STANDARDIZATION OF PROCESSING METHODS FOR PRODUCTION OF QUALITY WHITE PEPPER

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(2009-12-114)

THESIS

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2011

DECLARATION

I hereby declare that this thesis entitled "**Standardization of processing methods for production of quality white pepper**" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title, of any other University or Society.

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Shameena Beegum

Dedicated to

My

Family

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LIST OF ABREVIATIONS

%	-	per cent
CD	-	Critical difference
cm	-	centimetre
et al	-	And others
Fig.	-	Figure
g	-	gram
kg	-	Kilogram
m	-	metre
mg	-	milligram
min	-	minutes
ml	-	millilitre
mm	-	millimetre
°C	-	Degree Celcius
s	-	seconds
ppm	-	parts per million
i.e.	-	That is
viz.,	-	namely
CRD	-	Completely Randomized Design

Introduction

1. INTRODUCTION

Spices are intrinsically woven into the history of our nation. Among spices, black pepper is the one that captured the global attention. Columbus sailed in search of it, unfortunately landed in America. But Vasco de Gama was fortunate in discovering the land of spices as early as 1498. It was the spice that has inspired several expeditions and wars and remained a staple article of commerce between India and Europe. It has a great role in Indian system of medicine and over the years it enjoyed the reputation as the 'King of Spices'. Pepper is one of the oldest and most popular spices in the world. India is a major producer, consumer and exporter of black pepper in the world.

Black pepper (*Piper nigrum*) belongs to the family Piperaceae. The products developed from pepper broadly fall into three groups namely black pepper, white pepper and green pepper. Black pepper and white pepper are widely used for culinary purposes, flavouring of foods and for medicinal uses. White pepper has tremendous value in the world of food spices than the traditional black pepper because of charming creamy white colour , suitability to use in any food preparation and mild flavour (Purseglove, 1981). White pepper is the white inner corn obtained by removing the outer skin or pericarp of the ripe berries. It is widely used for making mayonnaise, salad dressing, sausages and for flavouring ice cream where black pepper is not suitable (Furia and Bellanca, 1991). In most of the European countries, white pepper is used traditionally and is preferred to black pepper. Considerable quantities of white pepper are also consumed in the United States, Canada, Australia and New Zealand in the belief that it is milder in pungency than black pepper.

White pepper of commerce is prepared by different methods viz., retting method, steaming or boiling technique, chemical method, decortication technique, microbial and enzymatic methods. Retting method being the traditional method of white pepper production is usually practiced by Indian farmers. Traditional method of white pepper production is by keeping the fully ripe pepper berries in running water for 10-14 days to soften the pericarp which then removed by scrubbing. White pepper thus obtained is cleaned, washed and sun dried. All the improved methods had advantages and disadvantages.

Time consuming retting process and the poor colour are the two main problems with the white pepper produced by traditional method. An attempt was made to reduce both these problems using some commonly using chemical bleaching agents and microbial fermentation using different isolates. The present investigation entitled "Standardizing of processing methods for production of quality white pepper" was carried out with the following objectives:

1. To produce good quality white pepper using promising bleaching agents.
2. To reduce the prolonged retting period by adopting microbial fermentation method
3. To evaluate the acceptability of developed white pepper products.
4. To analyse and popularize use of white pepper in domestic food preparations.

Review of literature

2. REVIEW OF LITERATURE

Spices contribute an important group of agriculture commodity that are considered indispensable for flavouring foods, beverages, pharmaceutical, perfumery and cosmetic industries (Sivaraman and Peter, 1999). They have played an important role in the history of civilization, exploration and commerce. Spices have been cultivated and used since ancient times from the beginning of human civilization in India, China, Babylon, Egypt, Greece and Rome (Senthikumaran and Thomas, 2011). The delightful flavour and pungency of spices make them indispensable in the preparation of palatable dishes. In addition, they are reputed to possess several medicinal and pharmacological properties and hence find position in the preparation of a number of medicines (Vijayan *et al.,* 2000). India has a unique position in global spice scenario as the largest producer of spices because of the wide range of agroclimatic regions specially suited for its cultivation (Ipe, 2002)

Indian economy is basically agrarian and hence exports of food and agricultural products assume crucial significance in our export efforts. The world consumption of spices is growing steadily year by year. It is therefore, important that we expand our exports of spices to increase or even to retain our share in the market.

Black pepper the king of spices is one of the oldest and most widely used spice by mankind and occupied a commanding position among all the spices (Ravindran *et al.,* 2006). It accounts for the lion's share of the spice exports from India. It is a perennial, climbing vine indigenous to the Malabar Coast of India.

The name pepper comes from the Sanskrit word 'pippali' meaning berry. Black pepper with its characteristic pungency and flavour is a major ingredient in wide category of food preparations. (Ravindran, 2000)

In India pepper is a known spice since ages. Even before the time of Alexander's conquest of India, Indians knew the flavor of pepper. This spice was prevalent in the biblical times as well. Pepper was much used by the Romans and in the Early Middle Ages became a status symbol of fine cookery. Pepper has secured a pivotal position in food, pharmaceuticals, perfumery and cosmetic industries (Nybe *et al.*, 2002).

The export share of pepper during the year 2004-05 to 2008-09 was 14% in terms of quantity and in value terms it came down to 8% (Parthasarathy *et al.*, 2009). Spices and spice products estimated at 433,455 tonnes valued Rs 5,485.40 crore has been exported from the country during April-January 2010-11. In the case of pepper and pepper products the increase is in trading was terms of value only. During April-January 2010-11, a total quantity of 15,700 tonnes of pepper valued Rs 307.47 crore have been exported as against 16,295 tonnes valued Rs 269.36 crore during the previous year. The unit value of pepper has increased from Rs 159.15 per kg in 2009-10 to Rs 195.84 per kg during 2010-11. (IPC, 2011).

According to the Vietnam Economic Times dated April 15th 2011, a report of the International Black Pepper organization said that the demand of pepper in the world is expected to grow by 5% in 2011.

2.1 QUALITY PARAMETERS OF BLACK PEPPER

An analysis of 23 types of black pepper from the various pepper growing tracts of Kerala, Kanara, Coorg and Assam gave the following ranges of values.

Component	Percentage
Moisture	8.7 - 14.15
Total nitrogen	1.55 -2.6
Non volatile ether extract	3.9 - 11.5
Starch	28-49
Crude fiber	8.7 -185
Piperine (by Spectrophotometric method)	1.7-7.4
Total ash	3.9 -5.7
Acid insoluble ash	0.03 -0.55

Chun *et al.* (2002) found that 88% of the polysaccharide of black pepper berries was glucose, followed by galactose, arabinose, galacturonic acid and rhamnose in smaller proportions.

2.2 VALUE ADDITION OF BLACK PEPPER

Value addition and product diversification aroused the need to increase the utilization of pepper. It will help the country to withstand the competition and threat from other pepper producing countries. Value addition is the one of the means to sustain the pepper producers in the field. Considerable advances have been achieved in the field of value addition and product diversification of black pepper. Such value added products can be classified as a) green pepper products b) black pepper products c) white pepper products d) pepper by products (Pruthi, 1997). Black pepper and white pepper are the two primary products that are internationally traded.

The demand for white pepper is on an increase because of its greater and worldwide appreciation in food preparations. Applied contaminants including pesticides, if any are removed by dismantling is an added attraction to these creamy white products (Manilal, 2008).

2.2.1 White pepper

White pepper is a major value-added product of black pepper (Pruthi, 1993). It possesses a mild flavour and pungency as compared to black pepper, which has a sharp, pungent aroma and flavour. Due to its mild flavour, pungency and light colour, there is a growing demand for white pepper in the markets all over the world. (Thankamani *et al.*, 2004). According to Gopinathan *et al.* (2005) white pepper is the most appreciated form of decorticated green or black pepper . White pepper berries are light yellow to grayish in colour, globular in shape and around five millimeter in diameter (Parry, 1969). It is prepared by dismantling the pericarp and outer portion of the mesocarp or in other words by removing outer skin or pericarp of ripe or fully matured berries (Varghese 1989).

It is preferred over black pepper in light coloured preparations such as sauces, cream soups etc. where dark coloured particles are undesirable. It imparts pungency and natural flavour to food stuffs (Sudarshan, 2000). According to Gopalam *et al.* (1991) it is preferred to black pepper in Europe, U.S.A and Japan because of the charming colour, stability to use in all food preparations, less microbial load, free from many contaminants and no substantial difference in pungent principle to that of black pepper. The world demand for white pepper is about 25% of the black pepper produced worldwide (Dhas *et al.*, 2003). China and Vietnam are the two major contributors to white pepper production and jointly share approximately 60 percent of the white pepper production (Spices Board,2011).

Annual white pepper contribution in India is less than 250 metric tonnes against the world demand of more than 1,50,000 metric tonnes. Indonesia is the largest white pepper producing country, converts about 50 % of its pepper to white. Malaysia and Brazil converting about 10 % and 5% of their pepper to white respectively (Annonymous, 2009).

2.2.1.1 Cultivars

Good quality white pepper can be obtained from pepper varieties having bold size berries (Sudarshan, 2000). Based on size, berries are classified into three main groups viz. large size (>4.25mm), medium size (3.25 to 4.25mm) and small size (<3.25mm). Among the varieties panniyur-1(large sized) and Balankotta (medium sized) are ideal for making white pepper. (Zachariah,2000). Out of more than 70 different cultivars that are cultivated in Kerala Panniyur-1 is considered to be the most outstanding one (Nybe *et al.,*1999). Panniyur-1 bear more spikes and berries, has higher mean weight higher than other cultivars (Mathai, 1986). Panniyur-1 is coming under the category of varieties having medium level of oil content. ie, 2.4 - 4.4 % (Gopalam *et al.*, 1987). According to Farooqi *et al.* (2005) the content of oleoresin, piperine and oil content in Panniyur -1 are 11.8%, 5.3% and 3.5% respectively and recovery of white pepper varies from 22-27% of green pepper.

2.2.1.2 Economics of white pepper production

On drying 100 kg mature pepper the yield of black pepper will be approximately 33 kg where as the white pepper obtained by the retting process will yield only 25 kg (Narayanan *et al.*, 2000)

The yield of white pepper from the bacterial fermentative method was 90 to 98% based on the amount of black pepper initially used. The creamy white pepper cores also retained its color without any microbial contamination (Thankamoni *et al.*, 2004).

2.2.1.3 Methods of white pepper production

2.2.1.3.1 Traditional method

White pepper is traditionally prepared by retting method in which the matured berries after despiking are filled loosely in gunny bags of 25- 50 kg capacity and are soaked in flowing water stream for two to three weeks (Natarajan *et al.,* 1967). If running water source is not available, other alternative is to use fermentation tanks wherein the water is changed every day (Nurdjannah *et al.,* 1998 and Sudarshan, 2000). Retting converts only ripe and fully mature berries to white pepper whereas green berries turn into black eventually after drying. White pepper so produced had microorganisms and mould greater than that of black pepper. Presence of black berries in white pepper is to the maximum of 5 percent. (Purseglove *et al.,* 1981).

According to Madhusoodanan *et al.* (1990) complete skin removal of mature pepper requires 15 days of soaking in flowing water. After retting the skin is mechanically or removed manually by trampling. After thorough washing the pepper is sundried to the moisture content of 8-12 percent.

Another study conducted by Varghese,(1999) revealed about the pit burial method of white pepper production in which the fully ripe berries were converted into white pepper after 7 days, the mature (green) and semi ripe (yellow) took 14 days. According to him, even mature berries get converted fully into white and the percentage of black pepper was less than 0.2% and this technology required vey less water and the chances of pollution was rather less.

2.2.1.3.2. Steaming or boiling Technique

Rathnawathie and Buckle, (1984) have experimented white pepper preparation by cooking of harvested berries in boiling water for five minutes and removing the pericarp by hand. The white pepper thus obtained is washed and dried in sun for two to three days.

Another method was patented by CFTRI, involves blanching of green pepper berries in steam or hot water for 10 to 25 minutes to soften the skin. The yield of white pepper by this method was 20%. But because of the gelatinization of starch by heat treatment, the colour of the ground pepper was not as white as that obtained by grinding white pepper by traditional retting process. This method however has the advantage considerably shortening the processing time and minimizing bacterial load. (Pruthi, 1993).

Another experiment done by Risfaheri and Hidayat, (1996a) revealed that, white pepper produced by boiling technique had stronger aroma than the traditional method.

2.2.1.3.3 Decortication method

Prototype of pedal and power operated pepper decorticators have been developed at RISMC (Research Institute for Spices and Medicinal Crops) by Risfaheri *et al.* 1996b. A power-operated black pepper decorticator was developed by Chitra *et al.* (2008) at Kelappaji College of Agricultural Engineering and Technology ,Tavannur and was evaluated by using various grinding surfaces. The principle of the power-operated decorticator was to subject the presoaked berries to compression and shearing between two abrasive surfaces, one stationary and the other rotating. The compressive forces crushed the skin of the berries and the shear forces separated the skin. White pepper produced by machine decortcation had higher oil content and better and stronger aroma than that obtained from traditional method. However, slight discolouration was observed due to the presence of phenol in the pericarp.

Conversion of black pepper to white by selective grinding has been attempted by Thomas *et al.* (1991). The difference in behavior of black skin and inner white core of dry black pepper to compressive forces was made use of to produce white pepper powder instead of the conventional retting and scrubbing. The process resulted in gray coloured berries. There was loss of aroma due to friction during decortications process. However, it saves time and avoids foul smell emanating due fermentation and contamination.

2.2.1.3.4 Enzymatic white pepper production

Enzyme application was proven to facilitate processing of white pepper with short processing time. Action of pectinases, the pectin degrading enzymes has been found effective in the smooth removal of pepper skin (Gopinathan *et al.*, 2003). By the actions of pectinases the bonding pectins are specifically removed, and there by the skin detatches easily from the core (Gopinathan and Manilal, 2004).

An enzyme company, Novozyme patented an enzyme (peelzyme) for white pepper production. Here threshed berries after subjecting to blanching in hot water (90-100 ^{0}C for 60 seconds.) were soaked in water and treated with enzyme dosage of 1000-4000ppm for about 1-3 days at room temperature. White pepper thus obtained had higher volatile oil, piperine content, better aroma and pungency (Ying, 2009).

Among various enzymes used for the decortication purpose, pectinase was found to be very effective for both dried and fresh pepper. Since enzyme is costly the process was not found to be economically feasible (Omanakutty, 2006)

2.2.1.3.5 Chemical white pepper production

Joshi (1962) developed a chemical process based on steeping whole dried black pepper in five times its weight of water for 4 days and treating with 4 percent sodium hydroxide solution and boiling the mixture. Then after removing the skin by agitation berries were bleached with 2.5 percent hydrogen peroxide solution and then subjected to drying. In another approach of white pepper preparation, commercially graded black pepper is soaked in water. Soaking leads to fermentative degradation and softening of skin in about 10-12 days. The partially degraded skin while soaking is removed using a fruit pulping machine. The deskinned pepper is subjected to chemical bleaching for improved whiteness. But long duration of water soaking and chemical bleaching are found unattractive in this process of white pepper production (Lewis *et al.*, 1969 a).

Another chemical method patented by Omanakutty (2006) comprises soaking the berries in a dilute solution of alkali, followed by blanching, decortication in a pulper, bleaching and drying. Here the advantage is that the decortication is made possible within 12-14 hours in the case of black pepper and less than an hour in the case of fresh green pepper.

Lime (calcium hydroxide) bleaching in ginger was reviewed by Prakashi *et al.* (2003) which consist of soaking of semi dried ginger in 2% lime solution for 8 hrs followed by sun drying. Prolonged contact with bleaching agents like sodium hydroxide with 5% concentration destroyed the pungency of ginger (Vikaas, 2006). Use of calcium hydroxide (1%) and calcium hypochlorite solution (0.2%) for white pepper bleaching was reviewed by Kumar (2006a) and revealed that in addition to better creamy white colour, it preserved the organoleptic qualities also.

Method of bleaching pepper and capsicum oleoresins by mixing directly with 6% and 3% benzoyl peroxide respectively followed by agitation and heating were described by (Sharratt *et al.*,1976) . Kuramoto *et al.* (1980) tried benzoyl peroxide for bleaching of milk and cream for blue cheese manufacture. Benzoyl peroxide used as a bleaching agent in flour with acceptable concentrations up to 40 mg/kg and found that was of no safety concern when used as a flavouring agent (WHO, 2002). It has been reported that benzoyl peroxide is typically used in the cheese manufacture at a level of 20 mg/kg to bleach milk used for the production of white Italian cheeses and the

FDA has affirmed benzoyl peroxide to be GRAS (Generally Recommended As Safe) when used as a bleaching agent in cheese making (U.S. FDA, 2003). Sodium perborate is an another bleaching agent used in active oxygen-type laundry bleaches, plastic de-staining and dish washing compounds, coffee-stain removers, neutralizers for cold-wave preparations, and a safe intra coronal bleaching agent (Jun 2002). Attin *et al.* (2003) recommended the use of sodium perborate solution for intracoronal bleaching

Turkun et al. (2003) reported that, sodium hypochlorite has no effect for cleaning root canal of teeth. Pretreatment of sodium hypochlorite and sodium metabisulphite in oyster mushroom produced lightest coloured products (Suhaila and Tok, 1994; Mohammed and Rosli (1994).

2.2.1.3.6 Microbial method

A study conducted by Thankamani *et al.* (2004) identified three *Bacillus* species viz, *Bacillus mycoides , B .brevis and B. licheniformis* as having skin degrading effect. White pepper was produced from black pepper by the fermentative method using the isolates in shake flaks as well as in a large-scale fermenter. Volatile oil and piperine contents of the product were 3.2% (v/w) and 4% (v/w) respectively. The moisture content was 15%. Microbial contamination was less than 10 per 100 g. The product also exhibited excellent storage stability. Another study done by Gopinathan and co-workers,identified a four member bacterial consortium consists of a *Xanthomonas sp, Pseudomonas sp,* and two spp. of Bacillus. Effective in the production of white pepper (Gopinathan and Manilal, 2005).

Application of bacterial consortium for degradation of complex biomolecules like cellulose, lignin, and pectin has been described by Khan *et al.* (1982) and Shivakumar *et al.(*1995). Pectins are important constituents of the cell wall of edible part of fruits and vegetables. They are the sole polysaccharide in the middle lamellae, functions as intracellular adhesive (Kertesz,1981). The degradation of pectin by bacterial consortium has been described by Breure *et al.* (1985) and Shivakumar and Nand, (1995). During the consortium fermentation , the pectin present in the pepper skin has reduced from 11.2% to 1.9% (Gopinathan and Manilal,2006).

The fermentative method of production of white pepper demonstrated a greater capability in providing a higher yield of superior quality white pepper at a relatively short time. The volatile oil and piperine contents, which are responsible for the aroma, were conspicuously enhanced in the fermentatively produced white pepper. Besides, the product had a lower microbial load as compared to that of the commercial white pepper (Thankamani *et al.*, 2005)

2.2.1.4 Quality parameters of white pepper

The quality of white pepper is directly dependant on the stage of maturity of the berries at harvest Sudarshan (2000). Spike with three to five ripe berries which have reached ripening (orange to reddish colour) are ideal and are harvested for preparing white pepper. White pepper is liked for its mellow flavour, mild pungency, low fibre and high starch content and above all, white colour and absence of black particle (Pruthi, 1993). The quality of white pepper in whole and ground form is imparted by appearance, aroma, and pungency (Risfaheri and Nurdjannah, 2000). For export purposes, the American Spice Trade Association (ASTA) standards are generally followed (Farooqui *et al.*, 2005).

	Moisture (%)	Ash (%)	Essential oil (%)	Starch (%)
Black pepper	12	7	2 (minimum)	30 (minimum)
White pepper	13	3.5	1.5 (minimum)	52 (minimum)

Chemical and physical specifications for international acceptance of ground white pepper were listed by Tainter and Grenis (1993) and are indicated below.

COMPONENT	COMPOSITION (%)
Moisture	14.0 (% max.)
Volatile oil	1.5 (% min.)
Starch	52.0 (% max.)
Total ash	1.5 (% max.)
Acid-insoluble ash	0.3 (% max.)

A study conducted by Omanakutty (2006) revealed that the organoleptic properties of white pepper remained rather stable on storage and chemically white pepper is more or less similar to black pepper. It was reported that white pepper possesses slightly high piperine content than black pepper (Lewis *et al.*, 1969a). The loss of skin does not affect the pepper oil content of pepper substantially (Mathew *et al.*, 1977).

Chemically white pepper is more or less similar to that of black pepper except certain parameters. Pepper pericarp contains fairly good number of oil bearing cells and fibers. Thus white pepper generally has lesser quality of volatile oil and crude fibres content than black pepper (Pruthi, 1992).

2.2.1.4.1 Physical parameters

2.2.1.4.1.1 Specific gravity

A study conducted at Sarawak indicated that pepper fruits having specific gravity greater than 1.12 g/cc are best for conversion to white pepper, and those having less than 1.12 are good for making black pepper (Anon.1995).

2.2.1.4.1.2 Size

The cleaning and removal of light berries from green and black pepper before microbial fermentation could result in white pepper with uniform size and better quality (Madan *et al.*, 2001).

