"*Lagenaria siceraria* juice extraction method optimization through pectinase enzyme"

A Dissertation submitted in partial fulfillment of

the requirement for the award of the degree of

MASTERS OF SCIENCE

IN

BIOTECHNOLOGY

By

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Certificate

This is to certify that the dissertation report entitled "***Lagenaria siceraria* juice extraction method optimization through pectinase enzyme"** submitted by Divyansha Bawa in the partial fulfillment of the requirement for the award of the degree of Masters of Science in Biotechnology, Department of Biotechnology, Thapar University, Patiala, is a record of the student's own work carried out by her under my supervision and guidance. This dissertation fulfills the requirements as per the regulations of the university and meets all the necessary standards for the submission. This report has not been submitted for the award of any other degree or certificate in this or any other university or institute.

Jyoti 29/8/17

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Candidate's Declaration

I, Divyansha Bawa (301501003), a bonafide student of Masters of Science in Biotechnology in Thapar University, Patiala, would like to declare that the dissertation report entitled "***Lagenaria siceraria*** **juice extraction method optimization through pectinase enzyme**" submitted by me in the partial fulfillment of the requirement for the award of the degree of Masters of Science in Biotechnology, is my original work which I have done in the period of six months from January 10 to July 15 under the supervision of Dr. Jyoti Rani, Assistant Professor (Food Technology), Department of Biotechnology, Thapar University, Patiala. This report has not been submitted for the award of any other degree or certificate in this or any other university or institute.

Place: Patiala

Date: 29.08.17

Divyansha

Divyansha Bawa

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ABSTRACT

The current research project was focused to develop a process which should eco- friendly and generate less waste or by-product with the complete utilization of the food itself. So, the pectinase assisted juice extraction method was optimizes by standardization of various parameters. The procedure involves incubating the bottle gourd pulp with commercial pectinase enzyme followed by filtration with muslin cloth. Certain parameters were optimized like the temperature of incubation was standardized at 45^{o}C, time of incubation was 4 hours, the concentration of enzyme added was 0.05 percent w/v and the blanching period was standardized at 15 minutes. Spiced version of pectinase assisted bottle gourd juice was prepared by addition of certain spices to enhance its overall acceptability.

All three juices: control juice, pectinase assisted juice and spiced version drink, were analyzed compared for various parameters like pH, Total soluble sugars, turbidity and percentage yield. The per cent acidity, vitamin C, tannins and flavonoids content of all three juices were determined by performing various assays. On evaluating these parameters, it was found that the tannins content (7.37µg/ml, 9µg/ml and 12.9µg/ml); per cent acidity (1.1per cent, 1per cent and 1.23per cent); Vitamin C content (0.187mg/ml, 0.491mg/ml and 0.516mg/ml) and flavonoids content (360mg/ml, 530mg/ml and 560mg/ml).

The pH of the juices was: 6.66 (control), 5.2 (pectinase assisted) and 5.0 (spiced version). pH decreased with the enzymatic treatment. Total Soluble Sugars was: 4.2 (control), 6.2 (pectinase assisted) and 8.1 (spiced version). TSS increased with the treatment. The turbidity decreased with the pectinase treatment as in control juice it was 1.0397 whereas in pectinase assisted juice it was 0.0977.

The formulated drink then undergone sensory analysis on Hedonic 9 point scale and it scored 8.76 which means it was liked very much by the panel. Statistical analysis of the obtained results was done to check that whether the results obtained are significant or not. ANOVA was performed and it was found that all the results were significant. Pearson correlation was used to determine the relationship between the parameters. It was observed that Total soluble solids and percent acidity; and TSS and pH were negatively correlated whereas the pH and per cent acidity were positively correlated.

Keywords: Control juice, pectinase assisted bottle gourd juice, spiced version of bottle gourd juice, pH, Total soluble sugars, Tannins, Vitamin C, Flavonoids, percentage yield, per cent acidity, pectinase, turbidity, pectin.

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CHAPTER – I

INTRODUCTION:

With the outbreak of chronic diseases, health has become the spearhead of scientific research for finding novel foods and strategies to tackle public health burden. Nutritional and pharmaceutical sciences have observed an increment in the scientific literatures concerned towards the use of food plants because of their numerous health benefits and potential clinical applications. Scientists now have understood that if nutrition science and drug therapy work together then they could confer optimum outcomes in fight against diseases. The prophylactic advantage of food plants is being investigated for their use as novel medicinal remedies because of the presence of pharmacologically active compounds. (Nelvana Ramalingum *et al*., 2014)

The classical notion of "adequate nutrition," that is, a diet that provides nutrients in sufficient quantities to satisfy particular organic needs, is being gradually replaced by the concept of "optimal nutrition" that is food components are having the potential to promote health, improve general well-being, and reduce the risk of developing certain illnesses. (Ramadan MF *et al*., 2012)

The rapidly growing interaction between the nutrition and medicine is the modern version of an ancient philosophy of food and medicine suggested by Hippocrates (460–370 BC) statement, **"Let food be thy medicine and medicine be thy food"**. (McGuire and Beerman, 2007)

It is believed that herbal remedies are less damaging and comparatively safer than synthetic drugs to the human body. Therefore the laboratories from all around the world are engaged in screening of the plants for biological activities with therapeutics potential. So, now the importance of the food in the management of diseases must be looked upon. (Kumar A. *et al*., 2012)

Bottle gourd (*Lagenaria siceraria*) belongs to the *Cucurbitaceae* family. It is an important vegetable crop of India and also known by other names in various parts of the world like calabash, opo squash etc. Since, bottle gourd belongs to a medicinal family; it plays a major role in the treatment of several diseases. It comprises of several organic chemical compounds including vitamin B complex, pectin, dietary soluble fibers, ascorbic acid, beta-carotene, amino acids and certain minerals. Therefore, it can be considered to have a great impact on therapeutic health benefits.

Phenotypic characteristics:

Bottle gourd is a popular vegetable crop and cultivated almost everywhere. This vegetable is extensively grown in India. Bottle gourd is a vigorous, annual and climbing vine having large leaves and a lush appearance. Bottle gourd is harvested young in three months and its flowers are solitary, chalky white in color and open at night. The vegetable has a light green and smooth skin with white colored flesh inside. They grow in a variety of shapes like they can be small and bottle shaped, huge and rounded, or slim and serpentine. The name is so due to its bottle like shape. It was first originated from tropical Africa and then spread across the world. (http://www.asiafarming.com/bottle-gourd-cultivation/)

Fig.1 Bottle gourd (round and short variety)

Health Benefits

- **Low in Calorie and Fat:** Bottle gourd has calories12-14kcal per 100g serving. It has negligible amount of fat (0.02g/100g). Thus, it is excellent for light and low calorie diet which makes it good for children and people with some digestive disorders.
- **Good source of vitamins:** It is an excellent source of vitamins. It is rich in vitamin B complex and vitamin C. It also has Vitamin A and E in adequate amounts.
- **Rich in antioxidants:** It is rich in antioxidants. Anti-oxidants neutralize the effect of free radicals which are produced in the body as a result of body's normal metabolism or by some environmental (UV rays) and chemical factors. These free radicals are also known as reactive oxygen species (ROS). They cause cell damage and diseases like cancer and cardiovascular problems. So, these must be neutralized or removed.
- **Cardiotonic**: Term used in Ayurveda for bottle gourd as it is good for heart. It reduces blood cholesterol and triglyceride levels.
- **Has Cooling Effect:** Bottle gourd comprises 96 per cent water and thus has a cooling, calming and diuretic effect on the body as described in Ayurveda. It is also effective against constipation.

- **Cures diabetes:** Bottle gourd has potential to lower the blood glucose levels because of good dietary fibre and as such good for diabetic patients.
- Effective in fever, asthma, cough and pain.
- **Cures digestive problems**: Bottle gourd is rich in dietary fibres. Both soluble and insoluble fibres are present and they help in curing in constipation, flatulence and even piles.
- Bottle gourd is recommended by Ayurvedic doctors for reducing and curing liver inflammation.

Occasional toxicity:

Bottle gourd contains **cucurbitacins**, like the other members of the gourd family, which is known to be a toxin at higher concentration. It is responsible for the bitter taste and may cause ulcers in the stomach. In extreme cases it could cause death too. But the plant on the other side is not so toxic and is safe to consume. The excessive bitterness and toxicity may be due to over ripening of the vegetable or improper storage.

