reported that the percentage of byproducts and waste in food processing industry is 30 per cent.

Shankar (2003) opined that there is much potential to enhance the world food supplies through waste utilization.

According to IASRI (2004), the total waste generated comes to 50 million tonnes per annum in India.

Okonko *et al.* (2009) defined waste as any material, which has not yet been fully utilized, *i.e.* the leftovers from production and consumption.

Agamuthu (2009) reported that globally, 998 million tonnes of agricultural waste is produced in a year.

2.1.1 Waste generation by the mango processing industry

Berh *et al.* (1976) reported that during processing of mango, by- products such as peel and kernel are generated. Stone contributes about 25-30 per cent of the fruit.

According to Sounder and Chandra (1987), during processing of mangoes, over 50 per cent waste is generated in the form of peel, stones and pulp fibres.

Mango is processed to a maximum extent, thereby producing high quality of solid and liquid wastes. Garg *et al.* (1994) reported that mango processing industry generates huge quantities of solid waste comprising mainly of peel and stones.

Among the processing wastes, kernel constitutes 15- 20 per cent and peels 25-30 per cent (Anand and Maini, 1997). In mango, solid wastes contribute about 40 to 50 per cent of total fruit waste out of which, 5 to 10 per cent is pulp waste and 15 to 20 per cent is kernel (Madhukara *et al.*, 1993; Pandey *et al.*, 2000).

Garg (2006) reported that 6-7 per cent of mango pulp is attached to the mango stone after processing. There is an immense potential for utilization of this pulp for value addition.

2.1.2 Waste generation by other fruits and vegetables

2.1.2.1 Jack fruit

During jack fruit processing, rind, perigones and seeds are left as wastes, which is about 45 per cent of total fruit weight (Anand and Maini, 1997).

Subburamu *et al.* (2003) reported that the non edible portion of the jack fruit was 59.2 per cent.

2.1.2.2 Pineapple

Core, peel and leaves are considered as waste portion in pineapple. Of the pineapple processing wastes, 40 to 50 per cent is constituted by peels, cores and pomace and cannery waste constitute 60 per cent (Srivastava and Sajeevkumar, 1994; Anand and Maini, 1997).

Shilpa and Theju (2011) reported that 40- 50 per cent of pineapple is considered as discarded portion.

2.1.2.3 Banana

Peels from the processing factories contribute the major waste of the fruit (Muhamed and Hassan, 1995; Girdharilal *et al.*, 1998) which accounts to about 20- 30 per cent (Anand and Maini, 1997).

2.1.2.4 Apple

Shah and Masoodi (1994) remarked that about 20,000 tonnes of apple pomace is generated in processing factories, after producing the major product *ie.* apple juice.

Apple pomace is a waste which is a byproduct of apple juice processing industry and creates environmental problem. It contains peel, seed and remaining solid parts and as estimated to be 20- 30 per cent of the total processed crop (Maini and Sethi, 2000).

A conventional process removes 75 per cent of fresh weight of apple as juice and 25 per cent is the pomace (Kaushal *et al.*, 2002).

In India, total production of apple pomace is about one million tonnes per annum and only approximately 10,000 tonnes of apple pomace is being utilized (Manimehalai, 2007).

According to Shalini and Gupta (2010), in large scale apple juice industry, about 75 per cent of apple is utilized for juice and the remaining 25 per cent is the byproduct, apple pomace.

2.1.2.5 Citrus

Bhalerao and Mulmuley (1989) reported that citrus juice sacs (lime and orange) and citrus pips, which are wasted by citrus juice industry, could be used in the production of powdered mixes for enhancing consumer appeal.

During the processing of citrus fruits, nearly 50 per cent of the fruit, comprising of peel and seeds evolve as waste, of which peels alone constitute 20 to 30 per cent (Khurdiya *et al.*, 1997).

Girdharilal *et al.* (1998) reported that, among the entire citrus waste, orange waste alone comes to about 50 per cent

Cohn and Cohn (2005) observed that citrus peel and rag constituted about 45 to 50 per cent of the waste.

2.1.2.6 Papaya

Chan (1979) reported that papaya seeds, which constituted about 30- 35 per cent of the waste derived from the fruit, were usually discarded.

2.1.2.7 Grape

According to Srivastava and Sanjeevkumar (1994) and Girdharilal *et al.* (1998), stems and pomace which constitute five to ten per cent of the fruit, are main waste products from grape processing factories.

Roberts *et al.* (2008) reported that depending on the condition of the grape at harvest and the type of press used, 13.5-14.5 per cent of the grapes crushed become grape pomace waste with extreme waste production as high as 20 per cent. Depending on the variety, grape seeds constitute approximately 26 per cent of the pomace.

2.1.2.8 Guava

El-Din and Yassen (2006) reported that seeds which contribute 6.0 per cent of fruit in guava were discharged as waste into the environment. In Brazil, around 202 thousand tonnes of guavas are processed every year and 12 thousand tonnes of seeds are discharged as waste every year.

Bourgeois *et al.* (2009) reported that in terms of weight, guava seeds represent less than 5.0 per cent of the fresh fruit and had a harder horny shell containing an oily kernel.

2.1.2.9 Tomato

Sogi *et al.* (2002) reported that during tomato processing about 7.07- 7.5 per cent solid waste of raw material was generated and pomace constituted about 71- 72 per cent of this waste.

2.1.3 Fruit and vegetable waste utilization

2.1.3.1 Pineapple

Devi and Ingale (1982) suggested that diversified uses of pineapple waste are possible, to produce vinegar, wine, candy and sugar syrup.

The waste from pineapple processing industries such as peel, core and pomace contain 7- 10 per cent sugar and could be utilized for vinegar production (Anand and Maini, 1997).

Hedge (1997) reported that peel and pulp waste from pineapple can be used for producing Nata-de-Pina. The bacterium culture of *Acetobacter xylenum* is found capable of bio converting pineapple juice into gel known as Nata-de-Pina, which can be eaten as a dessert or blending with fruit salad, ice cream and pudding.

Abdullah and Abdullah (2010) reported that pineapple waste can potentially be used as carbon source for fermentation to produce lactic acid. The production of lactic acid was found to be 79 per cent yield, while only 56 per cent yield was produced by using solid waste.

2.1.3.2 Jack fruit

In the processing of the edible portion of jack fruit into preserved products, the skin, peel and core are left as waste material. These wastes constitute about 45 per cent

of the total fruit weight and have been found to be a fairly good source of pectin (Jain and Girdhari, 1957).

Maini and Singh (2001) studied the composition and processing of jackfruit and its waste utilization and suggested that fruit wastes are potential sources of cattle feed due to richness of its carbohydrates and proteins.

2.1.3.3 Banana

The pulpy portion of banana peel is reported to be good for making banana cheese (Srivastava and Sanjeevkumar, 1994; Girdharilal *et al.*, 1998)

Mohammed and Hassan (1995) reported that pectin can be extracted from the banana peels.

According to Dhabekar and Chandak (2010), ethanol production from banana peels is 1.90 per cent equivalent to dextrose media.

Reddy *et al.* (2010) reported that banana waste is used as substrate for single step ethanol fermentation by thermophilic, cellulolytic, ethanologenic *Clostridium thermocellum.*

2.1.3.4 Apple

Apple pomace contains 26.4 per cent dry matter (DM), 4.0 per cent protein, 3.6 per cent sugars, 6.8 per cent cellulose, 0.38 per cent ash, 0.42 per cent acid and calcium, 8.7 mg/100 g (Vasil'ev *et al.*, 1976).

Ezhov *et al.* (1993) patented a method for extraction of pectin from apple pomace under acid conditions.

The pomace remaining after the extraction of juice and removal of core can be used for the preparation of pectin, cider and vinegar (Shah and Masoodi, 1994).

Efforts have been made to utilize apple pomace in the preparation of edible products like apple pomace jam and sauce (Kaushal and Joshi, 1995; Joshi *et al.*, 1996) or to make citric acid (Sharma and Joshi 2001; Kaushal *et al.*, 2002).

Kaushal and Joshi (1995) prepared cookies by incorporating different amounts (10–50%) of apple pomace powder in dough. Sensory evaluation of prepared cookies showed that 30 per cent of apple pomace powder could be incorporated in the preparation of cookies of good quality.

Sun *et al.* (2007) reported that apple pomace typically contains 66.4–78.2 per cent moisture and 9.5–22.0 per cent carbohydrates.

2.1.3.5 Citrus

Bawa and Saini (1988) found that the residue obtained after juice extraction from Kumkuat (wild citrus) fruit is a rich source of pectin (2.57%) and ascorbic acid.

Cohn and Cohn (2005) observed that citrus waste could be utilized for production of essential oil and carotenoid pigment.

The peel of citrus fruit is a rich source of flavanones and many polymethoxylated flavones, which are very rare in other plants (Ahmad *et al.*, 2006).

2.1.3.6 Grape

Hang and Woodams (1986) studied the possibility of citric acid production from grape pomace by solid state fermentation using *Aspegillus niger*. Under optimum fermentation conditions, this method reduced the pomace dry matter by about 43 per cent and yielded more than 90 g of citric acid per kg of grape pomace fermented. The yield was more than 60 per cent based on the amount of fermentable sugar consumed.

Srivastava and Sanjeevkumar (1994) reported that oil can be extracted from the seed and the cake can be used as a cattle feed.

2.1.3.7 Guava

Correia *et al.* (2004) reported that the bioconversion of soy flour-supplemented guava residue by *Rhizopus oligosporous* represents a novel strategy for the enhancement of phenolic antioxidant content and potential commercial value of guava wastes.

2.1.3.8 Tomato

Martínez-Valverde (2002) reported that the skin and seeds of tomatoes are richer sources of polyphenolic compounds than the pulp

George *et al.* (2004) found that the free polyphenolic content (expressed as mg catechin/100 g fresh weight) in pulp ranged from 9.2 to 27.0 mg/100 g, compared to 10.4 to 40.0 mg/100 g in skin, and also that for each genotype, the polyphenolic content in skin was higher than in pulp

2.2 Waste utilization through fermentation

2.2.1 Alcohol

Waste contains three primary constituents: cellulose, hemicellulose and lignin, and can contain other compounds (e.g. extractives). Cellulose and hemicellulose are carbohydrates that can be broken down by enzymes, acids, or other compounds to simple sugars and then fermented to produce ethanol renewable electricity, fuels, and biomass based products (Puri, 1984; Wyman and Goodman, 1993; Van Wyk, 2001).

The banana agrowaste can be utilized affectively to yield fermentable sugars which can be converted into other substances like alcohol (Baig *et al.*, 2003).

Akin-Osanaiye *et al.* (2008) evaluated the feasibility of production of ethanol from papaya agricultural waste, using dried active baker's yeast strain *(Saccharomyces cerevisiae)*. The results showed that the fermented papaya waste produced 2.82- 6.60 per cent ethanol. Moreover, that waste contained 90.82g/100g carbohydrate, 2:60g/100g lipid, 1.63g/100g crude protein and 4.95g/100g ash.

2.2.2 Organic acid

Fermentation of sugars by microbes is the most common method for converting sugars inherent within biomass. Value added products including ethanol (Ueda *et al.*, 1981; Lin and Tanaka, 2006), hydrogen (Buranakarl *et al.*, 1985; Chitradon *et al.*, 2008), fumaric acid (Carta *et al.*,1998; Carta *et al.*, 1999) and high fructose syrup lactic acid (Anuradha *et al.*, 1999; John *et al.*, 2006), citric acid (Pandey

et al., 2000), are obtained from microbial fermentation and enzyme utilization of waste.

Garg *et al.* (1998) reported that the pomace after extraction of pectin from guava fruits contained enough amount of cellulosic material which can be used for producing citric acid through microbial fermentation.

2.2.3 Enzymes

Biowastes are highly perishable materials and their disposal often is a problem in processing industries. Extraction of enzyme, pectinase, from biowastes using the technology of fermentation, which gained importance recently (Nottingham University, 1997), is one of the many ways of exploiting them profitably (Anand and Maini, 1997).

According to Gupta and Joshi (2000), the waste from the processing of fruit and vegetables includes water and various organic substances like simple and complex polysaccharides, vitamins and minerals. So, microbial fermentation is the best technology which can be applied to the food processing industry waste to convert them to valuable products.

Venkatesh *et al.* (2009) studied the possibility of producing pectinase utilizing fruit wastes of cashew, banana, pineapple, and grape under controlled fermentation with *Aspergillus foetidus*. Among the different wastes, enzyme production was seen maximum in the medium with grape waste.

2.2.4 Beverages

Gautam and Guleria (2007) reported that a number of beverages such as cider, beer, wine and brandy and vinegar can be obtained from the fermentation of fruit wastes.

2.3 Fruit waste as substrate for fermentation

Milson (1987) stated that glucose, maltose, lactose, and sucrose from cheap raw materials such as molasses, whey, cane and beet sugar and starch wastes have been used as substrate for fermentation process to produce lactic acid.

Dhanasekaran *et al.* (2011) reported that the pineapple skin waste was found to contain a good amount of reducing and non-reducing sugars (10 and 13%, respectively), which is most favourable for the growth of microorganisms.

2.4 Single cell protein from fruit waste

The fruit waste can be utilized as a substrate of choice to produce single cell protein (SCP) by fermentation process. A number of agricultural and agro industrial waste products have been used for the production of SCP and other metabolites, including orange waste, mango waste, cotton salks, kinnow-mandarin waste, barley straw, corn cops, rice straw, corn straw, onion juice and sugar cane bagasse, cassava starch, wheat straw, banana waste, capsicum powder and coconut water (Smith and Bull, 1976; Saquido *et al.,* 1981; Hamed, 1993; Tipparat and Kittikun, 1995; Nigam, 2000; Zhao *et al.,* 2010).

Studies have been conducted on the feasibility of utilizing different fruit wastes such as dates (Kamel, 1979), beet pulp (Ghanem, 1992), sugarcane bagasse (Azin and Moazami, 1989), banana skin (Enwefa, 1991) and guava peel (Moharib, 2003) as substrate for the production of single cell protein.

Batt and Sinskey (1987) reported that agricultural waste represents an inexpensive source of substrate for the production of single cell protein including baker's yeast.

Fruit pulp is one of the best substrates for yeast growth because of its high TSS and favourable acidity (Splittstoesser, 1994).

According to Anupama and Ravindra (2000), the protein from microbes is cheap, easy to obtain in crude form and nutritive. Moreover, bioconversion of agricultural and industrial wastes to protein-rich food and fodder stocks has an additional benefit of making the final product cheaper.

Pineapple waste has been found to contain upto 6.14 per cent of carbohydrate, minerals especially magnesium and 0.6% of crude protein (Hutagalung, 2002), thus undoubtedly a valuable fermentation substrate for both single cell protein and metabolite production (Rosma *et al.*, 2005).

Dhanasekaran *et al.* (2011) reported that agricultural wastes are useful substrate for production of microbial protein, but must meet the following criteria; it should be non toxic, abundant, totally regenerable, non-exotic, cheap and able to support rapid growth and multiplication of the organisms resulting in high quality biomass.

2.4.1 Culture conditions for yeast growth

Cruickshank *et al.* (1975) opined that most saprophytic bacteria have wide pH growth range and can thrive at pH as low as 4.4.

According to Pirt (1975), various factors are known to influence fermentation processes. These include carbon and energy source requirements, oxygen demand and supply, temperature, pH, nitrogen, phosphorus and potassium requirement.

Agitation is entailed during fermentation in order to warrant efficient nutrient transfer to the cell surface (Doran, 1997; Lee, 2008).

The energy requirement of yeast is considered to be minimal as it grows very well at room temperature (Adoki, 2002).

Ojokoh and Uzeh (2005) observed that the mean viable cell counts of *S. cerevisiae* in papaya medium decreased after 1 or 2 days. The decrease in growth was attributed to limiting nutrients and oxygen, arising from their exhaustion. Autolysis was enhanced by the exhaustion of nutrients and oxygen (Kays and Vanderzant, 1980).

Garg (2006) reported that the mango pulp with TSS as high as 22^0 Bx and low pH was most favourable for the growth of yeast.

In anaerobic conditions, as in alcoholic fermentation, yeasts do not grow efficiently, and sugars are converted to intermediate byproducts such as ethanol, glycerol and CO_2. Therefore, in yeast propagation, the supply of air is necessary for optimum biomass production (Bekatorou *et al.*, 2006).

Adoki (2008) reported that the pH determine the occurrence and types of particular contaminants in the culture medium. It is therefore necessary that the pH for

growth of any selected strain is optimal for the strain and at the same time reduces growth of contaminants.

According to Cardona *et al.* (2010), aeration is an essential factor for *S. cerevisiae* fermentation even though yeast has the ability to grow under anaerobic condition.

2.4.2 Growth supplements for yeast growth

Litchfield (1983) studied the effect of growth supplements such as molasses as the carbon source and ammonium salts as the nitrogen source on the multiplication of yeast *Saccharomyces cerevisiae*.

Camacho-Ruiz *et al.* (2003) reported that the growth of *Saccharomyces cerevisiae* was higher for the highest levels of nitrogen source concentration (20g/l) and air flow (3l/h) and lower levels of total sugar concentration (50g/l).

Ojokoh and Uzeh (2005) utilized glucose (2% w/v) and $(NH_4)_2HPO_4$ (0.25% w/v) as a nitrogen source supplement for the production of *Saccharomyces cerevisiae* biomass in papaya extract medium.

Adoki (2008) studied the factors affecting growth and protein yield of *Candida* sp. on substrate like orange, plantain and banana processing wastes. The organism multiplied faster at 37°C and the supplementation of media with 0- 15 per cent and 0-6.0 per cent (w/v) combination of dextrose and ammonium nitrate, respectively, resulted in optimal growth at a pH range of 3.0 – 5.8 after 6.0 hours. However, pH of 3.0 is required to reduce growth of saprophytic contaminants. Supplementation with phosphorus was not critical for growth of yeast.

A study conducted for the optimization of process parameters for the production of single cell biomass of *Candida utilis* in solid state fermentation by Irfan *et al.* (2011) showed that maximum cell biomass was obtained when wheat bran was supplemented with 2 per cent molasses as carbon and 0.25 per cent ammonium nitrate as nitrogen sources and 250mg/L of Biotin as inducer at pH 6.5. Also, the incubation period of 4 days was found suitable for maximum cell biomass of *Candida utilis* with 10 perecent (v/v) inoculum size.

Sankar *et al.* (2011) studied the effect of different nitrogen sources on the production of single cell protein by *Trichoderma harzianum* using waste banana peel. The study revealed that the supplementation of banana peel extract with sodium nitrate gave the highest protein yield of 0.78 ± 0.01g/l followed by 0.69 ± 0.03 g/l when supplemented with ammonium nitrate.

2.4.3 Nutritional value of single cell protein

Batt and Sinskey (1987) reported that yeast consist of 40- 60 per cent protein.

The microbial protein is reported to contain better percentage of essential amino acids and better chemical score than soya protein (Lyutskanov, 1990).

Sgarbieri *et al.* (1995) found that the protein content from dried biomass may range from 45- 50 per cent w/w and over 60 per cent w/w in yeast extract, thus making it an important SCP source.

A study by Wang *et al.* (2000) revealed that yeast cell matter is particularly rich in most of the B- group vitamins which ranged from 5.53 to 60.70 mg/kg dried cell and therefore constituted a potential source of enrichment for vitamin B- deficient diets.

Yeast extract from *Candida utilis* was found to contain high amount of glucose and total sugar content (Liong *et al.*, 2002).