The size of white pepper obtained from bacterial fermentation of black pepper was ranging from 3-6mm (Gopinathan and Manilal, 2005)

2.2.1.4.2 Chemical parameters

2.2.1.4.2.1 Essential oil

Essential oils are aromatic volatile components present in most spices (Menon, 2000). The characteristic aroma of black pepper is due to the presence of volatile oil which can be recovered by steam distillation or water distillation (Pruthi, 1997).

The essential oil of pepper is a mixture of a large number of volatile chemical compounds. The aroma is contributed by the totality of these components. More than 80 components have been reported in pepper essential oil (Gopalakrishnan *et al.*, 1993)

Pepper oil is used in perfumery and flavouring. The pepper oil derived from steam distillation is almost colourless to slightly greenish liquid with the characteristic odour of pepper. The taste of oil is mild, not at all pungent (Risfaheri and Nurdjannah, 2000). Prior to distillation the berries should be crushed to get maximum oil yield (Risfaheri and Hidayat, 1993).

The most abundant compounds in pepper oils were (E)-beta-caryophyllene (1.4-70.4%), limonene (2.9-38.4%), beta-pinene (0.7-25.6%), Delta-3-carene (1.7-19.0%), sabinene (0-12.2%), alpha-pinene (0.3-10.4%), eugenol (0.1-41.0%), terpinen-4-ol (0-13.2%), hedycaryol (0-9.1%), beta-eudesmol (0-9.7%), and caryophyllene oxide (0.1-7.2%) (Orav,2004)

Lewis *et al.* (1969b) studied 17 cultivars from Kerala and found that the oil content ranged from 2.4–3.8%. In the oils, monoterpene hydrocarbons ranged from 69.4-84%, sesquiterpene hydrocarbons 15– 27.6% and the rest were oxygenated constituents.

On a weight per corn basis, volatile oil formation is rapid, but decreases during ripening and this decrease coincides with the sharp drop in moisture (Mathew, 1992). In the case of milling process done by Thomas *et al.* (1991) revealed that due to the squeezing action on cells in roller milling and subsequent evaporation, loss of volatile oil was observed.

Sumathykutty *et al.* (1989) envisaged that the youngest fruits yielded the least oil content (1.75%), while in later developmental stages they yielded more oil (2.5-4.25%). Lewis (1982) examined various types of pepper originated from India such as bold pepper, white pepper, garbled pepper, light pepper and reported the oil content ranging from 1.5- 3.5%, the great light ,had registered the maximum oil content of 3.5%.

According to Gopinathan and Manilal, (2005) during the decortications process there is a slight decrease of volatile oil content in pepper. This is because of the loss of volatile oil bearing cells located on outer skin. The white pepper oil recovery from the process of microbial fermentation was 2-3.1%.

In a study conducted by Orav (2004) revealed that oil from ground black pepper contained more monoterpenes and less sesquiterprnes and oxygenated terpenoids as compared to green and white pepper oils. After 1 year of storage of pepper samples in a glass vessel at room temperature, the amount of the oils isolated decreased, the content of terpenes decreased, and the amount of oxygenated terpenoids increased.

2.2.1.4.2.2 Oleoresin

Oleoresins are concentrated products obtained by extraction of ground pepper using solvents like hexane, acetone, eththylene dichloride etc. (Pruthi, 1980). The oleoresin produced in influenced by the solubility of the solvent used (Risfaheri and Nurdjannah, 2000).

Normally a solid: solvent ratio of 1:3 is employed and a temperature of 55- 60 ^{0}C is maintained (Narayanan,.2000). It was found that yield of oleoresin and its quality are dependent on the raw material extracted. Even within a cultivar, variability has observed in chemical quality (Gopalam *et al.,* 1991).

The quality components are also reported to depend on maturity stage. (Sumathykutty *et al.,* 1989). Pepper oleoresin is a dark viscous liquid with a strong aroma and pungent taste. Oleoresin contains total pungency and flavour constituents of pepper (Dhas *et al.,* 2003). Oleoresin offered by some of the principal manufactures claimed that 1kg of oleoresin was obtained from 8 kg pepper (Purseglove *et al.,* 1981).

2.2.1.4.2.3 Ash content

Ash constitutes the non volatile uncombusted inorganic residues of combusted material. Ash determination of spices is of value since it is a good index of quality and helps to some extent in detection of adulteration in powder. Ashing recognized as a useful tool in determining the nature and extent of various constituents so vital in both human and animal health (Pruthi, 1997). The maximum limit of ash requirements for imported black and white pepper are 7.6% and 2.5% respectively.

2.2.1.4.2.4 Starch content

Rathnawathie and Buckle, (1984) have accounted higher starch content in white pepper. Starch is the predominant constituents of black pepper, ranging from 35-40% in black pepper and 53-38% in white pepper (Govindarajan, 1977). According to Frooqi, *et al.* (2005), starch content account for 34.85 % in black pepper and 63.2% in decorticated white pepper.

2.2.1.4.2.5 Piperine

The pungency of black pepper has been the subject of chemical investigations since the early 19th century. In 1819, Oersted isolated piperine, the most abundant alkaloid in pepper, as a yellow crystalline substance and its structure was later identified as the trans form of piperoyl piperidine (Narayanan, 2000). The alkaloid piperine generally is accepted as the active 'bite' component in black pepper.

The chemistry of pepper has been reviewed by Guenther (1982); Govindarajan (1977); Parmar *et al.* (1997) and Narayanan (2000) and described that it is the chemistry of its essential (volatile) oil and piperine.

Varietal variation of oleoresin and piperine was reported by Kurian *et al.* (2002) in black pepper (*Piper nigrum* L.) grown at Idukki District of Kerala. Mathew and Bhattacharyya,(1990) showed that a slightly immature grade of 'half pepper' was economically advantageous and contained the highest levels of piperine (6.8%). Among cultivars the piperine content varies from 0.4- 7 %.

Piperine can be estimated by UV spectrophotometry by measuring the absorption maximum at 342–345 nm of a solution in benzene or ethylene dichloride. As piperine in dilute solution is highly photosensitive the solution should not be exposed to direct light (Muggeridge *et al.*, 2002).

2.3 ORGANOLEPTIC QUALITY EVALUATION AND ACCEPTABILITY STUDIES

Scientific methods of sensory analysis of food are becoming increasingly important in evaluating the acceptability of the food product. When the quality of the food is assessed by means of human sense organs, the evaluation is said to be sensory analysis.Jellinick (1986) reported that the first impression of the food is usually visual and major part of our willingness to accept a food, depend upon its colour. Organoleptic qualities such as colour, flavour, taste, texture and appearance are assessed with a panel of selected judges (Watts *et al.*, 1989).The combination which got the highest scores was selected for formulation of products. According to Herrington, (1991) sensory evaluation technology is a method using skilled management and trained panelists to provide confirmation on the acceptability of the products in terms of product profile, consumer acceptability and consistency

Brue et al. (1991) had stated that the important factors in the marketing of products are its looking, eating and processing qualities. This includes the flavour, texture and appearance of the product. According to Mc Dermott (1992) sensory method in which palatability is evaluated by a panel of judges is essential to every standardization procedure because they answer all important questions of the food tastes, smells, looks and feels.

Rajaleshmi, (1993) described sensory analysis as a scientific discipline used to evoke, measure, analysis and interpret reaction to those characteristics on food materials as perceived by the sense of sight. Smell, taste, touch and hearing. Johns,(1993) had stated that, consumer the perceivable sensory attributes, colour, appearance, feel, aroma, taste and texture are the deciding factors of food acceptance.

According to Shanker, (1993) several factors such as raw material quality, storage temperature, and storage container process employed and the environment in which it is processed will have an effect on the quality of the food material.

Dorko and Penfield, (1993) reported that the aesthetic, safety, sensory characteristics and acceptability of foods are all affected by color. Almedia and Noguira, (1995) reported that organoleptic properties determine acceptance of food by the consumer with appearance being the first factor that determine the acceptance or rejection of a food.

Sharma *et al.* (1995) revealed that taste is the primary and most important quality among various attributes. They also reported that colour scores were significantly related with acceptability. Jack *et al.* (1995) reported that texture is a sensory attribute resulting from interaction between food and its consumer. It is the physical property of food stuffs apprehended by the eye, the skin and mouth.

Nikolaidias and Labuza (1996) opined that texture is an important sensory attribute for many cereal based foods and the loss of desired texture results in a loss of products quality and reduction in shelf life. Texture is the property of food which

Materials and methods

3. MATERIALS AND METHODS

The present investigation entitled "Standardization of processing methods for production of quality white pepper" was undertaken at the Department of Processing Technology, College of Agriculture, Vellayani during the period 2009-2011. Ripe berries of variety Panniyur-1 were selected for the study. The berries were obtained from Cardamom Research Station, Pambadumpara and Regional Agricultural Research Station, Ambalawayal.

The experiment was divided into three parts.

3.1 Chemical method of white pepper production

3.2. Microbial fermentation method

3.3 Organoleptic evaluation

3.1 CHEMICAL METHOD OF WHITE PEPPER PRODUCTION

3.1.1 Harvesting and collection of berries

Pepper matures in about 7-9 months after flowering. Harvesting season under Kerala condition starts from December which will extend upto March. Berries were harvested during the second week of January 2010 from Regional Agricultural Research Station, Ambalawayal and during the second week of March, 2010 from Cardamom Research Station ,Pampadumpara. Spikes with fully ripe berries were harvested, threshed, cleaned, sorted, packed in gunny bags and brought to College of Agriculture, Vellayani for starting the work.

3.1.2 Retting

The traditional retting process was chosen for production of white pepper. The berries were kept for retting for fourteen days. Water was changed daily and it was observed that within two days of commencement of retting, process of fermentation was started. It took fourteen days to complete retting. Then the berries were washed and cleaned thoroughly by gently rubbing with hands and were subsequently treated with chemicals (bleaching agents) selected for the study.

3.1.3 Chemical treatment

3.1.3.1 Preliminary experiment

The following seven bleaching agents were selected for chemical treatments.

T1 Calcium hypochlorite

T2 Calcium hydroxide

T3 Hydrogen peroxide

T4 Sodium hypochlorite

T5 Sodium hydroxide

T6 Sodium perborate

T7 Benzoyl peroxide

A preliminary trial was conducted to evaluate the performance of bleaching agents on decorticated pepper berries. White pepper berries was soaked with bleaching agents with different concentrations and time and changes due to the chemical reaction in the berries were noticed.

3.1.3.2 Standardization

The quantity of berries and water was standardized at 1:2 ratio. Based on the pre trials conducted, the time and concentration of the chemicals were standardized for the eight treatments were as follows,

T1 Calcium hypochlorite (0.2% for 6hrs)

T2 Calcium hydroxide (1.5%for 12 hrs)

T3 Hydrogen peroxide (2.5% for 12 hrs)

T4 Sodium hypochlorite (0.2% for 3hrs)

T5 Sodium hydroxide (0.5% for 6 hrs)

A) Calcium hypochlorite

B) Calcium hydroxide

C) Hydrogen peroxide

D) Sodium hypochlorite

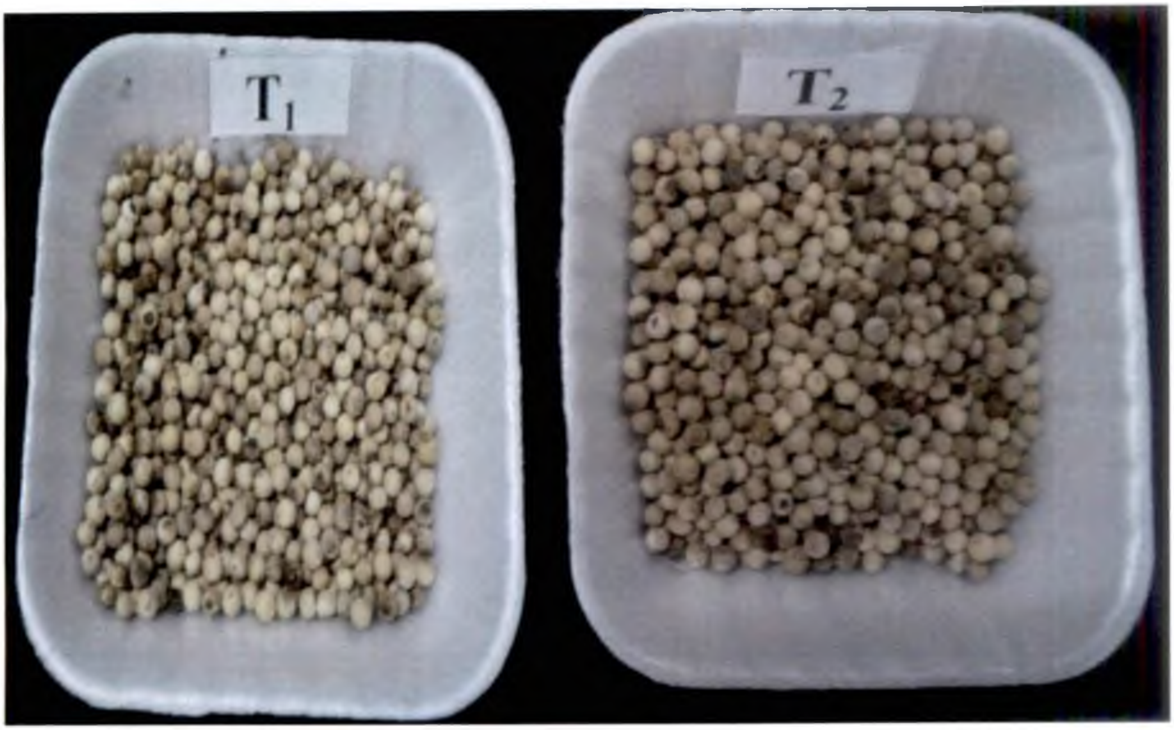
E) Sodium hydroxide

F) Sodium perborate

G) Benzoyl peroxide

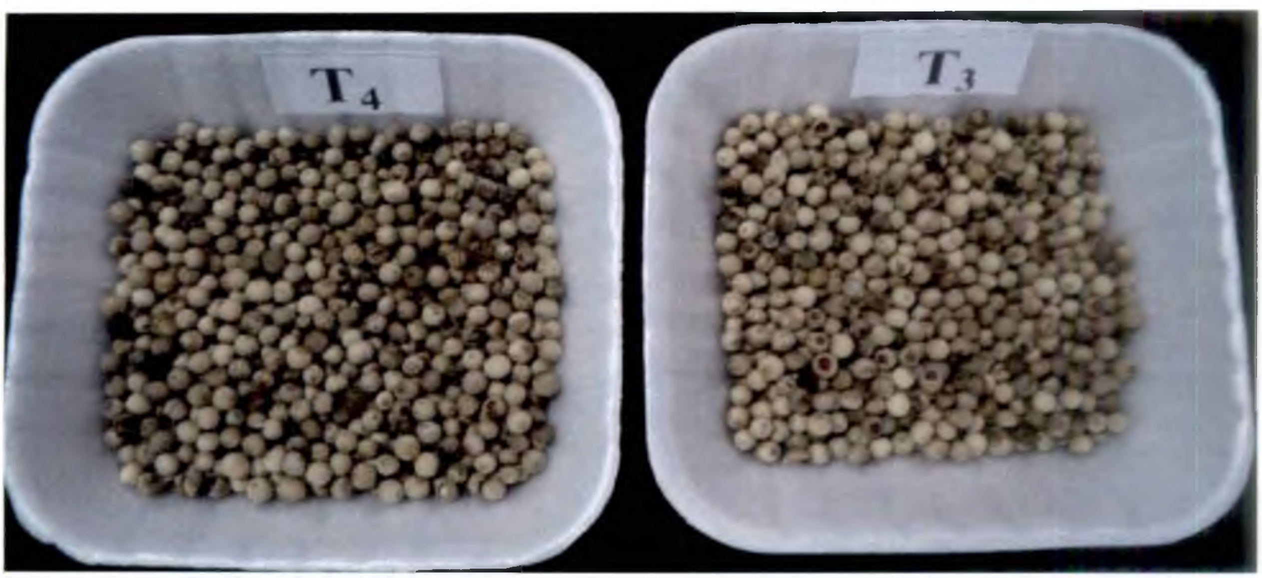
H) Control

Plate 1 Berries after treatment with bleaching agents



A) Treatment with calcium hypochlorite

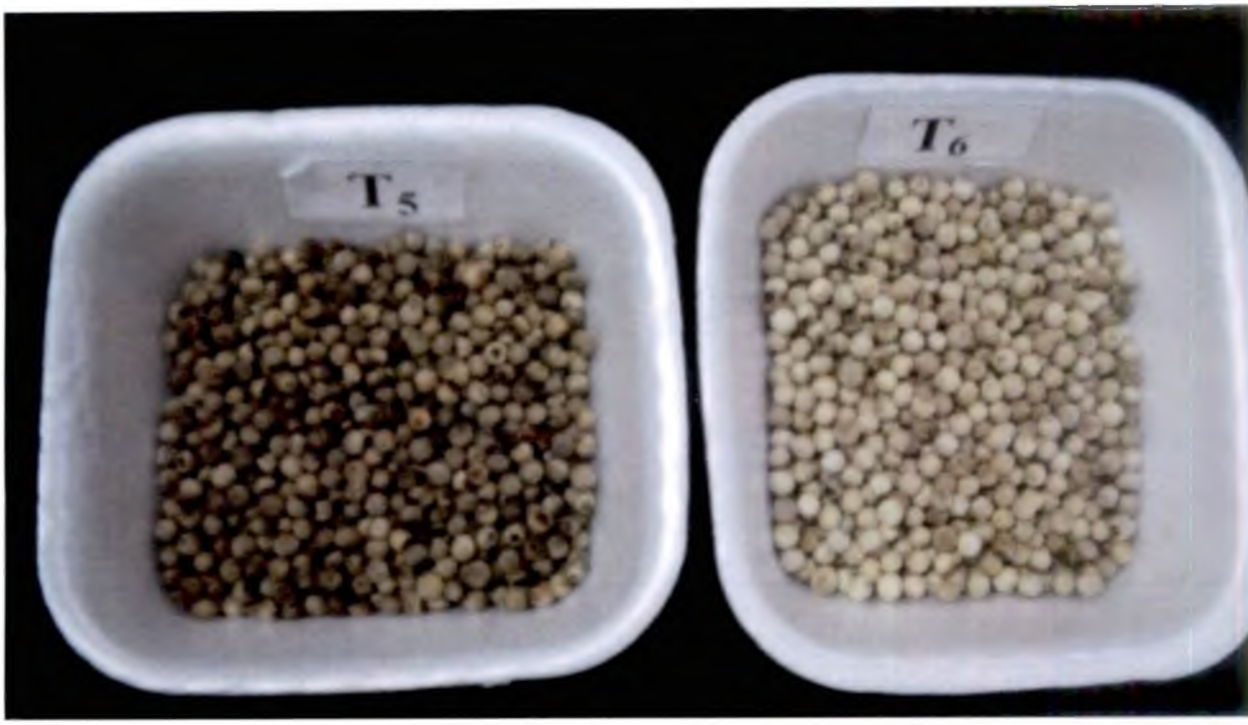
B) Treatment with calcium hydroxide



C) Treatment with sodium hypochlorite

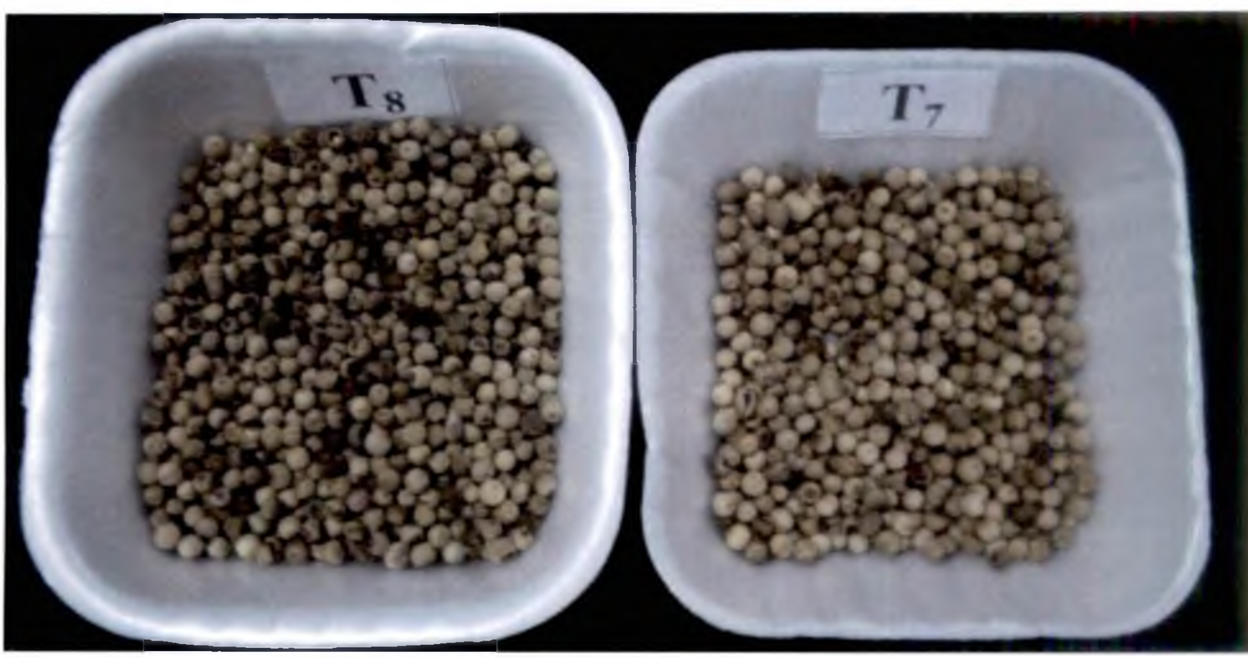
D) Treatment with hydrogen peroxide

Plate 2 Effect of different bleaching agents on white pepper berries



E) Treatment with sodium hydroxide

F) Treatment with sodium perborate



G) Conventional method (control)

H) Treatment with benzoyl peroxide

T6 Sodium perborate (3% for 6 hrs)

T7 Benzoyl peroxide (3% for 6 hrs)

T8 Conventional method (Control)

3.1.4 Drying

The pepper berries after chemical treatment were dried under sun for seven days, except those treated with hydrogen peroxide, which was kept under darkness for drying in order to prevent oxidation.