To avoid the poisoning one must taste a small piece of it before consuming ensuring not taste bitter. If it is found to be very bitter then it must not be consumed and discarded immediately. It should be ensured that the bottle gourd juice not to be mixed with any other juice which may cause masking effect on the flavor of the fruit juice because of cucurbitacin flavor dominance. (S. K. Sharma *et al.,* 2012)

PRESERVATION:

The moisture content of fresh fruits and vegetables is usually more than 80per cent; which makes them highly perishable in nature. The world fruit production was about 609 million MT in year 2010-11 (according to FAO, 2011). It is estimated that nearly 20-40 per cent of the fruits are lost due to spoilage, mishandling during transportation and lack of cold storage and processing techniques.

In India approximately 1.5 per cent of the total fruits and vegetables produced are processed and more than 20-25 per cent of the total harvest gets spoiled before utilization. Food preservation ensures conservation and better utilization of fruits and vegetables and utilizing the surplus during the off-season. It is necessary to employ modern techniques to extend the storage life for the better distribution and utilization even in the off-season. The fruits and vegetables can be preserved by converting it into products like jam, jelly, fruit bar, juice, pickle, murabba etc. to prolong their utilizable lifespan. Juice preparation is one of the easiest way to preserve fruits and vegetables (Kumar S, 2015).

JUICE:

The production of fruit and vegetable juices is important both from the human health and commercial standpoints. The availability of nutrition from fruits and vegetables to a wide range of consumers is facilitated throughout the year by means of marketing of juices. The demand of fruit juices is increasing day by day with the increase of health awareness among the people. Most of juice extraction processes are not producing satisfactory quality and quantity of juices. The traditional methods of bottle gourd juice extraction are through the use of mechanical presses like traditional rack and cloth press, screw presses, horizontal press, and the belt press. Juice extraction can also be done by using diffusion extraction, decanter centrifuge, screw type juice extractor, fruit pulper. But the yield of juice extraction by all these methods is not satisfactory enough. Hence, there is a great need for the modern and better methods to be explored for enhancing the yield and sensory qualities of the juice. Earlier in 1930s, when the juice production was initiated, the yield was very low. The industries faced a lot of difficulties in the filtration and clarification process. Then, these difficulties were replaced by introduction of the enzymes in the juice production process. Enzymes like pectinases, cellulases are used in juice production and these are known as macerating enzymes. About 96 per cent of water is present in it most of which is present in vacuole which is extracted by mechanically squeezing the pulp. (Bhardwaj R.L., *et al*., 2011).

Fig.2: Bottle gourd juice

Pectin:

The cell wall of fruits and vegetables is mainly composed of pectin. It is a complex plant polysaccharide which provides structure and firmness to the plant tissues. It is a polymer of galacturonan (α-galacturonic acid). During the ripening process, pectin's structure is altered by the enzymes which are naturally present in the fruits. These enzymes cleave the pectin chains along with the side chains attached to it, which creates the main chain. Pectin is converted from insoluble complex polysaccharide to small soluble polymers. The plant tissues becomes soft in ripening as the grip of pectin on the surrounding cell walls loosened. (Sharma H.P. *et al.,* 2014)

Pectinase:

Pectinases are also known as pectinolytic enzymes are a heterogeneous group of related enzymes widely distributed in plants and microorganisms that break down the pectic

substances. In plants, these enzymes play an important role in the fruit ripening. Pectinases are cell wall degrading enzymes secreted by pathogens. These enzymes are responsible for degradation of side chains of pectin and its complete degradation.

Pectins have fiber like structures which makes the clarification process more complicated and difficult. Pectinases are the enzymes which degrade these pectin fibres and cause the flocculation of pectin protein complexes. The resulting juice will be less viscous and short chains of pectin are suiTable for subsequent filtration process. Microbial pectinases are more advantageous over other ultrafilteration and other chemical processes as it makes the process cost- effective, eco-friendly and also provides remarkable results. Enzymatic degradation of biomaterials depends upon the type of enzyme, application temperature, incubation time, agitation, concentration, and pH. These parameters require optimization for maximum bioconversion. (Chadha R. *et al*., 2003)

Enzymatic extraction of juices results in higher yield increases the release of various phenolics and other nutritionally important components in the juice, reduced viscosity, decreased turbidity and improved filterability. Juice appearance is also improved by enzymatic clarification. Enzymes prevent darkening of juices. During the production of juice, use of enzymes increases both yield and process performance without additional capital investment. **Enzymes are generally used in two steps:**

(1) After crushing of the vegetable, increases the juice yield and reduces the processing time and also improves the extraction of valuable fruit components, and

(2) After the juice extraction, for clarification, increasing the filtration rate and stability of the final product. (Bhat M.K., 2000).

OBJECTIVES:

1. Optimization of enzyme assisted juice extraction method *via.* different factors standardization for enzyme activity and quality, sensory analysis of thesame.
2. Comparing the enzyme assisted juice extracted juice to normal juice for quality and sensory analysis.

CHAPTER – II

REVIEW OF LITERATURE:

2.1. Bottle Gourd (*Lagenaria siceraria*):

2.1.1. Plant profile and morphology:

Bottle gourd also known as Calabash and *Lagenaria siceraria* (scientific name) is a very popular crop in India and is yellowish-green in color. Its flesh is white in color and has white colored seeds embedded in its spongy flesh. It is one of the main culinary vegetables in many tropical and temperate regions of the world. It grows on a fast growing, annual climber vine that needs adequate sunlight for flowering and fruiting. It can be grown in a wide range of soils and need support for a spreading. Its highly branched stems bear deep green, broad leaves (just similar as that in pumpkins), and white flowers in the summer. After about 75 days from the plantation, the young and tender fruits are harvested. The surface of bottle gourd is marked by inconspicuous ridges that run lengthwise. It is cultivated in the humid weather of Sri Lanka, India, Pakistan, Indonesia, Malaysia, Philippines, China, tropical Africa and South America. It is also known by other names like bottle squash or white gourd or trumpet gourd (English); lauki or ghia (Hindi); ghiya (Urdu); alabu (Sanskrit); lauki or ghia (Hindi); dudhi or tumbadi (Gujarati); sorakkai (Tamil); chorakkaurdu (Malayalam); and calabash gourd. ("Bottle gourd (Calabash) nutrition facts")

L. siceraria (bottle gourd) is an important member of the Cucurbitaceae family and its taxonomical classification is depicted in **Table 1.** The word *Lagenaria* is originated from a Latin word lagenos/lagena which means a flask and cucurbita denotes gourd. The name of species *siceraria* probably refers to the fruit which is useful when it is mature and dry. This fruit contains six species which are classified into two groups, domestic and wild perennial. There is one domestic species which is regularly utilized by human beings and the rest of the five species are non-utilized source which are present in wild region.

The bottle gourd fruits and seeds have many medicinal values and are also considered as one of the predominate species in the southern nations of India. Based on the traditional uses of *L. siceraria*, it is clearly proven that the plant species possess a bio-active component in it. (Selvaraj *et al*., 2015)

Table 1: Scientific classification of Bottle Gourd

Kingdom:	**Plantae**
(unranked):	Angiosperms
(unranked):	Eudicots
(unranked):	Rosids
Division	*Magnoliophyta*
Class	*Magnoliopsida*
Order	Cucurbitales
Family:	Cucurbitaceae
Genus:	*Lagenaria*
Species:	*L. siceraria*

2.1.2 Composition of bottle gourd:

It is a rich source of vitamin B- complex and ascorbic acid along with pectin and also contains various saponins, fatty oils and alcohols. The fruit skin contains 17.5 per cent crude protein; 18.1 per cent cellulose; and 8.0 per cent lignin. (Gurpreet *et al*., 2014) The edible portion of *L. siceraria* contain carbohydrates, protein, fat, and minerals, including calcium and phosphorous, and is a good source of *β*-carotene, pectin dietary soluble fibres, and amino acids (Modgil et al., 2004). *L. siceraria* contains glucose and fructose (about 1:1 ratio) and trace amounts of sucrose and unidentified derivatives of mono- and di-caffeoylquinic acid (Calabrese *et al*., 1999). The seeds contain alkaloids, steroids, carbohydrates, fats, proteins, potassium, sodium, calcium, zinc, and iron etc.