Bekatorou *et al.* (2006) estimated the nutritional status of baker's yeast and their study revealed that fresh baker's yeast consists of approximately 30–33 per cent of dry materials, 6.5–9.3 per cent of nitrogen, 40.6–58.0 per cent of proteins, 35.0–45.0 per cent of carbohydrates, 4.0–6.0 per cent of lipids, 5.0–7.5 per cent of minerals and various amounts of vitamins, depending on its type and growth conditions.

Yeast cells contain the cell wall oligosaccharides, peptides and amino acids which may stimulate appetite and improve feed intake (Gao *et al.*, 2008).

Sivla *et al.* (2009) reported that the yeast protein extract also contains nucleotides which reportedly stimulated development of gastrointestinal tract.

According to Pandove *et al.* (2010), the SCP has 55- 60 per cent crude protein which has good amino acid balance except for a deficiency in sulphur containing amino acid.

Ofodile *et al.* (2011) reported that the SCP from orange peels by *Saccharomyces cerevisiae* had the highest reducing sugar (22.52%) and the least protein (3.89%) contents. Similarly, *Saccharomyces cerevisiae* on pineapple waste produced least yield of cholesterol (2.25 mg/100g).

2.4.4 Value addition of single cell protein

Reed (1981) reported that single cell protein is used as powder or tablet in the health food industry, as food additive in formulated food such as baby foods, soups, gravy, baked food etc. It is also used as food flavourant, source of enzymes and vitamins

Single cell protein from yeast using carbohydrate as a carbon source is utilized world wide on a large scale in animal nutrition (Oura, 1983).

Single cell protein (SCP) has attracted commercial interest due to the possible substitution of microbial protein for the conventional protein supplements currently being used in the dairy and poultry industries (Chanda and Chakrabarti, 1996).

SCP could be used directly as human food supplement or as an animal food supplement to at least partially replace the currently used protein-rich soybean meal and fish proteins and even cereals, which can be diverted for human consumption (Singh, 1998).

Anupama and Ravindra (2000) reported that SCP can be used for protein supplementation of a staple diet by replacing costly conventional sources like soymeal and fishmeal to alleviate the problem of protein scarcity.

Haldar *et al.* (2011) studied the effects of yeast (*Saccharomyces cerevisiae*) and yeast protein concentrate on production performance of broiler chickens exposed to heat stress and challenged with *Salmonella enteritidis.* The study revealed that dietary supplementation of YPC and YPC pellets improved body weight at 21 and 35 d of age and FCR (feed conversion ratio) in 35 d. Salmonella numbers decreased in

the pooled digesta and excreta due to dietary supplementation of yeast and YPC additives particularly in the YPC-pellets group.

Another study by Bob-Manuel and Alfred-Ockiya, (2011) on evaluation of yeast single cell protein (SCP) diets on growth performance, feed conversion and carcass composition of Tilapia *Oreochromis niloticus* (L.) fingerlings revealed that yeast SCP can successfully replace fishmeal up to 50 per cent level with 0.25 per cent dietary methionine supplementation in a 30% protein diet with no significant difference in fish performance.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The present investigation on "Process standardization for the production of baker's yeast from mango stones" was carried out in the Department of Processing Technology, College of Horticulture, Vellanikkara, Thrissur during 2010- 2012.

The project was carried out under three experiments *viz.*

1) Standardization of pretreatments of the substrate for baker's yeast production.
2) Optimization of culture conditions and growth supplements for baker's yeast production.
3) Utilization of SCP enriched flour for preparation of biscuit.

3.1 Physical and chemical composition of mango fruit

As a preliminary study, the physical composition of fruit and the chemical composition of pulp adhering to the mango stone were determined to explore the feasibility of using it for single cell protein production.

3.1.1 Physical composition of mango fruit

Weights of different components of mango fruit *viz.* pulp, peel, stone and adhered pulp were recorded separately and expressed in percentage.

3.1.2 Chemical composition of mango pulp

The chemical constituents of pulp *viz.* moisture, total, reducing and non reducing sugar, TSS, pH, ascorbic acid and acidity were estimated.

3.1.2.1 Moisture

The moisture content of the pulp was determined using a digital moisture meter.

3.1.2.2 Sugar

Reducing sugar was estimated by the method given by Lane and Eyon (Ranganna, 1986). Twenty five gram of the sample was ground thoroughly in a mortar and to that 50 ml distilled water was added, neutralized with NaOH and then clarified with neutral lead acetate. Excess lead was removed by adding potassium oxalate. The

volume was then made upto 250 ml. An aliquot of this solution was titrated against a mixture of Fehlings solution A and B using methylene blue indicator. The reducing sugar was estimated as percentage.

From the clarified solution used for the estimation of reducing sugars, 50 ml was taken and boiled gently after adding citric acid and water. It was later neutralized with sodium hydroxide and the volume was made up to 250 ml. An aliquot of this solution was titrated against Fehlings A and B. The total sugar content was expressed as percentage.

The percentage of non reducing sugar was calculated by deducting the value of reducing sugar from total sugar.

3.1.2.3 TSS

The TSS of the pulp was determined using hand refractometer.

3.1.2.4 pH

The pH of the pulp was determined using pH meter.

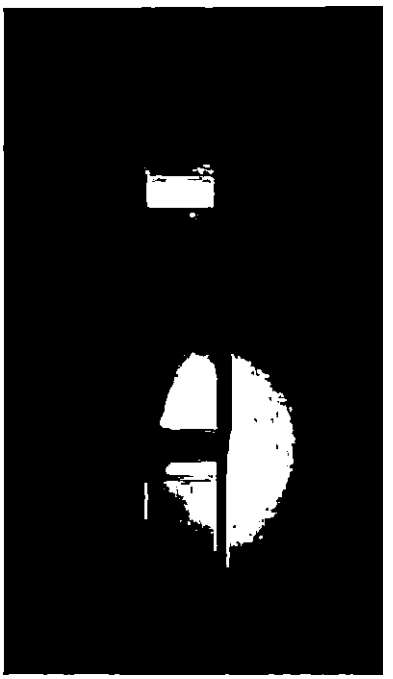
3.1.2.5 Acidity

Acidity of the pulp was determined by titration with standard sodium hydroxide (0.1N) and expressed as per cent of citric acid as per Ranganna (1997).

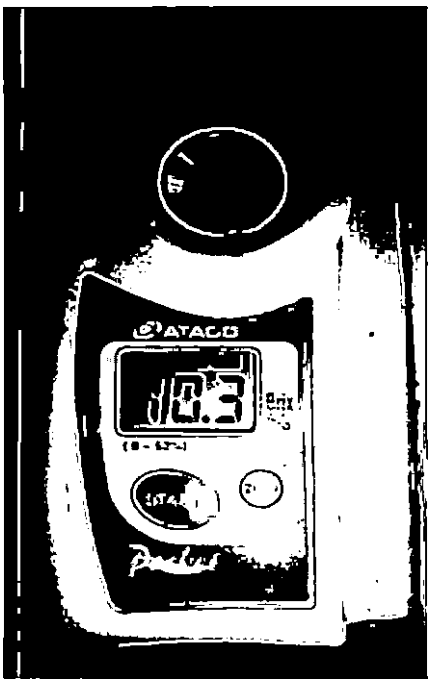
One gram of the sample was weighed accurately into a thimble and placed in 250ml conical flask. 100ml water was added and boiled for 15 minutes on the gas burner. Extract was cooled under tap water and made up to 250ml in a volumetric flask. It was mixed well and filtered through filter paper and 30ml supernatant was collected in 250ml volumetric flask. Few drops of phenolphthalein indicator was added and titrated with 0.1N NaoH. End point of titration was pink colour of solution in the beaker. Acidity was expressed in percentage.

3.1.2.6 Ascorbic acid

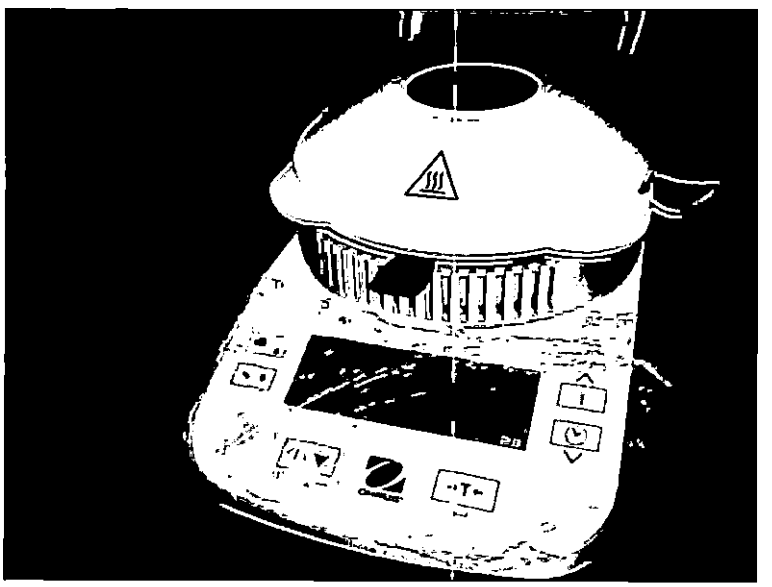
Five grams of pulp adhered on mango stones was taken and extracted with four per cent oxalic acid. Ascorbic acid content of the pulp was estimated by using



pH meter



Hand Refractometer



Moisture Meter

standard indicator dye 2, 6-dichlorophenol indophenol dye method and expressed as mg/100g of pulp (Sadasivam and Manikam, 1992)

3.2 Standardization of pretreatments of the substrate for baker's yeast production

3.2.1 Surface sterilization of mango stones

The pulp attached to the mango stones is not completely removed during processing. The mango stone with strongly adhering pulp can be utilized as medium for the growth of baker's yeast. But immediately after primary processing of mango, sugar present in the adhered pulp invites fungi, yeast and bacteria. So microbial contamination is the main problem encountered when the waste from the fruit processing industries is utilized as substrate for SCP production.

The ripe fruits of mango variety *Neelum* were selected, pulped and mango stones were extracted. Stones free from any visible weevil infestation were selected for the study. The stones with adhered pulp were subjected to the following treatments for surface sterilization.

3.1.3.1 Treatments

T_0- Control

T_1- Autoclaving at 121^{0}C and 15psi for 30 minutes

T_2- Steam blanching for 10 minutes

T_3- Hot water immersion for 10 minutes

3.1.3.2 Observations

3.1.3.2.1 Microbial count

The microbial count was enumerated after pretreatment. Serial dilution plating technique was used for isolation and enumeration of bacteria, yeast and fungi. One gram of adhered pulp was extracted by scraping using a sharp knife from the above mentioned pretreated mango stones under aseptic conditions. One gram of sample was suspended in 9 ml of distilled water which gave a dilution of 10^{-1}. The serial dilutions of 10^{-2} and 10^{-3} were made by pipetting 1 ml to the dilution blanks containing 9 ml

distilled water. Finally, these dilutions were added to sterile petridish to which sterilized media *viz.* Nutrient Agar for bacteria, Rose Bengal Agar for fungi and Sabourod Dextrose Agar for yeast were added. Dilution of 10^{-3} was selected for enumeration of bacteria, 10^{-2} and 10^{-1} were selected for fungi and yeast respectively.

Upon solidification, plates were incubated for 2-3 days at room temperature. After incubation, the plates were observed for growth of yeast, fungi and bacteria. Colony counts were taken and colony forming units (cfu) per gram of sample was calculated.

$$\text{cfu/g of sample} = \frac{\text{Number of colonies} \times \text{Dilution factor}}{\text{Volume of sample plated}}$$

3.2. Optimization of culture conditions and growth supplements for baker's yeast production

Lyophilized baker's yeast strain, *Saccharomyces cerevisiae* MTCC- 172 obtained from IMTECH, Chandigarh, was used for this study.

3.2.1 Preparation of primary inoculum

A small amount of a pellet of *Saccharomyces cerevisiae* was suspended in 50 ml yeast extract peptone dextrose (YEPD) broth. The composition of the YEPD media is given in Appendix I

MTCC culture was streaked on YEPD (Appendix I) agar media in a petridish and incubated at room temperature for 48 hours until visual growth appeared. For further use, the plates were stored in a refrigerator at 4^0C.

3.2.2 Standardization of working/ secondary inoculum

Yeast extract peptone dextrose broth (25ml) was prepared for the standardization of working inoculum. The prepared broth included 0.0075g yeast extract, 0.25g peptone, 0.5g dextrose and 25ml distilled water. The broth was sterilized by autoclaving at 121^0C and 15psi pressure for 30 minutes. The pH of the medium was adjusted to 4.5. A single colony was inoculated into the broth and incubated for 48 hours at room temperature. Serial dilution plating technique was used

Lyophilized bakers yeast

↓

50 ml YEPD Broth

↓

Incubation for 48 hours

↓

Primary inoculum

↓

Single Colony

↓

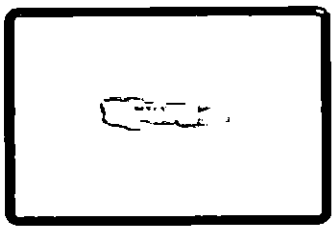
25ml YEPD Broth

↓

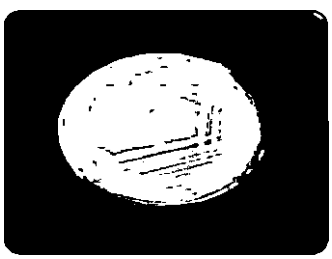
Incubation for 48 hours

↓

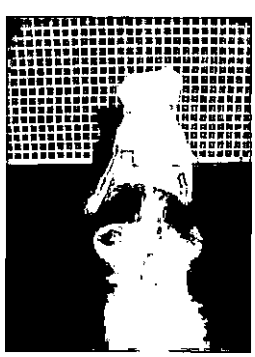
Working inoculum



Lyophilized bakers yeast



Primary inoculum



Working inoculum

Plate.2: Preparation of working inoculum/standard

to enumerate the viable yeast colonies present on the broth. The yeast population in 1ml of YEPD broth was counted in three different dilutions of 10^{-4}, 10^{-5} and 10^{-6} after 48 hours.

3.2.3 Preparation of working/ secondary inoculum

A single colony was scooped out from the surface of agar into 25ml YEPD broth in 50ml conical flask and incubated for 48 hours. After 48 hours, white colonies were found to sediment at the bottom of the conical flask which was used for inoculating the substrate after shaking.

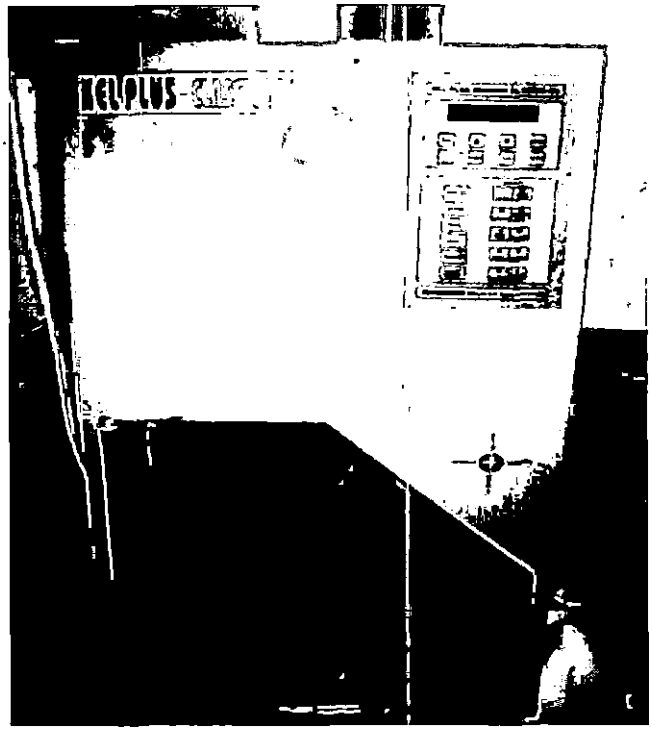
3.2.4 Optimization of culture conditions for baker's yeast production

3.2.4.1 TSS

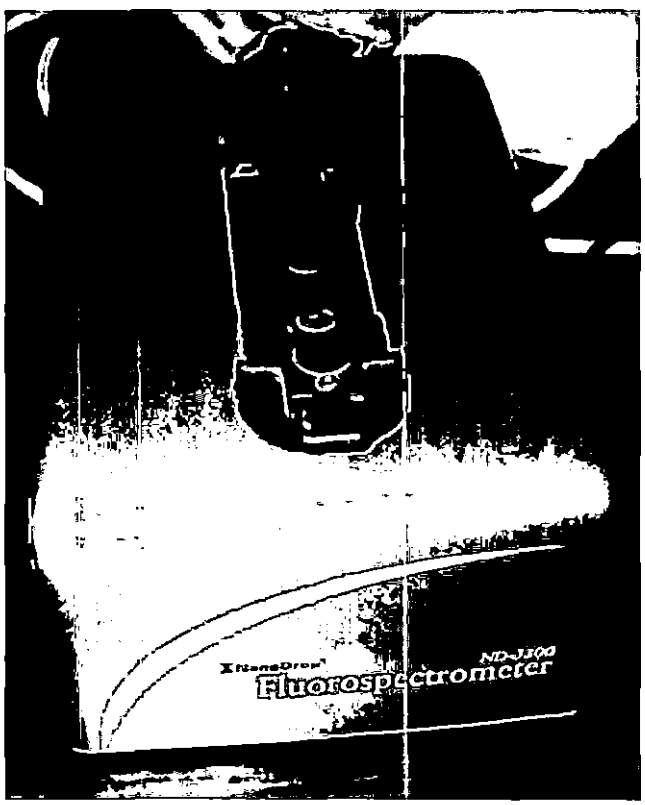
Ripe *Neelum* mangoes were selected, pulped and stones were extracted. The stones were weighed and equal amount of distilled water added to it. After thorough mixing, the initial TSS of the solution was recorded using hand refractometer. The TSS of the different solutions was adjusted to 20^0Bx, 30^0Bx and 40^0Bx respectively. One control sample was also maintained. All the above mentioned treatments were sterilized by autoclaving at 121^0C and 15psi pressure for 30 minutes. A known volume of secondary inoculum of baker's yeast *Saccharomyces cerevisiae* MTCC 172 strain was added to the fermenting media to get a cell volume of 10^6 cells/ml of substrate solution. The solution was capped air tight and kept for one week. After one week, the population of baker's yeast in each replication was isolated and enumerated by serial dilution plating technique. The final TSS of the solution was also determined using refractometer. The best TSS for the growth of baker's yeast was identified and maintained in the subsequent studies.

3.2.4.2 pH

Substrate for fermentation containing mango stones was prepared as detailed under 3.2.4.1. The TSS identified as best in the previous experiment was also maintained by adding sugar. The pH of the different solutions was adjusted to 3, 3.5 and 4 respectively. The substrate solution was sterilized by autoclaving at 121^0C and 15psi pressure for 30 minutes. The solution was capped air tight and kept for one



Kelplus instrument



Spectroflouri meter

Plate. 3: Instruments used for nutrient analysis

week. After one week, the population of baker's yeast in each replication was isolated and enumerated by serial dilution plating technique. The best pH for the growth of baker's yeast was identified and maintained in the subsequent studies. The change in pH during fermentation was also determined by recording the initial and final pH.