3.1.5 Packing and storage

The dried berries were cleaned well by winnowing and packed in low density polyethylene covers (LDPE) and stored for conducting physical and chemical analysis.

3.1.6 Quality parameters of white pepper.

The treated white pepper berries were subjected to the following physical and chemical analysis.

3.1.6.1 Physical parameters

3.1.6.1.1 Specific gravity

Specific gravity of 10g berries were expressed in g/cc by the method outlined by Pruthi (1999). The specific gravity is measured using specific gravity bottles and was calculated with the given formula,

$$\text{Specific gravity} = \frac{m_3 - m_1}{m_2 - m_1}$$

Where,

m_1 = mass in g rams of empty specific gravity bottle

m_2= mass in g rams of specific gravity bottle filled with water at ambient temperature.

m_3= mass in g rams of specific gravity bottle filled with material under test at ambient temperature.

3.1.6.1.2 1000 berry weight

The net weight of 1000 berries was weighed using an electronic weighing balance and the average expressed in grams.

3.1.6.1.3 1000 berry volume

1000 berry volume was calculated in ml by measuring volume displaced in a measuring cylinder when 1000 berries were immersed.

3.1.6.1.4 Sizes of berries

Sizes of ten berries were measured using vernier caliper scale and the average was expressed in millimetre (mm).

3.1.6.1.5 Yield of white pepper berries (%)

The percentage yield of white berries obtained by chemical method was carried out to analyse the effectiveness of chemical treatment in yield recovery.

3.1.6.2 Chemical characteristics

3.1.6.2.1 Volatile oil

Volatile oil content of pepper berries was estimated by modified Clevenger's method and was expressed in percentage (Pruthi,1999). The volatile constituents have much lower boiling points than that of water and hence they volatalise and get distilled over before and with water vapour. This is the universally accepted official method for estimation of essential oils.

The percentage of oil is calculated by

$$\text{Volatile oil (v/w)} = \frac{\text{volatile oil (ml)}}{\text{Weight of the sample (g)}} \times 100$$

3.1.6.2.2 Non volatile ether extract (NVEE)

Non volatile ether extract (NVEE) was estimated using the method of soxhlet extraction with diethyl ether as solvent and expressed in percentage. (Pruthi,1999).

3.1.6.2.3 Piperine

Piperine content was determined by U.V. Spectrophotometric method using ethylene dichloride as the solvent (AOAC, 1996). After refluxing in alcohol to extract the piperine, absorbance was compared to a standard in a spectrophotometer at 342–345 nm . The percentage piperine content was estimated using the formula,

$$\text{Piperine (\%)} = \frac{(AxFxV)}{(Wx10)} \times 100$$

Where,

A= Absorbance of sample

F= Factor derived from piperine standard

V=Dilution volume

W=Weight of sample (g)

3.1.6.2.4. Oleoresin

Oleoresin content in white pepper berries was determined by soxhlet extraction using acetone as solvent (Sadasivam and Manikam, 1992) and expressed in percentage.

3.1.6.2.5 Moisture

Moisture content in the berries was determined using Dean and Stark apparatus (AOAC, 2000) and expressed in percentage.

$$\text{Moisture content (\% by weight)} = \frac{100\,V}{M}$$

Where ,

V = Vol in ml of water collected

M = Weight of sample

3.1.6.2.6 Starch

Starch will be converted to reducing sugars on hydrolysis with concentrated hydrochloric acid. The total reducing sugars were estimated by direct titration against fehling's solution using methylene blue as indicator (Sadasivam and Manikam (1992).

3.1.6.2.7. Total Ash

The total ash content was determined by ashing method using muffle furnace at a temperature of 550^0 C for 2-3 hours (AOAC, 2000) and expressed in percentage. The calculation was done using the formula,

$$\text{Total ash on (dry basis) \% by wt} = \frac{(W2 - W)}{W1 - W} \times 100 \times \frac{100}{100 - M}$$

Where,

W = Weight in g of empty dish

W1 = Weight in g of dish + sample

W2 = Weight in g of dish + total ash

M = Percent moisture content

3.1.7 Analysis for residues of chemicals

As far as the berries were treated with chemical agents, there will be chemical residues exist in them. The residue level of calcium, sodium and chlorine content were estimated using suitable methods described below.

3.1.7.1 Calcium content in berries treated with calcium

Calcium in the treated berries were estimated by nitric and perchloric acid (9:3) digestion and Versanate titration method with standard EDTA (Ethylene Dichloride Tetra Acetic acid) (Tandon, 1993) and expressed in percentage.

3.1.7.2 Sodium content in berries treated with sodium

Sodium content (percent) in berries treated with sodium hydroxide was determined using sodium chloride solution as standard by flame photometer method (Jaiswal, 2003). Sodium on being heated in air-propane blue flame gets heated, and begin to dissipate energy by emission of 5890A wave length. The emission is directly proportional to the Na concentration, and it is measured by a galvanometer connected to photocell set behind sodium filter facing blue flame. The content was calculated using the given formula,

$$\text{Percent sodium content in sample} = (S-B)\,\frac{dt \times T \times 100 \times ME}{W \times 1000}$$

Where,

S = Milliequivalent sodium per litre in dilute sample test solution estimated by reference to standard graph for Na

B = Milliequivalent sodium per litre in diluted blank test solution estimated by reference to standard graph

dt = Dilution times of the sample test solution

T = Total volume of the sample test solution in ml

W = Weight of sample taken in grams for the preparation of sample test solution

ME = Milligram equivalent weight of sodium (0.023g)

3.1.7.3 Chlorine content in berries treated with chlorine

The sample test solution containing chlorine was titrated by standard silver nitrate ($AgNo_3$) in the presence of chromate (CrO_4) in an alkaline medium (Chapman and Pratt,1961). As long as some chlorine persists in the solution, formation of red silver chromate (Ag_2CrO_4) is momentary. When chlorine in solution was exhausted through precipitation as silver chloride (AgCL), the red precipitate of silver chromate ($Ag_2 CrO_4$) sharply signals the end point.

3.2. MICROBIAL FERMENTATION

A fermentation process was developed for the retting of berries which involved three steps viz. a) Isolation of organisms through enrichment culture technique b) Evaluation of isolates and cultures obtained from the Department of Microbiology c) Standardization of retting process for white pepper production.

3.2.1 Isolation of organism following enrichment culture technique

Samples were collected from various sources and the following six pooled samples were subjected for isolation.

T1- Garden soil

T2- Surface soil

T3- Cowdung

T4- Mud

T5- Rhizosphere

T6- Organic waste.

Ripe pepper samples of 50g each were taken in 250 ml conical flask and 1gm complex inoculum source was added and mixed well. 10 ml distilled water was also added and then kept for incubation at 30^0C for 12 days. Bacteria and fungi associated with retting were isolated from the enrichment flask following serial dilution and plating and brought into pure culture following standard technique. After analyzing eight cultures available at the Department of Microbiology, best two cultures were also included in the experiment. They were denoted as Ay1 and *Mycophyta.*

The isolates obtained were as follows,

Treatments	Source	Bacterial isolates	Fungal isolates
T1	Garden soil	IsB1	ISF1
T2	Surrface soil	IsB2	IsF2
T3	Cowdung	IsB3	IsF3
T4	Mud	IsB4	IsF4
T5	Rhizosphere	IsB5	IsF5
T6	Organic waste	IsB6	IsF6
T7	Organic waste	IsB7	-

*T8	Ayurvedic herbal waste	-	Ayl
*T9	Vegetable waste	-	*Mycophyta*

*Selected cultures obtained from the Department of Agricultural Microbiology

3.2.2. Evaluation of available isolates

Evaluation was done by taking the cellulolytic and pectinolytic activities as the major criteria.

3.2.2.1 Assessment of cellulolytic and pectinolytic activities of isolates

Pectin and cellulose, being the major constituents of pepper berry, their degradation capacity were tested. The cultures were inoculated on cellulolytic (asparagine) and pectinolytic (MP-5) media. The plates were kept for incubation at 30 ^{0}C for 5 days. After incubation the fungal and bacterial growth were examined and were noted for presence of hydrolytic zone formation, width of zone (mm) and diameter of growth (mm).

3.2.2.2. Determination of reducing sugar

The reducing sugar content of cellulose medium due to hydrolytic activity of different isolates were estimated by modified Dinitrosalicylic acid (DNS) method (Miller,1972).

3.2.2.2.1 Procedure

The culture filtrate was collected from the cellulolytic enrichment media. 0.5ml culture filtrate was taken in test tube and the volume was made up to 1 ml by adding distilled water accordingly. 1ml of dinitrosalicylic acid reagent was added to each of the test tube. The test tubes were kept in a boiling water bath for 5 min and cool to room temperature .1ml of 40% of rochelle salt (sodium potassium tartarate) solution was added. The final volume was made upto 4 ml by adding distilled water and mixed well. Then OD was read at a visible range of 540 nm in a spectrophotometer. The amount of reducing sugar was determined using a standard graph.

3.2.2.2.2. Standardization of glucose

Graded volume of (0.2 to 1ml) standard glucose solution were pipette out into a series of clean dry test tubes marked S_1 to S_5 and the volume was made up to 1ml by adding distilled water accordingly. 1 ml of dinitrosalicylic acid reagent was added to each of the test tube. The test tubes were kept in a boiling water bath for 5 min and cool to room temperature. 1ml of 40% rochelle salt was added. The final volume was made up to 4ml by adding distilled water and mixed well. Then OD was read at a visible range of 540nm. Blank containing 1ml distilled water was also treated in the same manner. A graph was plotted along the X-axis and OD values along the Y-axis. From the graph, the concentration of unknown was calculated.

3.2.3. Standardization of retting process for white pepper production

All the bacterial and fungal isolates were subjected for evaluating their retting ability. For that, 25 g each berries were taken in conical flasks and the microbial inoculum was sprayed and mixed well and kept for incubation at room temperature. The two promising cultures (Ay1 and *Mycophyta*) were also used for evaluating the retting ability with the same manner. The rate of decortication was noticed every day and the percentage of decortication was calculated.

3.2.4 Effects of microbial fermentation on physical parameters of white pepper

Physical parameters like size, specific gravity, 1000 berry weight and 1000 berry volume were observed for berries obtained by the fermentation method using different microbial isolates.

Amongst all the isolates, considering the retting capacity and quality of resultant product, best two isolates were selected and recommended for white pepper processing.

3.3 ORGANOLEPTIC EVALUATION

Organoleptic evaluation was done primarily to assess the colour along with other characters like appearance, flavour, texture and taste. Colour was compared with the colour of white pepper produced by National Institute for Interdisciplinary Science and Technology (NIIST), Trivandrum (Appendix I). For comparing taste, cucumber was flavoured with white pepper powder and made into a salad and was evaluated by the team consisting of selected group of students and research associates (Plate 8).

For scoring, a nine point scale was used, nine representing the optimum for all quality characteristics and one representing poorest quality. The major quality attributes included in the score card were colour, flavour, texture, taste and appearance (Appendix II).

3.4 EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

Experiments were laid out in Completely Randomized Design (CRD) and analysed using analysis of variance technique (Gomez and Gomez, 1984). Analysis of organoleptic evaluation was done using Kruskal-Wallis one way analysis of variance technique (Kruskal and Wallis, 1952).

A) Samples for organoleptic evaluation

B) Judging panel

C) White pepper flavoured cucumber salad

D) White pepper powder

E) Crushed white pepper

F) Whole white pepper

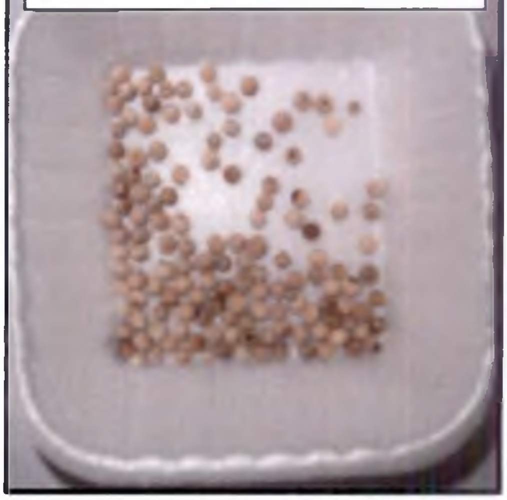


Plate 3 Organoleptic evaluation

Results

4. RESULTS

The results of the investigation on "Standardization of processing methods for production of quality white pepper" are presented under the following heads.

4.1 Chemical method of white pepper production

4.2 Microbial white pepper production

4.3 Organoleptic evaluation

4.1 CHEMICAL METHOD OF WHITE PEPPER PRODUCTION

Eight chemical treatments were evaluated. The data collected on physical and chemical parameters were analysed statistically and presented below.

4.1.1 Effect of bleaching agents on physical parameters of pepper berries

Physical parameters such as yield, specific gravity, 1000 berry weight, 1000 berry volume and sizes of berries were analysed. All the physical parameters except size were significantly influenced by bleaching agents. The data pertaining to these characters are presented in Table 4.

4.1.1.1 Specific gravity

Pepper berries treated with sodium perborate (Table 4) recorded the highest specific gravity (1.12g/cc) followed by those with sodium hypochlorite (1.11g/cc), hydrogen peroxide (1.08g/cc). Treatment with sodium hydroxide resulted in berries with lowest specific gravity (0.89g/cc).

4.1.1.2 1000 berry weight

1000 berry weight was significantly different for treatments with a level of 5% significance. Berries treated with sodium hypochlorite recorded highest 1000 berry weight of 37.34g were on par with berries treated with sodium hypochlorite, sodium

Table: **4 Effect of bleaching agents on physical parameters of white pepper berries**

Treatments	**Specefic gravity (g/cc)**	**1000 berry weight**	**1000 berry volume (ml)**	**Sizes of berries (mm)**
Calciumhypochlorite	1.03	34.51	35	4.00
Calcium hydroxide	1.07	36.06	34	4.10
Hydrogen peroxide	1.08	35.30	32	4.00
Sodiumhypochlorite	1.11	37.34	35	4.17
Sodium hydroxide	0.98	36.77	34	4.03
Sodium perborate	1.12	34.82	33	4.03
Benzoyl peroxide	1.08	35.08	33	3.90
Conventional method	1.1	36.67	32	4.07
Mean	1.07	35.82	33	4.04
F	2.80*	3.32 *	23.65 **	0.42 NS
CD	8.56	1.72	0.79	0.367

*Signicant at 5% level ** Signicant at 1% level NS- not significant hydroxide, calcium hydroxide and conventional method. The lowest value was found from berries treated with calcium hypochlorite (34.51g).

4.1.1.3 1000 berry volume

There was a wide variation among treatments in berry volume. The different treatments showed a high level of significance (at 1%). Treatment with calcium hypochlorite and sodium hypochlorite were having the highest value of 35 ml. Other treatments differed significantly in 1000 berry volume.

4.1.1.4 Sizes of berries (mm)

There were no significant effects of chemical treatments on sizes of berries (Table 4). Berries treated with sodium hypochlorite recorded the maximum sized berries (4.17mm) followed by calcium hydroxide (4.10mm). Berries treated with benzoyl peroxide had the lowest size (3.90mm).

4.1.1.5 Yield of white pepper berries (%)

Yield of white pepper obtained from various treatments differed significantly (Table 5). Berries treated with calcium hypochlorite recorded the highest yield (81.18%). This was closely followed by treatments with benzoyl peroxide (79.03%), sodium perborate (77.96%) and calcium hydroxide (76.34%) which were found to be on par. The lowest yield was obtained from white pepper berries produced by conventional method (64.48%) and it was found to be on par with sodium hypochlorite (69.89%).

Table 5 **Percentage Yield of white pepper berries**

Treatments	**Yield (%)**
Calcium hypochlorite	81.18
Calcium hydroxide	76.34
Hydrogen peroxide	74.30
Sodium hypochlorite	69.89
Sodium hydroxide	73.65
Sodium perborate	77.96
Benzoyl peroxide	79.03
Conventional method	64.47
Mean	74.60
F	8.68 **
CD	5.51

** Signicant at 1% level

4.1.2 Effect of bleaching agents on chemical parameters of pepper berries

The results of chemical parameters such as volatile oil ,non volatile ether extract (NVEE), piperine, oleoresin, moisture, starch and ash content were given in Table 6 and 7. All chemical parameters except moisture, ash and starch content, were significantly influenced by treatments with bleaching agents.

4.1.2.1 Volatile oil

Different treatments had significant influence on volatile oil content of white pepper (Table 6). The berries prepared by conventional method exhibited the maximum volatile oil content (2.80%) which was on par with the berries treated with benzoyl peroxide, calcium hypochlorite and hydrogen peroxide (2.80, 2.60 and 2.53 % respectively). Lowest volatile content was found to be in berries treated with sodium hypochlorite (1.20%).

4.1.2.2 Non volatile ether extract (NVEE)

There was significant difference in non volatile ether extract content among treatments (Table 6). White pepper berries produced from conventional method recorded maximum non volatile ether extract content (8.53%) which was found to be on par with berries treated with calcium hypochlorite (8.47%), sodium hypochlorite (7.80%), benzoyl peroxide(7.60%), calcium hydroxide(7.60%) and hydrogen peroxide(7.47%).

4.1.2.3 Piperine

Percentage piperine content ranged from 1.23 to 2.91 (Table 6). White pepper berries prepared by conventional method was found to be the superior to all other treatments and it was on par with the berries treated with calcium hydroxide (2.83%), hydrogen peroxide (2.63%), calcium hypochlorite (2.49%) and sodium hypochlorite

Table 6 **Effect of bleaching agents on chemical parameters of white pepper berries**

Treatments	**Volatile oil (%)**	**Non volatile ether extract (%)**	**Piperine(%)**	**Oleoresin (%)**
Calcium hypochlorite	2.53	8.47	2.49	10.27
Calcium hydroxide	1.99	7.60	2.82	10.67
Hydrogen peroxide	2.06	7.47	2.63	7.27
Sodium hypochlorite	1.20	7.80	2.44	10.33
Sodium hydroxide	2.32	4.87	1.4	7.73
Sodium perborate	2.35	5.53	2.04	6.07
Benzoyl peroxide	2.60	7.60	1.23	8.73
Conventional method	2.80	8.53	2.91	10.83
Mean	16.24 **	11.67 **	16.14 **	19.09 **
F	0..37	1.17	0.475	1.245

(2.44%). Treatment with sodium hydroxide exhibited the lowest piperine content (1.23%).

4.1.2.4 Oleoresin

There was significant difference in percentage of oleoresin among treatments (Table 6). For this aspect also conventionally prepared berries recorded the highest value (10.83%). This was found on par with treatment containing calcium hydroxide (10.67%), sodium hypochlorite (10.33%) and calcium hypochlorite (10.27%). The berries treated with sodium perborate indicated the lowest value (6.07%) followed by those treated with hydrogen peroxide (7.27%).

4.1.2.5 Moisture

Moisture content was found to be non significant for all the treatments (Table7). The berries treated with benzoyl peroxide (15.01%) and those with sodium hypochlorite (13.75%) were having the highest and lowest moisture contents respectively.

4.1.2.6 Starch

There was no significant effect of treatments on starch content of white pepper (Table 7). The values ranged from 58.59% (calcium hypochlorite treated berries) to 64.38% (conventionally prepared berries).

4.1.2.7 Total ash content

No significant differences in ash content were observed among treatments (Table 7). However the highest value was obtained for berries treated sodium hydroxide (1.09%) and the lowest for berries treated with hydrogen peroxide (0.56%).

Table 7 **Effect of bleaching agents on chemical parameters of white pepper berries (continued)**

Treatments	Moisture (%)	Starch (%)	Total ash (%)
Calcium hypochlorite	14.62	58.59	0.57
Calcium hydroxide	14.43	62.67	0.58
Hydrogen peroxide	14.40	61.82	0.56
Sodium hypochlorite	13.75	60.82	0.74
Sodium hydroxide	14.66	62.67	1.09
Sodium perborate	14.27	58.99	0.72
Benzoyl peroxide	15.01	61.81	0.86
Conventional method	14.19	64.38	0.99
Mean	14.42	61.47	0.76
F	14.19 NS	1.80NS	1.47NS
CD	1.036	4.37	0.50

NS- not significant

4.1.3 Aanalysis for residue of chemical treatments

The residual level of calcium, sodium and chlorine in treated berries were analysed and the results are given below (Table 8).

4.1.3.1 Ca content in white pepper berries treated with calcium

The residue analysis in berries treated with calcium hypochlorite and calcium hydroxide indicated that significant variation exist in the calcium content of various treatments containing calcium (Table 8.1). Treatment with calcium hydroxide was having the highest residue of calcium (1.58%) which was on par with the berries treated with calcium hypochlorite (1.52%). The content of calcium in berries prepared by conventional method was found to be the lowest (1.2%).