Table 2: Phytochemical profile of bottle gourd

S.No.	**Tests**	**Methanolic Extract**	**Water Extract**
1	Alkaloids	-	-
2	Carbohydrates	+	+
3	Phytosterols	+	-
4	Fixed oils and fats	-	-
5	Saponins	+	+
6	Phenolic compounds and tannins	+	+
7	Proteins and amino acids	+	+
8	Gums and mucilage	-	-
9	Volatile oils	-	-
10	Flavonoids	+	+

2121 Nutritional Composition:

The bottle gourd has very low energy value and very low fat content. It has zero cholesterol and its regular consumption is known to reduce the blood cholesterol levels. It is rich in vitamins, minerals and other compounds like alkaloids, phenols, essential fixed oils, tannins, flavonoids and steroidal compounds. It has very high water content (about 96per cent) which makes it very light, easy to digest and has a cooling effect on the body.

Table 3: General composition of bottle gourd (Source: USDA)

Nutrient Components	Value	Percentage of RDA (%)
Water moisture	96.1g	-
Protein	0.6g	1
Total Fat	0.02g	0.5
Cholesterol	0mg	0
Fibre	1.2g	1
Carbohydrates	3.39g	2.5
Energy	12-14Kcal	<1

2122 Flavonoids:

Flavonoids are large class of plant pigments and are composed of polyphenols. They have anti-oxidant and anti-inflammatory properties. These are reported to cure cancer and cardiovascular diseases. Four C-glycosylflavones were isolated by Miroslawa and Cisowski in bottle gourd. These were- 7-0-glucosyl-6-C-glucoside apigenin, 6-C-glucoside apigenin, 6-C-glucoside luteolin, and 7, 4'-O-diglucosyl-6-C-glucoside apigenin. These were then identified by the spectroscopic analysis: UV, FD-MS, LSI-MS, H-NMR, C-NMR, melting point, and enzymatic hydrolysis. (Miroslawa and Cisowski, 1995)

2123 Vitamins:

Bottle gourd is rich in vitamins especially vitamin B and C. The 100g edible portion of bottle gourd contains vitamin A, B complex and vitamin C in following amount given in the **Table 4**. Bottle gourd is known to have very high amount of choline16.02mg/g (dry basis). Choline is a lipotropic factor and is required for the normal brain development and nerve functioning. It is also known to be required for cure of mental disorders. The amount of choline present in the bottle gourd is the highest among all the vegetables known to man till date.

Table 4: **Vitamin content of bottle gourd (Source: USDA)**

Vitamins	**Value**	**Percentage of RDA (%)**
Folate (B_9)	6 µg	1.5
Niacin (B_3)	320 mg	2
Pantothenic acid (B_5)	0.152 mg	3
Pyridoxine (B_6)	0.04 mg	3
Riboflavin (B_2)	0.022mg	2
Thiamin (B_1)	0.029 mg	2.5
Vitamin A	16 IU	0.5
Vitamin C	10.1 mg	17

2124 Minerals:

As vitamins, the minerals are also required for proper growth and development of the body and these can be acquired *via* food. All five minerals that humans require for normal growth and development are calcium, magnesium, potassium, phosphorous and sodium are present in the bottle gourd in sufficient amounts. These are listed in the **Table 5**. Some trace elements are also present like zinc, iron, selenium, and copper. These trace elements have some specific biochemical function in the body. All essential minerals and the trace elements that are present in 100g edible portion of bottle gourd are listed in the following Table. The RDA per cent is the Recommended Dietary Allowance which is the average daily level of intake sufficient to meet the nutrient requirements of nearly all (97-98 per cent) healthy people.

Table 5: Mineral composition of Bottle Gourd (Modgil, M *et al*., 2004)

Minerals	**Value (mg)**	**Percentage of RDA (%)**
Calcium	26	2.6
Magnesium	11	3
Phosphorus	13	2
Iron	0.25	2.5
Sodium	2	<1
Potassium	150	3
Copper	0.034	4
Selenium	0.2	<1
Zinc	0.7	6.5

2125 Proteins and amino acids:

A novel protein, named as *langenin*, was isolated from the seeds of bottle gourd. This protein is documented to be a Ribosome-Inactivating Protein (RIP). RIP is reported to be a potent active phytochemical that shows ribonucleolytic activity. This protein catalytically cleaves the N-glycosidic bonds of adenine in specific RNA sequences which results in the inhibition of protein synthesis. Mock *et al*. (1996) reported the RNase activity of RIPs. Byers *et al.,* reported that these RIPs can be used as anti-HIV agents. Other biological activities of RIPs like anti-tumour, anti-proliferative, immunomodulatory and anti-fertility activity were reported by Ng *et al.* (1992) The potential application of RIPs, they can be conjugated with antibodies to form immunotoxins for cancer therapy. (B. V. Ghule *et al*., 2009). The hydroalcohlic extract of bottle gourd showed strong and dose dependent inhibition of lung cancer cell line (A549). These proteins were when expressed in the transgenic plants; the plants gain resistance against certain viral and fungal infections. (Irfan Ahmad *et al*., 2011).

Table 6: Amino acid content of bottle gourd (Source: USDA)

S.No.	Amino Acid	Value (in mg/g)
1	Leucine	0.8
2	Phenylalanine	0.9
3	Valine	0.3
4	Tyrosine	0.4
5	Alanine	0.5
6	Threonine	0.2
7	Glutamic acid	0.3
8	Serine	0.6
9	Aspartic acid	1.9
10	Cystine	0.6
11	Cysteine	0.3
12	Arginine	0.4
13	Proline	0.3

2126 Carbohydrates:

The total amount of carbohydrates present in 100 g edible portion of dry bottle gourd is 8.29 g. From that amount only 7.92 per cent are reducing sugars, 0.029 per cent are non-reducing sugars, 1.57 per cent is starch, 5.58 per cent hemicelluloses and 16.40per cent

cellulose is present. A water-soluble polysaccharide, isolated from fruiting bodies of *Lagenaria siceraria*, is composed of three units: methyl-α-d-galacturonate, 3-O-acetyl methyl-α-d galacturonate, and β-d-galactose and present in a ratio of nearly 1:1:1. This polysaccharide has been reported to show cytotoxic activity *in-vitro* against human breast adenocarcinoma cell line (MCF-7).

Table 7: Carbohydrates and dietary fibre constituents in bottle gourd (g/100g dry weight basis) (Madhu Modgil *et al*., 2004)

Attributes	**Bottle Gourd**	
	With peel	**Without peel**
Total Sugar	5.84	8.29
Reducing Sugar	5.22	7.92
Non- Reducing Sugar	0.65	0.29
Starch	1.31	1.57
Crude fibre	4.45	3.40
NDF	22.71	21.16
ADF	16.26	15.67
Hemicelluloses	6.45	5.58
Cellulose	16.07	16.40
Lignin	0.193	0.167

2127 Triterpenes:

Four new triterpenes were isolated:-

- 3 b-*O*-(*E*)-feruloyl-D:*C*-friedooleana-7, 9 (11)-dien-29-ol(1),
- 3b-*O*-(*E*)-coumaroyl-D:*C*-friedooleana-7,9(11)-dien-29-ol(2)
- 3b-*O*-(*E*) coumaroyl-d:*C*-friedooleana-7,9 (11)-dien-29-oic acid (3),
- methyl 2 b,3 b-dihydroxy-D:C-friedoolean-8-en-29-oate (6),

Following five triterpenes were already known with the sameskeleton,

- 3-epikarounidiol (4),
- 3-oxo-d:C-friedoolena-7, 9 (11)-dien-29-oic acid (5),
- bryonolol (7),
- bryononic acid (8),
- 20-epibryonolic acid (9)

These were isolated from the methanol extract from the stems of plant. Out of all these nine triterpenes, the compounds 3 and 9 have reported to show significant cytotoxic activity against the SK-Hep 1 cell line. (RP Prajapati *et al*., 2010).

2.12.8 Sterols:

Sterols are the precursors of the fat soluble vitamins (vitamin A, D, E and K). Phytochemical analysis of the bottle gourd has revealed that two sterols are present: Fucosterol and Campesterol. Fucosterol lowers the cholesterol absorption. It has anti–oxidant and anti-diabetic activity. Campesterol reduces the cholesterol absorption in the intestines (Shirwaikar and Sreenivasan, 1996).