3.2.4.3 Duration of fermentation

The mango stone substrate for fermentation was prepared as detailed under 3.2.4.1. The TSS and pH identified as best for fermentation were also maintained. Each treatment was sterilized by autoclaving at 121^0C and 15psi pressure for 30 minutes. The flasks were taken out at 3, 5 and 7 days intervals. Baker's yeast population was enumerated by serial dilution technique after the specified duration of fermentation. The best duration of fermentation for the growth of baker's yeast was identified and maintained in the subsequent studies.

3.2.4.4 Aeration

The mango stone substrate for fermentation was prepared as detailed under 3.2.4.1. The TSS and pH standardized in the previous experiment were also maintained. Each treatment was sterilized by autoclaving at 121^0C and 15psi pressure for 30 minutes. One treatment was kept in stationery conditions and the other one was kept in an Orbital shaker at 100 rpm for 5 days. The substrate for fermentation was kept for the duration identified as best in 3.2.4.3. The yeast population was enumerated by serial dilution technique after the fermentation. The best treatment for the growth of baker's yeast was identified and maintained in the subsequent studies.

3.2.5 Optimization of growth supplements for the production of baker's yeast

The effect of the following supplements on biomass yield was evaluated. The substrate was prepared as detailed in 3.2.4.4. The different treatments were as follows

3.2.5.1 Treatments

T_0- Mango stone (1kg) + Distilled water (1 litre)

T_1- Mango stone (1kg) + Distilled water (1 litre) + Broken wheat (20g)

T_2- Mango stone (1kg) + Distilled water (1 litre) + Ammonium Sulphate (0.8%)

T_3- Mango stone (1kg) + Distilled water (l litre) + KH_2PO_4 (0.75%)

T_4- Mango stone (1kg) + Distilled water (l litre) + Biotin (0.04mg/l)

T_5-Mango stone (1kg) + Distilled water (l litre) + Ammonium Sulphate (0.8%) + KH_2PO_4 (0.75%) + Biotin (0.04mg/l)

The treatments were replicated thrice

3.2.5.2 Observations

3.2.5.2.1 Growth of baker's yeast

The population of baker's yeast was determined by serial dilution plating technique as detailed in 3.2.4.1

3.2.5.2.2 Yeast biomass yield

The wet and dry biomass yield of yeast in different treatments was determined. 40ml substrate solution was poured into sterilized Oakridge tube (centrifugation tube) and centrifuged at 11000 rpm for 10 minutes. The biomass was scooped out from the tube using well sterilized spatula. Then, the yeast cells were mechanically disintegrated at pH 9.5 with 10 per cent NaCl and heated at 80^0C for 10 minutes for reducing the nucleic acid content. The precipitated yeast biomass separated and wet weight of the biomass was recorded. The biomass was dried in a vacuum drier and dry weight of the biomass was determined.

3.2.5.2.3 Protein

The protein content of yeast biomass was analyzed by Kjeldal method using Kelplus instrument. Vacuum dried yeast biomass of 0.2g weight was used for the analysis of protein content. The weighed sample was digested for 2 hours by using concentrated H_2SO_4 in a digestion chamber. The digested sample was transferred to 50ml volumetric flask and the volume was made upto 50ml. Then, the digested sample (10ml) was distilled by 40 per cent NaOH and 4 per cent boric acid in Kelplus instrument. The extract after distillation was titrated against 0.05N H_2SO_4 till the colour changed from blue to pink. The percentage of protein in the yeast biomass was calculated by using the formula.

$$\text{Percentage of protein} = \frac{TV \times 14 \times 0.05 \times 100 \times 50 \times 6.25}{1000 \times 0.2 \times 10}$$

3.3. Utilization of SCP enriched flour for preparation of biscuit

3.3.1 Enrichment of refined wheat flour

Powdered dry yeast was incorporated into the refined wheat flour so as to raise the protein content to 15 and 20 per cent.

3.3.1.1 Observations

3.3.1.1.1 Protein

Protein content of refined wheat flour and enriched refined wheat flour was determined as detailed in 3.2.5.2.3

3.3.2 Preparation of biscuit

Biscuits were prepared using both refined wheat flour and enriched refined wheat flour by standard procedure (Appendix. II). The dried biomass contained 39.38 per cent of protein and biomass of 0.156 and 0.257g was incorporated into one gram of refined wheat flour to raise its protein content from 11.75 per cent to 15 and 20 per cent respectively.

3.3.2.1 Observations

3.3.2.1.1 Protein

Same as 3.2.5.2.3

3.3.2.1.2 Thiamine

Thiamine content was estimated as suggested by Sadasivam and Manikam (1992). Dissolved 50 mg of thiamine hydrochloride in 500 ml of 0.1N sulphuric acid containing 25 per cent alcohol to get stock solution and from this working standard was prepared.

Five gram of finely ground sample was taken in a 250 ml conical flask in duplicate. Slowly added 100 ml 0.1N sulphuric acid without shaking and was kept overnight. After shaking vigorously, filtered through Whatman No.1 filter paper and discarded the first 10-15 ml of the filtrate. Pipetted out 10 ml of the extract in duplicate into 100 ml separating funnels. Pipetted out 10 ml of working standard and

added 3 ml of 15 per cent sodium hydroxide into each separating funnel immediately followed by four drops (0.2 ml) of ferricyanide solution. 10 ml of the extract without the addition of ferricyanide solution was set as the sample blank. After shaking gently for exactly 30 seconds, added 15 ml of isobutanol rapidly from a quick delivery burette. Stoppered immediately, shook vigorously for 60 seconds and allowed the layers to separate. Drained off the bottom layer carefully and discarded it and added one spatula full of sodium sulphate directly into the separating funnel, stoppered and swirled gently to clarify the extract. The clear extract was collected from the top into a clean dry test tube and read at an excitation wave length of 365 nm and emission wave length 435 nm. The fluorescence of the sample was noted. The thiamine content was expressed as mg per 100g of the sample.

3.3.2.1.3 Riboflavin

Riboflavin content was estimated as suggested by Sadasivam and Manikam (1992).

Dissolved five milligram of riboflavin standard in 100 ml standard flask with five per cent acetic acid. The flask was then covered with aluminum foil to prevent decomposition of riboflavin. It was further diluted to give 10 ppm with five per cent acetic acid. Blank was set at 5 per cent acetic acid.

Weighed two gram of the sample into 250 ml conical flask and added 75 ml 0.1N H_2SO_4 and autoclaved the mixtures for 30 minutes. Cooled and added five ml of 2.5 molar sodium acetate solution and kept for one hour. Transferred to volumetric flask and made up to 100 ml. Filtered and discarded the first 10-15 ml. Ten ml of the sample solution was taken and added two ml of water and one ml of potassium permanganate (4%) solution, kept for two minutes and then added one ml of hydrogen peroxide solution and read immediately in the spectroflurimeter with an excitation wave length of 390nm. The riboflavin content was expressed as mg per 100g of the sample.

3.3.3 Sensory evaluation

3.3.3.1 Selection of judges

A series of sensory evaluation were carried out using hedonic scale at laboratory level to select a panel of ten judges between the age group of 18- 40 years as suggested by Jellinek (1985)

3.3.3.2 Preparation of score card

Score card including the quality attributes like odour, colour, texture, taste, after taste and overall acceptability was prepared for sensory evaluation of biscuits. Each of the above mentioned qualities were assessed by a 9 point hedonic scale. Overall acceptability was calculated separately using the average of above mentioned quality attributes. The score card used for the evaluation of biscuits is given in Appendix III.

3.3.3.3 Organoleptic evaluation

Organoleptic evaluation of biscuits was carried out using the score card by a panel of ten selected judges.

3.3.3.4 Analysis of data

The observations recorded were tabulated and the data analyzed statistically. The scores of organoleptic evaluation were analyzed by Kruskal Wallis test.

4. RESULTS

The results of the study on "Process standardization for the production of baker's yeast from mango stones" are presented in this chapter.

4.1. Physical and chemical composition of mango fruit and stone

4.1.1 Physical composition of fruit

The physical composition of fruit was determined by computing percentage of peel, pulp and stone. Studies on physical composition revealed that the content of the pulp, peel, stone and adhered pulp in mango ranged from 65.22- 73.69 per cent, 8.32- 12.18 per cent, 13.50- 21.94 per cent and 5.32- 8.89 per cent respectively (Tab. 1). Pulps had highest contribution to the fruit composition followed by stone, peel and adhered pulp.

4.1.2 Chemical composition of mango pulp

The chemical constituents of the pulp adhering to mango stone viz. moisture, reducing sugar, non reducing sugar, acidity and ascorbic acid were estimated and results are presented in Table 2.

4.1.2.1 Moisture

Moisture content of the pulp ranged from 78.8 to 80.10 per cent.

4.1.2.2 Sugar

The total sugar content in mango pulp adhered on mango stone ranged from 13.05-13.77 per cent. The reducing and non-reducing sugar content in mango pulp ranged from 2.80-3.52 and 10.25 per cent respectively.

4.1.2.3 TSS

The TSS of pulp adhering to mango stone was ranged from 9.13- 15. 80^0B.

4.1.2.4 pH

The pH of the pulp adhering to mango stone was determined by pH meter. It ranged from 1.90- 3.50

Tab. 1: Physical composition of mango fruit

Physical composition	Percentage
Pulp	65.22- 73.69
Peel	8.32- 12.18
Stone	13.50- 21.94
Adhered pulp	5.32- 8.89

Tab.2: Chemical composition of mango pulp

Chemical constituents	Content
Moisture (%)	78.8- 80.1
Reducing sugar (%)	2.80-3.52
Non reducing sugar (%)	10.25
Total Sugar (%)	13.05-13.77
TSS (^{0}B)	9.30- 15.80
pH	1.90- 3.50
Acidity (%)	0.26- 0.37
Ascorbic acid (mg/100g)	16. 94-21.01

Ripe *Neelum Mangoes*

Proportion of pulp, peel and stone

Plate.4: Physical composition of mango fruit

4.1.2.5 Acidity

The acidity of the pulp adhering to mango stones ranged from 0.26- 0.37 per cent.

4.1.2.6 Ascorbic acid

The ascorbic acid in the pulp ranged from 16.94-21.01 mg/100g.

4.1.3 Standardization of pretreatments of the substrate for baker's yeast production

4.1.3.1 Surface sterilization of mango stones

The presence of pulp on the mango stones after primary processing invites microbes. The effect of autoclaving, steam blanching and hot water immersion for surface sterilization was studied. Microbial growth was detected three days after pretreatment. Population of bacteria, yeast and fungi after pretreatments was enumerated by serial dilution plating technique and the data is presented in the Table 3

4.1.3.1.1 Bacteria

T_1 (autoclaved samples) was found to be the best pretreatment without bacterial population. The population of bacteria in T_2 was 15.25×10^3 cfu/g which was on par with T_3 (16.00×10^3 cfu/g). Highest population of bacteria was observed in T_0 (40.50×10^3 cfu/g).

4.1.3.1.2 Fungi

Population of fungi was not observed in T_1. Maximum fungus population was observed in T_0 (51.75×10^2 cfu/g). The fungus population of T_3 was 16.50×10^2 cfu/g which was on par with T_2 (13.75×10^2 cfu/g).

4.1.3.1.3 Yeast

Yeast growth was observed on third day except in autoclaved samples. T_1 was the best pretreatment for surface sterilization without yeast population. T_0 (109.50×10^1 cfu/g) was found to be the highest yeast populated treatment. The treatment, T_2 (28.50×10^1 cfu/g) was on par with T_3 (30.00×10^1 cfu/g) with respect to yeast population.

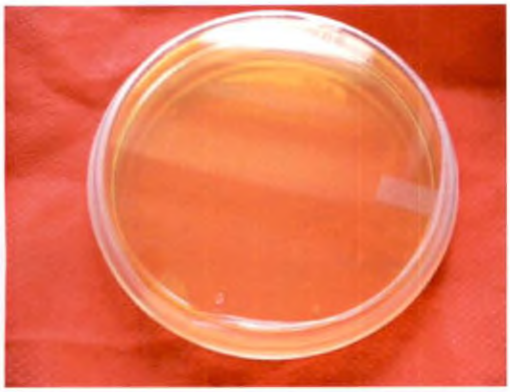
Control (T_0)

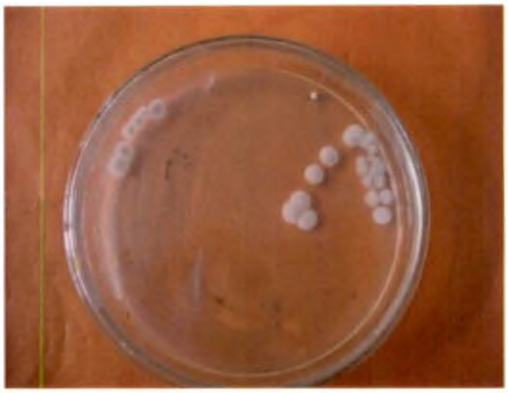
Autoclaving (T_1)

Steam blanching (T_2)

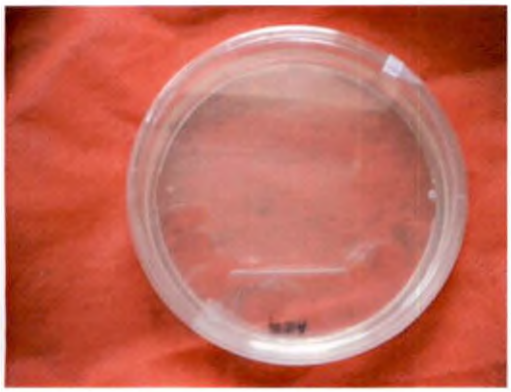
Hot water immersion (T_3)

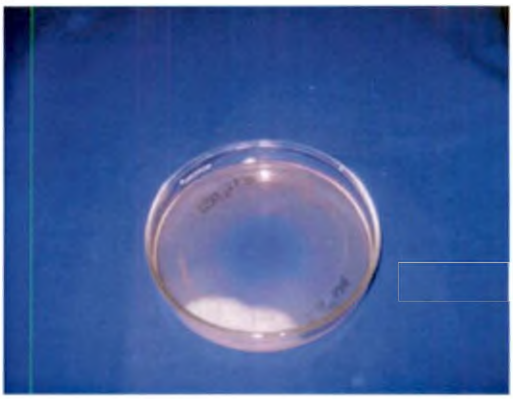
Plate.5: Surface sterilization treatments of mango stones



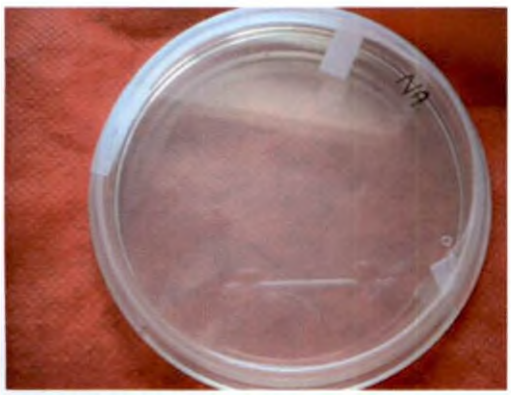


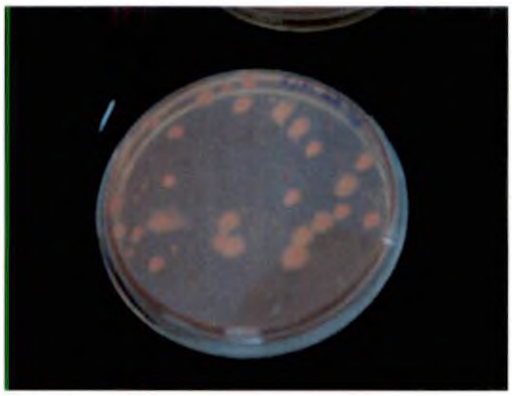
Yeast





Fungus





Bacteria

Autoclaved treatment **Control**

Plate.6: Effect of surface sterilization on microbial decontamination

The results revealed that autoclaving (T_1) was the best pretreatment for surface sterilization of mango stones without microbial population

4.2 Optimization of culture conditions and growth supplements for baker's yeast production

4.2.1 Standardization of secondary inoculum

The secondary inoculum was standardized by inoculating a single colony into 25ml YEPD broth. The yeast population was enumerated after 48 hours by serial dilution plating technique. Yeast colonies observed in 1ml of broth was 106×10^6 cfu/ml. This result indicated that the addition of 0.009ml of secondary inoculum to the substrate solution gives cell volume of 10^6 cells/ml of the substrate solution.

4.2.2 Culture conditions

4.2.2.1 TSS

The effect of TSS on yeast growth was studied and the results are presented in Table 4. The different treatments were T_0 (control), T_1 (20^0B), T_2 (30^0B) and T_3 (40^0B). The yeast population after adjusting the TSS was enumerated by serial dilution plating technique. Milky white yeast colonies were detected two days after inoculation. Highest yeast count (176.00×10^6cfu/g) was observed in T_2 (30^0 B). The least yeast count was found in control sample (T_0) *ie.* substrate without sugar supplementation (70.00×10^6cfu/g) which was on par with T_3 (104.50×10^6cfu/g) and T_1 (76.50×10^6cfu/g).

The final TSS of treatments was measured after one week of fermentation. The initial TSS of 20.00^0 B in T_1 decreased to 17.20^0B one week after fermentation (Table 4). The sugar utilization of T_1 was 14.00 per cent. Highest sugar utilization (46.00%) was noticed in T_2 where the initial TSS of 30.00^0 B decreased to 16.20^0 B, followed by T_3 with initial TSS 40.00^0 B, final TSS 29.90^0 B and 25.25 per cent sugar utilization. Sugar utilization was least in T_0 (7.60%).

4.2.2.3 pH

The effect of pH on the growth of yeast and the change in pH during fermentation was studied and the results are presented in Table 5. T_3 (pH 4) which

Tab.3: Effect of surface sterilization on microbial population (cfu/g)

Treatments	Bacteria (10^3 cfu/g)	Fungi (10^2 cfu/g)	Yeast (10^1 cfu/g)
T_1 (Autoclaving)	0.00^a	0.00^a	0.00^a
T_2 (Steam blanching)	15.25^b	13.75^b	28.50^b
T_3 (Hot water immersion)	16.00^b	16.50^b	30.00^b
T_0 (Control)	40.50^c	51.75^c	109.50^c

Value with different alphabets as superscripts are significantly different (<P 0.05)

DMRT column wise comparison

Tab.4: Effect of TSS on yeast growth and sugar utilization

Treatment	Initial TSS (^{0}Bx)	Final TSS (^{0}Bx)	Percentage of sugar utilized	Yeast count (10^6 cfu/g)
T_1	20.00	17.20	14.00	76.50^b
T_2	30.00	16.20	46.00	176.00^a
T_3	40.00	29.90	25.25	104.50^b
T_0	5.20	4.80	7.60	70.00^b

Value with different alphabets as superscripts are significantly different (<P 0.05)

DMRT column wise comparison

recorded the highest yeast count (225×10^6cfu/g), was the best pH for the growth of baker's yeast. The minimum yeast count was observed in T_0 (49.5×10^6cfu/g). The yeast count observed in T_2 was 116×10^6cfu/g which was on par with T_1 (65×10^6cfu/g).

The change in pH one week after fermentation was evaluated. The initial and final pH was measured by using digital pH meter. The results revealed that during fermentation, the substrates become more acidic. The change in pH was maximum in T_3 where the pH changed from 4.00 to 3.10 followed by T_2 where initial pH of 3.50 decreased to 3.05 one week after fermentation. The initial pH of T_1 decreased from 3.00 to 2.85 one week after fermentation. The least change in pH was observed in control sample where the initial pH 2.30 decreased to 2.20.