4.1.3.2 Sodium content in berries treated with sodium

There was no significant variation existed among treatments (Table 8.2). Berries treated with sodium perborate recorded the highest residual sodium content (0.04%) followed by berries with sodium hypochlorite (0.03%) and sodium hydroxide (0.01%). Conventionally prepped berries showed sodium content of 0.008%.

4.1.3.3 Chlorine content in berries treated with chlorine

Berries treated with sodium hypochlorite left out significantly higher residual level of chlorine (3.49ppm) and those prepared by conventional method registered a residual chlorine content of 2.70 ppm.

Table 8 RESIDUE ANALYSIS OF CHEMICAL TREATMENTS

Table 8.1 **Calcium content in white pepper berries treated with calcium**

Treatments	Ca content (%)
Calcium hypochlorite	1.52
Calcium hydroxide	1.58
Conventional method	1.20
Mean	1.43
F	32.95 **
CD	0.11

Table 8.2 **Sodium content in white pepper berries treated with sodium**

Treatment	Na content (%)
Sodium hypochlorite	0.030
Sodium hydroxide	0.010
Sodium perborate	0.040
Conventional method	0.008
Mean	0.022
F	1.65NS
CD	0.03

Table 8.3 **Chlorine content in white pepper berries treated with chlorine**

Treatment	Cl content (ppm)
Sodium hypochlorite	3.49
Conventional method	2.70
CD	0.17
F	103.21**

*Signicant at 5% level ** Signicant at 1% level NS- not significant

4.2. MICROBIAL FERMENTATION

Microbial fermentation method was done by three steps i.e., a) Isolation of organisms through enrichment culture technique, b) Evaluation of the available cultures, c) Standardization of retting process for white pepper production.

4.2.1 Isolation of organisms through enrichment culture technique

Bacterial and fungal isolates were obtained from different sources following enrichment culture technique as explained in materials and methods. Seven bacterial isolates numbered as IsB1 to IsB7 and six fungal isolates numbered as IsF1 to IsF6 were obtained. These isolates were subjected for further evaluation along with two selected fungal cultures, Ay1 and *Mycophyta* obtained from the Department of Agricultural Microbiology.

4.2.2. Evaluation of available cultures

The cellulolytic, pectinolytic, activities were taken as the major criteria for assessing the efficiency of isolates for retting black pepper berries.

4.2.2.1 Assessment of pectinolytic activity of isolates

The hydrolytic zone formation and the width of zone formed in MP-5 media inoculated with culture, recorded for assessing the pectinolytic activity. The results are presented in Table 9.

The data showed that, IsF4 was having the maximum zone formation with a width of 2.2mm. This was followed by IsF1 with 1.5 mm width and IsF3. The highest growth diameter was achieved by isolate IsF1 (58mm) followed by isolate IsF4 (47mm), IsF3 (45mm) and Ay1. There was no hydrolytic zone formation observed in the case of bacterial isolates.

Table 9 **Assessment of pectinolytic activity of the fungal isolates**

Isolates	**Zone formation**	**Zone width (mm)**	**Growth diameter(mm)**
IsF1	+ +	1.5	58
IsF2	++	1	38
IsF3	++	1	45
IsF4	+++	2.2	47
IsF5	+	1.4	41
IsF6	+	1.4	42
Ay1	+	1.6	44
Mycophyta	+	1.6	43

Table 10 **Assessment of cellulolytic activity of fungal isolates**

Isolates	**Zone formation**	**Zone width(mm)**	**Growth(mm)**
IsF1	+	<1	34
IsF2	+	<1	25
IsF3	++	1	25
IsF4	+	<1	33
IsF5	+	<1	35
IsF6	++	1	29
Ay1	++	1	30
Mycophyta	++	1	32

4.2.2.2 Assessment of cellulolytic activity of isolates

As in the case of pectin assay, the zone formation and width of zone developed with the isolates in cellulose medium (asparagine) were assessed for evaluating the cellulolytic activity. The isolate IsF3, Ay1 and *Mycophyta* showed highest zone formation with a zone width of 1mm (Table 10). Other isolates showed very poor zone formation around the colony. In the case of colony growth, IsF5 with a diameter of 35 mm was found to be the superior one among all the isolate followed by IsF1 (34mm). The growth of IsF3, Ay1 and *Mycophyta* 25, 30 and 32 mm respectively. None of the bacterial isolates showed zone around the colony.

4.2.2.3 Estimation of reducing sugar

The reducing sugar content of the cellulose medium due to the hydrolytic activity of different isolates were estimated as explained in material and method. Among the different bacterial isolates (Table 11), IsB3 and IsB6 recorded the highest in glucose production (0.07%) followed by IsB1 and IsB2 (0.06%). In the case of fungal isolates, IsF5 recorded a reducing sugar percentage of 0.09 and IsF3, IsF4, IsF6 and IsF8 showed a reducing sugar content of 0.08 %(Table 12)

4.2.3. Standardization of retting process

All the bacterial and fungal isolates were further subjected for evaluating their retting ability as explained in materials and methods. The bacterial cultures showed poor multiplication rate and fermentation. Most of the bacterial isolates took five days for complete retting except IsB2 which took only four days.

Amongst the bacterial isolates IsB2 (completed in four days) was the most promising one followed by IsB3 with five days (Table 13)

Table 11 **Glucose formation as influenced by different bacterial isolates**

Isolates	**Glucose formed (%)**
IsB1	0.06
IsB2	0.06
IsB3	0.07
IsB4	0.05
IsB5	0.05
IsB6	0.07
IsB7	0.03

Table 12 **Glucose formation as influenced by different fungal isolates**

Isolates	**Glucose formed (%)**
IsF1	0.06
IsF2	0.06
IsF3	0.08
IsF4	0.08
IsF5	0.09
IsF6	0.08
Ay1	0.07
Mycophyta	0.07

Table 13 **Retting pattern as influenced by different bacterial isolates**

Percentage berries retted at different periods of incubation										
	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day	11th day
IsB1		56	71	92	100					
IsB2		50	100							
IsB3		63	86	100						
IsB4		50	86	82	87	100				
IsB5		38	71	91	90	100				
IsB6		83	91	91	100					
control		25	33	45	58	62	75	87	89	90

A) IsB2 on fourth day of inoculation B) IsB3 on fifth day of inoculation

Plate 4 Bacterial isolates showing complete removal of pericarp on 4^{th} and 5^{th} day of inoculation

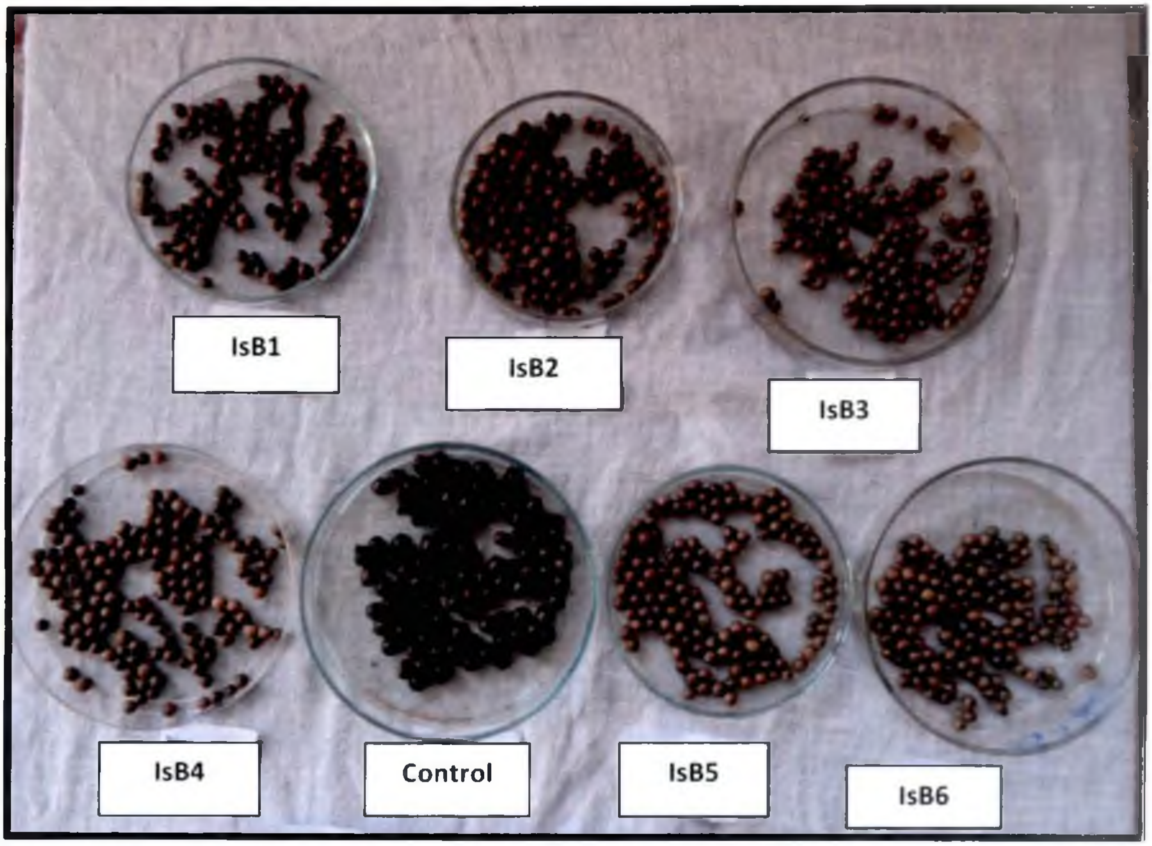


Plate 5 **Retting pattern as influenced by different bacterial isolate on 6^{th} day of inoculation**

Table 14 **Retting pattern as influenced by different fungal isolates**

	Percentage berries retted at different periods of incubation									
	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day	11th day
IsF1	67	75	100							
IsF2	33	86	90							
IsF3	40	100								
IsF4	50	57	100							
IsF5	25	43	100							
IsF6	50	71	100							
Ay1	78	100								
Mycophyta	60	100								
Control		25	33	45	58	62	75	87	89	90

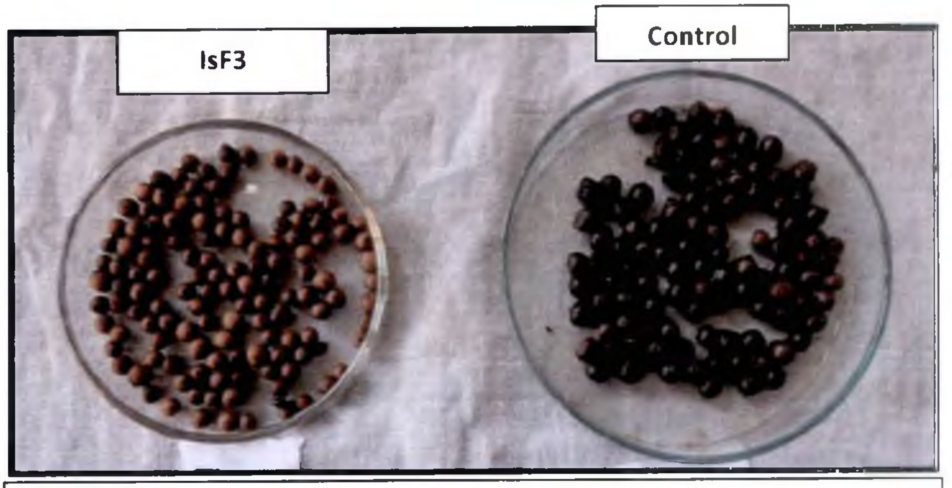


Plate 6 **Fungal isolate IsF3 completed retting of berries on 3rd day of inoculation**

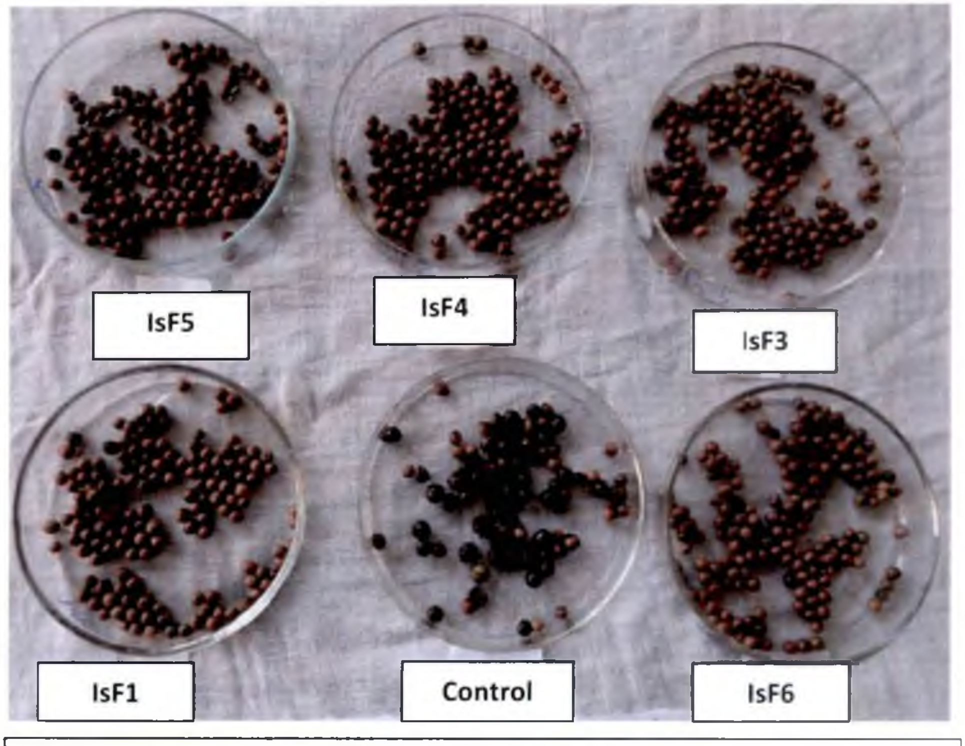


Plate 7 **Retting pattern as influenced by different fungal isolates on 4th day of inoculation**

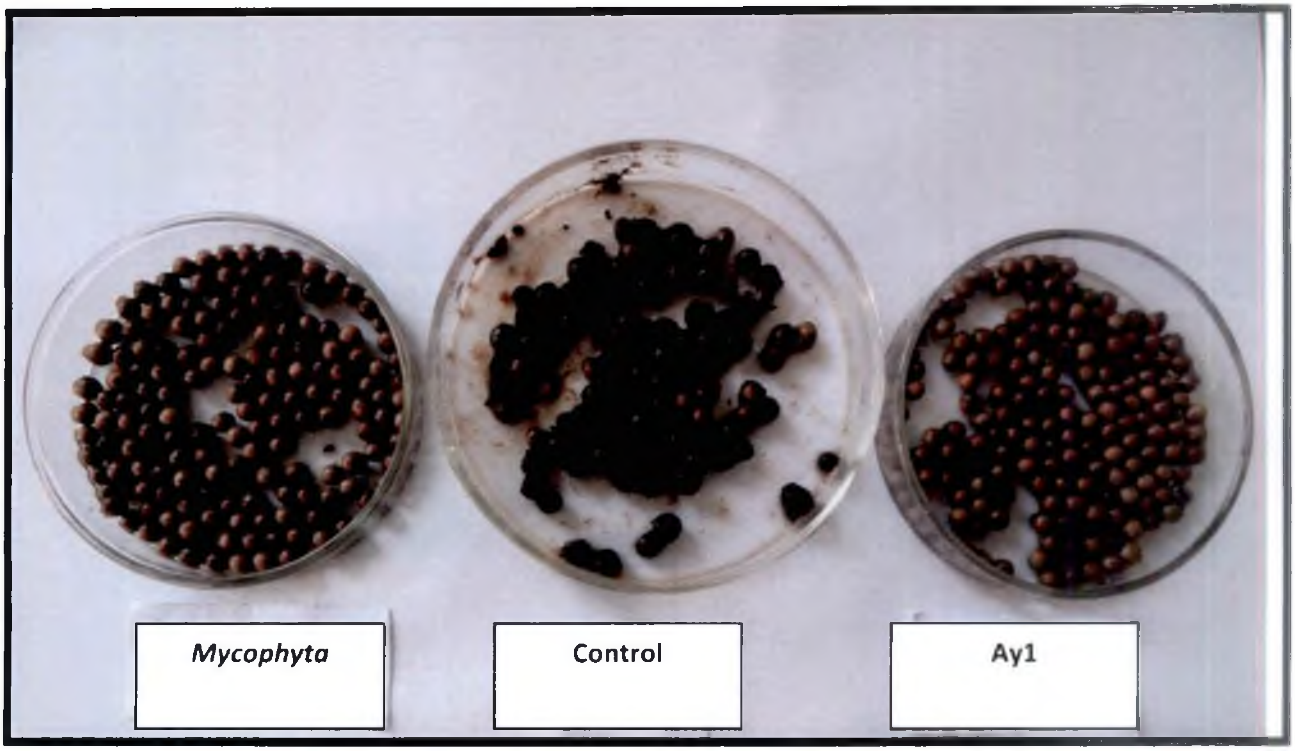


Plate 8 **Retting pattern as influenced by fungal isolates Mycophyta and Ay1 on 3th day of inoculation**

Amongst the fungal isolates IsF3 completed retting of all the berries by third day. The selected cultures Ay1 and *Mycophyta* also recorded 100 percentage setting by third day (Table 14). The IsF1, IsF4, IsF5and IsF6 also showed complete retting of berries by fourth day. The control treatment showed 98% retting by ninth day. It was noted that in the control, berries started rotting by ninth day and the recovery was poor. Amongst the isolates the fungal isolates IsF3, Ay1 and *Mycophyta* were the most promising and recorded 100 % retting without any damage by third day itself.

4.2.4 Quality parameters of white pepper

4.2.4.1 Specific gravity

White pepper berries produced by microbial fermentation had a specific gravity in the range of 0.99 to 1.12g/cc. This was in range with the control. Fungal isolate IsF3 was having the highest specific gravity of 1.1.2g/cc.

4.2.4.2 1000 berry weight

1000 berry weight for all the fifteen isolates were in the range of 34.81 to 37.5g.

4.2.4.3 1000 berry volume

The highest value for 1000 berry volume was observed from IsF2 (36 ml) and all other isolates were falling in 34 and 35 ml .The control and IsF5 got the lowest value (43 ml).

4.2.4.4 Sizes of berry

The berry size of all fifteen isolates was falling in between 4.0 and 4.3mm. All the berries were equally effective for having similar size.

Table 15 **Physical parameters of white pepper berries produced by microbial fermentation**

Isolates	Specific gravity	1000 berry weight (g)	1000 berry volume (ml)	Size (mm)
IsB1	1.10	35.07	35	4.0
IsB2	1.08	36.67	35	4.3
IsB3	1.03	37.50	35	4.1
IsB4	1.10	35.55	34	4.3
IsB5	1.10	36.25	35	4.1
IsB6	1.00	36.90	34	4.2
IsF1	1.03	36.00	35	4.1
IsF2	1.01	36.10	36	4.3
IsF3	1.12	35.52	35	4.1
IsF4	1.01	36.00	35	4.2
IsF5	1.02	36.78	34	4.3
IsF6	0.99	34.81	35	4.2
Ay1	1.10	36.75	35	4.0
Mycophyta	1.07	35.88	35	4.2
Control	1.08	36.25	34	4.3

4.3. ORGANOLEPTIC QUALITY EVALUATION

The resultant products obtained from both chemical and microbial method were evaluated organoleptically to assess the level of acceptability. Organoleptic scoring for quality characters like appearance, colour, flavour, texture, and taste were analysed statistically and the results are presented below. The rank means proved that treatments showed a significant influence on organoleptic qualities (Table 16).

4.3.1 Organoleptic evaluation of white pepper produced by chemical method

4.3.1.1 Effect of treatments on appearance of whole white pepper

The rank mean values obtained for appearance was ranged from 78.02 (berries treated with sodium hypochlorite) to 166.73 (berries with sodium hypochlorite). Berries treated with calcium hypochlorite was superior in appearance (166.73) followed by those with sodium perborate (159.8) followed by calcium hydroxide (147.18) and hydrogen peroxide(132.2) (Table 16.1)

4.3.1.2 Effect of treatments on appearance of white pepper powder

The highest rank mean value for appearance in powdered white pepper sample was found for berries treated with sodium perborate (165.00) and it was on par with treatment of berries with benzoyl peroxide (154.20), calcium hydroxide (139.03) and calcium hypochlorite (131.37). Berries with sodium hypochlorite was the most inferior (57.73) (Table 16.1).