2.12.9 Glycosides:

Glycosides are the compounds which are derived from the simple sugars. Many drugs and poisons of plant origin are commonly made of this. Plants usually store their chemicals in this form. The glycosides present in the bottle gourd are: Cucurbitacin B, D, G and H. Plants usually made them in the adverse conditions and use them as a defence against herbivores. These toxins are bitter in taste and are not dangerous for the human beings when these are administered in small amounts. These cucurbitacins helps in the removal of cholesterol from the body. Due to its sticky properties, it sticks to the cholesterol rich bile and remove out from the body. It also helps in removing lead and mercury toxins from the body.

2.12.10 Tannins:

Tannins are polyphenols. These are bitter tasting brownish compounds and are water soluble in nature. The tannins are widely distributed in many plants species. These compounds play a major role in the protection of the plant from the predation. Hence, now a day's these are also used as pesticides and in plant growth regulation. These are mainly present in the seeds of bottle gourd. Tannins recorded in bottle gourd have its highest content in the aqueous extract (12mg/100 g) and the lowest in ethyl acetate extract (2.5mg/100g). Tannins are known to be useful for the prevention of cancer as well as treatment of inflamed or ulcerated tissues. (Antia *et al*., 2013)

2.1.3 Traditional uses of various parts of the plant:

2.1.3.1 Plant as a whole: The fruits, stem, leaves, oil and seeds of the bottle gourd are traditionally used for the treatment of various disorders like hypertension, skin diseases, ulcers, piles, colitis, insanity, cardiac failure, diabetes and jaundice.

2.1.3.2 Fruit: The pulp is used as sedative, purgative, emetic, diuretic, antibilious and pectoral. It has very few calories so these are used for the preparation of curries and pickles.

2.1.3.3 Flowers: The flowers of bottle gourd are antidote of certain poison.

2.1.3.4 Stem bark and rind: The stem and the bark of the plant have a diuretic effect. It was rich in tannins.

2.1.3.5 Leaf: Crushed leaves are applied on the head for headache and also used for baldness. In Ayurveda, the leaves of bottle gourd are known to prevent premature greying and improve hair growth. It will not change the already grey hair to black but will stop more greying of the hairs as it contain B vitamins and has a cooling effect on the scalp. The decoction of the leaves is taken to cure flatulence. They are also used as poultices for mange, skin irritation and tumors.

2.1.3.6 Seeds: The seeds of the bottle gourd are vermifuge i.e. they are used to expel worms from the body.

2.1.3.7 Extract: The extract of the plant has antibiotic activity

2.1.3.8 Juice: The juice of LS is a wonderful remedy for heart problems as it reduces the cholesterol and triglyceride levels in the blood. It cures digestive and urinary disorders. It has a lot of dietary fibres which helps in curing constipation and flatulence. It even cures piles. The juice shows better effect in the treatment of insomnia, epilepsy and other nervous disorders due to the presence of high amount of choline.

It helps in breaking of calculus (stones) in the body. In summers it has a cooling effect on the body and is good to have as it prevents excessive loss of sodium from the body thus satiating thirst. This vegetable is good for heath of female reproductive system. It is extremely popular for weight loss when taken in noon.

2.1.3.9 Hard dried shells: Dry hard shells of the bottle gourd were used by tribal people for various purposes. They were used for making domestic utensils like pots, spoons, bottles, bowls and containers of specific types. These were also used for making stringed and wind instruments and pipes. At some places some tribal people uses them as floating material on the water bodies. (Kumar A. *et al*., 2012)

2.1.4 Pharmacological profile of *L. siceraria* (Table 8)

S. no.	Pharmacological benefits	Action	References
1	Hepatoprotective activity	Hepatotoxicity was induced in rats by intraperitoneal injection of CCl_4. The ethanolic extracts of *L. siceraria* juice extract were administered to the experimental rats. The hepatoprotective effect of these extracts was evaluated by the assay of liver. The toxic effect of CCl_4 was significantly controlled. The levels of serum bilirubin, protein and enzymes were significantly restored as compared to the standard drug Silymarin - treated groups.	Lakshmi BVS, *et al.*, 2011
2	Central nervous	Various extracts of the juice showed analgesic activity. It successfully reduced the nociception in dose dependant manner which is induced by acetic acid.	Pawar JC *et al*., 2010
3	Antioxidant activity	The juice extract of *Lagenaria siceraria* showed maximum antioxidant activity against *in vitro* models against DPPH. The fresh juice of the fruit also showed antiradical activity. This is because of the presence of ellagitannins.	Deshpande *et* al*., 2007*
4	Cardioprotective activity	Doxorubicin induced cardiotoxicity was significantly reduced by hydroalcohlic extract of bottle gourd.	Singh *et al.*, 2012
5	Diuretic Activity	The diuretic activity was evaluated by measuring various parameters like sodium, potassium and chloride concentration of urine, total urine volume.	Ghule *et al.*, 2007
6	Antihyperglycemic activity	Sterptozotocin induced hyperglycemia was treated by administration of MELS in rats. Glibenclamide was used as reference drug.	Ghule *et al.*, 2009
7	Antihyperlipidemic effect	Fat rich diet induced hypolipidemic rats were administered with the methanolic extract to evaluate its antihyperlipidemic activity. Atorvastatin was used as a standard drug. Significant reduction in the lipid levels was observed after 30 days.	Ghule *et al.*, 2009

8	Anticancer activity	EAC cells after treatment with MELS were inoculated into mice. A standard drug: 5-Fluorouracil was administered in another group of mice. Tumour growth response was studied and the results revealed that the MELS posses significant anticancer activity which might be due to its cytotoxic and antioxidant activity.	Saha P *et al.*, 2011
9	Antidepressant activity	The antidepressant activity of MELS was evaluated using forced swim model. Imipramine was used as a reference standard drug. The dose dependent reduction in duration of immobility in behaviour despair test. Experimental results revealed that *L. siceraria* fruits posses significant antidepressant activity.	Prajapati R *et al.*, 2011
12	Immunom odulatory activity	Oral administration of MELS significantly inhibited delayed-type hypersensitivity reaction in rats. A dose-dependent increase in both primary and secondary antibody titer was observed. Fractions also significantly increased both white blood cell and lymphocyte count.	Gangwal A *et al.*, 2008
13	Antistress and adaptogenic property	The antistress activity of the ethanolic extracts was evaluated on the albino rats against the standard reference drug, Withania somnifera. Extract treated animals showed both antistress and adaptogenic properties.	Lakshmi B V , *et al.*, 2009
14	Antimicrobial activity	The antimicrobial activity of MELS was evaluated. The results revealed that it shows activity against *Pseudomonas aeruginosa* and *Streptococcus pyogenes*, but not against clinical isolates of *S. aureus* and *E. coli*. Thus LS can be used to treat various skin disorders.	Goji M, *et a.*,2006

2.2 Preservation by juicing:

Fruits and vegetables are important source of nutrients. The moisture content in them is more than 80 per cent which made them perishable. Like other fruits and vegetables, bottle gourd is also perishable in nature and have high activity of peroxidases due to that its post-harvest shelf life is very limited. There should be adequate processing of these fruits and vegetables to prevent their spoilage. Food spoilage has become a major problem. According to FAO in 2010, it was estimated that in the year 2010-11, the world fruit production was about 609 million MT and out of which nearly 20-40 per cent were lost due to spoilage, improper storage, mishandling and lack of processing techniques. India is the second largest producer of fruits and vegetables in the world, but only 1.5 per cent of total fruits and vegetables produced were processed. Food preservation ensures better utilization and conservation of food. Processed food can be preserved and used even in off-season. So, to ensure minimum spoilage and maximum utilization and distribution, proper processing techniques must be developed. There are numerous ways with which food can be preserved like jam, jelly, juice, fruit bar, pickle etc. Fruit juice production is one of the easiest and most efficient one. By making juice out of bottle gourd, one can ensure that the nutritional components from it would be available to a wide range of consumers throughout the year. (Kumar S, 2015)