4.2.2.4 Duration of fermentation

The population of yeast was recorded on third, fifth and seventh day of fermentation and the results are presented in Table 6. The yeast count on third day (T_1) of fermentation was 12.00×10^7cfu/g which increased to 34.00×10^7cfu/g on 5th day (T_2) of fermentation. However, the yeast population decreased to a level of 23.00×10^7cfu/g when observed on 7th (T_3) day of fermentation.

4.2.2.5 Aeration

The effect of aeration on yeast growth was observed by keeping the flasks containing substrates in stationery and shaking conditions (100rpm) for 5 days. Significant variation was observed between the two treatments. Substrates kept in stationary conditions were observed to have the least population of yeast (30.75×10^7 cfu/g). The maximum count was recorded in substrates which were kept in an orbital shaker (38.00×10^7 cfu/g) during fermentation.

Tab.5: Effect of pH on yeast growth

Treatments	Initial pH	Final pH	Yeast count (10^6 cfu/g)
T_1	3.00	2.85	65.00^{bc}
T_2	3.50	3.05	116.00^{b}
T_3	4.00	3.10	225.00^{a}
T_0	2.30	2.20	49.50^{c}

Value with different alphabets as superscripts are significantly different (<P 0.05)

DMRT column wise comparison

Tab.6: Effect of duration of fermentation on yeast growth

Treatment	Yeast count (10^7cfu/g)
T_1 (3^{rd} day)	12.00^{c}
T_2 (5^{th} day)	34.00^{a}
T_3 (7^{th} day)	23.00^{b}

Value with different alphabets as superscripts are significantly different (<P 0.05)

DMRT column wise comparison

4.2.3 Growth supplements

4.2.3.1 Effect of growth supplements

The influence of different growth supplements on yeast growth was studied under this experiment and the results are presented in Table 7. The maximum yeast count (49.00×10^7 cfu/g) was found in T_2 *ie.* media supplemented with ammonium sulphate followed by the media supplemented with a combination of ammonium sulphate, KH_2PO_4 and Biotin *ie.* T_5 (44.50×10^7 cfu/g). The control treatment had the least yeast count (39.00×10^7 cfu/g) which was on par with T_4 (39.50×10^7 cfu/g). Yeast count in T_3 was 41.00×10^7 which was on par with T_1 (41.00×10^7 cfu/g).

4.2.3.2 Biomass yield

4.2.3.2.1 Wet biomass yield

The substrate solution was centrifuged at 11000rpm for 10 minutes. The precipitated yeast cells were scooped out and weighed to obtain the wet biomass yield and the results are presented in Table 7. The highest wet biomass yield was noticed in T_2 (13.67g/l) followed by T_5 (9.31g/l) and T_1 (9.02g/l). The least wet yield was in T_0 (5.80g/l) and T_4 (6.11g/l). The wet yield of T_3 was 7.43g/l.

4.2.3.2.2 Dry biomass yield

The wet biomass was dried in a vacuum drier, the dry weight recorded and the results are presented in Table 7. T_2 recorded the maximum dry biomass yield (2.18g/l) followed by T_5 (1.38g/l) and T_1 (1.33g/l). Minimum dry biomass yield was observed in T_0 which was on par with T_3 (0.94g/l) and T_4 (0.88g/l).

4.2.3.2.3 Protein content of biomass

Weighed dry yeast biomass (0.2 g) was used for the estimation of protein in Kelplus instrument. The result showed that the protein content of dry yeast was highest in the media supplemented with ammonium sulphate *ie.*T_2 (39.38%) which was on par with T_1 *ie.* media supplemented with broken wheat (38.28%) and T_5 *ie.* media supplemented with a combination of ammonium sulphate, KH_2PO_4 and biotin

(38.83%). Least protein content (31.17%) was recorded in control treatment which was on par with T_3 (33.90%) and T_4 (32.81%).

4.2.3.2.4 Protocol for production of baker's yeast

Based on the results of the second experiment, the protocol for production of baker's yeast using mango stones as substrate was developed (Plate. 7). The optimum culture condition for maximum growth of *Saccharomyces cerevisiae* MTCC- 172 were TSS (30^0B), pH (4) and providing aeration by keeping in an orbital shaker at 100rpm for 5days. Supplementation of the substrate with a nitrogen source (NH_2SO_4 0.8%) is essential for high biomass yield and protein content.

4.3 Utilization of SCP enriched flour for preparation of biscuit

Single cell protein from baker's yeast rich in protein, vitamins and enzymes is used both in human and animal feed industry. The single cell protein could be used in foods replacing the conventional protein sources. Hence, the feasibility of utilizing refined wheat flour enriched with single cell protein for preparation of biscuit was studied.

4.3.1 Enrichment of refined wheat flour

Powdered yeast of 0.257g was incorporated to the refined wheat flour to raise the protein content of 1g refined wheat flour to 20 per cent and 0.156g was incorporated to raise the protein content to 15 per cent.

4.3.1.1 Protein

The protein content of refined wheat flour and enriched refined wheat flour was analyzed using Kelplus instrument. The protein content recorded in refined wheat flour was 11.75 per cent and in enriched refined wheat flour was 15.02 and 19.41 per cent.

4.3.2 Preparation of biscuit

Biscuits were prepared by incorporating refined wheat flour and enriched refined wheat flour as per standard recipe (Appendix I). The results of protein, riboflavin and thiamin content in the biscuits are presented in this section.

Plate.7:Protocol for production of bakers yeast

Mango stones + Distilled water

↓

TSS 30^0B, pH 4, NH_2SO_4

↓

Autoclave (121^0C, 15psi, 30 min)

↓

Yeast inoculation

↓

Agitate @100rpm for 5 days

↓

Centrifuge @11000rpm for 10 min.

↓

10% NaCl, pH 9.5, 80^0C for 10 min

↓

Centrifuge @ 11000rpm for 10 min.

↓

Wet Biomass

↓

Vacuum freeze drying

↓

Dry yeast

Mango stones

Autoclave

Orbital Shaker

Centrifuge

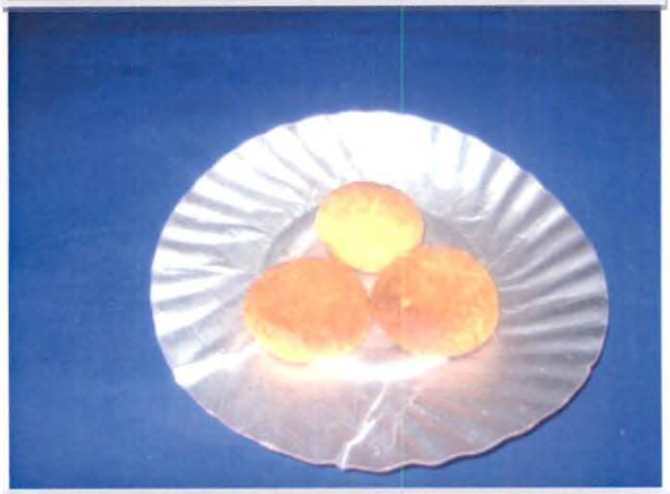
Wet Biomass

Vacuum Freeze Drier

Dry Yeast

Plate. 8:Protein enrichment of refined wheat flour

Refined Wheat Flour Biscuits



15% Protein Enriched Biscuits

20% Protein Enriched Biscuits

Plate. 9:Protein enrichment of biscuits

4.3.2.1 Protein

Biscuits from enriched refined wheat flour were found to be good source of protein. The protein content in biscuit enriched with single cell protein was 14.85 (T_1) and 19.14 (T_2) per cent whereas the biscuits made from refined wheat flour had a protein content of 11.21 per cent.

4.3.2.2 Thiamine

Thiamine was not detected in biscuits made from both refined and enriched refined wheat flour.

4.3.2.3 Riboflavin

Riboflavin was not detected in biscuits made from both refined and enriched refined wheat flour.

4.3.3 Sensory evaluation

The biscuits prepared from refined wheat flour and enriched refined wheat flour was evaluated by sensory method. The attributes evaluated were odour, colour, texture, taste, after taste and overall acceptability. The biscuits were ranked for all these quality attributes based on mean scores. The results of organoleptic evaluation of biscuits are presented in this section.

The mean scores obtained for various quality attributes like colour, odour, texture, taste, after taste and overall acceptability of biscuits are presented in Table 8.

Overall acceptability was highest for T_0 (7.7) which was followed by T_1 (6.6) and least in T_2 (6). The mean rank on the basis of Kruskal-Wallis Test was found to be 22.20, 14.55 and 9.75. The statistical analysis showed that all the treatments were acceptable in sensory attributes.

Biscuits made from refined wheat flour obtained the highest mean score (8) and mean rank (23.30) for taste. The biscuits made from 20 per cent enriched flour had recorded a mean score 5.2. The 15 per cent protein enriched biscuits recorded mean rank 14.7 and mean score 6.5.

Tab.7: Effect of different growth supplements on yeast growth, biomass yield and protein content

Treatment	Yeast count (10^7cfu/g)	Wet biomass yield (g/l)	Dry biomass yield (g/l)	Protein content (Percent)
T_1	41.00^{bc}	9.02^{b}	1.33^{b}	38.28^{a}
T_2	49.00^{a}	13.67^{a}	2.18^{a}	39.38^{a}
T_3	41.00^{bc}	7.43^{bc}	0.94^{c}	33.90^{b}
T_4	39.50^{c}	6.11^{c}	0.88^{c}	32.81^{b}
T_5	44.50^{b}	9.31^{b}	1.38^{b}	38.83^{a}
T_0	39.00^{c}	5.80^{d}	0.85^{c}	31.17^{b}

Value with different alphabets as superscripts are significantly different (<P 0.05)

DMRT column wise comparison

Tab.8: Mean scores for the organoleptic qualities of biscuits

Sensory Attributes	T_0	T_1	T_2
Colour	8.1 (23.35)	6.3 (12.25)	6.0 (10.90)
Texture	7.4 (17.40)	6.9 (15.50)	6.7 (13.60)
Odour	7.6 (21.60)	6.6 (15.25)	6.0 (9.65)
Taste	8.0 (23.30)	6.5 (14.70)	5.2 (8.50)
After taste	7.0 (21.85)	6.0 (14.55)	4.9 (10.10)
Overall Acceptability	7.7 (22.20)	6.6 (14.55)	6.0 (9.75)

The biscuits made from refined wheat flour obtained the highest mean score (8.1) and rank (23.35) followed by T_1 with mean score (6.3) and rank (12.25) and T_2 with mean score (6.0) and rank (10.90) for colour.

All the three treatments were found to be acceptable for the quality attribute, texture. Among these, the refined wheat flour biscuits recorded the highest mean score (7.4) and rank (17.40). T_1 and T_2 obtained the mean scores 6.9 and 6.7 respectively.

In case of sensory attribute after taste and taste, 20 per cent enriched biscuits were found to be below acceptable level due to a slight bitterness evolved from the yeast powder. The highest mean score (7) was recorded in biscuits made from refined wheat flour (T_0).

Out of three treatments, T_0 recorded highest mean score for odour (7.6) and mean rank (21.60) followed by T_1 (6.6) (15.25) and T_2 (6) (9.65). All the treatments had an acceptable odour.

DISCUSSION

5. DISCUSSION

Proteins are the essence of life processes and are important for proper growth and development of all the living beings. Protein deficiency may lead to a number of health disorders in an individual. There is a big gap between the demand of protein rich food and its supply to the ever increasing population. The global shortage of food and feed protein has prompted researchers to seek protein production from both conventional and non conventional sources. One promising unconventional source is the production of microbial protein by fermentation of agricultural waste products.

Fruit and vegetable processing industries generate waste to the extent of 20-50 per cent of raw materials utilized. The fruit waste can be utilized as a substrate of choice to produce single cell protein (SCP) by fermentation process. Moreover, bioconversion of agricultural and industrial wastes to protein-rich food and fodder stocks has an additional benefit of making the final product cheaper (Anupama and Ravindra, 2000). The yeasts are extensively used as a microbe of choice for production of single cell protein because of its ease of isolation and growth on carbohydrate containing media. The yeasts are also used as sources of high nutritional value proteins, enzymes and vitamins, with applications in the health food industry as nutritional supplements, as food additives, conditioners and flavouring agents.

Mango is one of the most important fruits used by the processing industry in India for the production of wide variety of products like pickles, jam, beverages, fruit bar, powder *etc.* During processing of mangoes, more than 50 per cent waste is generated in the form of peel, stones and pulp fibres (Sounder and Chandra, 1987). Among these, stone contributes about 25-30 per cent of fruit (Berh *et al.*, 1976). The high TSS and low pH of pulp adhering to the stones makes it one of the best substrates for baker's yeast production. Hence, the present study was undertaken to study the feasibility of utilizing mango stones as a substrate for multiplication of yeast *Saccharomyces cerevisiae* MTCC 172 and optimizing the culture conditions for maximum biomass and protein yield. The results of experiments conducted are discussed in this chapter.

5.1 Physical and chemical composition of mango fruit and pulp

Study of the proximate composition of the pulp adhering to stone is a prerequisite for single cell protein production from mango stones. Hence under this experiment the physical and chemical composition of mango fruit and pulp were evaluated.

5.1.1 Physical composition of mango fruit

The physical composition of mango fruit was determined by computing the percentage of pulp, peel and stone. The content of pulp, peel, stone and adhered pulp in mango ranged from 65.22- 73.69 per cent, 8.32- 12.18 per cent, 13.5- 21.94 per cent and 5.32- 8.89 per cent respectively. The result is in general agreement with Warade *et al.* (2009) who reported that the pulp, stone and peel content of mango ranged from 48.69- 78.75 per cent, 8.24- 26.22 per cent and 11.11- 40.38 per cent respectively. Hanmant (2010) also reported that the content of pulp, peel and stone in mango ranged from 30.70- 70.00 per cent, 15.20- 28.47 per cent and 11.96- 40.83 per cent respectively. Garg (2006) reported that pulp strongly attached to the mango stones ranged from 6-7 per cent.

5.1.2 Chemical composition of mango pulp

The chemical composition of mango pulp adhering to the mango stones viz. moisture, sugar, TSS, pH, acidity and ascorbic acid were analyzed. The TSS ranged from 9.1 to 15.8^{0}B and the moisture content from 78.8 to 80.1 per cent. The pH ranged from 1.90- 3.50. The acidity and ascorbic acid ranged from 0.26- 0.37 per cent and 16.94-21.01mg/100g respectively. The sugar in terms of total, reducing and non-reducing sugar was noted and the content ranged from 13.05-13.77, 2.80-3.52 and 10.25 per cent respectively. The results corroborates with the findings of Anila and Radha (2003) who reported that the TSS, total and reducing sugar in mango pulp ranged from 10- 24^{0}B, 6- 20.66 per cent and 2.23- 2.97 per cent respectively. The preliminary study conducted on physical and chemical composition of mango indicates the possibility of utilizing mango stones as a substrate for single cell protein.

5.1.3 Microorganism

The strain of *Saccharomyces cerevisiae* MTCC 172 was selected for single cell protein production. The high content of microbial protein having similar amino acid profile with vegetable protein, rapid growth and high biomass are the advantages of using *Saccharomyces* to obtain single cell protein. They can use a wide range of raw materials as organic carbon source, mainly waste or byproducts from other industries (Begea *et al*, 2008).

5.1.4 Standardization of pretreatments of the substrate

One of the main problems associated with mango stones is that during processing the pulp attached to the stones are not removed completely. The remaining pulp adhering to mango pulp attracts microorganisms especially fungi and yeasts. As the fungi grow, they utilize the sugar present in the pulp and after that produce spores which further disperse in the medium. If by chance, this fungus happens to be pathogenic in nature it may cause nuisance. When the waste from fruit processing industries is utilized as substrate for single cell protein production, microbial contamination is the main problem encountered. Hence the experiment was carried out to study the effect of different surface sterilization methods such as autoclaving at 121^0C and 15psi for 30 minutes, steam blanching for 10 minutes and hot water immersion for 10 minutes on reduction of microbial load on the mango stones.

Significant decrease in microbial population (yeast, bacteria and fungi) was observed after each treatment and the data on results shown in figure 2. The population of microorganisms (yeast, bacteria and fungi) was not observed in autoclaved samples. Venkatesh *et al.* (2009) also reported that autoclaving at 121^0 for 20 minutes was effective in killing the native microorganisms present in fruit waste. Similar results were reported by Rosma *et al.* (2005) in single cell protein production by *Candida utilis* utilizing pineapple waste, where the substrate was autoclaved at 121^0C and 15 psi for 30 minutes. It is obvious that autoclaving is effective in reducing surface microbial contamination and the best surface sterilization method for fruit waste.

5.2 Optimization of culture conditions and growth supplements for baker's yeast production

Various factors are known to influence fermentation processes. These include carbon and energy source requirements, oxygen demand and supply, temperature, pH, nitrogen, phosphorus and potassium requirement (Pirt, 1975). Hence in this study some factors affecting the cell biomass production from mango stones by *Saccharomyces cerevisiae* MTCC 172 were evaluated.

5.2.1 TSS

Pulp adherence on mango stones makes it one of the best substrates for yeast growth because of its high TSS. The mango pulp adhering to the stones was found to contain a good amount of reducing (2.80-3.52%) and non reducing sugars (10.25%). Fermentable sugars in the substrate which can be used as a substrate for the growth of microorganisms and production of single cell protein are indicated by the TSS (Khan and Dahot, 2010). Hence the effect of TSS on yeast growth was evaluated after adjusting the TSS of the fermenting media by adding sugar to raise the TSS to 20^0B, 30^0B and 40^0B.