4.3.1.3 Effect of treatments on colour of whole white pepper

There was a positive effect on colour of whole white pepper berries on different treatments (Table 16.1). Highest organoleptic quality was obtained for berries treated with sodium perborate (181.85) followed by calcium hypochlorite

Table 16 ORGANOLEPTIC QUALITY EVALUATION

Table 16.1 **Organoleptic evaluation white pepper produced by chemical method**

Treatments	Appearance (whole)	Appearance (powder)	Colour (whole)	Colour (powder)
Calcium hypochlorite	166.73	131.37	160.85	137.02
Calcium hydroxide	147.18	139.03	144.47	118.97
Hydrogen peroxide	132.20	116.20	138.58	114.72
Sodium hypochlorite	78.20	57.73	89.62	75.97
Sodium hydroxide	78.02	84.42	88.27	89.48
Sodium perborate	159.80	165.00	181.85	170.62
Benzoyl peroxide	102.33	154.20	79.55	151.35
Conventional method	99.53	115.82	80.82	105.88
χ^2	58.03	59.69	76.18	46.53

Table 16.2 **Organoleptic evaluation white pepper produced by chemical method (continued)**

Treatments	Flavour (crush)	Flavour (powder)	Taste	Texture	Overall acceptability
Calcium hypochlorite	137.18	125.93	126.85	142.12	143.65
Calcium hydroxide	130.50	113.05	135.37	112.62	134.55
Hydrogen peroxide	108.35	120.42	125.98	119.15	124.67
Sodium hypochlorite	96.65	75.27	85.67	87.10	76.62
Sodium hydroxide	96.47	98.05	125.60	118.17	84.60
Sodium perborate	116.83	137.48	98.63	96.77	145.57
Benzoyl peroxide	120.83	142.97	124.32	128.55	121.55
Conventional method	157.18	150.83	141.58	159.53	132.8
χ^2	19.85	28.47	16.89	25.145	29.6395

(160.85). Berries trated with benzoyl peroxide recorded the poorest colour (79.55) followed by berries produced by conventional method (80.82).

4.3.1.4 Effect of treatments on colour of white pepper powder

The rank mean values for colour of powdered white pepper showed marked variation with different treatments (Table 16.1). Berries with sodium perborate recorded the highest rank mean value (170.62) and was on par with benzoyl peroxide treated berries (151.35). It was significantly different from all other chemical treatments.

4.3.1.5 Effect of treatments on flavour of crushed white pepper

The chemical treatments significantly influenced the flavour of white pepper both in crushed and powdered forms of white pepper (Table 16.2). Berries prepared by conventional method recorded highest flavour characteristics with a rank mean of 157.18 and it was found to be on par with calcium hypochlorite (137.18), calcium hydroxide(130.50).

4.3.1.6 Effect of treatments on flavour in white pepper powder

Chemical treatments significantly influenced the flavour white pepper powder. (Table 16.2). Conventionally prepared white pepper berries were having maximum flavour characteristics (150.83) and was on par with the flavour in berries treated with benzoyl peroxide (142.97), sodium perborate (137.48) and calcium hypochlorite(125.93).

4.3.1.7 Effect of treatments on taste characteristics

The rank mean values obtained for taste qualities ranged from 85.67 (berries treated with sodium hypochlorite) to 141.58 (berries produced by conventional method) (Table 16.2). Berries produced by conventional method did not show any significant difference with treatment of berries containing calcium hydroxide (135.37), calcium hypochlorite (126.85), hydrogen peroxide (125.98), sodium hydroxide (125.60) and benzoyl peroxide (124.32).

4.3.1.8 Effect of treatments on textural qualities

Different treatments showed significant influence on texture of crushed white pepper berries (Table 16.2). Berries prepared by conventional method (159.53) recorded the highest rank mean value for textural characteristics and it was on par with berries containing calcium hypochlorite (142.12) and those with benzoyl peroxide (128.55). The lowest value was obtained by the berries with sodium hypochlorite (87.1).

4.3.1.9 Effect of treatments on over all acceptability

Treatment with sodium perborate recorded the highest acceptability among all the treatments (Table 16.2) and recorded a rank mean value of 145.57 followed by berries with calcium hypochlorite (143.65) and calcium hydroxide (134.55).

4.3.2 Organoleptic evaluation of white pepper produced by microbial fermentation

Organoleptic evaluation of the white pepper produced by microbial fermentation was analysed statistically and the results are given below. The result (Table 16.3) showed that, the pepper produced by conventional method (control) was the poorest in all the parameters.

Table 16.3 **Organoleptic evaluation white pepper produced by microbial fermentation**

Isolates	Appearance (whole)	Appearance (powder)	Colour (whole)	Colour (powder)
IsB1	129.40	133.90	128.10	132.50
IsB2	50.50	50.50	50.50	50.50
IsB3	50.50	50.50	50.50	50.50
IsB4	129.30	125.20	125.10	128.20
IsB5	50.50	50.50	50.50	50.50
IsB6	50.50	50.50	50.50	50.50
IsF1	119.60	112.30	118.40	124.90
IsF2	50.50	50.50	50.50	50.50
IsF3	125.80	125.01	129.90	108.80
IsF4	50.50	50.50	50.50	50.50
IsF5	50.50	50.50	50.50	50.50
IsF6	50.50	50.50	50.50	50.50
Ay1	123.50	143.595	126.20	133.30
Mycophyta	50.50	50.5	50.50	50.50
Control	50.50	50.5	50.50	50.50
χ^2	142.24	131.10	142.31	144.51

4.3.2.1 Effect of isolates on appearance of whole white pepper

White pepper berries obtained by fermentation with bacterial isolate IsB1 was found superior (Table 16.4) in appearance (129.4). This was on par with bacterial isolate IsB4 (129.30) and fungal isolates IsF3 (125.80) and Ayl (123.50).

4.3.2.2 Effect of isolates on appearance of white pepper powder

Among different isolates, IsB1 was found to be the superior in terms of appearance in powder form with a rank mean value of 133.90 (Table 16.3.1) followed by Ayl (131.10), IsB4(125.20) and IsF3(125.01).

4.3.2.3. Effect of isolates on colour of whole white pepper

The fungal isolate IsF3 was found to be the superior one with a rank mean value of 129.9 and was on par with berries treated with bacterial isolate IsB1(128.10), Ayl(126.20) and with IsB4 (125.10). The control berries was having poorest colour.

4.3.2.4. Effect of isolates on colour of white pepper powder

The fungal isolate Ayl (Table 16.3) got the highest rank mean value for colour in the form of white pepper powder (133.30). The isolates IsB1(132.50), B4(128.20), IsF1(124.90) and IsF3(108.80) were found to be on par with Ayl.

4.3.2.5. Effect of isolates on flavour of crushed white pepper

The fungal isolate IsF3 got the highest rank mean value (129.3). It was found to be on par with isolates IsB4 (126.80), IsB1 (126.25), Ayl (124.90) and IsF1 (120.35).

Table 16.4 **Organoleptic evaluation white pepper produced by microbial fermentation (continued)**

Isolates	Flavour (crush)	Flavour (powder)	Taste	Texture	Overall acceptability
IsB1	126.25	129.05	124.00	130.90	129.30
IsB2	50.50	50.50	50.50	50.50	50.50
IsB3	50.50	50.50	50.50	50.50	50.50
IsB4	126.80	129.10	132.20	128.85	131.90
IsB5	50.50	50.50	50.50	50.50	50.50
IsB6	50.50	50.50	50.50	50.50	50.50
IsF1	120.35	120.55	118.70	120.00	124.20
IsF2	50.50	50.50	50.50	50.50	50.50
IsF3	129.20	126.80	129.20	119.50	114.65
IsF4	50.50	50.50	50.50	50.50	50.50
IsF5	50.50	50.50	50.50	50.50	50.50
IsF6	50.50	50.50	50.50	50.50	50.50
Ay1	124.90	122.00	123.40	128.25	127.45
Mycophyta	50.50	50.50	50.50	50.50	50.50
Control	50.50	50.50	50.50	50.50	50.50
χ^2	141.92	142.06	142.57	142.595	142.53

4.3.2.6 Effect of isolates on flavour of white pepper powder

Bacterial isolate IsB4 (Table 16.4) was having the highest flavour characteristics (129.10), which was found to be on par with IsB1 (129.05), IsF1 (120.55), IsF3 (126.80) and Ay1 (122.00).

4.3.2.7 Effect of treatments on taste characteristics

The bacterial isolate IsB4 was dominating in taste characteristics (132.2) followed by IsF3 (129.2), IsB1 (124), Ay1 (123.4) and IsF1 (118.7) (Table 16.4).

4.3.2.8 Effect of treatments on textural qualities

Isolate IsB1 recorded the highest rank mean value for textural qualities (130.9). IsB1 was on par with IsB4 (128.85), Ay1 (128.25), IsF1 (120.00), and IsF3(119.50)

4.3.2.9 Effect of treatments on over all acceptability

The maximum score for overall acceptability was obtained for bacterial isolate IsB4 (though it was not that appreciable in retting character) with a rank mean of 131.90. The isolates, IsB1 (129.30), IsF1 (124.20), IsF3 (114.65) and Ay1 (127.45) were found to be on par with IsB1 (Table 16.4)

Amongst all the isolates, considering the retting process and quality of the product, the isolated IsF3 and Ay1 were found to be the most superior.

Discussion

5. DISCUSSION

White pepper is the most remunerative value added form of black pepper, which is an elegant culinary agent. White pepper is preferred over black pepper by the people of certain countries as its colour matches with wide categories of light coloured food preparations like sauces and soups. It gives a modified natural flavour to the food stuff and it imparts a medium level of pungency. The present study entitled as "standardization of processing methods for production of quality white pepper", was conducted to find out a better method of white pepper production along with its quality attributes. The major problems with regard to white pepper produced by Indian farmers is the inferior colour and off odour developed through traditional method of retting. Attempt has been made to reduce the time taken for retting and also to improve the whiteness of berries. The experiment was conducted in three parts, 1) chemical method of white pepper production 2) microbial method and 3) organoleptic evaluation of the developed products. The results gathered from of the experiments conducted are discussed in this chapter.

5.1 CHEMICAL METHOD OF WHITE PEPPER PRODUCTION

Pepper variety Panniyur-1 was used for production of white pepper, since it produces bold sized berries (>4.25mm) which is the most important factor influencing the quality. Similar results were reported by Zachariah (2000). Effects of bleaching agents on physical and chemical parameters of white pepper are discussed below.

5.1.1 Effect of bleaching agents on physical parameters of white pepper berries

Physical parameters of white pepper viz., specific gravity, 1000 berry weight, 1000 berry volume and yield except size were significantly influenced by different bleaching agents.

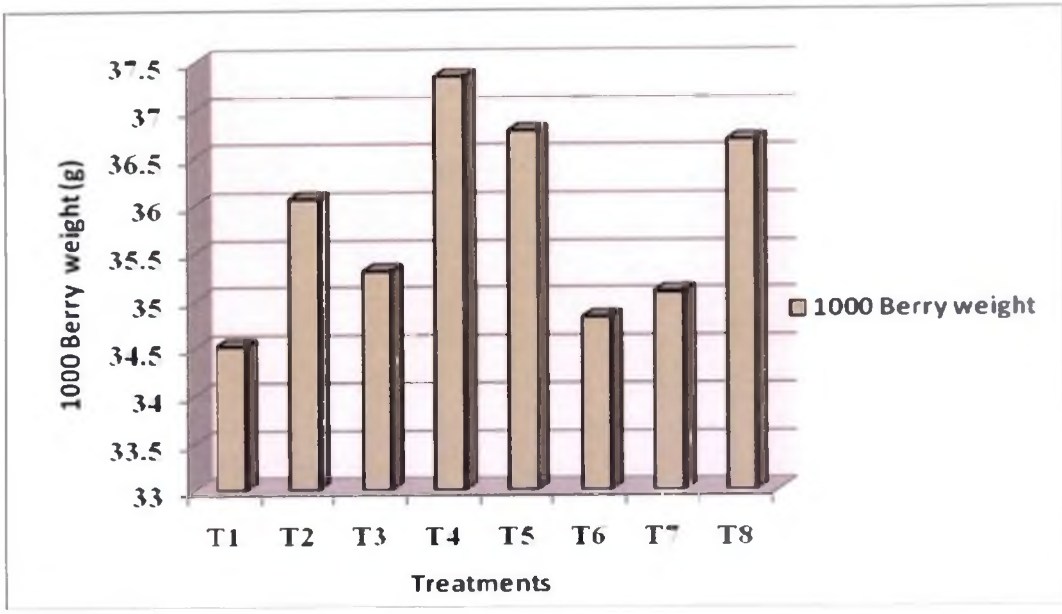


Fig.1 Effect of bleaching agents on 1000 berry weight of white pepper

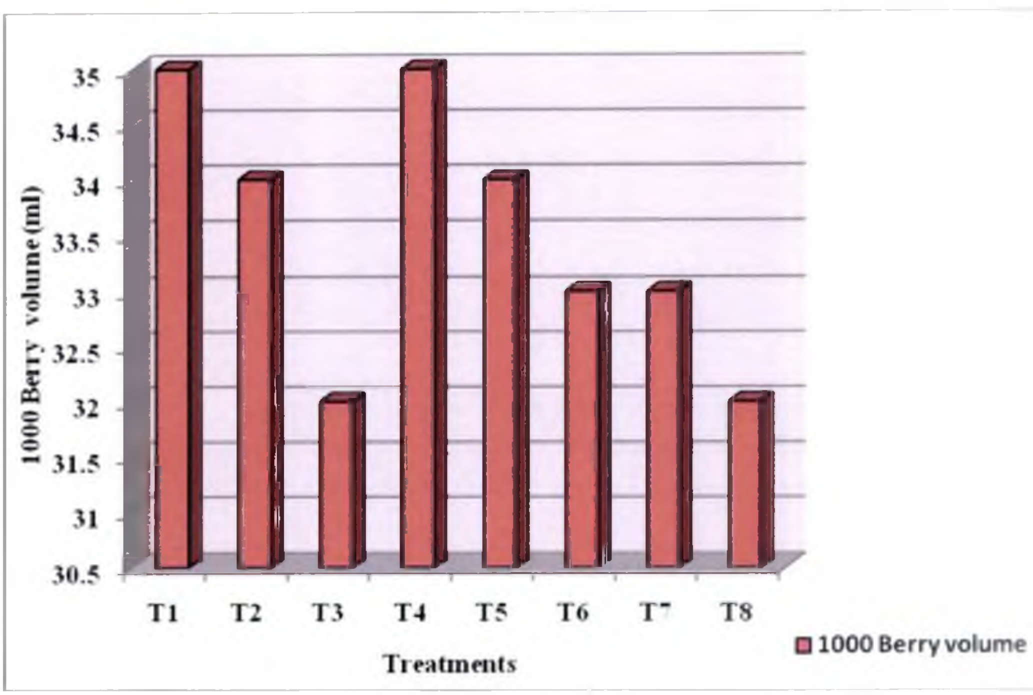


Fig.2 Effect of bleaching agents on 1000 berry volume of white pepper

The values of 1000 berry weight were ranged from 34.51 to 37.34g and there was significant difference between treatments (5%) (Fig. 1). White pepper berries treated with sodium hypochlorite and calcium hypochlorite recorded the maximum and minimum 1000 berry weight respectively.

Different treatments showed significant influence for 1000 berry volume. The values were ranged from 32- 35ml. The highest value was obtained for both the berries treated with calcium hypochlorite and sodium hypochlorite. Since there is weight difference among berries there will be significant difference in 1000 berry volume also (Fig.2).

Different treatments showed a significant influence on specific gravity of white pepper berries (Fig. 3). The result showed that berries treated with sodium perborate exhibited the maximum specific gravity of 1.12 g/cc and the lowest was noticed in berries treated with sodium hydroxide (0.98g/cc). Weiss (2002) reported that good quality white pepper required a specific gravity ranging from 0.88 to 0.905 g/cc. White pepper berries produced by chemical methods had satisfied the required range of specific gravity. Another study conducted at Sarawak indicated that pepper fruits having specific gravity greater than 1.12g/cc are best for conversion to white pepper (Anon, 1995). This observation is also supporting the findings of present study.

Different treatments did not show any significant influence for berry size. White pepper berries treated with calcium hypochlorite got the maximum sized berries. The berry size was varying from 3.9 to 4.1 mm. This was in conformity with the findings of Kumar (2006b). This was also supported by the findings of Gopinathan and Manilal (2001).

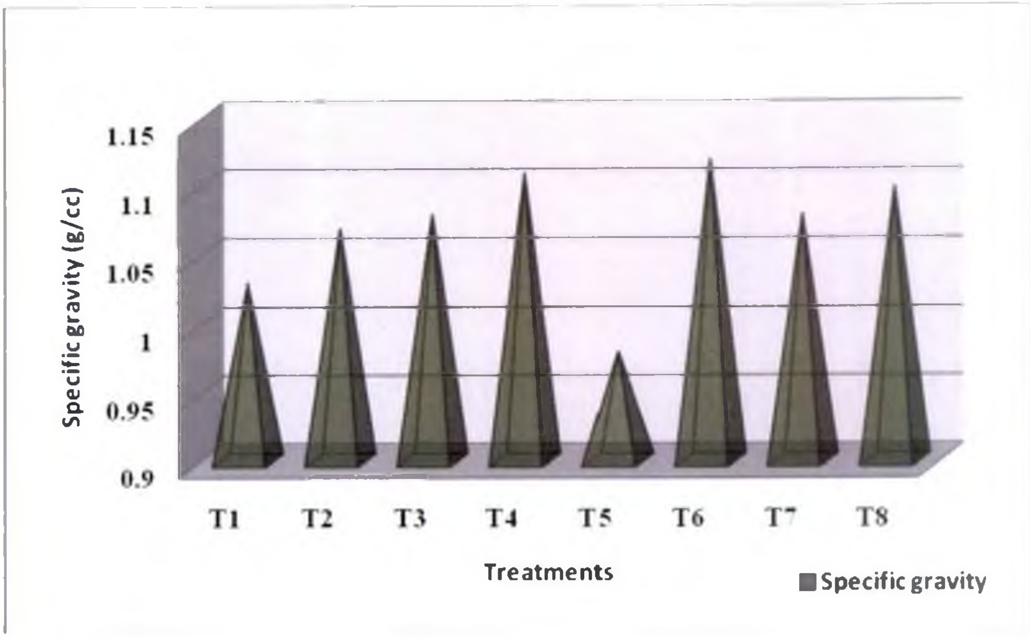


Fig.3 Effect of bleaching agents on specific gravity of white pepper berries

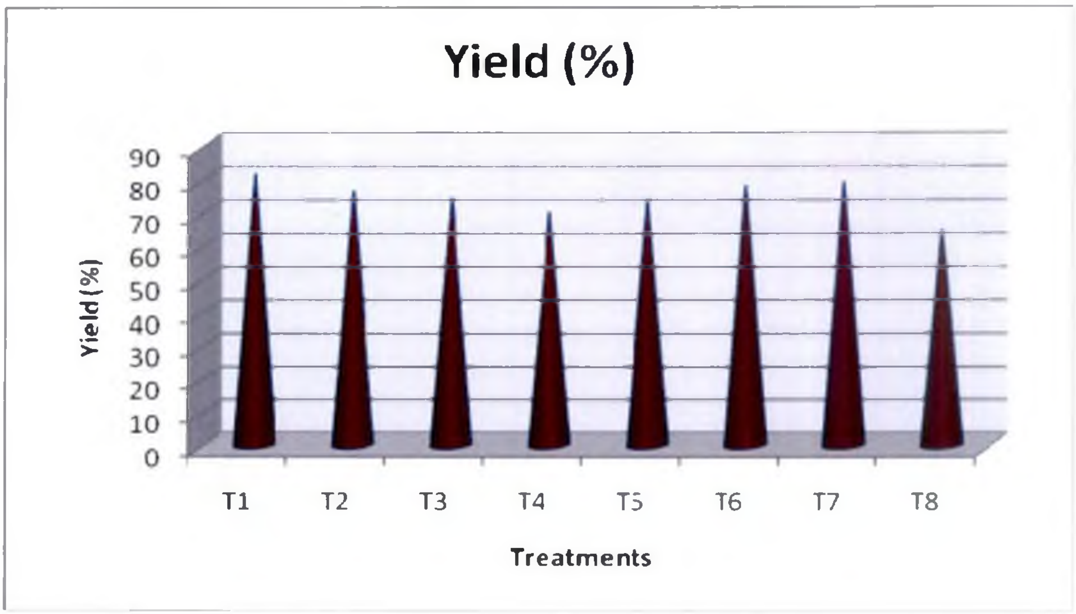


Fig. 4 Effect of bleaching agents on percentage yield of white pepper berries

The yield obtained from different treatments (Fig.4) differed significantly. White pepper berries treated with calcium hypochlorite recorded the maximum yield percentage of 81.18%. The percentage of black pepper berries in chemical treated white pepper was very less. The conventional method indicated the lowest value (64.48%) because of the presence of dark berries which were later removed by cleaning and winnowing. The yield obtained from different treatments was in conformity with the findings of Gopinathan and manilal, (2005) in bacterial decortications of white pepper.

5.1.1 Effect of bleaching agents on chemical parameters of pepper berries

Changes in the chemical qualities of white pepper consequent to the treatments with bleaching agents were discussed below. The result showed that different treatments significantly influenced the various chemical characteristics of white pepper. The results indicating changes in the content of volatile oil, non volatile ether extract (NVEE), piperine, oleoresin, moisture content, starch and total ash are discussed hereunder.

5.1.2.1. Volatile oil

The oil recovery was more in the case of powdered form than using crushed berries. This is due to the reason that, in powered form the particle size will be very small and therefore maximum surface area will be available for the extracting medium. The highest volatile oil content was found in berries produced by conventional method (2.8%). The oil content of white pepper berries treated with benzoyl peroxide (2.60%) and calcium hypochlorite (2.53%) were comparable with the oil content of conventionally prepared berries (Fig.5). This was supported by the findings of Lewis (1979), in which he obtained oil content in the range of 1.5- 3.5%. This was further supported by the findings of Gopinathan and Manilal, (2005).