Juice is made up of water; soluble solids such as sugars and organic acids; vitamins and minerals; pectic substances; pigments; flavor and aroma components and very little amount of proteins and fats. The juice consumption has always been associated with a healthy diet. The demand for juice has increase with the increase in awareness for health among the people. But the supply is limited and could not meet with high demands. The reason for this is that the traditional method of juice production has low yields. The production of fruits and vegetable juice include three main steps: extraction, clarification and stabilization. The traditional methods for juice extraction include mechanical presses like screw press; rack and cloth press; and belt press etc. The yield of juice by such mechanical presses was less efficient as fruits and vegetables contain a lot of fibres. The cell wall is made up of a complex polysaccharide called pectin. So the fruits and vegetables must be pre-treated with some enzymes like pectinases which breaks down this pectin, releasing the moisture from the vacuoles of the plant cells and thus increasing the yield. (H. P. Sharma *et al.,* 2014)

2.3 Pectin:

Pectin is a complex plant polysaccharide which gives the firmness and structure to the plant tissues. It comprises of one-third of the macromolecules of primary cell wall and is the major component of middle lamellae. It acts as a binding material. In the unripe fruits and vegetables, insoluble pectin is bound to the cellulose microfibrills. This provides rigidity to the cell wall. During the process of ripening, the structure of the pectin is altered by some enzymes naturally present in the fruits and vegetables. These enzymes cleaves the pectin chains along with the side chains thus as a result the pectin becomes more soluble and its grip on the surrounding cell walls gets loosened. As a result the plant tissue gets soften. (http://shodhganga.inflibnet.ac.in/bitstream/chapter_2.pdf)

2.3.1 Structure of pectin:

Pectin is a polymer of α-D-galacturonan units which are linked to each other by 1,4 glycosidic bonds. The carboxylic groups of α-D-galacturonan are partially esterified by methylgroups. These are sometimes partially or fully neutralized by sodium, potassium or ammonium ions. (Sharma B.R. *et al*., 2006)

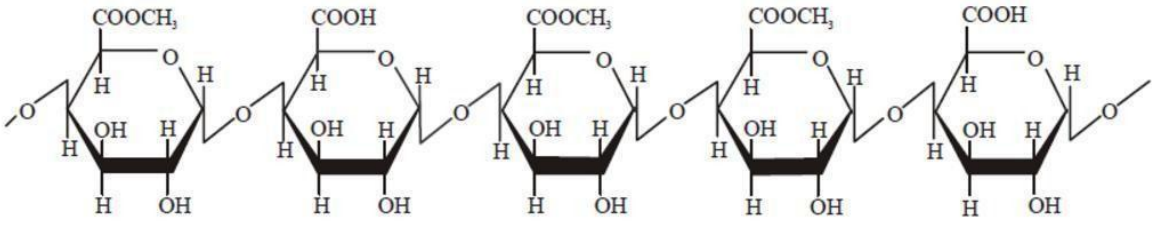


Fig.3: Pectin with a variable number of methyl ester groups (Pawar H A *et al.*, 2015)

These are generally classified into two types: Homogalacturonan and Heterogalacturonan

1. **Homogalacturonan:** It is a linear polymer and referred as the smooth or region of pectin. It is composed of α-D-galacturonan units linearly linked by 1,4 glycosidic bonds. These can be acetylated or methyl esterified. They have been isolated from sunflower head and apple pectin but these are less common in nature. (Sharma BR *et al*., 2006)
2. **Heterogalacturonan:** These are further of three types: (Sharma B.R. *et al*., 2006)

- Xlyogalacturonan: It is α-1,4 linked D-galacturonan chain which is substituted by β-D xylose at C-3 position.
- Rhamnogalacturonan I: The D-galacturonic acid units in the backbone are interrupted at various places by α-1,2-linked L-rhamnose residues. To these residues, long galactan and arabinan chains can be attached at O-4 position.
- Rhamnogalacturonan II: In this, cluster of comlex side chains are attached at O-2 or O-3 position in the backbone.

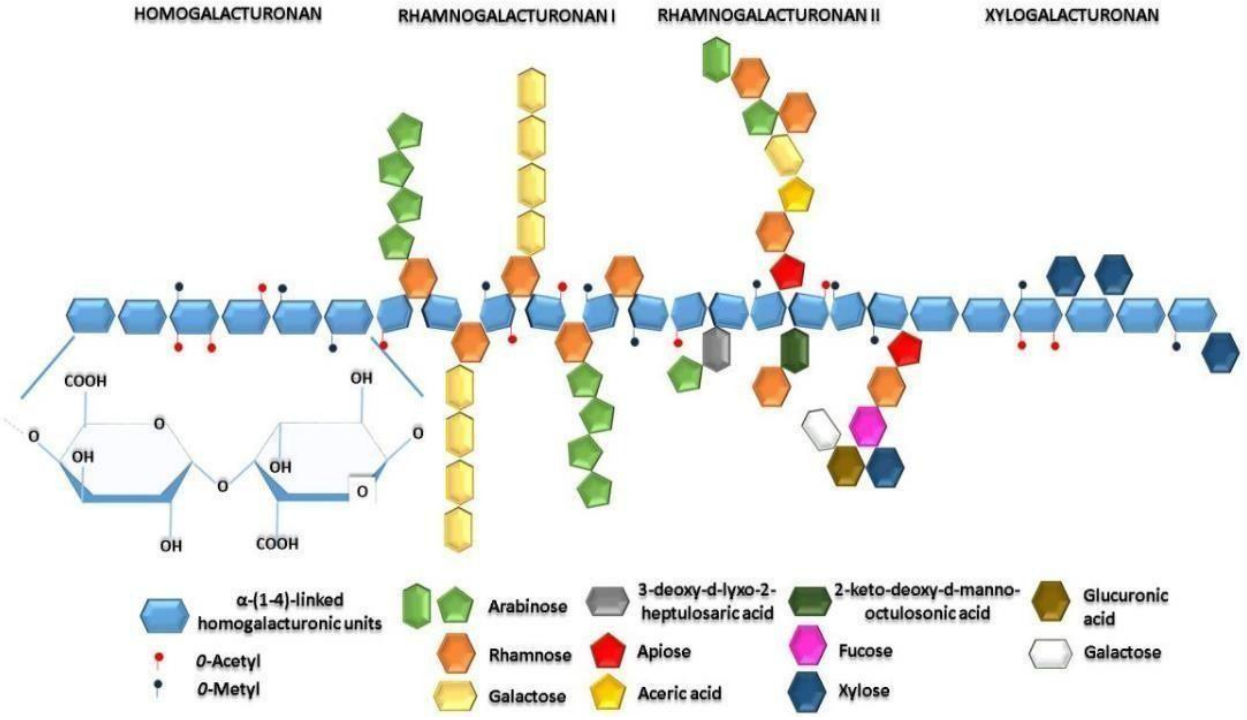


Fig.4: Structure of pectin chain (Albuquerque, P *et al.*, 2016)

2.3.2 Pectin hinders the extraction process:

When the pectin rich fruits and vegetables are mechanically crushed, a highly viscous puree is obtained from which juice extraction is quite difficult by pressing. When the fruits and vegetables were mechanically crushed, the juice remains bound to the pulp and forms a jellified mass. The pectin increases the viscosity of the juice and it also increases the water retention. The viscous juice was not appealing. Thus to improve the yield of the juice along with the enhancement of sensory parameters like taste, aroma and flavor etc.; improving the nutritional quality of the juice and finally to reduce the processing time; this pectin needs to

be degraded. The pectin can be degraded with the help of pectin degrading enzymes and also known as pectinolytic enzymes, example- pectinase. The crush fruits and vegetables should be pre-treated with pectinase prior to pressing to enhance the yield and to improve other parameters mentioned above. (Kumar S, 2015)

2.4 Pectinase:

Pectinase is a pectinolytic enzyme which breaks down the pectic substances. These are widely distributed in plants and micro-organisms. In plants, these are naturally present and plays important role in ripening. In microbes, these are cell wall degrading enzymes and are released by pathogens. In non-pathogenic microbes, these enzymes help in decaying of dead plant material and thus recycle the carbon compounds. (Bhat M. K., 2000)

2.4.1 Classification of pectinases:

These are of three types based on their specificity in action on pectic substances: Polygalacturonase, Pectate Lyase and Polymethylesterases

Polygalacturonase (PG):

It cleaves the α-D-galacturonan linkages in the homogalacturonan. These enzymes prefer non-methylated esterified pectins (pectic acids). These are commonly found in *Aspergillus niger*, *Erwina* and *Bacillus subtilis.* The function of this enzyme is to soften and sweeten the fruits and vegetables during the ripening process. The rate of hydrolysis of pectin depends upon the length of polysaccharide chain. They can be further of two types: (1) Endopolygalacturonase: It hydrolyses the chain in random fashion and results in oligogalacturonans. (2) Exopolygalacturonase: They hydrolyse the ends of the non-reducing polymers and generates monosaccharide galacturonans.