Significant variation was observed between substrates of different TSS. The results of the study revealed that there was a gradual increase in yeast population with increase in TSS upto 30^0B (176.00×10^6cfu/g), thereafter a decline in the population was observed (Fig.2). The yeast growth in control (70×10^6cfu/g), T_1 (76.50×10^6cfu/g) and T_3 (104.50×10^6cfu/g) are found to statistically equal. Garg (2006) reported that the mango pulp has TSS as high as 22^0B and low pH (5.0- 5.5) which is most favourable for the growth of yeast. Similarly, Rosma *et al.* (2005) evaluated the growth of yeast in pineapple waste for 1, 3 and 5^0B where the maximum yeast was obtained from the combination of 5^0B and inoculum size six per cent showing an increment of 216.79 per cent compared to non-optimized fermentation process. The added sugar acts as carbon source which would have lead to peak growth in substrate with 30^0B. Adoki (2008) also reported that supplementation with sugar (dextrose) had a positive effect on the growth of the organisms. The significant decrease of yeast growth at 40^0B may

Fig.1 Effect of surface sterilization on microbial count (cfu/g)

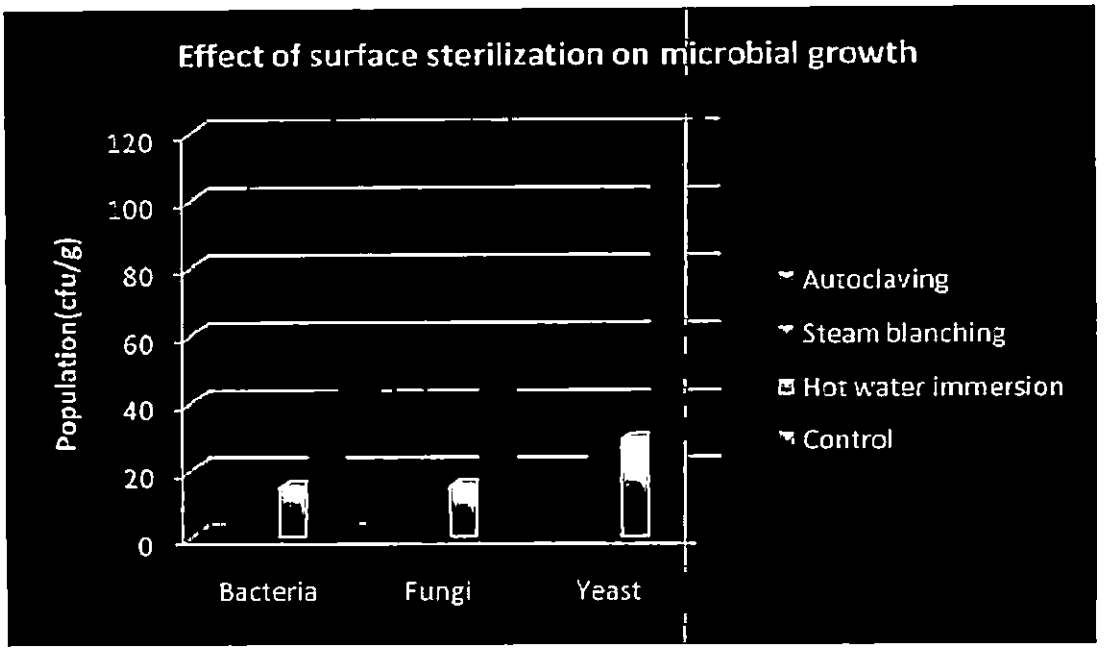
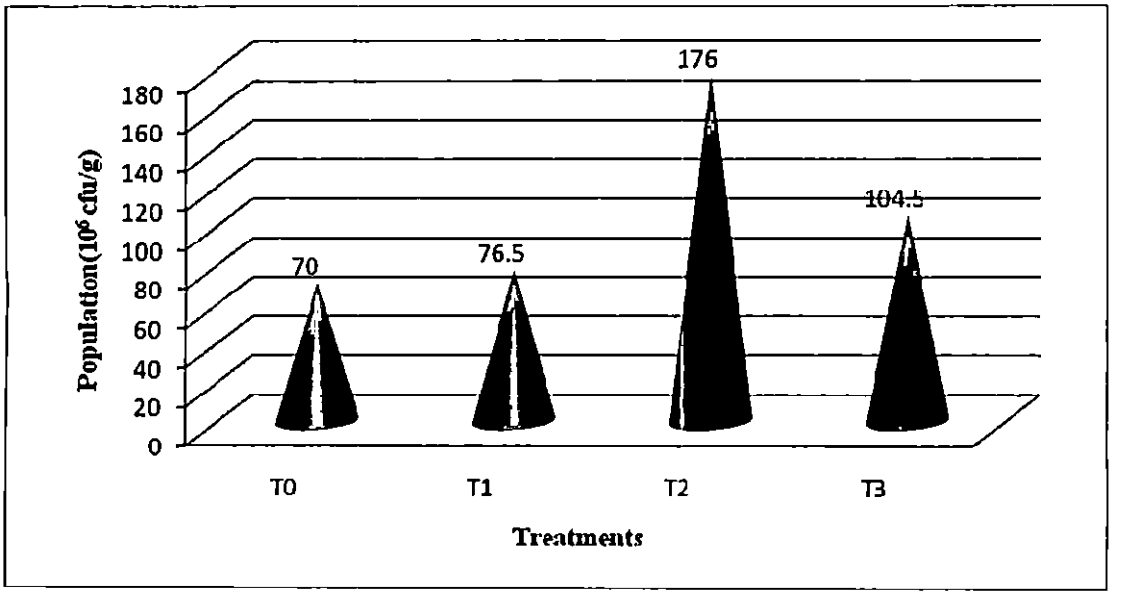


Fig.2: Effect of TSS (^{0}B) on yeast growth



be due to the substrate sugar inhibition. Deken (1966) reported that in batch cultivation of yeast, high sugar concentration in the culture can result in catabolic repression which inhibits respiratory enzymes.

The results clearly indicated that the sugar utilization increased with increase in rate of yeast growth. *Saccharomyces cerevisiae* MTCC 172 registered the highest sugar utilization in 30^0B (46.00%) followed by 40^0B (25.25%). The results are in general agreement with El-Diwani *et al.* (1992) who reported that the growth of *Saccharomyces cerevisiae* in beet molasses increased with the increment of total sugar consumed.

5.2.3 pH

One of the factors controlling the rate of growth in culture media of any selected strain is the pH. The pH would also determine the occurrence and types of particular contaminants in the culture medium. It is, therefore, necessary that the pH for growth of any selected strain be such that it is optimal for the strain and at the same time reduce growth of contaminants.

The effect of pH of culture media on yeast growth was studied by adjusting the substrate pH to 3 (T_1), 3.5 (T_2) and 4 (T_3). The yeast growth was found to increase from pH 3 to 4 and maximum yeast growth was observed at pH 4 (225.00×10^6cfu/g) (Fig.3). Control sample (T_0) was found to have yeast count of 49.50×10^6cfu/g. According to Ramesh (2007), the optimum pH for yeast growth was 4-5. Similarly, Skountzou *et al.* (2003) reported that optimum yeast growth was obtained from beet molasses at pH range of 4.5- 5.0. Dhanasekaran *et al.* (2011) conducted a study on production of single cell protein from pineapple waste extract using *Saccharomyces cerevisiae* MTCC 463 and reported that maximum yeast growth was at pH of 5.5. The optimum pH for growth of yeast, *Candida utilis* strain determined by fruit waste suspension was found to be 3.0- 6.2 (Adoki, 2008). Li *et al.* optimized the culture conditions for the production of yeast (*Candida utilis*) using bamboo as substrate and reported the optimum pH as 6.1. Gbologade (2006) obtained maximum production of *Lepiota procera* at pH of 6.5. Hence it is evident that pH is a crucial factor effecting

yeast growth and the optimum pH for growth depends on the different species and strains of yeast. The ideal pH for biomass production of *Saccharomyces cerevisiae* MTCC 172 as identified from the present study is 4.

The change in pH was also evaluated one week after fermentation using digital pH meter and a significant decrease was observed in all the treatments. Highest pH change was observed in T_3 where the initial pH of 4 decreased to 3.10. The pH change in T_2 was 3.5 to 3.05 whereas change in T_1 was 3.00 to 2.85. Least change in pH was observed in control sample where the pH decreased from 2.30 to 2.20. Ojokoh (2007) also observed significant decrease in pH during fermentation.

5.2.4 Duration of fermentation

To optimize the duration of fermentation, substrates were inoculated with *Saccharomyces cerevisiae* and TSS and pH adjusted to 30^0B and 4 respectively. The optimum duration for single cell protein production was determined by recording yeast growth after 3 (T_1), 5 (T_2) and 7 (T_3) days of fermentation. Data on results of duration of fermentation test as shown in Figure 4 indicated that maximum yeast growth was observed after 5 days of fermentation (34.00×10^7cfu/g). The results of the study revealed that there was a gradual increase in yeast population with increase in duration upto 5 days thereafter a decline in the population was observed. In *Saccharomyces cerevisiae* MTCC 463, maximum growth was attained after 7 days of fermentation (Dhanasekaran *et al.*, 2011). *Saccharomyces cerevisiae* culture isolated from rotten papaya was found to have maximum growth in papaya fruit waste extract after 2^{nd} day of fermentation. Khan *et al.* (2009) utilized papaya fruit waste for SCP production and reported highest mycelial growth of *Rhizopus oligosporus* after 5^{th} day of fermentation. Li *et al* (2009) reported that the fermentation period of 69h was best for maximum cell biomass production of *Candida utilis*. Ravinder *et al.* (2003) studied effect of fermentation period on the production of mutant *Aspergillus oryzae* SCP from deoiled rice bran and obtained maximum SCP after 72 hrs. The duration of fermentation for maximum biomass yield is therefore species and strain specific. The

Fig.3: Effect of pH on yeast growth

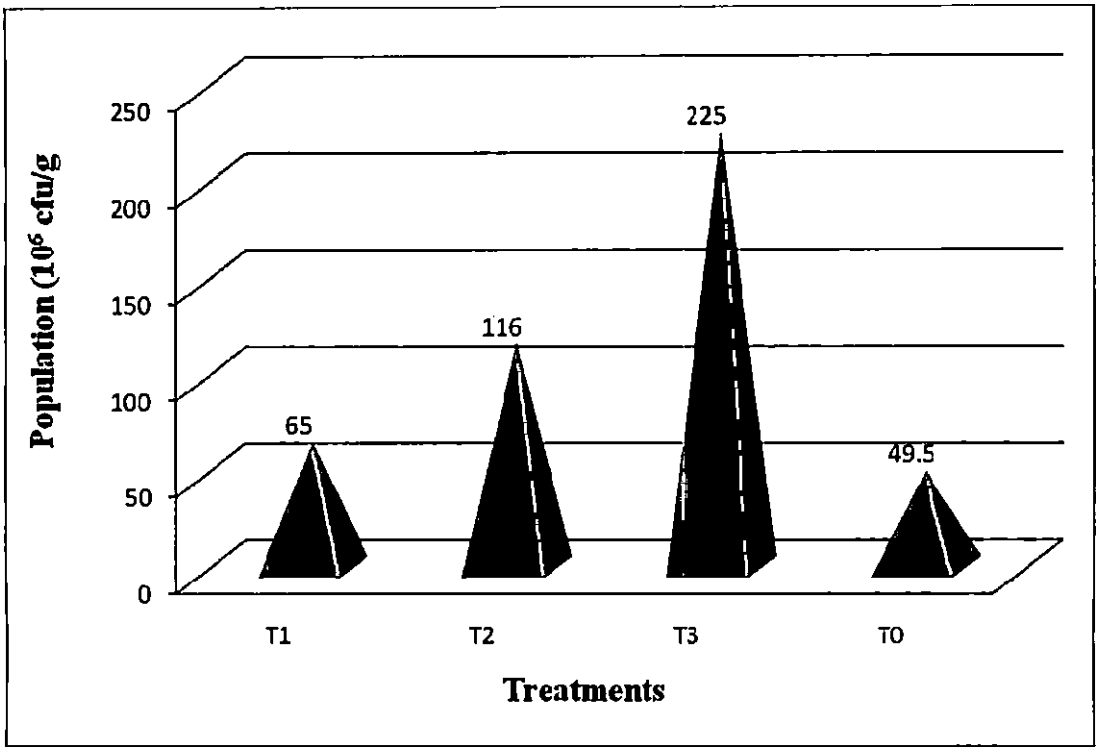
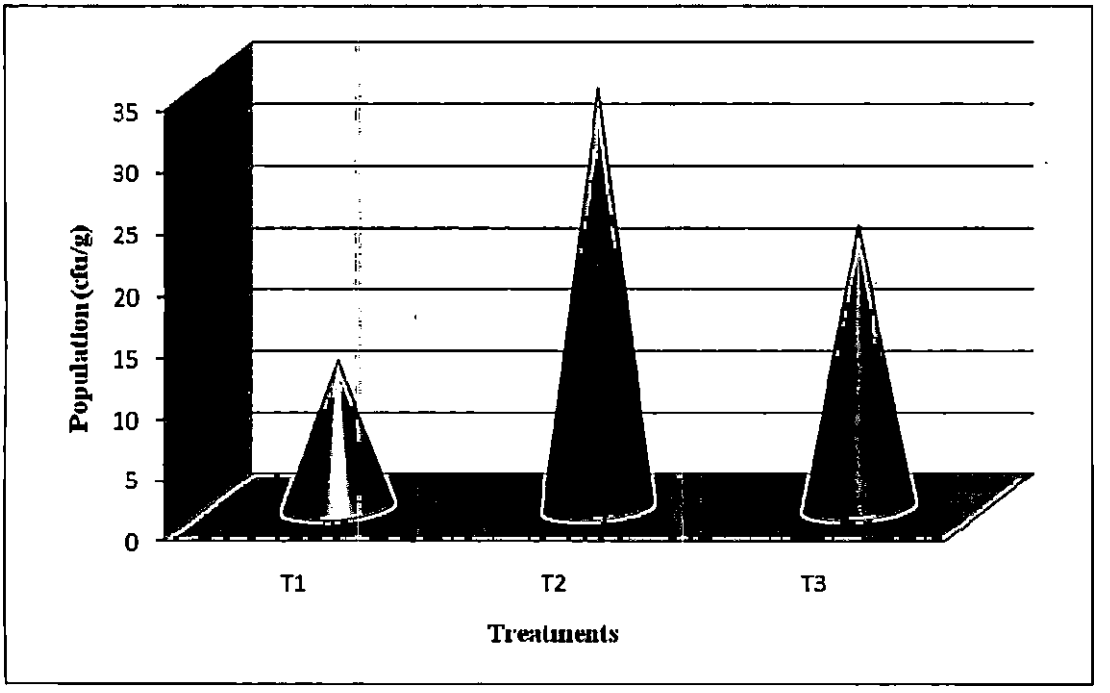


Fig.4: Effect of duration of fermentation on yeast growth



optimum duration of *Saccharomyces cerevisiae* MTCC 172 when grown on mango stone substrate was 5 days.

5.2.5 Aeration

Yeasts are facultative anaerobes and can grow with or without oxygen. In the presence of oxygen, they convert sugars to CO_2, energy and biomass. In anaerobic conditions, as in alcoholic fermentation, yeasts do not grow efficiently and sugars are converted to intermediate by-products such as ethanol, glycerol and CO_2. Therefore, in yeast propagation, the supply of air is necessary for optimum biomass production. Aeration is the main factor affecting dissolved oxygen concentration, which in turn affect the biomass and intracellular protein content (Lee *et al.*, 2001). The effect of aeration on yeast growth was studied by keeping the fermentation flasks on a shaker. It was observed that aerated samples enabled maximum yeast growth (38.00×10^7cfu/g) whereas yeast population in non aerated samples maintained in stationary conditions was 30.75×10^7cfu/g (Fig.5). In stationery conditions, yeast population slightly decreased towards the end of fermentation. This is due to the shortage of O_2 and limitation of substrate that lead to degradation of cells. Because of agitation in shaker, more O_2 was incorporated into the substrate media which lead to progressive growth of *Saccharomyces cerevisiae* MTCC 172. It also increased the consumption of reducing sugar in the substrate. This result is in agreement with the results obtained by Rosma and Ooi (2006) who observed that biomass production increased with the increase in aeration rate.

5.2.6 Growth supplements

Many workers in their investigations have used growth supplements for promoting the yeast growth on waste materials. Hence, the effect of supplementation with carbon, nitrogen, phosphorus and inducers were determined by supplementing media with broken wheat, NH_2SO_4, KH_2PO_4 and Biotin. The culture conditions standardized in the first experiment was used for this study.

The statistical analysis of data obtained showed that the growth supplements have significant effect on growth of *Saccharomyces cerevisiae* MTCC 172. The

Fig. 5: Effect of aeration on yeast growth

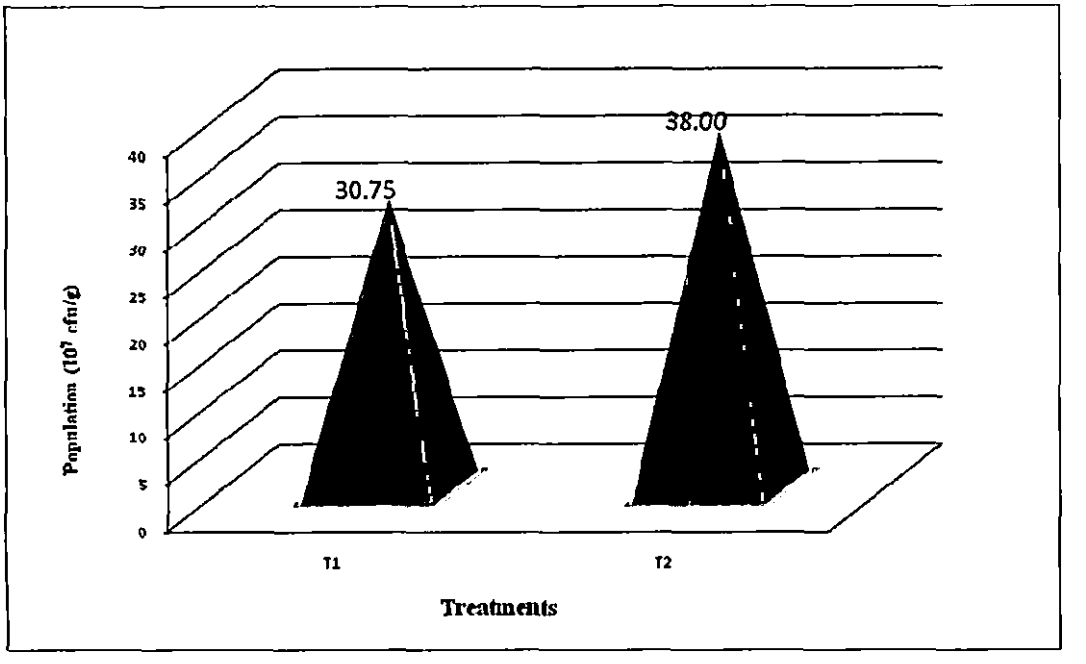
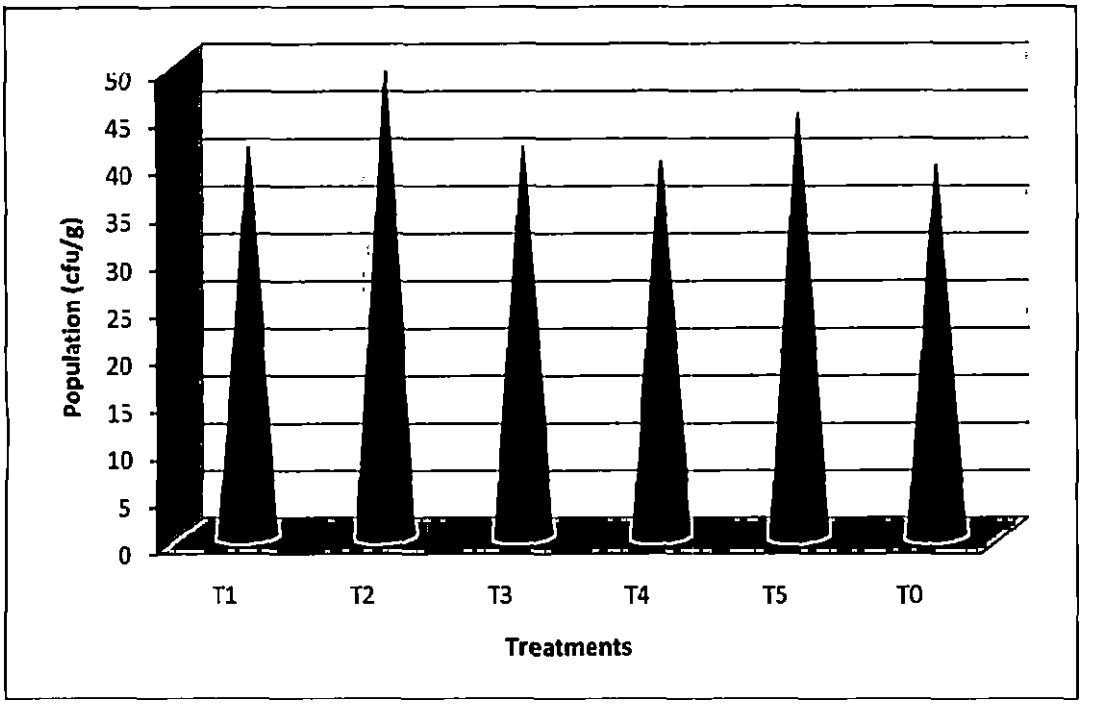


Fig. 6: Effect of growth supplements on yeast growth



results indicated that supplementation with NH_2SO_4 (T_2) had a positive effect on the growth of the organism (49.00×10^7cfu/g). Growth in the substrate supplemented with combination of NH_2SO_4, KH_2PO_4 and Biotin (T5) was higher compared to that recorded in the treatments T_0 and T_4 (Fig. 6). The statistical data showed that the growth in T_1 and T_3 was on par. The results of the study corroborate with that obtained by Moeini *et al.* (2004). Cristiani-Urbina *et al.* (2000) and Ojokoh and Uzeh (2005) also reported that rate of SCP production can be improved by addition of NH_2SO_4 as nitrogen supplement. Similarly Adoki (2008) evaluated the effect of supplements on growth of *Candida utilis* and found that supplementation with nitrogen source resulted in an increase in growth of the test strain, no such marked effect was observed when the production medium was supplemented with a source of phosphorus. Irfan *et al.* (2011) reported that biotin enhanced the growth of *Candida utilis*. The test strain was capable of meeting its amino acid supplements in culture when supplied with inorganic nitrogen sources.