5.1.2.2 Non volatile ether extract (NVEE)

Non volatile extract content in treated white pepper berries were falling in a range between 4.87 to 8.53 percentage. Non volatile ether extracts in berries produced by conventional method was the superior one and those treated with treated with calcium hypochlorite (8.47 %), sodium hypochlorite (8.47%), benzoyl peroxide (7.6%), calcium hydroxide, (7.6%) and hydrogen peroxide (7.47%) were found be comparable with the traditionally produced berries(Fig.6). This was supported by the findings of Geisler *et al*, (1990). According to him, White pepper should contain a minimum of 7.5 percentage of non volatile extract. This was further supported by the minimum requirement for non volatile ether extract laid under Brazilian quality specifications. There was a negative influence of sodium on white pepper berries. Presence sodium in the berries affected the non volatile content present in them. This reduction in non volatile contents could be attributed to certain chemical reactions on the berries.

5.1.2.3 Piperine

Maximum piperine content was obtained from the berries prepared from conventional retting process with a percentage of 2.91(Fig.5). The berries treated with calcium hydroxide (2.82%), hydrogen peroxide (2.63%), calcium hypochlorite (2.49%) and sodium hypochlorite (2.44%) was also found superior in piperine content. Berries with sodium hydroxide gave the lowest level of 1.40%. According to Farooqi *et al.* (2005) the maximum piperine content in white pepper is 5.3 %. Chemical treatment resulted in white pepper berries with much lower piperine contents due to the chemical reaction occurred by the addition of bleaching agents. When the berries were soaked in sodium hydroxide, the colour of water became darker, which gave an indication that some quality attributes might have been lost by the action of sodium hydroxide on white pepper berries. The reactions of benzoyl

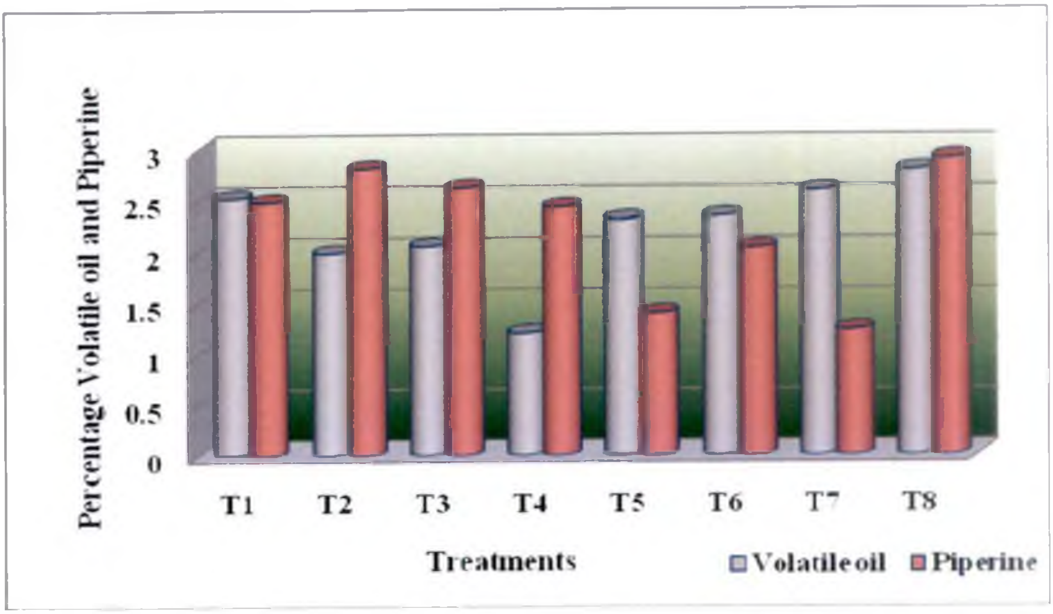


Fig. 5 Effect of bleaching agents on volatile oil and piperine contents of white pepper berries

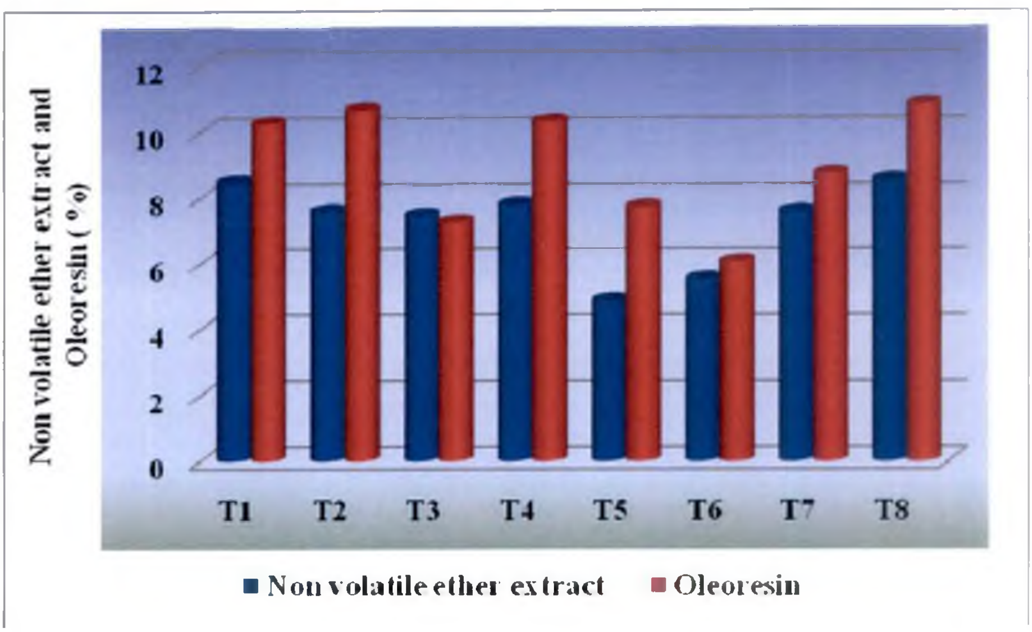


Fig. 6 Effect of bleaching agents on non volatile ether extract and oleoresin contents of white pepper berries

peroxide and sodium hydroxide although its concentrations were low, found to be most affected on piperine content of white pepper berries.

5.1.2.4 Oleoresin

The percentage oleoresin content obtained was ranged from 6.07 to 10.83(Fig.6). Similar result was obtained by Farooqui (2005). Among the chemical treatments, the white pepper prepared from conventional method resulted in higher content of oleoresin (10.67%) followed by treatment with calcium hydroxide (10.67%) and the lowest was obtained from berries treated with sodium perborate. (6.07%). Reaction of sodium in berries with sodium perborate was evident. But those treated with calcium hydroxide, hydrogen peroxide and calcium hypochlorite were found to be comparable with those produced by conventional method.

5.1.2.5. Moisture

Since all the treatments were dried under identical conditions , significant changes in moisture was not observed. The moisture content was in range of 13.75 % (treatment with sodium hypochlorite) to 15.01% (treatment with benzoyl peroxide). American Spice Trade Association (ASTA) standards specified a moisture content of 13% for white pepper. Yet no quality deterioration occurred to the treated white pepper.

5.1.2.6. Starch

There was no significant influence of different treatments on the starch content. White pepper prepared using conventional method resulted in higher starch content of 64.38% and the lowest was reported by the berries treated with calcium hypochlorite (58.59%). Precisely no detrimental effects were observed consequent to the treatment of chemical agents on the starch content of white pepper.

Analysis for residues in the experiment I (chemical treatments)

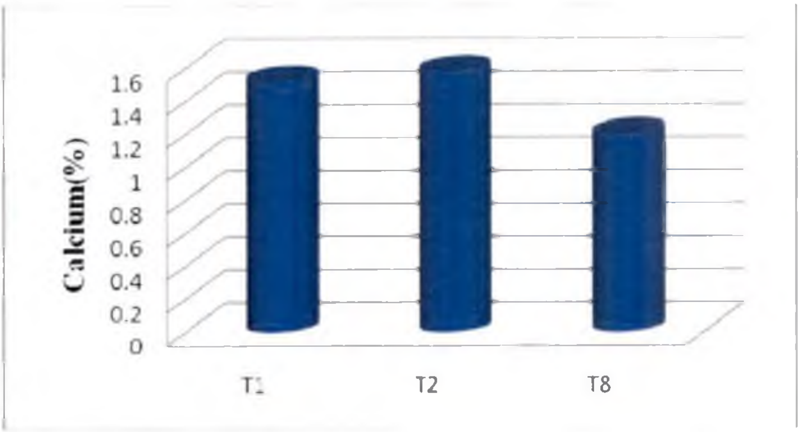


Fig. 7 Percentage calcium content in berries treated with calcium

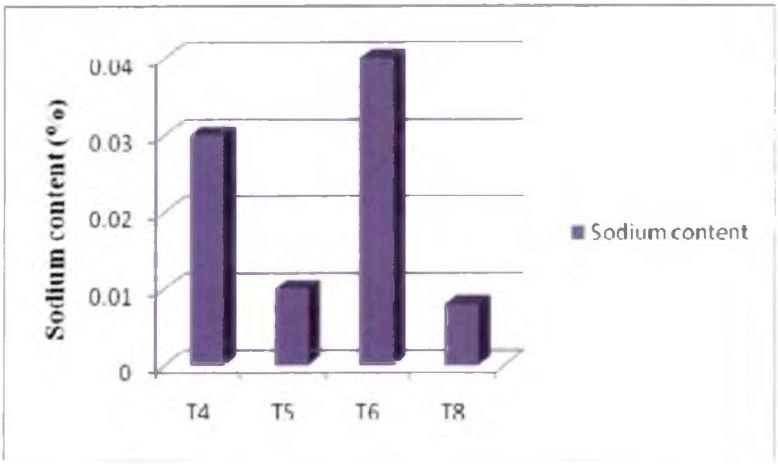


Fig. 8 Percentage sodium content in berries treated with sodium

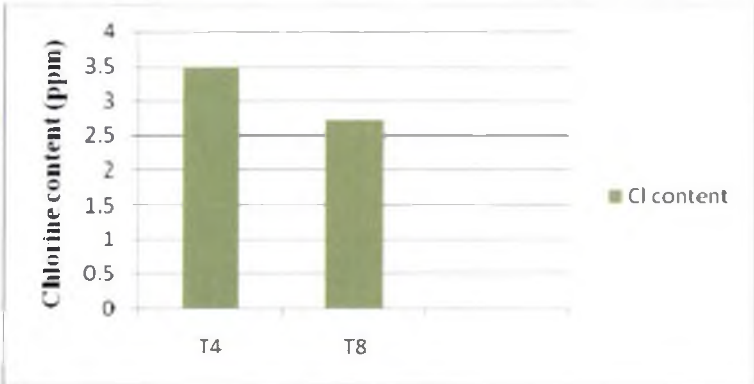


Fig. 9 Chlorine content in berries treated with chlorine

5.1.2.7 Total ash content

Ash content in white pepper berries showed no significant difference among different treatments. However the highest value was obtained for white pepper berries treated with sodium hydroxide (1.09%) and the lowest by berries with hydrogen peroxide (0.56 %).

5.1.2 Analysis for residues of chemical treatments

5.1.2.1 Calcium content in white pepper berries treated with calcium

There was significant variation in residual calcium content of various treatments (Fig.7). Berries treated with calcium hydroxide were found to have the highest residue of (1.58%) which was on par with berries treated with calcium hypochlorite (1.52%). Findings of Jackson (1973) opined that calcium level of less than 1.75% considered to be low and safe. So the calcium contents detected in the treated berries were also of low levels and were in a safe range for uses in food and flavour sector. Similar results were findings were reported by Cheng and Bray, (1985).

5.1.2.2 Sodium content in berries treated with sodium

The treatments did not reveal any change in residual sodium content (Fig.8). However the highest residual of sodium was analysed from berries treated with sodium perborate . Since the sodium content was rather low, there will not t be any harm. This was in conformity with the findings of Chun *et al.* (2002) in tooth bleaching.

5.1.3.3 Chlorine content in berries treated with chlorine

Treatment with sodium hypochlorite left out a significantly high residual level of chlorine in berries (3.49ppm), yet the residue level was considered to be low. and safe for consumption. This was supported by the findings of Eaton, (1987) in tobacco and corn.

5.2 MICROBIAL FERMENTATION

The microbial fermentation of pepper berries with isolates of bacteria IsB1 to IsB7 and fungal isolates IsFI to IsF6 obtained under the study along with selected cultures Ay1 and *Mycophyta* were conducted. The findings are discussed hereunder.

5.2.2. Evaluation of isolates for retting ability.

Evaluation of isolates was done by assessing the pectinolytic and cellulolytic activities and amount of reducing sugar produced by isolates in the invitro studies.

5.2.2.1 Assessment of pectinolytic activity of isolates

Observations recorded on the zone formation and zone width, it could be concluded that, bacterial isolate IsF4 was having the highest pectin degrading capacity. The isolates IsF1 and IsF3 were also capable for degrading pectin.

When considering the growth of cultures, IsB5 was having the highest growth rate in MP-5 media followed by IsF4 and IsF1. The pectin degrading ability of bacterial isolates was very poor. The fugal isolates had greater activity against pectin than bacteria. Hence the fungal isolates are better option for retting process as pectin is a major component of pericarp of berry.

5.2.2.2 Assesment of cellulolytic activity of isolates

Here also, the fugal isolates were having higher cellulose degrading activity than bacteria. Bacterial isolates did not produce any zone in the asparagine media, indicating the poor cellulolytic activity. The better cellulolytic activity of fungal isolates is an indication that the pericarp can be utilized as a carbon source for the isolates, there by effective retting can be achieved.

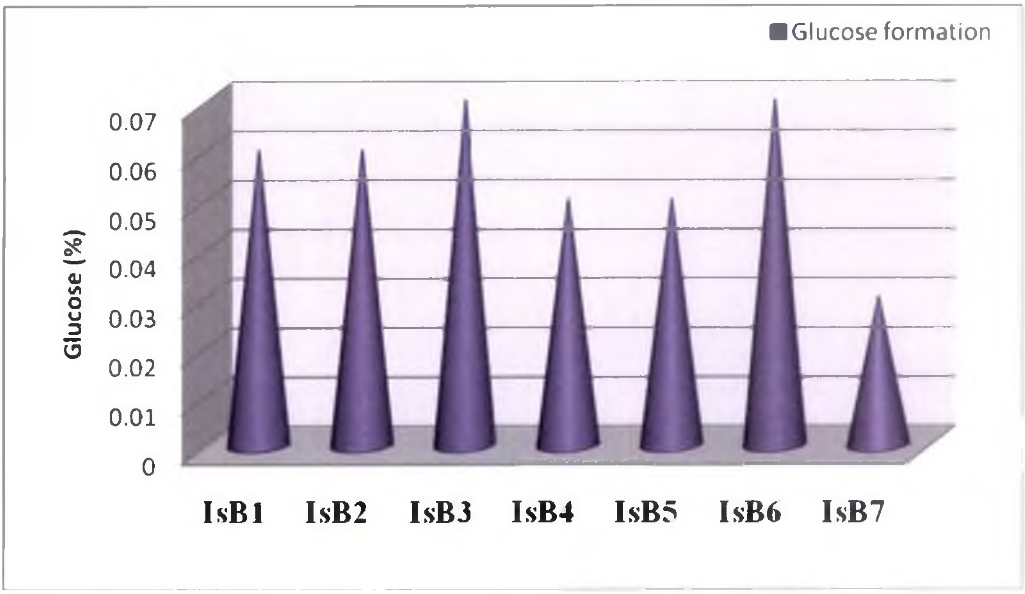


Fig.10 Glucose formation as influenced by different bacterial isolates

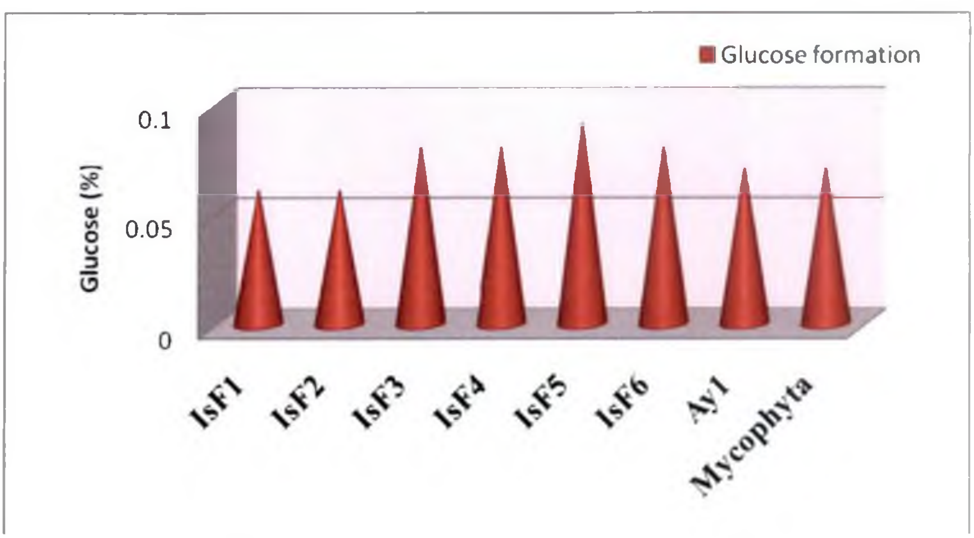


Fig. 11 Glucose formation as influenced by different fungal isolates

5.2.2.3 Estimation of reducing sugar

The amount of reducing sugar formed was studied by dinitrosalicylic acid method. Isolate IsF5 showed the highest level of activity (0.09%), followed by IsF3, IsF4, IsF6 and *Mycophyta* with glucose content of 0.08% (Fig.10 and 11). The higher glucose formation is an indication of effective cellulose degradation. Cellulose being one of the major components of pericarp, these cultures may hasten the retting process.

5.2.3. Standardization of retting process

Retting process was standardized using different isolates after assessing their pericarp degrading activity. The pepper berries inoculated with microbial cultures after incubation were tested for the degree of decortication. . Comparatively bacterial cultures showed a poorer degree of fermentation. Compared to bacterial isolates fungal isolates were faster in retting. Fungal isolate IsF3, Ay1 and *Mycophyta* showed 100 percentages retting in three days. The degree of retting was fastest in the case of Ay1 followed by *Mycophyta* and IsF3. All other isolates completed within four days. The better pectinolytic and cellulolytic activity noticed with fungal cultures reflected on the retting process. This could be due to the presence of cellulose and pectin present in the pericarp which was effectively utilized by the isolates as its carbon source.

The cleaned, packed white pepper after microbial retting were analysed for quality parameters and are discussed below.

5.2.4 Quality parameters of white pepper

5.2.4.1 Specific gravity

Fungal isolate IsF3 had showed superior in specific gravity (1.12 g/cc) and all other isolates were also equally effective in producing berries of similar specific gravity (Fig.12 and 13).

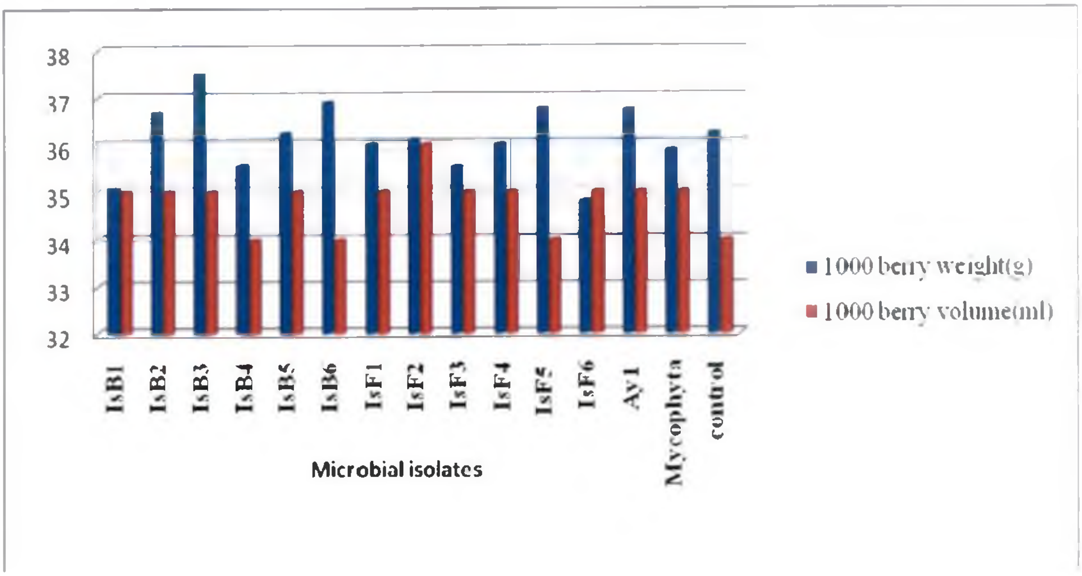


Fig. 12 Effect of microbial fermentation on physical parameters of white pepper berries

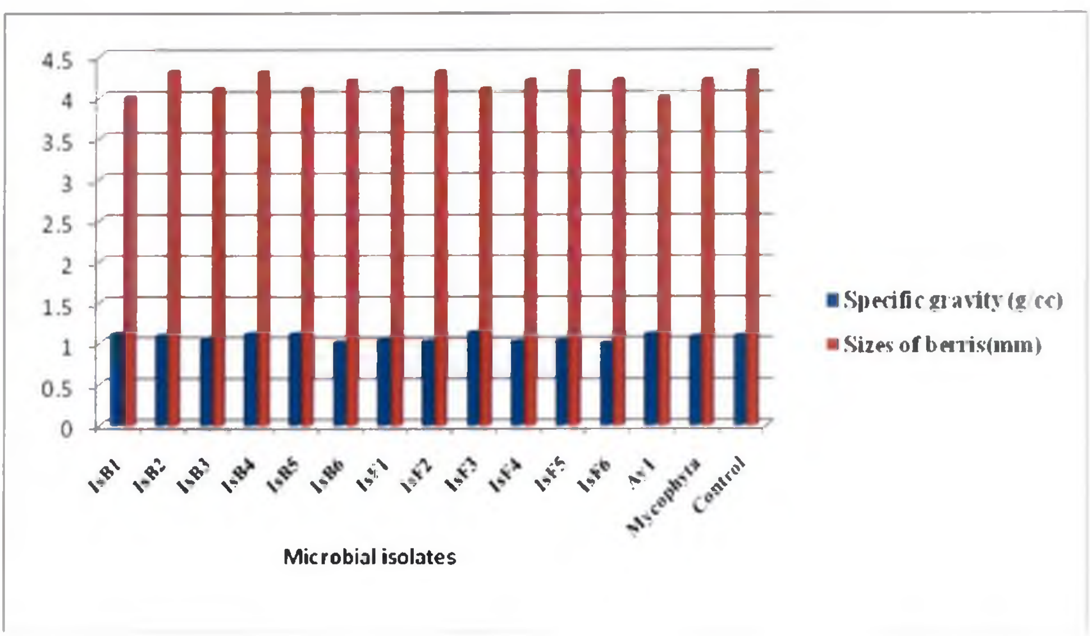


Fig. 13 Effect of microbial fermentation on physical parameters of white pepper berries (continued)

5.2.4.2. 1000 berry weight

Bacterial isolate IsB3 was superior for 1000 berry weight (37.50g). Other isolates were also having similar 1000 berry weights (Fig.12).