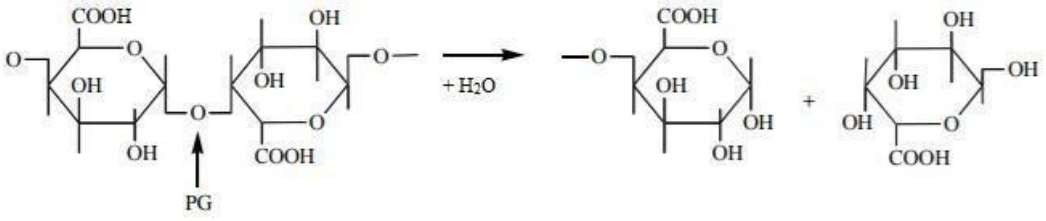


Fig.5: Mode of action of Polygalacturonase (PG)

Table 9: Properties of some polygalaturonases (Dhembare, A. J *et al.*, 2015)

Source	Enzyme	Optimum pH	Optimum Temperature
Aspergillus carbonarius	Endo-PG I	4.0	55
	Endo-PG II	4.1	50
	Endo-PG III	4.3	55
Streptomyces lydicus	Exo-PG	6.0	50
Aspergillus giganteus	Exo-PG	6.0	55
Aspergillus kawakii	Endo-PGI	4.5	50
Aspergillus niger	PG	4.6	40
Bacillus sp.	Exo-PG	7.0	60
Fusarium moliniforme	Endo-PG I	4.8	45
	Endo-PG II	5.3	40
Mucor flavus	Endo-PGL	3.5-5.5	45
Pectinase CCM*	PG	4.0	50
Pectinex 3XL*	PG	4.7	50
Penicillium frequentans	Exo-PG I	3.9	50
	Exo-PG II	5.0	50
	Exo-PG III	5.8	50
Rapidase C80*	PG	4.0	55
Rhizopus oryzae	Endo-PG	4.5	45
Termoascus aurantiacus	Endo-PG	5.5	60-65

Pectate Lyase (PAL):

It is also known as pectin lyase. It breaks the α-D-galacturonan linkage in homogalacturonan and heterogalacturonan by trans or β-elimination which results in a double bond formation between C4 and C5 on the non-reducing end of the chain. These enzymes act on highly methylated pectins. (Sharma H. P., *et al*., 2014)

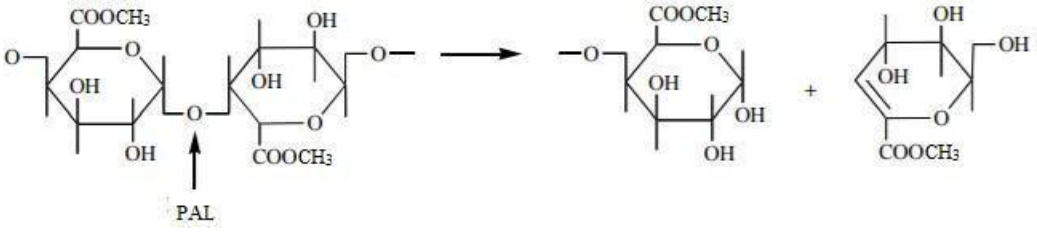


Fig.6: Mode of action of Pectate Lyase (PAL)

Some examples of Pectate lyase, the microbes producing them and optimum conditions for their action are listed in the **Table10**.

Table10: Properties of some Pectate lyases (Dhembare, A. J *et al.*, 2015)

Source	Enzyme	Optimum pH	Optimum Temperature (°C)
Aspergillus japonicus	PL	4.5-5.5	-
Aspergillus giganteus	PL	8.5	50
Aspergillus niger	PGL (PeL I)	6.0	50
	PGL (PeL II)	4.6	50
	PGL (PeL III)	4.2	35
Bacillus macerans	PGL	8.0-8.5	63-67
Bacillus pumilus	PGL	8.5	70
Bacillus sp. DT7	PL	8.0	60
Bacillus sp. KSM-P7	PGL (Pel-7)	10.5	60-65
Bacillus subtilis	PGL (Pel C)	10.0	65
Debaryomyces nepalensis	PL	6.4	35
	PGL	7.5	32
Grindamyl 3PA*	PL	6.0	40
Paenibacillus barcinonensis	PGL (Pel A)	10.0	55
Pectinase CCM*	PL	6.0	40
Pectinex 3XL*	PL	5.0-6.5	35
Penicillium canescens	PL A	5.0-5.5	60
Penicillium italicum	PL (PNL)	6.0-7.0	50
Rapidase C80*	PL	6.0	40-45

Polymethylesterases (PME):

This enzyme cleaves the methyl and acetyl groups from pectin molecule. They act toward high methylated pectin. PME de-esterifies the methyl groups on the galacturonic acid backbone and creates charged regions which forms complexes with calcium ions. These complexes forms gels and clarify the juice. (Sharma H. P., *et al*., 2014)

2.5 Advantages of using pectinase in juice extraction:

The use of enzymes in food processing has a greater impact of biotechnology. Pectinases were one of the first enzymes to be commercially applied in processing of juices and wines.

- **Juice recovery**: Extraction of juice using pectinases claimed to enhance the yield of juice from fruits and vegetables. The condition should be optimized prior to treatment so that the enzyme could function maximally. Singh *et al.,* (2012) had shown that the

yield increased by 17.5 per cent in bael fruit. Similarly Joshi *et al.*, (2011) had shown increased yield from 50 per cent to 80 per cent in apricot, 52 per cent to 78 per cent in plum and 38 per cent to 63 per cent in peach.

- **Clarification of juice**: Juice are usually cloudy because of presence of certain polysaccharides like pectin, cellulose, hemicellulose, lignin and starch. Clear juices are more appealing to the consumers. High concentration of pectin makes the juice cloudy. Thus pectinases are required to cleave this insoluble pectin into soluble oligo saccharides which are digestible. Brown and Ough, (1981) used pectinase enzymes in grape juice maceration & increased the juice clarity and filterability by 100per cent.
- **Total Soluble Solids (TSS):** TSS of the juice was also increased by enzymatic treatment. Yusof and Ibrahim (1994) reported that by using pectinases for soursop significantly increased the soluble solids content from 6.8 to 7.3°Brix within the first hour of incubation. Increasing the incubation time to two or three hours did not cause any significant increase in the TSS content. Vijayanand P. *et al.,* (2010) reported an increase in TSS in litchi juice and as shown in the following figure:-

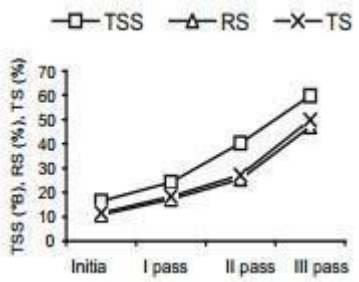


Fig.7: Effect of pectinase treatment on TSS

- **Decrease in viscosity:** Pectinases reduced the viscosity of the juices and made them more appealing. Abdullah *et al.* (2007) reported reduction in viscosity of carambola juice treating it with 0.1 per cent enzyme concentration for 20min at 30°C incubation temperature.
- **pH:** The pH of the juice decreased with the increase in enzyme concentration. Yusof and Ibrahim (1994) observed that for each level of enzyme used, decrease in pH was not significant for the first hour of incubation. As the incubation time was increasing (2-3h), the decrease in pH values was significantly observed. The values after 2 and 3hr of incubation were almost the same. According to Woodroof and Phillips (1981) a decrease in pH from 4.5 to 3.0 could increase the shelf life of juice to about 3 times.