5.2.7 Biomass yield

A comparison of biomass production (wet and dry) by *Saccharomyces cerevisiae* MTCC 172 in different supplemented growth media revealed that the wet (13.67g/l) and dry (2.18g/l) biomass production was highest for the substrate supplemented with NH_2SO_4. Compared to control sample, significant increase was recorded in the substrate supplemented with a combination of NH_2SO_4, KH_2PO_4 and biotin (Fig.7). On the other hand, the lowest biomass yield was obtained in the substrate supplemented with biotin, KH_2PO_4 followed by unsupplemented substrate. The results clearly indicated that the yeast growth and biomass yield were influenced by growth supplements. The studies conducted by Moeini *et al.* (2004) revealed that there was significant increase in yeast growth in whey supplemented with NH_2SO_4. The use of NH_2SO_4 as a nitrogen source either alone or in combination with other growth supplements resulted in high biomass (wet and dry) yield.

5.2.8 Protein content of biomass

Various growth amendments are known to influence the yeast growth and protein content in yeast biomass. Hence, the effect of different growth amendments on protein content of biomass was studied. There was a significant difference in the protein content among the treatments and the results are presented graphically in figure 8. The protein content was higher in media supplemented with ammonium sulphate (39.38%). The protein content in media supplemented with broken wheat (38.28%) and combination of ammonium suplhate, KH_2PO_4 and biotin (38.83%) were on par with media supplemented with ammonium sulphate alone. However, the lowest protein content was observed in unsupplemented media (31.17%) which was on par with media supplemented with KH_2PO_4 (33.90%) and biotin (32.81%). The results obviously indicated that the supplementation of substrate with nitrogen and carbon sources can enhance the protein content and yeast growth. Oshoma and Ikenebomeh (2005) found that addition of ammonium sulphate improved the protein production. Dhanasekaran *et al.* (2011) also reported that the protein content increased with increase in concentration of carbon source in the media.

5.2.9 Protocol for production of baker's yeast

The protocol for production of baker's yeast using mango stones as substrate was developed. The optimum culture condition for maximum growth of *Saccharomyces cerevisiae* MTCC- 172 were TSS (30^0B), pH (4) and providing aeration by keeping in an orbital shaker at 100rpm for 5days. Supplementation of the substrate with a nitrogen source (NH_2SO_4 0.8%) is essential for high biomass yield and protein content. SCP for human consumption should be free from nucleic acids as purine bases are metabolized to uric acid, creating problems to humans that do not possess the enzyme uricase (Jay, 1996). Nucleic acid content in SCP was reduced by addition of sodium chloride (10%) to wet biomass, adjusting the pH to 9.5 and heating at 80^0C for 10 minutes.

Fig.7: Effect of growth supplements on biomass yield

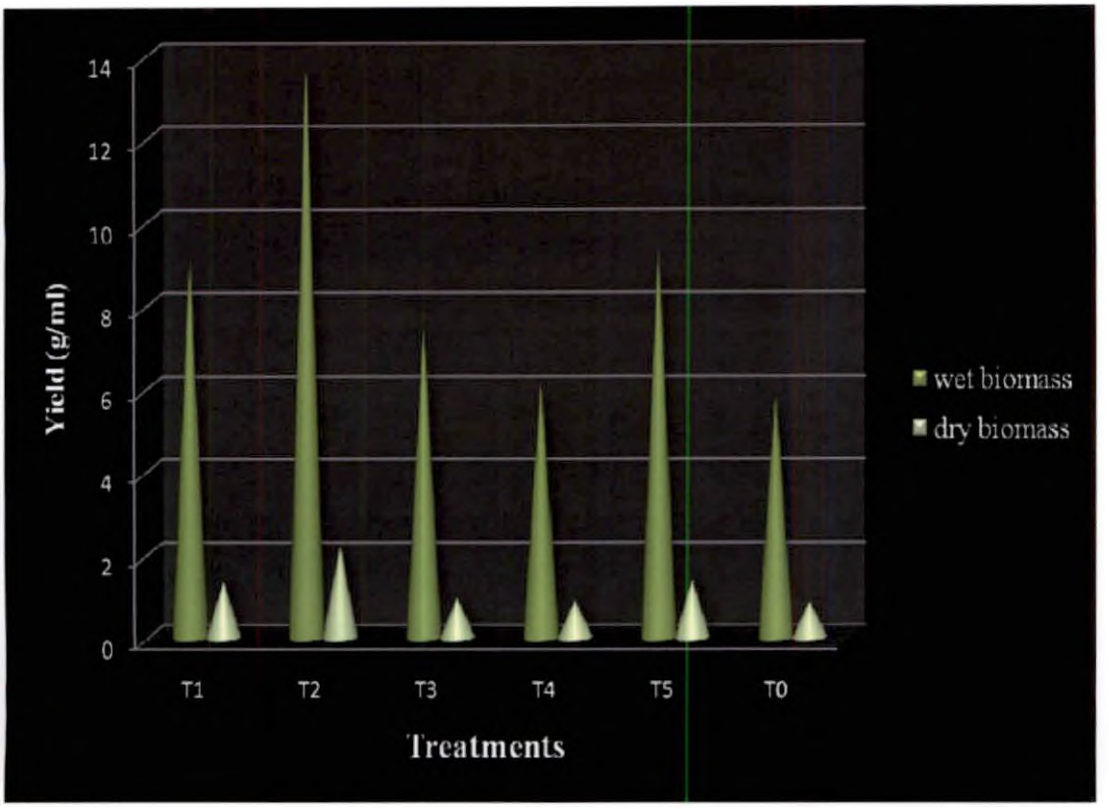
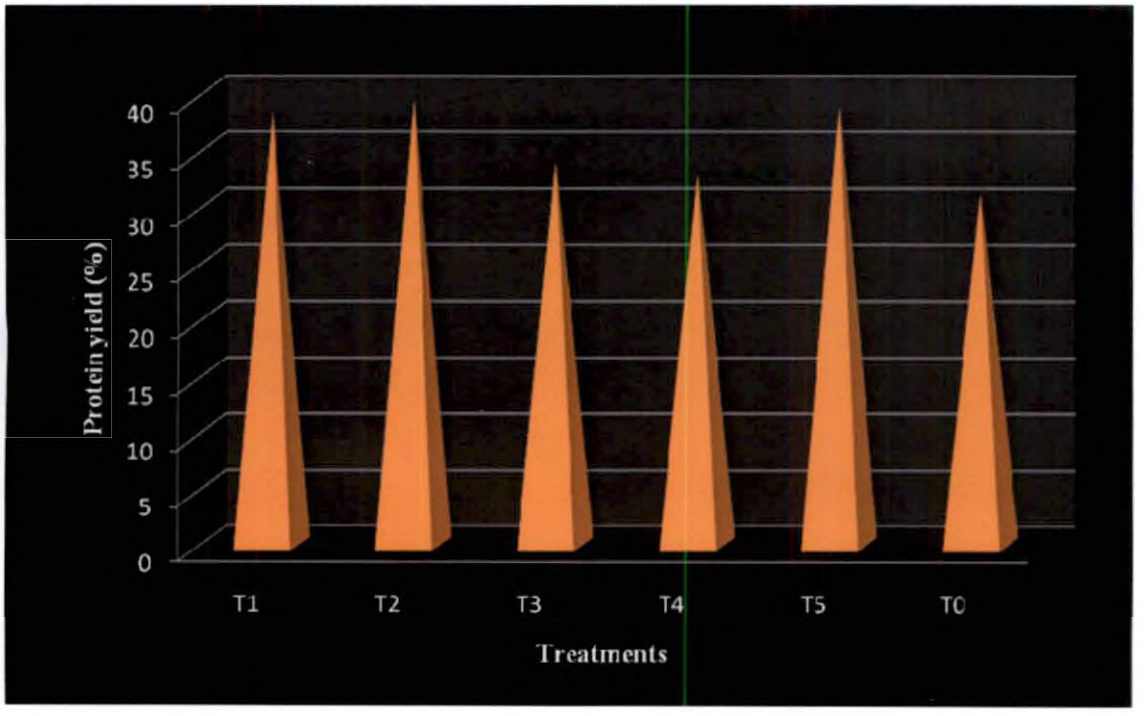


Fig.8: Effect of growth supplements on protein content



5.3 Utilization of single cell protein enriched flour for preparation of biscuit

Yeasts have been known to human beings for thousands of years as they have been used in traditional fermentation processes like wine, beer and bread making. The shortage of food and feed protein necessitated the enrichment of these foods and feed items with non conventional protein sources like single cell protein. The protein obtained from the microorganisms is not only cheap but also provide balanced nutrition and is a potential supplemental source for feeding poultry, livestock and human (Singh *et al.*, 1991; Pacheco *et al.*, 1997). The enriched flour could be used as protein supplement and functional ingredient in human diet. So, the feasibility of enriching refined wheat flour with single cell protein and quality of developed product was evaluated in this experiment.

5.3.1 Protein content of refined wheat flour and enriched refined wheat flour

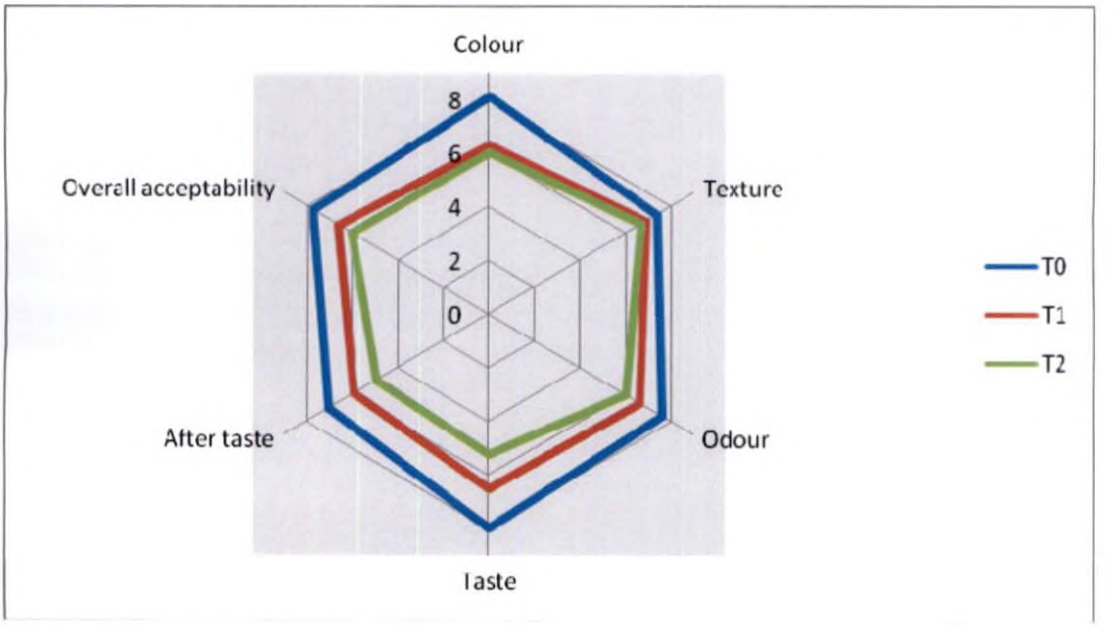
The protein content of the yeast biomass as observed from the previous experiment was 39.38 per cent. The refined wheat flour was enriched by adding a known amount of dried yeast biomass to raise its protein to 15 and 20 per cent respectively and the protein content of both refined wheat flour and enriched refined wheat flour was evaluated. The estimated protein content of the refined wheat flour was 11.75 per cent and this result is in agreement with Gopalan *et al.* (1999) who reported that the protein content of refined wheat flour was 11.00 per cent. The refined wheat flour was fortified with dry yeast biomass so as to improve its nutritive value. The protein content in the enriched refined wheat flour was estimated to be 15.02 and 19.41 per cent.

5.3.2 Preparation of biscuit

Biscuits were prepared using both refined wheat flour and enriched refined wheat flour. Further, the quality attributes of both types of biscuits like protein, riboflavin and thiamine were evaluated in this experiment.

The protein content of biscuits prepared from refined wheat flour and the enriched biscuits were found to be 11.21, 14.85 (T_1) and 19.14 per cent (T_2) respectively. The protein content of enriched wheat flour was 15.02 per cent (T_1) and

Fig. 9: Organoleptic evaluation of biscuits



19.41 per cent (T_2) and the biscuits prepared from it were 14.85 per cent and 19.14 per cent respectively indicating that there is no significant loss of protein content during baking of biscuit.

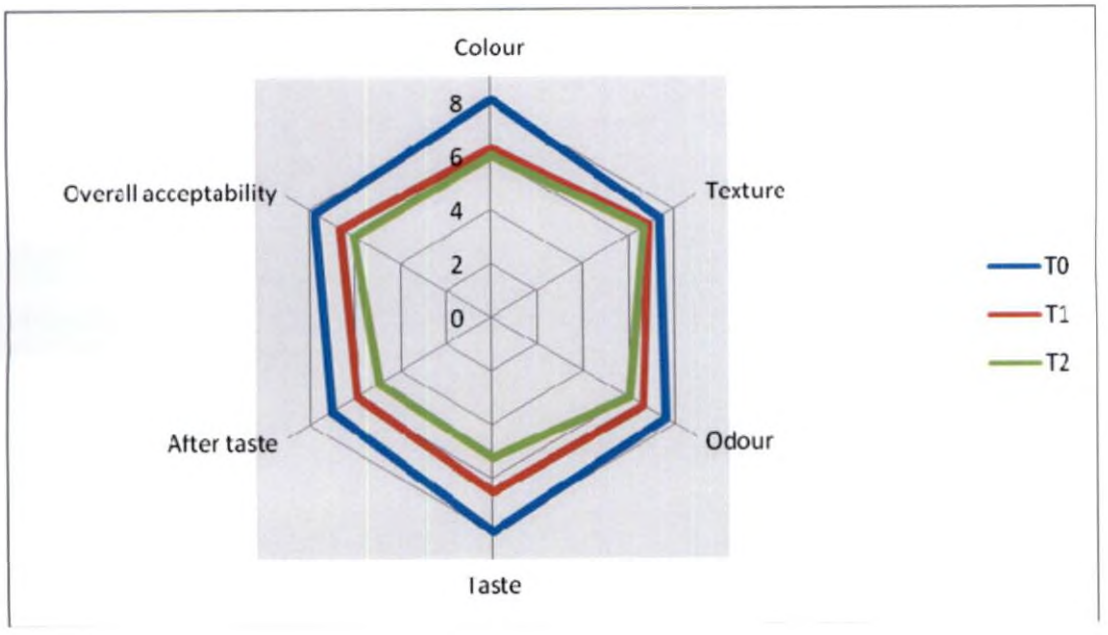
In the present study, thiamine and riboflavin content was not detectable in the biscuits made from refined wheat flour and enriched refined wheat flour. This may be due to the photosensitive nature of thiamine and riboflavin and the heat generation at the time of drying and baking. According to Beizadea (2009), thiamine is one of the most unstable B vitamins and heat treatment drastically reduces the thiamine content in foods. Rosado *et. al.* (2005) also reported that storage time affected the stability of riboflavin and thiamine in fortified and non fortified corn masa flour, while the cooking process resulted in considerable losses of both vitamins.

5.3.3 Sensory evaluation

The organoleptic qualities of biscuits were evaluated by 9 point hedonic scale. The colour and odour were found to be better in biscuits made from refined wheat flour followed by the biscuits made from enriched refined flour with 15 per cent protein content. The biscuits made from enriched refined wheat flour (20%) obtained lowest mean score of 6.0 for these attributes indicating that the biscuits were only slightly acceptable to the panel members (Fig.9). Biscuits prepared from refined wheat flour recorded the highest score for taste and after taste followed by T_1 and the lowest mean score was recorded in T_2. The texture of biscuits (both plain and enriched) was found to be acceptable may be because of higher gluten content of the refined wheat flour.

The results of the study revealed that the biscuits prepared from refined wheat flour were superior in all the sensory qualities. The biscuits enriched with protein at 15 per cent level recorded higher score for organolepic qualities than 20 per cent protein enriched biscuits. The score recorded for all the sensory attributes was above six indicating that attribute was acceptable. The scores obtained for T_2 for the parameters, taste and after taste were below the acceptable level which may be due to the predominant yeasty flavour in 20 per cent protein enriched biscuits. The biscuit

Fig. 9: Organoleptic evaluation of biscuits



prepared out of enriched refined wheat flour (15%) was acceptable to the participants of the study indicating the scope for commercialization. The protein content of the biscuits was enhanced due to incorporation of yeast biomass thereby improving its nutritive value also. When compared to the non enriched biscuits, the scores recorded for most of the organoleptic qualities were comparatively lower in enriched biscuits. This indicates that further studies have to be carried out for the improvement of organoleptic qualities of the enriched biscuits to make it more palatable and acceptable to consumers. Garg (2006) reported that refined wheat flour enriched with baker's yeast could be successfully used in bakery industry for making protein enriched biscuits and protein supplement for fish and poultry meal.

5.3.4 Conclusion

Mango processing industries generate enormous quantities of waste mainly in the form of stones and peels. Effective disposal of this waste is the main problem faced by the processing industries. Microbial fermentation is the best technology which can be applied to the food processing industry waste to convert them to value added products. The exploitation of mango processing waste for microbial protein production will greatly eliminate the immense cost of waste pollution control. The high TSS and low pH of pulp adhering to mango stones render it an ideal material for baker's yeast production. Yeast, *Saccharomyces cerevisiae* is a good source of protein (39.38%) as revealed from the present study and has high potential for protein enrichment of foods.

The raw materials used as substrate for industrial yeast biomass production are usually agricultural, forestry and food waste byproducts. The most widely used substrate for baker's yeast production is cane or beet molasses, the main byproduct of the sugar industry. In the present study, the bioconversion effect of mango processing waste into single cell protein (SCP) using yeast was evaluated. The findings of the study indicated that mango stones with adhering pulp can be used as an alternate carbon source for SCP production. However, under laboratory conditions, the cost of production for one gram dry yeast came to Rs. 4.50. Further studies have to be carried

out using additional carbon, nitrogen and phosphorus supplements and inducers for maximizing the biomass production and protein content so as to make the process economically viable. Scaling up of the technology is vital for commercial production of baker's yeast.