5.2.4.3 1000 berry volume

White pepper berries with fungal isolate IsF2 shown highest 1000 berry volume and all the other was falling in 34 and 35 ml (Fig.12). Since berries with different isolates were having similar weights, berries would be with similar volume.

5.2.4.4 Sizes of berry

The berry size of all fifteen isolates was falling in between 4.0 and 4.3mm (fig.12). Similar findings were reported by Gopinathan and Manilal, (2005); Thankamani *et al.* (2004). All berries were equally effective for having similar sizes. There was no difference among berries produced from different isolates.

5.3. ORGANOLEPTIC QUALITY EVALUATION

The third part of the experiment was the organoleptic evaluation of developed products from both chemical and microbial methods. The scoring for organoleptic qualities like appearance, colour, flavour, texture, taste and over all acceptability was done by a team consisted of a group of students and research associates.

5.3.1 Organoleptic evaluation of white pepper produced by chemical method

5.3.1.1 Effect of different chemical treatments on appearance of whole white pepper

The berries treated with calcium hypochlorite resulted in better appearance. Effective reaction of calcium hypochlorite on white pepper berries was clearly understood. The findings of Kumar (2006b) support that 0.2% calcium hypochlorite was effective for white pepper bleaching, which resulted in better appearance and

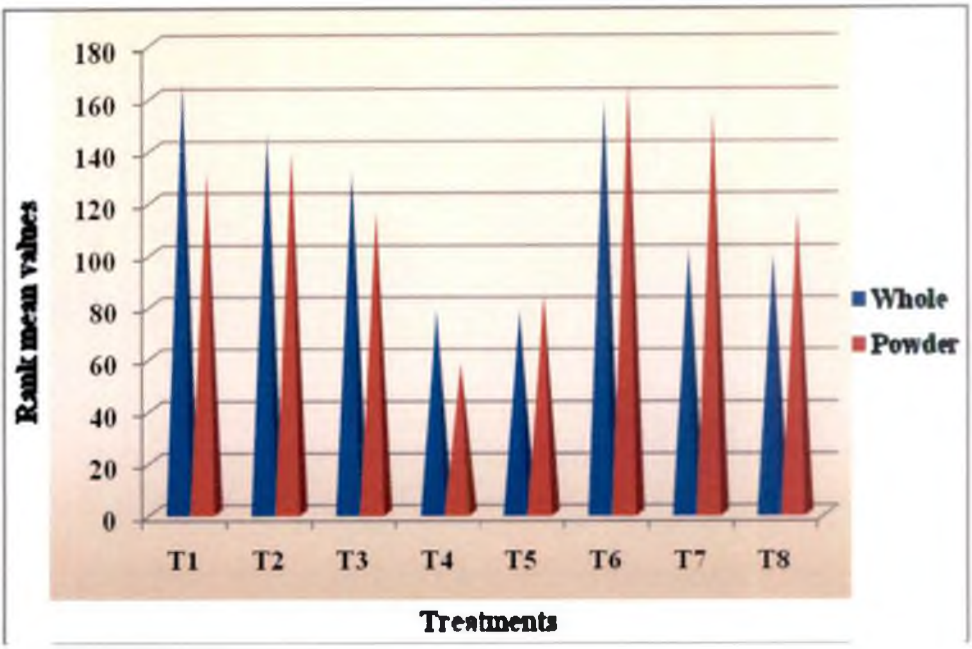


Fig. 14 Effect of bleaching agents on appearance of white pepper

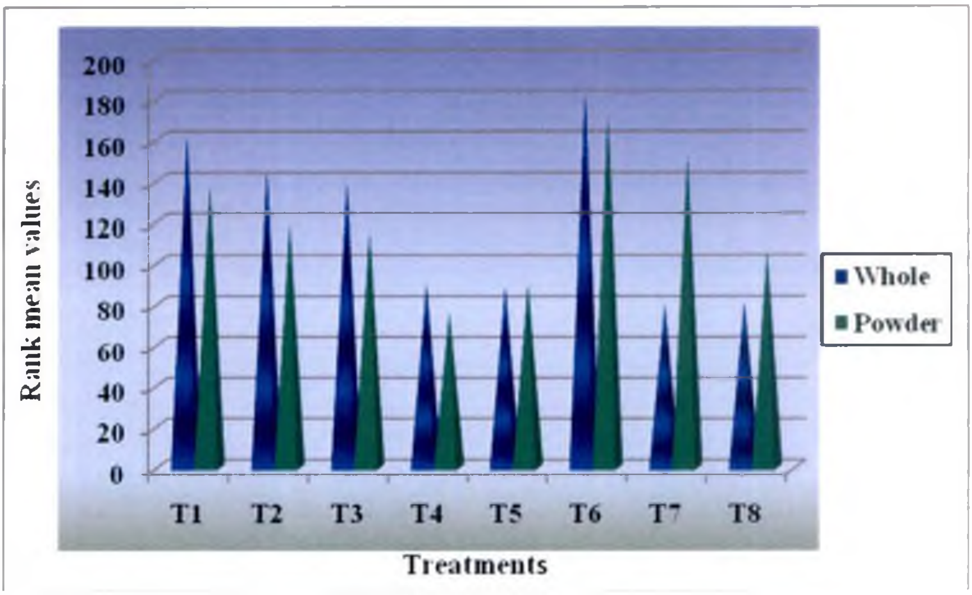


Fig. 15 Effect of bleaching agents on colour of white pepper

colour. The berries treated with sodium perborate, calcium hydroxide and hydrogen peroxide were equally effective for exhibiting acceptable colour (Fig.14). The better colour developed by berries treated with sodium perborate might have influenced the appearance of white pepper in both whole and powder forms. It was further supported by the findings of Vachon *et al.* (1998). The appearance of conventionally prepared berries was very poor. This was supported by Dhas, *et al.* (2003), reported that due to the reaction of alkali, the developed berries resulted in poor appearance.

5.3.1.2. Effect of treatments on appearance of white pepper powder

Appearance in powder form is a very important quality characteristic because, use of white pepper is mainly in powder form. Berries with sodium per borate succeeded in producing a better appearance of white pepper powder. This was in conformity with the findings Vachon *et al.* (1998). Berries treated with benzoyl peroxide, calcium hydroxide and calcium hypochlorite were also equally acceptable for kitchen use (Fig.14).

5.3.1.3. Effect of treatments on colour of whole white pepper

There was a positive effect on colour by the different treatments. Among different treatments (Fig.15), berries treated with sodium hypochlorite was having the superior colour (creamy white). This was supported by the findings of Ho and Goerig (1989); Warren *et al.* (1990); Rotstein, (1991); Rotstein *et al.* (1993) in intracoronal bleaching. Berries produced by conventional retting method gave a darkish coloured product. Similar results were obtained reported by Natarajan *et al.* (1967); Lewis (1982). The chemical treatments were highly effective for improvement of whiteness of berry.

5.3.1.4 Effect of treatments on colour of white pepper powder

Bleaching of white pepper berries resulted in better colour development in products (Fig.15). Nathoo *et al.* (2001) and Li *et al.*(2003) reported that bleaching was capable of restoring teeth to their original colour. Berries treated with sodium perborate could able to influence the colour of white pepper powder. This was in conformity with the findings Vachon *et al.* (1998). Attin *et al.* (2003) recommended the use of sodium perborate solution for intracoronal bleaching . Berries with calcium hypochlorite and benzoyl peroxide was equally effective in producing better colour. Kumar (2006b) reported the bleaching effect of calcium hypochlorite on colour of white pepper colour. Benzoyl peroxide used for bleaching liquid whey resulted in whiter colour (Croissant *et al.*, 2009). Berries produced by conventional method resulted in inferior colour as reported by Risfaheri and Hidayat (1996a).

5.3.1.5. Effect of treatments on flavour of crushed white pepper

Highest flavour characteristics were found in berries prepared from conventional retting process (Fig.16). Berries with calcium hypochlorite and calcium hydroxide were also having acceptable flavour. This was in conformity with the findings of Kumar (2006b) in which he reported that, white pepper berries bleached with 1% calcium hydroxide and 0.2% calcium hypochlorite resulted in preserving characteristic pepper flavour. All the other treatments were having inferior flavour characteristics. This might be due to greater chemical reaction resulted by bleaching.

5.3.1.6. Effect of treatments on flavour in white pepper powder

Conventionally prepared berries showed superior flavour in powder form also. (Fig 16). This was on par with berries treated with benzoyl peroxide, sodium perborate, calcium hypochlorite and hydrogen peroxide. The findings on flavour of whey protein concentrate by Croissant et *al.* (2009) revealed that type of bleaching

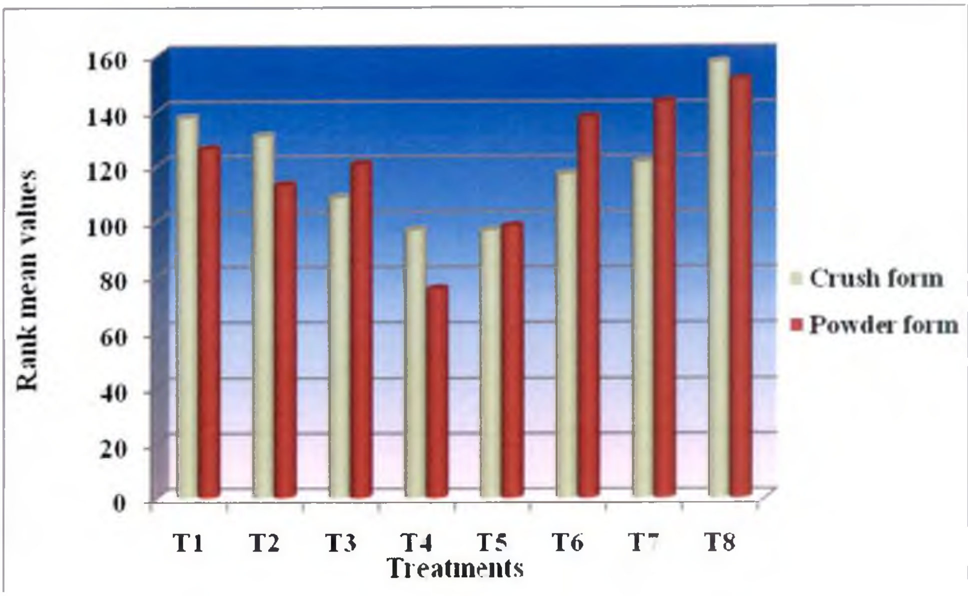


Fig. 16 Effect of bleaching agents on flavour of white pepper

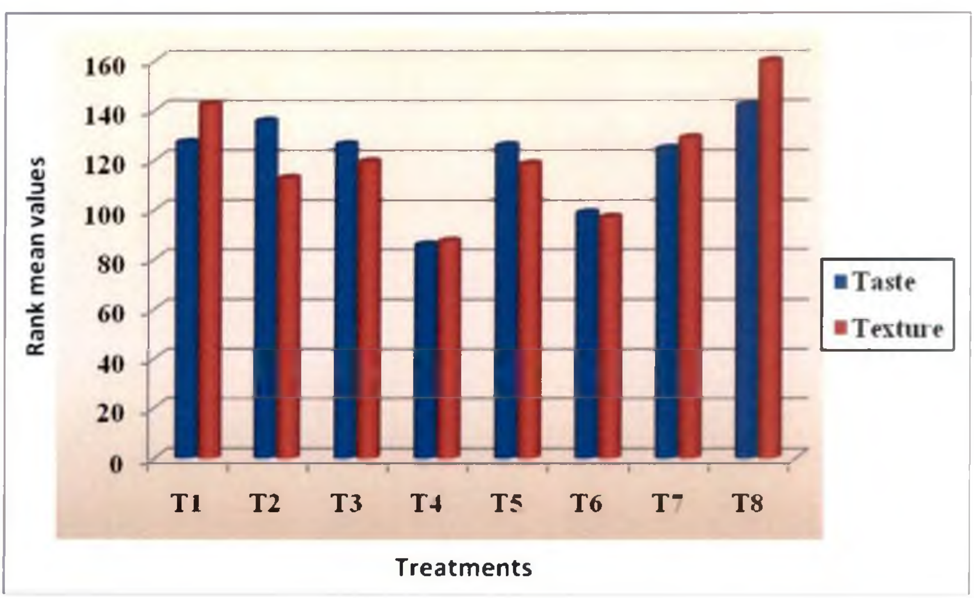


Fig. 17 Effect of bleaching agents on taste and texture of white pepper

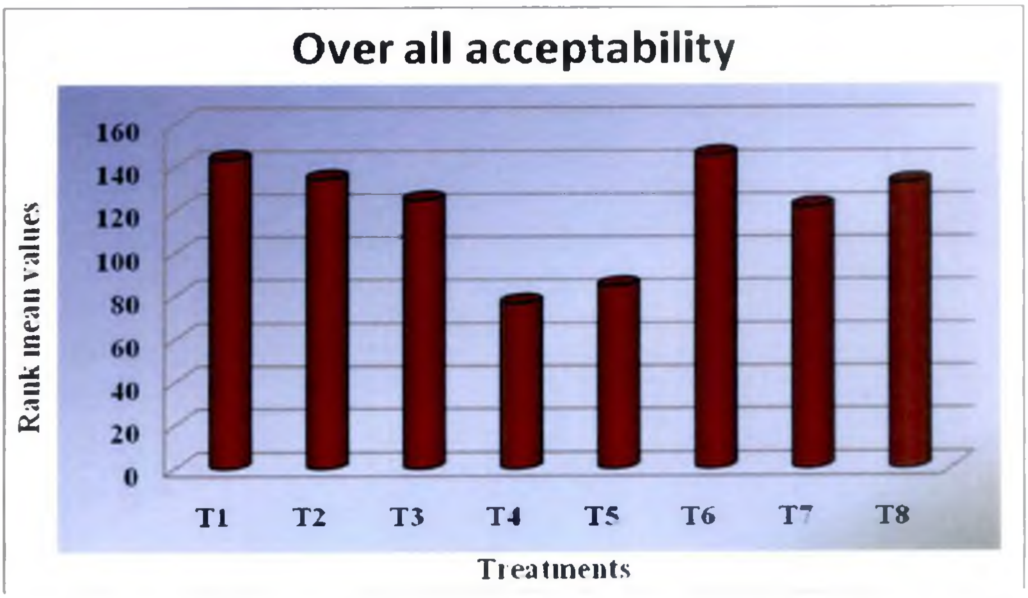


Fig. 18 Effect of bleaching agents on over all acceptability of white pepper

agent used influenced the flavour of whey protein concentrate. Alkali treatment adversely affected the flavour properties and was the most inferior among all treatments. Comparatively lower influence of bleaching agents might be the chemical reactions on white pepper berries.

5.3.1.7. Effect of treatments on taste characteristics

Among the different treatments, white pepper berries prepared by conventional retting resulted with best taste characteristics. White pepper berries treated with calcium hydroxide, calcium hypochlorite, hydrogen peroxide, sodium hydroxide and benzyl peroxide were equally acceptable (Fig.17). Taste characteristics were not much affected by chemical bleaching agents. Improved Appearance, colour, and flavour of product resulted from calcium hypochlorite bleaching might had influenced the taste characteristics also.

5.3.1.8.. Effect of treatments on textural qualities

Conventionally retted berries stood superior with in textural qualities. Berries with calcium hypochlorite and benzoyl peroxide were equally effective in providing better texture to the berries. (Fig.17). Study conducted by Suhaila and Tok (1994) reported that ,calcium treatment were most effective in preserving the textural properties of rehydrated dried oyster mushroom. Beneficial effect of benzoyl peroxide was supported by the findings of Croissant et *al.* (2009) in whey protein concentrate.

5.3.1.9 Effect of treatments on over all acceptability

Among the different treatments, overall acceptability was rated for berries treated with sodium perbotrate. This might be due to its brilliant appearance and creamy white colour that influenced in over all acceptability (Fig18).