Vijayanand, P, *et al.,* 2010, reported an decrease in pH in litchi juice and shown in the following figure:

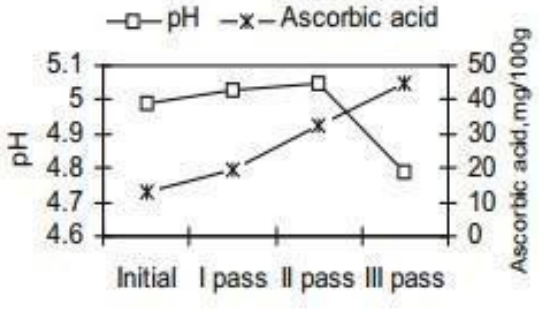


Fig.8: Effect of pectinase treatment on pH and Ascorbic acid (Vijayanand P. *et al.*, 2010)

- **Ascorbic acid:** The vitamin C content in the litchi juice was increased when treated with pectinase as shown by Vijayanand P *et al.*, (2010) in figure8. They also postulated that:
- Treatment of pulp with enzymes increased the release of various phenolics and other nutritionally important components in the juice.
- Enzymatic treatment prevents darkening and browning of juices.
- **Removal of bitterness of juice:** Some juices like citrus juices get bitter during storage or open left for some time and which is a great hindrance. The bitter taste was mainly because of formation of limolin. It is a highly oxygenated derivative of triterpene. Some enzymes could degrade this compound and was found to remove bitterness of the juice.
- **Titrable acidity:** It is the amount of developed acid because of reducing sugars. It was found by Vijayanand P *et al.,* (2010) that titrable acidity increased with the treatment with pectinase. Yusof and Ibrahim (1994) reported that the titraTable acidity for soursop juice increased from 0.41 per cent to 0.49 per cent after 1st , 2nd and 3rd hours of incubation with 0.025 per cent enzyme concentration but not at 0.05 per cent,0.075 per cent and 0.1 per cent concentrations.

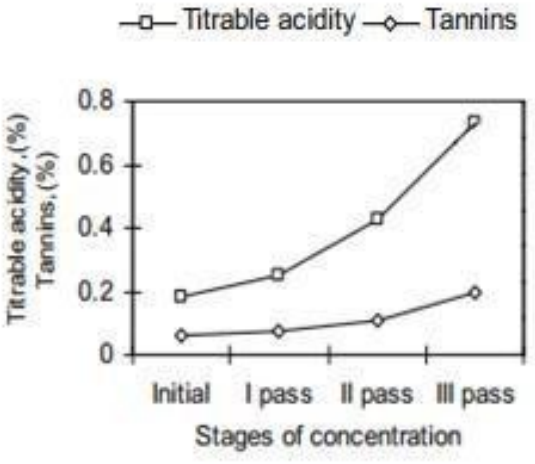


Fig.9: Effect of pectinase treatment on per cent acidity and tannins content

- **Tannins:** Tannins are bitter tasting polyphenols and are very useful in treating diseases like cancer. It was reported by Vijayanand, P, *et al.,* 2010 that tannins content also increased by treatment with pectinase. Landbo *et al.,* (2007) found that the total tannins content increased with the increase in incubation temperature.

2.6 Optimum parameters for pectinase activity:

The enzymatic treatment is one of the pre-treatments which can significantly enhance the juice yield economically. The pure enzyme preparations are highly specific and uncontaminated. But these are very costly because of extremely expensive downstream processing. The crude enzyme preparation is a complex system of many different enzymes with less purity and activity. It requires minimum downstream processing and thus is less costly than pure enzyme preparation. The synergetic effect of different enzymes like cellulase and pectinase enhances the yield and reduces the cost.

Enzymatic degradation of biomaterials depends upon various parameters like the type of enzyme, treatment temperature, incubation time, enzyme concentration, agitation and pH. These parameters are to be optimized for achieving a quality product with maximum bioconversion. (Chadha, R, *et al*., 2003)

- **The pH:** A. J. Dhembare *et al.,* (2015) has shown that the optimum pH for functioning of pectinase was 5. The results are shown in the Table 11 which showed the activity of the enzyme at various pH.

Table 11: Effect of pH on the activity of pectinase

Sr No.	Time (Hour)	Enzyme activity (µg/ml/sec) 10^{-4}			
		pH 4	pH 5	pH 6	pH 7
1	24	13.21	71.23	62.21	59.31
2	48	41.31	113.71	101.12	98.24
3	72	80.56	285.24	209.57	170.95
4	96	105.86	340.56	234.51	201.58
5	120	63.39	21.58	138.17	171.53

- **2.6.2 Incubation time:** The time for which the treatment of enzyme is given is referred as incubation time. H P Sharma *et al.,* (2014) has shown the incubation time for different fruits and vegetables in the Table.
- **2.6.3 Enzyme concentration:** H. P. Sharma *et al.,* (2014) has shown the concentration of enzyme used for various fruits and vegetables in the Table 12.

Table12: Optimum parameters for pectinase activity.

Fruit/ Vegetable	Incubation Time[a]	Incubation Temperature[b]	Enzyme Concentration[c]	Juice Recovery[d]	References
Bael (*Aegle marmelos correa*)	425	47	20 mg/100g	86.6	Singh *et al.* (2012).
Guava (*Psidium guajava* L.)	436.2	43.3	0.70 mg/100g	62.2	Kaur *et al.* (2009).
Elderberry *(Sambucus nigra L)*	50	60	0.34 mg/100g	77.0	Landbo *et al.* (2007).
Tamarind (Variety *Ajanta*)	360	37	5 mg/100g	92.4	Joshi *et al.* (2012).
Mayhaw (*Crataegus opaca* Hook.)	60	32	0.20%	75.7	Trappey *et al.* (2008).
Plum (variety *Titrone*)	300	45	0.5%	82	Chauhan *et al.* (2001).
Mango (variety *Amrapali*)	360	45	0.9%	59	Chauhan *et al.* (2001).
Mango	120	50	2%	65	Gupta and Girish (1988).
Apricot (variety *Charmagz*)	300	45	0.5%	78	Chauhan *et al.* (2001).
Pear	240	40	2.5%	72	Joshi *et al.* (2011).
Black currant (*Ribes nigrum*)	30	60	0.18%	66-78	Landbo and Meyer (2004).
Banana *(Musa sapientum* cv Berangan)	240	44	0.4%	69.4	Shahadan and Abdullah (1995).
Soursop *(Annona muricata* L.)	180	35-40	0.05%	67.2	Yusof and Ibrahim (1994).
Apricot	240	40	2.5%	80	Joshi *et al.* (2011).
Pineapple	30	40	0.02%	63-64	Dzogbefia *et al.* (2001).
Date (*Phoenix dactylifera L.*)	300	50	50U	72.25	Abbes *et al.* (2011).

[a]Incubation time in min, [b]Incubation temperature in °C, [c]Enzyme concentrations in mg/100g : mg per 100 g of pulp, % Percentage on pulp basis, U : Enzyme Unit, [d] Juice recovery in Percentage (%)

- **2.6.4 Temperature:** A. J. Dhembare *et al.,* (2015) has also reported the optimum temperature for the pectinase as shown in the Table 13. At 45^0C, pectinase had the maximum activity. Thus it can have maximum activity at this temperature.

Table 13: Effect of temperature on enzymatic velocity

Sr. N0.	Temperature (^{0}C)	Velocity (µm/min/mg) 10^{-4}
1	30	2.2
2	35	3.3
3	40	8.5
4	45	9.5
5	50	2.0
6	55	1.0

With the all above cited facts it has been clarified that enzymes are eco-friendly and less detrimental to the nutritional value rather they enhance the nutritional value of the product. Hence, the present study was focused on deriving a product from the food with the use of enzymes by changing temperature, incubation time, pH and the other parameters.

CHAPTER – III

MATERIALS AND METHODS:

3.1 Pre-requisites for the study

3.1.1 Collection of the Bottle gourd: The bottle gourd was obtained from the vegetable vendors from the local market of Patiala near Thapar University. Two types of varieties of bottle gourd were available: round and short one and cylindrical. The round and short variety were selected and used for the study.

3.1.2 Pectinase: The enzyme was of HIMEDIA brand. It is polygalacturonase enzyme and is isolated from *Aspergillus niger*. The enzymatic activity is 8000-12000U/g i.e.(571.2-856.8 U/ml). Mass of this enzyme is 43.0 KDa. The storage temperature is 2-8 ^{o}C. It was provided by the Department of Biotechnology, TU, Patiala. The enzyme classification number: 3.2.1.15.

3.1.3 Hand blender: Glen Gl 4044 Hand Blender was used to obtain puree of steam blanched bottle gourd. It has 300W power and was bought from shop near Thapar University, Patiala.