SUMMARY

6. SUMMARY

The study on "Process standardization for the production of baker's yeast from mango stones" was undertaken at the Department of Processing Technology, College of Horticulture during 2010-12. The main objectives of the study were to optimize the culture conditions for production of single cell protein (baker's yeast) using mango stones as the substrate, explore the feasibility of enriching refined wheat flour with single cell protein (SCP) and evaluate the quality of the product developed.

The possibility of using the mango stones with adhered pulp as a substrate for the production of baker's yeast was studied. The physical composition of the mango fruit was determined by computing the percentage of pulp, stone and peel which ranged from 65.22- 73.69 per cent, 8.32- 12.18 per cent, 13.5- 21.94 per cent respectively. The pulp attached to the stone was 5.32- 8.89 per cent. The adhered mango pulp recorded 78.8-80.1 per cent moisture, 2.8-3.52 per cent reducing sugar, 10.25 per cent non reducing sugar, 13.77-13.05 per cent total sugar, 9.3-15.8^{0}B TSS, 1.9- 3.5 pH, 16.94-21.01 mg/100g ascorbic acid and 0.26- 0.37 per cent acidity. The preliminary study revealed that mango stones with adhered pulp could be used as a substrate for baker's yeast production.

The effect of different surface sterilization methods, *viz.* autoclaving at 121^{0}C and 15psi for 30 min, hot water immersion for 10 min and steam blanching for 10 min on surface decontamination was studied. Significant variation was observed between treatments for surface sterilization. Autoclaving (T1) of substrate resulted in complete microbial decontamination whereas fungal, bacterial and yeast growth was noticed in the treatments T_2 and T_3.

Lyophilized baker's yeast, *Saccharomyces cerevisiae* MTCC 172 strain obtained from IMTECH, Chandigarh, was used for the study. The secondary/working inoculum was standardized to get a uniform cell volume of 10^6 cells/ml of substrate solution.

The culture conditions for SCP production using mango stone as substrate was standardized and the best identified culture conditions were maintained for subsequent studies. Factors like TSS, pH, duration of fermentation, aeration affecting the cell biomass production from mango stones by *Saccharomyces cerevisiae* MTCC 172 were evaluated. A gradual increase in yeast population with increase in TSS up to 30^0B (176.00×10^6cfu/g), and thereafter a decline in the population was observed. The yeast growth was found to increase from pH 3 to 4 and maximum yeast growth was observed at pH 4 (225.00×10^6cfu/g). The yeast population was found to increase upto 5 days of fermentation, thereafter a decline in the population was noticed. Aeration of samples enabled maximum yeast growth (38.00×10^7cfu/g) whereas yeast population in non aerated samples maintained in stationary conditions was 30.75×10^7cfu/g. The optimum conditions of the substrate for maximum yeast growth were TSS (30^0B), pH (4) and provision of aeration by keeping in a orbital shaker at 100 rpm for five days.

The effect of supplementation with carbon, nitrogen, phosphorous and inducers on yeast growth, wet biomass yield, dry biomass yield and protein content were determined by supplementing media with broken wheat, NH_2SO_4, KH_2PO_4 and Biotin. Significant variation was observed between treatments. Maximum yeast growth (49.00×10^7 cfu/g) was observed in media supplemented with ammonium sulphate (0.8%). Wet (13.67g/l) and dry (2.18g/l) biomass yield was found to be highest in media supplemented with ammonium sulphate. Highest content of protein (39.38%) was observed in media supplemented with ammonium sulphate which was on par with media supplemented with broken wheat (38.28%) and combination of ammonium sulphate, KH_2PO_4 and biotin(38.83%).

The protocol for production of baker's yeast from mango stones was developed which includes extraction of mango stones from mango, addition of equal amount of distilled water, maintaining the optimized culture conditions and growth supplement, centrifuging at 11000 rpm for 10 min, removal of nucleic acid, drying the wet biomass by using vacuum freeze dryer and sieving to yield yeast powder.

The possibility of protein enrichment of biscuits was evaluated by incorporating baker's yeast to refined wheat flour so as to raise the protein to 15 and 20 per cent level. The enriched biscuits recorded protein content of 14.85(T_1) and 19.14 (T_2) per cent whereas the biscuits made from refined wheat flour had a protein content of 11.21 per cent. Vitamins, thiamin and riboflavin were not detected in both enriched and non enriched biscuits which may be due to the heat degradation of vitamins during drying and baking of biscuits.

Highest scores for organoleptic properties of biscuits were obtained for T_0 (biscuits made from refined wheat flour) followed by T_1 (15% protein enriched biscuits). The biscuits enriched with protein at 15 per cent level recorded higher scores for organoleptic qualities than 20 per cent protein enriched biscuits. The scores obtained for T_2 for the parameters, taste and after taste were below the acceptable level which may due to the predominant yeasty flavour in 20 per cent protein enriched biscuits. The biscuit prepared out of enriched refined wheat flour (15%) was acceptable to the participants of the study indicating the scope for commercialization.

REFERENCES

REFERENCES

Abdullah and Abdullah. 2010. *Solid and liquid pineapple waste utilization for lactic acid fermentation using Lactobacillus delbrueckii. Reaktor* 11 (1): 50-52.

Adoki, A. 2002. Culture characteristics of *Candida* sp. in waste conversion. *J. Appl. Sci.* 6: 49-58.

Adoki, A. 2008. Factors affecting yeast growth and protein yield production from orange, plantain and banana waste processing residues using *Candida* sp.. *Afr. J. Biotechnol.* 7 (3): 290-295.

Agamuthu, P. 2009. Challenges and opportunities in agro-waste management: an Asian perspective. In: *Report of Inaugural Meeting of First Regional 3R Forum in Asia*; 11-12 Nov 2009, Tokyo, Japan, p.25.

Ahmad, M. M., Salim-ur-Rehman, Z., Iqbal, F. M., Anjum and Sultan, J. I. 2006 Genetic variability to essential oil composition in four citrus fruit species. *Pakist. J. Bot.* 38(2): 319-324.

Akin-Osanaiye, B. C., Nzelibe, H. C., and Agbaji, A. S. 2008. Ethanol production from *Carica papya* fruit waste. *Asian J. Biochem.* 3(3): 188- 193.

Anand, J. C. and Maini, S. B. 1997. Utilization of fruit and vegetable wastes. *Indian Food Packer* 51(2): 45- 63.

Anila, R. and Radha, T. 2003. Physico-chemical analysis of mango varieties under Kerala conditions. *J. Trop. Agric.* 41: 20-22.

Anupama and Ravindra, P. 2000. Value-added food: Single cell protein. *Biotechnol. Adv.* 18(6): 459-479.

Anuradha, R., Sumesh, A. K., and Venkatesh, K. V. 1999. Simultaneous saccharification of starch to lactic acid. *Process Biochem.* 35: 367–375.

Azin, M. and Moazami, N. 1989. Production of SCP from sugarcane bagasse. In: *Second Congress of Forest and Technology*; 19- 21 Dec 1989, Faculty of Engineering, Tehran University, pp. 19.

Baig, M. M. V., Mane, V. P., More, D. R., Shinde, L. P., and Baig, M. I. A. 2003. Utilization of agricultural waste of banana: production of cellulases by soil fungi. *J. Environ. Biol.* 24: 173 -176.

Batt, C. A. and Sinskey, A. J. 1987. Single cell protein: production, modification and utilization. In: Knorr, D. (ed.), *Food Biotechnology*. Marcel Dekker, Inc., New York, pp. 347- 362.

Bawa, A. S. and Saini, S. P. S. 1988. Studies on processing and utilization of kumkaut (wild ctrus) fruit. *Indian Food Packer* 42(2): 7- 9.

Begea,M., Sticescu, C., Baldea, G.,Vladeescu, M. and Baron, E. 2008. Isolation and selection of yeast for single cell protein biomass production, *Seria Agron.* 51 (3): 67-70.

Beizadea , A. E. 2009. Fortification of wheat flour. *Rom. Biotechnol.* 14(2): 4300-4306.

Bekatorou, A., Psarianos, C., and Koutinas, A. A. 2006. Food grade yeasts. *J. Food Technol. Biotechnol.* 44 (3): 407–415.

Berh, O. P., Raghuramaiah, B., Krishnamurthy, G. V., and Giridhar, N. 1976. Utilization of mango waste: recovery of juice from fruit waste pulp and peel. *J. Food Sci. Technol.* 13: 138-141.

Bhalerao, S. D. and Mulmuley, G. V. 1989. Dehydrated citrus pips: a valuable adjunct for dry beverage mixes. *Indian Food Packer* 43(6): 22-23.

Bob-Manuel, F. G. and Alfred-Ockiya, J. E. 2011. Evaluation of yeast single cell protein (SCP) diets on growth performance, feed conversion and carcass composition of Tilapia *Oreochromis niloticus* (L.) fingerlings. *Afr. J. Biotechnol.* 10(46): 9473-9478.

Bourgeois, P., Aurore, G. S., Abaul, J., and Joseph, H. 2009. Processing guava seeds: kernel oil and abrasive shell powder [online]. Available: http://www.cabi.org [05-10-2011].

Buranakarl, L., Fan, C. Y., Ito, K., Izaki, K., and Takahashi, H. 1985. Production of molecular hydrogen by photosynthetic bacteria with raw starch. *Agric. Biol. Chem.* 49: 3339–3341.

Camacho-Ruiz, L., Perez-Guerra, N., and Perez-Roses, R. 2003. Factors affecting the growth of *Saccharomyces cerevisiae* in batch culture and in solid state fermentation. *Electr. J. Environ. Agric. Food Chem.* 8(1): 22- 27.

Cardona, C. A, Sánchez, O. J., and Gutiérrez, L. F. 2010. *Process Synthesis for Fuel Ethanol Production* [e-book]. Taylor and Francis Group, LLC., Florida. Available: http://www.crcnetbase.com/page/engineering_ebooks [10-10-2011]

Carta, F. S., Soccol, C. R., Machado, L., and Oates, G. C. 1998. Prospect of using cassava bagasse waste for producing fumaric acid. *J. Sci. Indian Res.* 57: 644–649.

Carta, F. S., Soccol, C. R., Ramos, L. P., and Fontana, J. D. 1999. Production of fumaric acid by fermentation of enzymatic hydrolysates derived from cassava bagasse. *Bioresource Technol.* 68: 23–28.

Chan, H. T. 1979. The chemistry and biochemistry of papaya. In: Inglettv, G. E. and Charalambous, G. (eds.), *Tropical Foods: Chemistry and Nutrition: Vol. 1.* Academic Press, New York, pp. 33- 53.

Chanda, S. and Chakrabarti, S. 1996. Plant origin liquid waste: A resource for single cell protein production by yeast. *Bioresourse Technol.* 57: 51–54.

Chitradon, L., Channarong, W., Ngmjarearnwong, M., and Dechprae, N. 2008. Biohydrogen from phototrophic bacteria, a renewable energy potentially combine with fuel cell. *J. Res. Eng. Technol.* 5: 183–201.

Cohn, R. and Cohn, L. 2005. The by-products of fruit processing. In: Arthey, D. and Ashurst, P. R. (eds.), *Fruit Processing Nutrition, Products and Quality Management.* Springer India, New Delhi, pp. 225- 248.

Correia, R. T. P., Miccue, P., Magalhaes, M. M. A., Macedo, G. R., and Shetty, K. 2004. Phenolic antioxidant enrichment of soy flour-supplemented guava waste by *Rhizopus oligosporus* -mediated solid-state bioprocessing. *J. Food Biochem.* 28: 404–418.

Cristiani-Urbina, E., Netzahuatl-Munoz, A. R., Manriquez-Rojas, F. J., Juarez-Ramirez, C., Ruiz-Ordaz, N., and Galindez-Mayer, J. 2000. Batch and fed-batch cultures for the treatment of whey with mixed yeast cultures, *Process Biochem.* 35: 649–657.

Cruickshank, R., Duguid, J. P., Marmion, B. P., and Swain, R. H. A. 1975. *Medical Microbiology: The Practice of Medical Microbiology: Vol. II.* Churchill Livingstone, London, 587p.

Deken, H. 1966. The Crabtree effect: a regulatory system in yeast. *J. General Microbiol.* 44: 149-156.

Devi, D. K. and Ingle, U. M. 1982. Utilization of pineapple by-products and wastes- a review. *Indian Food Packer* 6(5): 15- 22.

Dhabekar, A. and Chandak, A. 2010. Utilization of banana peels and beet waste for alcohol production. *Asiatic J. Biotech. Res.* 1: 8-13.

Dhanasekaran, D., Lawanya, S., Saha, S., Thajuddin, N., and Panneerselvam, A. 2011. Production of single cell protein from pineapple waste using yeast. *Innovative Romanian Food Biotechnol.* 8: 26- 32.

Doran, P. M. 1997. *Bioprocess Engineering Principles.* Academic Press Ltd. London, 578p.

El-Din, M. H. A. S. and Yassen, A. A. E. 2006. Evaluation and utilization of guava seed meal (*Psidium guajava* L.) in cookies preparation as wheat flour substitute. *Nahrung Fd.* 41(6): 344- 348.

El-Diwany, A. I., El-Abyad, M. S., El-Refai, A. H., and Sallam, L. A. 1992. Effect of some fermentation parameters on ethanol production from beet molasses by *Saccharomyces cerevisiae. Bioresourse Technol.* 42: 191- 195.

Enwefa, C. 1991. Biomass production from banana skins. *Appl. Microbiol. Biotechnol.* 36(2): 283-284.

Ezhov, V. N., Lebedev, V. V., Lukanin, A. S, Petrushevskii, V. V, and Romanovskaya, T. I. 1993. Wasteless processing of apple pressings. USSR Patent SU 1:785 639

Fehr, M., Calcado, M. D. R., and Romano, D. C. 2002. The basis of a policy for minimizing and recycling food waste. *Environ. Sci. Policy* 5(3): 247- 253.

Gao, J., Zhang, H. J., Yu, S. H., Wu, S. G., Yoon, I., Quigley, J., Gao, Y. P., and Qi, G. H. 2008. Effects of yeast culture in broiler diets on performance and immunomodulatory functions. *Poult. Sci.* 87: 1377–1384.

Garg, N. 2006. Production of Baker's yeast from mango stones. *Indian Food Packer* 61(1): 61- 62.

Garg, N., Tandon, D. K., and Kalra, S. K. 1994. Utilization of fruit industry waste. *Beverage Food World* 21(1): 16- 20.

Garg, N., Tandon, D. K., and Kalra, S. K. 1998. Potential of using guava jelly processing waste for citric acid production through fermentation. *Indian Food Packer* 52(3): 7- 9.

Gautam, H. R. and Guleria, S. P. S. 2007. Fruit and vegetable waste utilization. *Sci. Tech. Entrepreneur* 11(2): 8- 10.

Gbolagade, J. S. 2006. The effect of different nutrient sources on biomass production of *Lepiota procera* in submerged liquid cultures. *J. Biotechnol.* 5: 1246-1249.

George, B., Kaur, C., Khurdiya, D. S., and Kapoor, H. C. 2004. Antioxidants in tomato (*Lycopersicon esculentum*) as a function of genotype. *Food Chem.* 84: 45-51.

Ghanem, K. M. 1992. Single cell protein production from beet pulp by mixed culture. *Microbiologia* 8(1): 39-43.

Girdharilal, Siddappa, G. S., and Tandon, G. L. 1998. *Preservation of Fruit and Vegetables.* ICAR, New Delhi, 256p.

Gopalan, C., Sastri, B.V.R., and Balasubramanian, S. C. 1999. *Nutritive Value of Indian Foods.* NIN, Hyderabad, 117p.

Gupta, K. and Joshi, V. K. 2000. Fermentative utilization of waste from food processing industry. In: Verma, L. R. and Joshi, V. K. (eds.), *Postharvest Technology of Fruits and Vegetables: General Concepts and Principles.* Indus Publishing Company, New Delhi. pp. 1171-1193.

Haldar, T. K., Ghosh, Toshiwati, M. R. and Bedford. 2011. Effects of yeast (*Saccharomyces cerevisiae*) and yeast protein concentrate on production performance of broiler chickens exposed to heat stress and challenged with *Salmonella enteritidis. Anim. Feed Sci. Technol.* 168: 61– 71.

Hamed, A. S. A. A. 1993. Bioconversion of wheat straw by yeast into single cell protein. *Egyptian J. Microbiol.* 28(1): 1-9.

Hang, Y. D. and Woodams, E. E. 1986. Utilization of grape pomace for citric acid production by solid state fermentation. *American J. Enol. Vitic.* 37: 2141-2142.

Hanmant, G. P.2010. *Process Standardization for Value Addition of Mango (Mangifera indica* L.*) Seed Kernal.* MSc (Hort) thesis, Kerala Agricultural University. Thrissur, 82p.

Hedge, S. V. 1997. Nate-de-pina: a novel delicacy and value added product from pineapple. *Indian Food Packer* 51(3): 44- 45.

Hutagalung, R. I. 2002. Use of carbohydrate residues in Malaysia[Malaysia]. Available: http://www.unu.edu.unipress/unupbooks/80362e/80362ob.htm [10- 10-2010]

IASRI [Indian Agricultural Statistics Research Institute]. 2004. Indian Agricultural Research Data Book. Indian Agricultural Statistical Research Institute, New Delhi, 280p.

Irfan, M., Nazir, M. I., Nadeem, M., Gulsher, M., Syed, Q., and Baig, S. 2011. Optimization of process parameters for the production of single cell biomass of *Candida utilis* in solid state fermentation. *American-Eurasian J. Agric. Environ. Sci.* 10 (2): 264-270.

Jain, N. L. and Girdhari, L. 1957. Some Studies in the Utilization of Jack Fruit Wastes as a Source of Pectin. *Indian J. Hort.* 14(4): 213- 222.

Jay, J. M. 1996. *Modern Food Microbiology,* Chapman and Hall, New York, USA, 127p.

John, R. P., Nampoothiri, K. M., and Pandey, A. 2006. Simultaneous saccharification and fermentation of cassava bagasse for L-(+)- lactic acid production using *Lactobacilli. Appl. Biochem. Biotechnol.* 134: 263–272.

Joshi, G. D., Relekar, P. P., Pawar, C. D., and Magdum, M. B. 1996. Preliminary studies on utilization of nutmeg fruit spice rind. *Indian Cocoa Arecanut Spices J.* 20(1): 5- 9.

Joshi, V. K., Kaushal, N. K., and Thakur, N. S. 1996. Apple pomace sauce development, quality of fresh and stored products. *J. Food Sci. Technol.* 33: 414–419.

Kamel, B. S. 1979. Dates as a potential substrate for single cell protein production. *Enzyme Microbial Technol.* 1(3): 180- 182.

Kaushal, N. K. and Joshi, V. K. 1995. Preparation and evaluation of apple pomace based cookies. *Indian Food Packer* 49(5): 17–24.

Kaushal, N. K., Joshi, V. K., and Sharma, R. C. 2002. Effect of stage of apple pomace collection and the treatment on the physical-chemical and sensory qualities of pomace papad (fruit cloth). *J. Food Sci. Technol.* 39: 388–393.