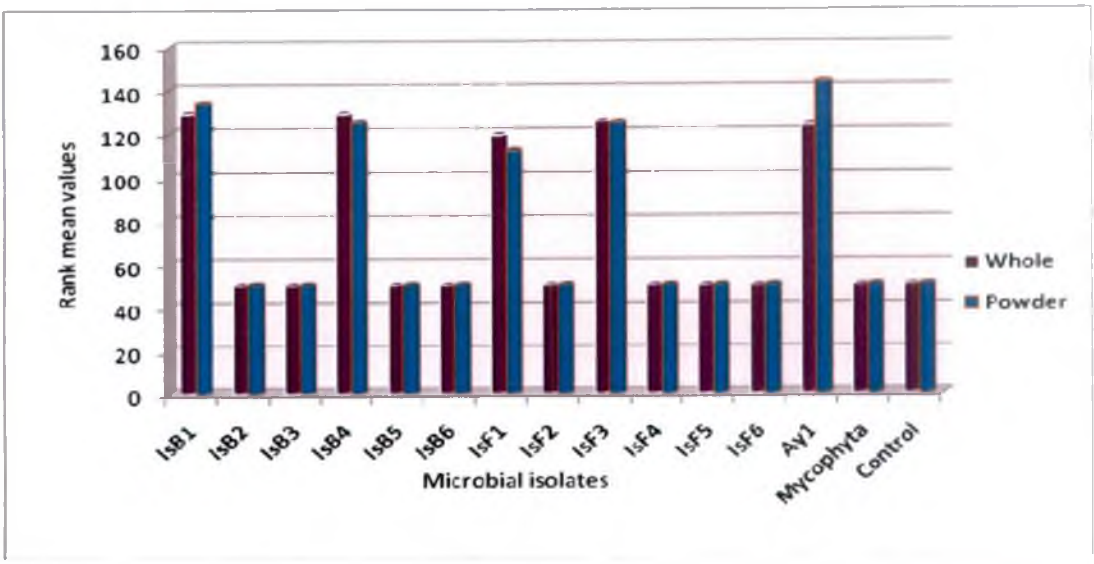


Fig.19 Effect of microbial fermentation on appearance of white pepper

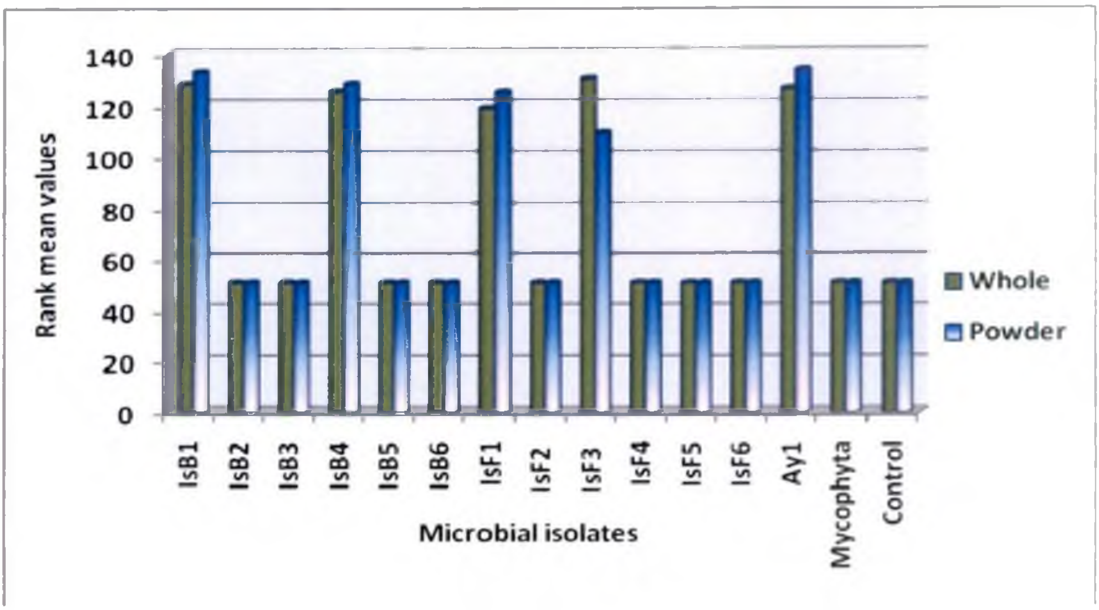


Fig.20 Effect of microbial fermentation on colour of white pepper

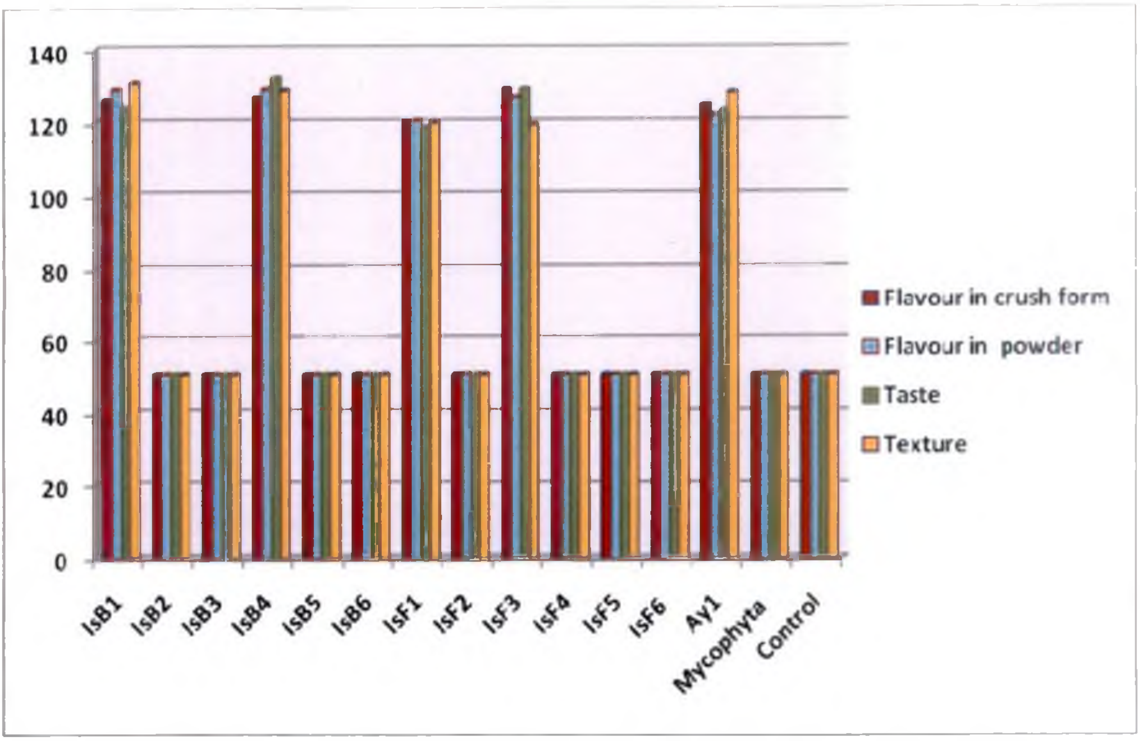


Fig.21 Effect of microbial fermentation on flavour, taste and texture of white pepper

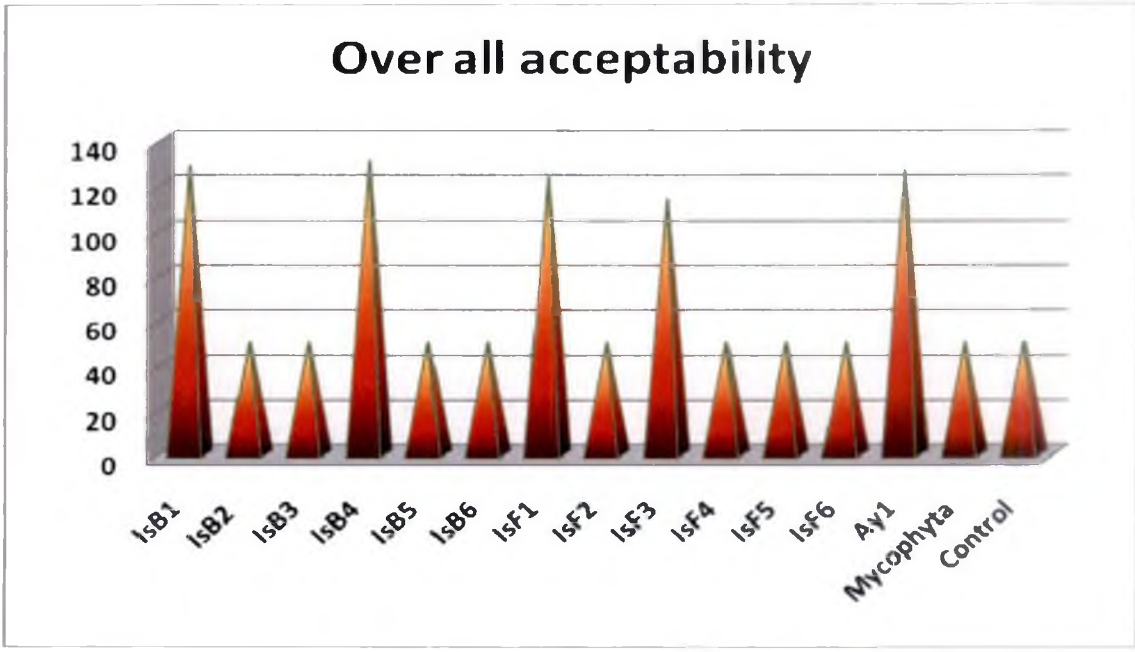


Fig.22 Effect of microbial fermentation on over all acceptability of white pepper

When considering the organoleptic qualities of white pepper produced by chemical methods, it could be concluded that the berries made from conventional method was superior in flavour, taste and textural characteristics. But when evaluating the appearance and colour, the score went in favour of berries treated with sodium perborate. Berries treated with calcium hypochlorite stood on par with the highest score in all the parameters.

5.3.2 Organoleptic evaluation of white pepper produced by microbial fermentation

The isolates IsB1, IsB4, IsF1 IsF3 and Ay1 were found to be superior in all the quality attributes like appearance (Fig.19), colour (Fig.20), flavour, texture, taste (Fig.21) and over all acceptability (Fig.22). These were the most acceptable for kitchen use.

Problem with chemically produced white pepper is the persistence of chemical residue resulting in loss of quality attributes. But in case of berries treated with calcium hypochlorite, both the residue level and loss of chemical qualities were significantly very less and it possessed good organoleptic qualities or was on par with the superior one. Most importantly, it gave better colour to white pepper berries. Hence calcium hypochlorite with a concentration of 0.2% can be recommended for bleaching of white pepper.

Prolonged retting period of 10-14 days can be shortened to 3-4 days using effective microbial isolates. Among the isolates, the fungal isolate number IsF3, Ay1 and *Mycophyta* can be recommended for fast retting due to their advantages like higher cellulose and pectin degradation activities and higher degree of retting. When the organoleptic qualities are considered, isolate IsF3 and Ay1 were found to be the best. Development of poorer colour was the only problem with microbial fermentation method.

In this era of organic farming, customers would prefer to avoid chemically bleached white pepper. In this context, white pepper produced by the microbial method is of paramount importance from the safety regulations of food laws. When the colour of white pepper is taken into account, in such cases customer can go for white pepper produced by chemical treatments, but with caution of chemical residues in safe levels for consumption.

FUTURE LINE OF WORK

1. Isolation of promising microbes for providing better colour to the white pepper.
2. Popularization of value added products of white pepper in the domestic markets of India and also augmenting export of white pepper.

Summary

6. SUMMARY

The present investigation entitled "Standardization of processing methods for production of quality white pepper" was carried out to produce good quality white pepper using improved processing techniques. The experiment was conducted in the Department of Processing Technology, College of Agriculture, Vellayani during the period 2009-11. Major findings of the study are summarized in this chapter.

The experiment consisted of chemical method of white pepper production, microbial fermentation method and organoleptic evaluation of the resultant product.

The effect of chemical treatments on physical parameters of white pepper, such as yield, specific gravity, 1000 berry weight, 1000 berry volume and size of berries were analysed. All the physical parameters except size were significantly influenced by bleaching agents. Berries treated with sodium perborate had the highest specific gravity followed by those with sodium hypochlorite. Sodium hypochlorite treated berries were having the highest 1000 berries weight. Berries treated with calcium hypochlorite were having the highest value 1000 berry volume and yield percentage.

Chemical parameters such as volatile oil , non volatile ether extract (NVEE), piperine, oleoresin, moisture, starch and ash content were analysed and the results revealed that all chemical parameters except moisture, ash and starch content, were significantly influenced by treatments with bleaching agents. White pepper berries prepared by conventional method (control) was found to be superior in all the chemical qualities. The berries treated with calcium hypochlorite were found equally effective for all the chemical parameters.

There was significant variation in the calcium and chlorine residue among treated berries. Berries treated with calcium hydroxide was found to be having highest calcium residue ,which was on par with berries treated with bleaching powder. Both the treatments showed significant difference with the conventionally prepared white pepper. Berries with sodium hypochlorite left out significantly a higher residual level of chlorine. Published reviews on similar lines are in support that, levels of these chemical residues are considered to be low and they are safe to use.

Experiment on microbial fermentation consisted of isolation of organisms through enrichment culture technique, evaluation of available cultures and standardization of retting process for white pepper production. Seven bacterial isolates and eight fungal isolates were obtained, each assigned with specific isolate number. Evaluation of isolates was done by assessing the pectinolytic and cellulolytic activities. The fungal isolate IsF4 was having the highest pectin degrading capacity. The isolates IsFl and IsF3 were also capable of degrading pectin. IsF5 was having the highest growth rate in MP-5 media followed by IsF4. Bacterial isolates did not produce any hydrolytic zone for both asparagine and MP-5 media. There was a greater activity of fungal isolates against cellulose and pectin. Isolate IsF5 showed the highest level of reducing sugar content followed by IsF3, IsF4, IsF6 and *Mycophyta* . Retting process was standardized using different isolates after assessing their activity for degradation of pericarp. The better pectinolytic and cellulytic activities noticed with fungal cultures reflected on the retting process. Amongst all the isolates, the fungal isolates IsF3, Ayl and *Mycophyta* recorded 100 percentage retting without any damage by third day itself.

The isolate IsF3 had the highest specific gravity of 1.1.2g/cc and all the other isolates were also equally effective in producing berries of similar specific gravity. 1000 berry weight was in the range of 34.81 to 37.5g for all the isolates. The highest value for 1000 berry volume was observed for IsF2 with 36 ml and all the others had

34 and 35 ml respectively. The berry size of all fifteen isolates was in between 4.0 and 4.3mm. All the berries were equally effective for having similar sizes.

The third part of the experiment was organoleptic evaluation of the products obtained from both chemical and microbial methods. The scoring for organoleptic qualities like appearance, colour, flavour, texture, taste and over all acceptability were done by a team consisting of a group of students and research associates.

Berries treated with calcium hypochlorite and sodium perborate were superior in appearance in whole and powdered forms. Superior colour was obtained for berries treated with sodium perborate followed by calcium hypochlorite. Berries treated with calcium hydroxide, hydrogen peroxide, sodium hydroxide sodium hypochlorite and those by conventional method were also having good colour in powder forms. Berries produced from conventional retting method found to be superior in flavour, taste and texture characteristics. Treatment with calcium hypochlorite also found equally acceptable. Over all acceptability was maximum for the berries treated with sodium perborate. But berries treated with sodium perborate were inferior in chemical qualities.

Amongst all the eight treatments, considering quality parameters and quality of developed product, treatment with calcium hypochlorite was found to be the most superior.

White pepper produced by microbial fermentation revealed that, the pepper produced by conventional method (control) was the poorest in all the five organoleptic parameters. The isolates IsB1, IsB4, IsF1, IsF3, and Ay1 were found to be superior in all the organoleptic quality attributes.

Amongst all the isolates, considering the retting process and quality of the product, the isolates IsF3 and Ay1 were found to be the most superior.

CONCLUSION

The time consuming traditional retting can be shortened to three to four days using microbial fermentation method with promising isolates IsF3 and Ay1. While opting for a better colour, bleaching with a promising chemical bleaching agent like calcium hypochlorite can be recommended.

FUTURE LINE OF WORK

1. Isolation of promising microbes for providing better colour to the white pepper
2. Popularization of value added products of white pepper in the domestic markets of India and also augmenting export of white pepper.

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Abstract

STANDARDIZATION OF PROCESSING METHODS FOR PRODUCTION OF QUALITY WHITE PEPPER

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(2009-12-114)

ABSTRACT

of the thesis submitted in partial fulfillment of the requirement for the degree of

MASTER OF SCIENCE IN HORTICULTURE

(Processing Technology)

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF PROCESSING TECHNOLOGY

COLLEGE OF AGRICULTURE

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ABSTRACT

The research entitled "Standardization of processing methods for production of quality white pepper"was conducted at the Department of Processing Technology, College of Agriculture, Vellayani . The study was undertaken with the objective of producing good quality white pepper using improved processing methods.

The experiment was divided into three parts viz., chemical method of white pepper production, microbial fermentation method and organoleptic evaluation of the resultant products. The experiment was laid out in CRD with three replications. Seven promising bleaching agents were selected for the chemical experiment. Berries with calcium hypochlorite, calcium hydroxide, hydrogen peroxide, sodium hypochlorite, sodium hydroxide, sodium hydroxide, benzoyl peroxide and the conventional retting (control) constituted the eight treatments.

Effects of these treatments on the physical and chemical properties of white pepper berries were analysed. All the physical properties except sizes of berries were significantly influenced by the treatments. Treatment with sodium perborate recorded maximum specific gravity followed by sodium hypochlorite, hydrogen peroxide. The highest value for 1000 berry weight was reported from berries treated with sodium hypochlorite. Treatment with calcium hypochlorite and sodium hypochlorite had registered a significantly higher value for 1000 berry volume. Calcium hypochlorite treated berries were having the highest yield.

Berries produced by conventional retting process (control) found to be superior in volatile oil, oleoresin, piperine and non volatile ether extract and treatment with calcium hypochlorite was on par with control in all chemical parameters. Residual level of bleaching agents was found to be in a safe range for consumption.

The microbial fermentation work was consisted of, isolation of organisms through enrichment culture technique, evaluation of available cultures and standardization of retting process for white pepper production. Seven isolates of bacteria IsB1 to IsB7 and eight fungal isolates IsF1 to IsF6 were obtained. Evaluation of isolates was done by assessing

the pectinolytic and cellulolytic activities. The isolates IsF1 and IsF3 were also capable for degrading pectin. Degradation of cellulose was maximum in IsF3, Ayl and *Mycophyta.* Bacterial isolates did not produce any zone for both asparagine and MP-5 media. Compared to bacterial isolates fungal isolates was faster in retting. Isolate IsF3, Ayl and *Mycophyta* were the most promising and recorded 100 percentage retting without any damage by third day itself. Amongst all the isolates, considering the retting process and quality of the product, the isolates IsF3 and Ayl were found to be the most superior.

Berries treated with sodium perborate recorded maximum over all acceptability for Organoleptic qualities eventhough it resulted poor chemical qualities. Amongst all the eight treatments, considering quality parameters and quality of developed product, treatment with calcium hypochlorite was found to be the most superior.

The above study could be concluded with the findings that, prolonged retting period can be shortened to three to four days using microbial fermentation method with promising isolates IsF3 and Ayl. While opting for a better colour, bleaching with a promising chemical bleaching agent like calcium hypochlorite (0.2%) can be recommended.

Appendices

APPENDIX-I
KERALA AGRICULTURAL UNIVERSIY
COLLEGE OF AGRICULTURE, VELLAYANI
Department of Processing Technology

Title of thesis: Standardization of processing methods for production of quality white pepper

Name of student: Shameena Beegum.P.P (2009-12-114)

SCORE CARD FOR ASSESSING QUALITY PARAMETERS OF WHITE PEPPER

Criteria	Samples											
	1	2	3	4	5	6	7	8	9	10	11	12
Appearance (whole berry)												
Appearance (powder)												
Colour (Whole berry)												
Colour (powder)												
Flavour (crushed berry)												
Flavour (powder)												
Taste (salad)												
Texture (crushed berry)												
Overall acceptability												

<u>Score</u>

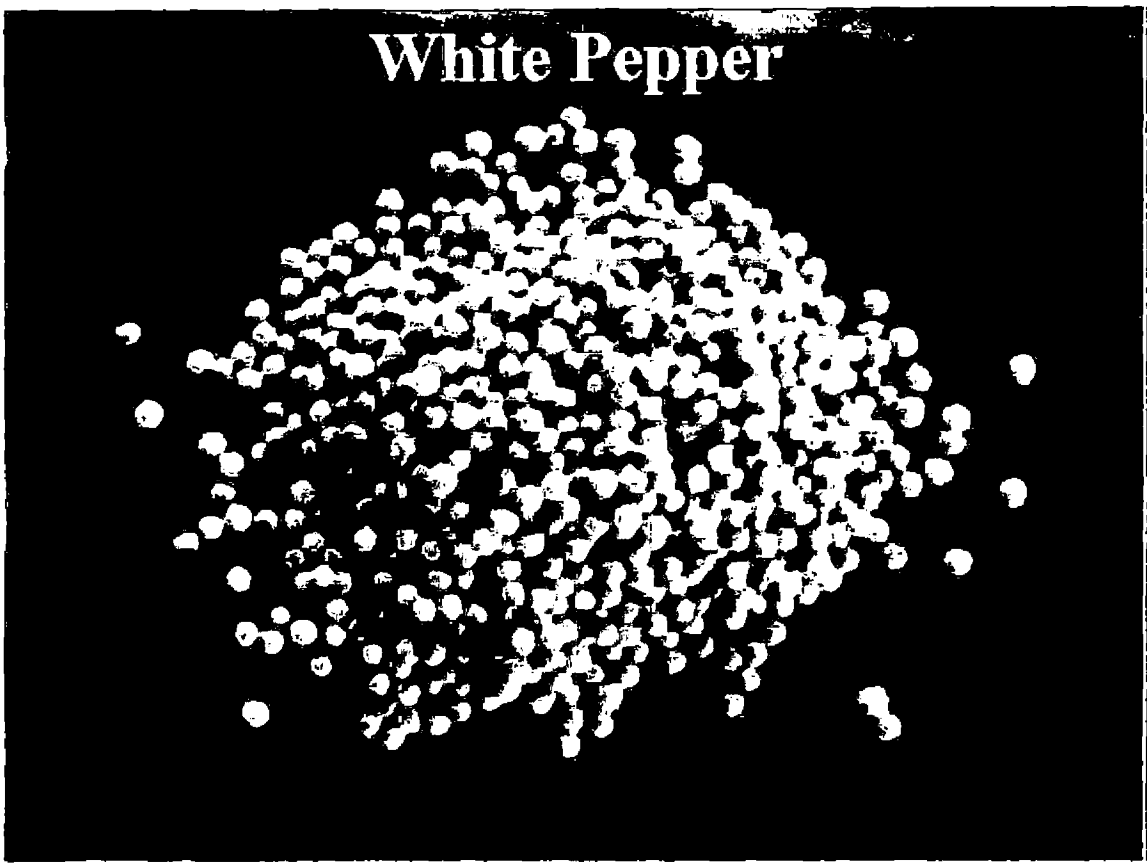
Like extremely - 9
Like very much - 8
Like moderately - 7
Like slightly - 6
Neither like or dislike - 5
Dislike slightly - 4
Dislike moderately - 3
Dislike very much - 2
Dislike extremely - 1

Date:

Name :
Signature:

APPENDIX-II

White pepper produced by microbial fermentation by NIIST (National Interdisciplinary Institute of Science and Technology), Trivandrum.



APPENDIX-III

MEDIA COMPOSITION

Potato Dextrose Agar (Fungi)

Potato	– 200gm
NaCl	– 5gm
Beef Extract	– 3.0 g
Agar	– 20.0g
Distilled water	– 1000mL
P^H	- 7.0

Asparagine Medium (cellulolytic medium)

$(NH)_2SO_4$	– 0.5g
L. Asparagine	– 0.5g
K_2HPO4	– 0.5g
KCl	– 05g
$MgSO_4.7H2O$	– 0..5g
$CaCl_2$	– 0.1g
Yeast Extract	– 0.5g
Cellulose	– 10.0 g
(Filter Paper strips for enrichment)	
Distilled Water	– 1000Ml

APPENDIX-IV

MEDIA COMPOSITION CONTINUED

Nutrient agar (Bacteria)

Peptone- 5g

Nacl-5g

Beef extract- 3g

Agar- 20g

Distilled water -1000ml

P H – 7.0

MP-5 Medium(Pectinolytic medium)

Pectin -5.000g

Monopotassium phosphate- 4.000g

Diosodium phosphate- 6.000g

Ammonium sulphate- 2.000g

Yeast extract - 1.000g

Ferrous sulphate - 0.001g

Calcium chloride -0.001

Megnesium silphate - 0.200

Calcium chloride - 0.001

Boric acid -0.0001g

Agar - 15.000