Kays, T. M. and Vanderzant, C. 1980. Factors affecting yeast growth. *Dev. Ind. Microbiol.* 21: 481-487.

Khan, M. Y. and Dahot, M. U. 2010. Effect of Various Agriculture Wastes and Pure Sugars on the Production of Single Cell Protein by *Penicillium Expansum. World Appl. Sci. J.* 8: 80- 84.

Khan, M., Khan, S. S., Ahmed, Z., and Tanveer, A. 2009. Production of fungal single cell protein using *Rhizopus oligosporus* grown on fruit wastes. *Biological Forum*1(2): 32-35.

Khurdiya, D. S., Islam and Sen, N. 1997. Production of carbonated beverages from phalsa, lime and grape juice and pomace extract. *Indian Food Packer* 51(6): 55- 57.

Lee, Bum-Kyu, and Kim, J K. 2001. Production of *Candida utilis* biomass on molasses in different culture types. *Aquacultural Eng.* 25: 111-124.

Lee, J. M. 2008. *Biochemical Engineering. Albright's Chemical Engineering Handbook.* Taylor & Francis Group, Florida, 436p.

Li, X., Xu, O., Chen, M., Song, X., Yong, Q., and Yu, S. 2009. Optimization of culture conditions for production of yeast biomass using bamboo wastewater by response surface methodology. *Bioresource Technol.* 100: 3613-3617.

Lin, Y. and Tanaka, S. 2006. Ethanol fermentation from biomass resources: Current state and properties. *Appl. Microbiol. Biotechnol.* 69: 627–642.

Liong, M. T., Rosma, A., Azemi, M. M. N., and Nadiah, W. W. A. 2002. Yeast extracts of *Saccharomyces cerevisiae* and *Candida utilis* from agrowaste. In: *Proceedings of 25th Malaysian Microbiology Symposium and 5th UNESCO National Workshop for the Promotion of Microbiology in Malaysia*, Malaysian Society of Microbiology, Kuala Lumpur.

Litchfield, J. H. 1983. Single cell protein. *Science* 219: 740-746.

Lyutskanov, N. 1990. Protein extracts for nutritional purposes from fragile strain of *Saccharomyces cerevisiae*, reduction of the nucleic acid content and applicability of the protein extracts. *J. Basic Microbiol.* 30: 523-528.

Madhukara, K., Krishnanad, N., and Srilatha, H. R. 1993. Ensilage of mango peel for methane generation. *Process Biochem.* 28: 119-123.

Maini, S. B and Sethi, V. 2000. *Utilization of Fruit and Vegetable Processing Waste.* Indus Publishing Company, New Delhi (India), 252p.

Maini, S. B. and Singh, R. 2001. Utilisation of processing waste from tropical and subtropical fruits. *Indian J. Hort.* 58(12): 183- 190.

Manimehalai, N. 2007. Fruit and waste utilization. *Beverage Food World* 34(11): 53– 56.

Martínez-Valverde, I., Periago, M. J., Provan, G., and Chesson, A. 2002. Phenolic compounds, lycopene and antioxidant activity in commercial varieties of tomato (*Lycopersicum esculentum*). *J. Sci. Food Agric.* 82: 323-330.

Milson, P. E. 1987. *Organic Acid by Fermentation: The Food Biotechnology.* Elsevier Applied Science. London, 367p.

Moeini, H., Nahvi, I., and Tavassoli, M. 2004. Improvement of SCP production and BOD removal of whey with mixed yeast culture. *Electr. J. Biotechnol.* 7(3): 249- 255.

Mohamed, S. and Hassan, Z. 1995. Extraction and characterization of pectin from various tropical agro wastes. *Asean Food J.* 10(2): 43- 50.

Moharib, S. A. 2003. Utilization of guava peel waste as substrate for protein and protease production by *Saccharomyces cerevisiae*. *Adv. Food Sci.* 25(2): 49-55.

NHB [National Horticulture Board]. 2011. *Indian Horticulture Database-2011.* National Horticulture Board, Gurgaon, 278p.

Nigam, N. M. 2000. Cultivation of *Candida langeronii* in sugarcane bagasse hemi cellulose hydrolysate for the production of single cell protein. *W. J. Microbiol. biotechnol.* 16: 367- 372.

Nottingham University. 1997. *Uses of enzymes in food industry. Biotechnology awareness, skill education and training*, Nortingham University, England, 34p.

Ofodile, L. N., Doherty, F., Oyelola, O. T., Oguntuyi, B. L., and Kolawole-Joseph, O. S. 2011. Biodegradation of orange and pineapple peels for production of single cell protein. *J. Environ.* 1(11): 31- 36.

Ojokoh, A. O. 2007. Effect of fermentation on the chemical composition of mango (*Mangifera indica* L.) peels. *Afr. J. Biotechnol.* 6 (16): 1979-1981.

Ojokoh, A. O. and Uzeh, R. E. 2005. Production of *Saccharomyces cerevisiae* biomass in papaya extract medium. *Afr. J. Biotechnol.* 4(11): 1281-1284.

Okonko, I. O., Adeola, T., Aloysius, F. E., Adejoye, Damilola, O., and Adewale, O. A. 2009. Utilization of food wastes for sustainable development. *EJEAFChe.* 8 (4): 263-286.

Oshoma, E. A. and Ikenebomeh, M. J. 2005. Production of *Aspergillus niger* biomass from rice bran. *Pakistan J. Biochem. Eng.* 13: 215- 218.

Oura, E. 1983. Biomass from carbohydrate. In: Dellweg (ed.), *Biotechnology: Vol. 3.* Verleg Xhemie, Weinheim, pp. 3- 41.

Pacheco, M. T., Caballero-Cordoba, G. M., and Sgarbieri, V.C. 1997. Composition and nutritive value of yeast biomass and yeast protein concentrates. *J. Nutr. Sci. Vitaminol.* 43: 610–612.

Pandey, A., Soccol, C. R., Nigam, P., and Soccol, V. T. 2000. Biotechnological potential of agro-industrial residues. I: Sugarcane bagasse. *Bioresourse Technol.* 74: 69-80.

Pandey, A., Soccol, C. R., Nigam, P., Soccol, V. T., Vanderberghe, L. P. S., and Mohan, R. 2000. Biotechnological potential of agro-industrial residue. II : Cassava bagasse. *Bioresource Technol.* 74: 81–87.

Pandove, G., Sharma, S., Bajwa, U., and Achal, V. 2010. Single cell protein: A potential food protein alternative. *Indian Food Ind.* 29(3): 17- 27.

Pirt, S. J. 1975. *Pninciples of Microbe and Cell Cultivation.* Black-well Scientific Publications, Oxford, 375p.

Puri, V. P. 1984. Effect of crystallinity and degree of polymerization of cellulose on enzymatic saccharification. *Biotechnol. Bioeng.* 26: 1219–1222.

Ramesh, V. 2007. *Food Microbiology.* MJP Publishers, Chennai, 802p.

Ranganna, S. 1986. *Handbook of Analysis and Quality Control of Fruit Vegetable Products.* Second edition, Tata McGraw Hill Pubishing Company Ltd., New Delhi, 594p.

Ranganna, S. 1997. Handbook of Analysis and Quality Control for Fruits and Vegetable Products (3rd ed.), Tata Mc Graw and Hill Publication Co. Ltd., NewDelhi, 634p.

Ravinder, R., Raol, L. V., and Ravindra, P. 2003. Production of SCP from deoiled rice bran, *Food Technol.Biotechnol.* 41: 243- 246.

Reddy, H. K., Srijana, M., Reddy, M. D., and Reddy, G. 2010. Coculture fermentation of banana agro-waste to ethanol by cellulolytic thermophilic *Clostridium thermocellum* CT2. *Afr. J. Biotechnol.* 9 (13): 1926-1934.

Reddy, L. V., Reddy, O. V. S., and Young-Jung, W. 2011. Production of ethanol from mango (*Mangifera indica* L.) peel by *Saccharomyces cerevisiae* CFTRI 101. *Afr. J. Biotechnol.* 10(20): 4183-4189.

Reed, G. 1981. Use of microbial cultures: yeast products. *Food Technol.* 35: 89.

Roberts, J. S., Kidd, D. R., and Padilla-Zakour, O. 2008. Drying kinetics of grape seeds. *J. Food. Eng.* 89: 460- 465.

Rosado, J. L., Cassís, L., Solano, L., and Duarte-Vázquez, M. A. 2005. Nutrient addition to corn masa flour: effect on corn flour stability, nutrient loss, and acceptability of fortified corn tortillas. *Food Nutr. Bull.* 26(3): 266-272.

Rosma, A. and Ooi, K. I. 2006. Production of *Candida utilis* biomass and intracellular protein content: Effect of agitation speed and aeration rate. *Malaysian. J. Microbiol.* 2: 15-18.

Rosma, A., Liong, M. T., Azemi, M. M. N., and Nadiah, W. W. A. 2005. Optimization of single cell protein production by *Candida utilis* using juice extracted from pineapple waste through response surface method. *Malaysian J. Microbiol.* 1(1): 18- 24.

Sadasivam, S. and Manikam, A. 1992. *Biochemical Methods for Agriculture Sciences.* Eighth edition. Wiley Eastern Ltd., New Delhi and Tamil Nadu Agricultural University, Coimbatore, 422p.

Sankar, N. R., Kumar, V. K., Shailaja, R., Saritha, K., and Naidu, N. V. 2011. Single cell protein production from *Trichoderma harzianum* using waste banana peel. *Int. J. Microbiol. Res.* 2(1): 78- 81.

Saquido, P. M. A., Cayabyab, V. A., and Vyenco, F. R. 1981. Bioconversion of banana waste into single cell protein. *J. Appl. Microbiol. Biotechnol.* 5(3): 321-326.

Sgarbieri, V. C., Alvim, I. D., Vilela, E. S. D., Baldidni, B. L. S., and Bragagnolo, N. 1995. Pilot plan production of yeast *Saccharomyces cereviseae* derivatives for use as ingredient in food formulations. *Brazilian J. Food Technol.* 2(1): 119- 125.

Shah, G. H. and Masoodi, F. A. 1994. Studies on the utilization of the wastes from apple processing plants. *Indian Food Packer* 48(5): 47- 51.

Shalini, R. and Gupta, D. K. 2010. Utilization of pomace from apple processing industries: a review. *J. Food Sci. Technol.* 47(4): 365–371.

Shankar, T. N. B. 2003. How Eco-friendly is food processing? [Abstract]. In: *5th International Food Convention*; 5- 8, December, 2003, Mysore. Association of Food Scientists and Technologists (India), Mysore, pp: 152- 153.

Sharma, R. C and Joshi, V. K. 2001. Processing. In: Chadha, K. L., Awasthi, R. P. (eds.), The Apple: Improvement, production and postharvest management, Malhotra Publ, New Delhi, pp. 428–483

Shilpa, R. and Theju, C. 2011. Waste utilization of pineapple fruit- enhancement of economic value of waste. BE thesis, Sri Jayachamarajendra Engineering College, Mysore, 52p.

Silva, V. K., da Silva, J., Torres, K. A. A., de Faria, F. D., Hirota, H. F., and de Moraes, V. M. 2009. Humoral immune response of broilers fed diets containing yeast extract and prebiotics in the prestarter phase and raised at different temperatures. *J. Appl. Poult. Res.* 18: 530–540.

Singh, A., Abidi, A. B., Agrawal, A. K., and Darmwal, N. S. 1991. Single cell protein production by *Aspergillus niger* and its evaluation. *Zentralbl Microbiol.* 146: 181–184.

Singh, B. D. 1998. *Biotechnology* (1st Ed.). Kalyani Publishers, New Delhi, 500p.

Skountzou, P., Soupioni, M., Bekatorou, A., Kanellaki, M., Koutinas, M. M., Marchant, R., and Banat, I. M. 2003. Lead uptake during baker's yeast production by aerobic fermentation of molasses. *Process Biochem.* 38: 1479–1482.

Smith, M. E. and Bull, A. T. 1976. Protein and other compositional analysis of *Saccharomyces fragilis* grown on coconut water waste. *J. Appl. Bacteriol.* 41: 97-107.

Sogi, D. S., Sidhu, J. S., Arora, M. S., Garg, S. K., and Baw, A. S. 2002. Effect of tomato seed meal supplementation on the dough and bread characteristics of wheat (PBW 343) flour. *Int. J. Food. Properties* 5(3): 563-571.

Sounder, S. and Chandra, J. S. 1987. Cellulose degradation by a mixed bacterial culture. *J. Indus Microbiol.* 2: 257- 265.

Splittstoesser, D. F. 1994. Biodeterioration of processed foods. In: Garg, K. L., Garg, N., and Mukerji, K. G. (eds.), *Recent Advances in Biodeterioration and Biodegradation (Vol. II)*, pp. 153- 177.

Srivastava, R. P. and Sanjeevkumar. 1994. *Fruit and Vegetable Preservation- Principles and Practices.* International Book Distributing Co.. Lucknow, 227p.

Subburamu, K., Singaravelu, M., Nazar, A., and Irulappan, I. 2003. A study on the utilization of jack fruit waste. *Bioresource Technol.* 40(1): 85- 86.

Sun, J., Hu, X., Zhao, G., Wu, J., Wang, Z., Chen, F., and Liao, X. 2007. Characteristics of thin layer infrared drying of apple pomace with and without hot air pre-drying. *Food Sci. Technol. Int.* 13(2): 91–97.

Tipparat, H. and Kittikun, A. H. 1995. Optimization of single cell protein production from cassava starch using *Schwanniomyces castellii. W. J. Microbiol. Biotechnol.* 11: 607-609.

Ueda, S., Zenin, C. T., Monteiro, D. A., and Park, Y. K. 1981. Production of ethanol from raw cassava starch by a non-conventional fermentation. *Biotechnol. Bioeng.* 23: 291–299.

Van-Wyk, J. P. H. 2001. Biowaste as a resource for bioproduct development. *Trends Biotechnol.* 19 (5): 172-177.

Vasil' ev, Yu. I, Morozov, A. N., Zaiko, G. M., and Moiseeva, V. G. 1976. The utilization of waste from vegetable and fruit canning plants. *Konservnaya-i-Ovoshchesushil' naya- Promyshlennost* 3:32–34.

Venkatesh, M., Pushpalatha, P. B., Sheela, K. B., and Girija, D. 2009. Microbial pectinase from tropical fruit wastes. *J. Trop. Agric.* 47 (2): 67-69.

Wang, J. H., Liu, Y. Y., Yao, B., and Wang, Y. R. 2000. Study on high fructose syrup production with inulase and nutrition value of yeast cell. *Food Fermentation Technol.* 26(2): 1- 4.

Warade, S. D., Patil, S. P., Kulkarni, S. S. and Garad, B. V. 2009. Physiochemical characters of different mango (*Mangifera indica L.*) cultivars grown under Western Maharashtra conditions. In: Balasubramanyan, S. (ed.), *Proceedings of the National Seminar on Production, Postharvest Technology and Marketing;* 23-25 September, 2009; Periyakulam, pp. 37- 40.

Wyman, C. E. and Goodman, B. J. 1993. Biotechnology for production of fuels, chemicals and materials from biomass. *J. Appl. Biochem. Biotechnol.* 39: 39–59.

Zhao, G., Zhang, W., and Zhang, G. 2010. Production of single cell protein using waste capsicum powder produced during capsanthin extraction. *Lett. Appl. Microbiol.* 50: 187-191.

APPENDICES

APPENDIX - I

Composition of YEPD

Yeast extract	: 3.0g
Peptone	: 10.0g
Dextrose	: 20.0g
Distilled water	: 1.0L
Agar	: 15.0g

APPENDIX - II

Standard recipe for preparation of biscuit

Ingredients

Refined wheat flour	: 50g
Powdered sugar	: 30g
Butter	: 25g
Salt	: a pinch
Baking powder	: a pinch

Refined wheat flour, salt and baking powder were mixed together and sieved twice in a fine mesh sieve. Sugar and butter were creamed together and folded with the sieved ingredients and rolled. The biscuit was cut using a mould and baked at 180^0C for 12 minutes.

Number of biscuits : 15

APPENDIX - III

Score card for organoleptic evaluation of biscuits

Characteristics	Score		
	T_0	T_1	T_2
Colour			
Texture			
Odour			
Taste			
After taste			
Overall acceptability			

9 point Hedonic scale

Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

Name of the judge: **Signature:**

Date:

PROCESS STANDARDIZATION FOR THE PRODUCTION OF BAKER'S YEAST FROM MANGO STONES

By

NIKHIL V. M.
(2010-12-111)

ABSTRACT OF THE THESIS

Submitted in partial fulfillment of the requirement for the degree of

Master of Science in Horticulture

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Abstract

The study on "Process standardization for the production of baker's yeast from mango stones" was undertaken at the Department of Processing Technology, College of Horticulture. The main objectives were to optimize the culture conditions and growth supplements for baker's yeast production and to explore the feasibility of enrichment of biscuits with single cell protein (SCP).

The physical composition of mango fruit and chemical constituents of mango pulp were evaluated. The contribution of pulp, peel and stone in mango ranged from 65.22- 73.69 percent, 8.32- 12.18 percent and 13.5- 21.94 percent respectively. The pulp adhered to mango stones recorded 78.8-80.1 per cent moisture, 2.8-3.52 per cent reducing sugar, 10.25 per cent non reducing sugar, 13.77-13.05 per cent total sugar, 9.3-15.8^{0}B TSS, 1.9- 3.5 pH, 16.94-21.01 mg/100g ascorbic acid and 0.26-0.37 percent acidity.

The effect of different surface sterilization methods *viz.* autoclaving, hot water immersion and steam blanching on surface decontamination of mango stones were studied. Autoclaving of the substrate at 121^{0}C and 15psi for 30 minutes was found to be the best surface sterilization method.

Lyophilized baker's yeast *Saccharomyces cerevisiae* MTCC 172 strain was used for the study. The secondary/working inoculum was standardized by inoculating this strain into YEPD broth. The culture conditions for SCP production using mango stone as substrate was standardized. Factors like TSS, pH, duration of fermentation, aeration affecting the cell biomass production from mango stones by *Saccharomyces cerevisiae* MTCC 172 were evaluated. The optimum conditions of the substrate for maximum yeast growth were TSS (30^{0}B), pH (4) and provision of aeration by keeping in a orbital shaker at 100 rpm for five days.

The effect of supplementation with carbon, nitrogen, phosphorus and inducers on yeast growth, wet biomass yield, dry biomass yield and protein content were

determined by supplementing media with broken wheat, NH_2SO_4, KH_2PO_4 and Biotin. Significant variation was observed between treatments. Maximum yeast growth (49.00×10^7 cfu/g) was observed in media supplemented with ammonium sulphate (0.8%). Wet (13.67g/l) and dry (2.18g/l) biomass yield were found to be highest in media supplemented with ammonium sulphate. Highest content of protein (39.38%) was observed in media supplemented with ammonium sulphate (0.8%), broken wheat (20g) and combination of ammonium sulphate, KH_2PO_4 and biotin.

The possibility of protein enrichment of biscuits was evaluated by incorporating baker's yeast in biscuits so as to raise the protein to 15 and 20 percent level. The enriched biscuits recorded protein content of 14.85(T_1) and 19.14 (T_2) percent whereas the biscuits made from refined wheat flour had 11.21 percent protein content. Highest scores for organoleptic properties of biscuits was obtained for T_0 (biscuits made from refined wheat flour) followed by T_1 (15% protein enriched biscuits).