3.1.4 Muslin cloth: Muslin cloth with pore size of 7-22 mm was used in the filtration step. The slurry was squeezed to obtain juice. It was provided by the BTD of Thapar University.

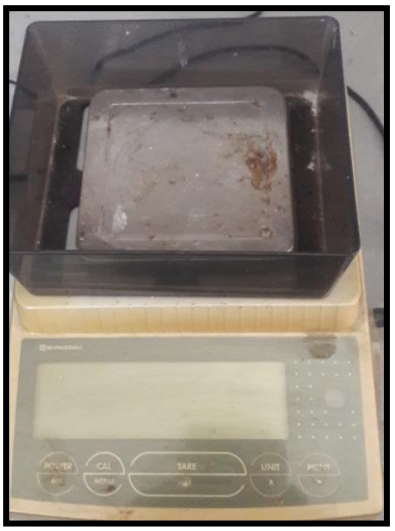
3.1.5 Filter paper: The juice obtained through passing the slurry from muslin cloth is then filtered through filer paper. The quantitative filter paper sheets were used and these were provided by DBT, Thapar University.

3.1.6 Weighing balance: Electronic weighing balance was used for measuring pectinase and spices. This weighing balance has the least count of 0.01g and capacity of 600g. It was present in the STEP, Thapar University.

3.1.7 Refrigerator: LG refrigerator was used for storage of bottle gourd and produced juice samples. It was also present in STEP.

3.1.8 Aluminium foil: *Freshwrapp* Aluminium foil was used for wrapping vegetables and juice bottles. By covering juice bottles, the oxygen was prevented to enter the bottle. Thus oxidation of juice is prevented. It was provided by DBT, Thapar University.

3.1.9 Refractometer: Hand refractometer with range of 0-32^{o}Brix was used to measure the TSS of the juice. It was present in the STEP.



[B]

[A] **[C]**

Fig.10: Weighing balance [A], Aluminium foil [B], Refractometer [C].

3.1.10 Digital thermometer: Digital thermometer was used to measure temperature of the slurry in water bath. It was provided by the project guide.

3.1.11 Spectrophotometer: Spectrophotometer was used to measure the turbidity/clearance of the juice or to check the amount of dispersed solid material. It was also used in the analytical tests to record the absorbance of the standards and samples. It was present in the STEP.

3.1.12 Steamer: Steam was created by steamer to give steam blanch to bottle gourd. It was present in the STEP.

3.1.13 The pH meter: Cyberscan pH tutor was used to measure pH of the bottle gourd juices. It was present in STEP.

3.1.14 Spices: The spices like salt, cumin powder and black pepper powder of *Catch* brand were used in the bottle gourd juice formulation. These were bought from the market near Thapar University.

3.1.15 Glass Bottles: Glass bottles were used to store the bottle gourd juice. These bottles were properly sterilized before use. It was present in STEP.

3.1.16 Autoclave: Autoclave was used for the sterilization of glassware used in the work. It was available in STEP, Thapar University.

3.1.17 Water bath: Water bath is required to provide optimum temperature and incubation conditions to the pectinase enzyme. It was present in STEP, TU.

[A] [B] [C] [D]

[E] [F]

Fig.11: Pectinase enzyme from HIMEDIA [A], Glen hand blender [B], pH meter [C], digital thermometer [D], water bath [E], spectrophotometer [F].

3.2 EXPERIMENTAL PLANNING:

3.2.11 Processing of Bottle gourd for juice extraction:

Fresh bottle gourd was taken. It was first properly washed under running tap water and then it was peeled. After peeling the outer skin, the gourd was then chopped into small pieces with the help of a knife. They were weighed to calculate the amount of pectinase required for enzymatic treatment. Then these pieces were steam blanched to inactivate the peroxidase enzyme which causes browning in the juice. The inactivation of peroxidase was tested by hydrogen peroxide test.

After blanching, the bottle gourd pieces were pureed with the help of a hand blender to obtain uniform slurry. The procedure for processing of bottle gourd is similar in both the

control juice and the juice which has undergone the enzymatic pre-treatment before processing. The processing procedure in pictorial representation is shown in the figure 12.

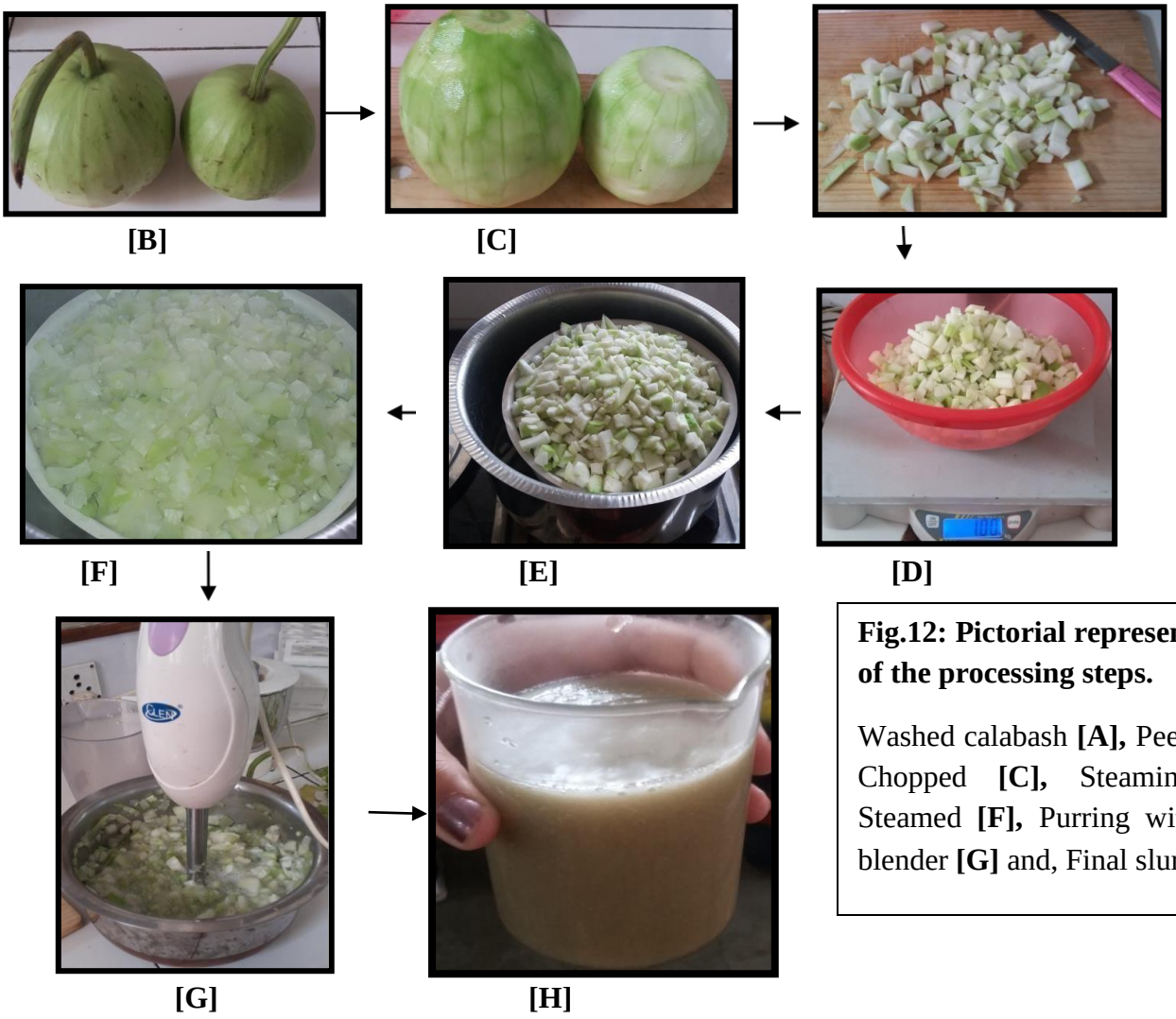


Fig.12: Pictorial representation of the processing steps.

Washed calabash **[A],** Peeled **[B],** Chopped **[C],** Steaming **[E],** Steamed **[F],** Purring with hand blender **[G]** and, Final slurry **[H].**

3.2.12 Process standardization: Both, the process for control juice and pectinase assisted juice production were standardized.

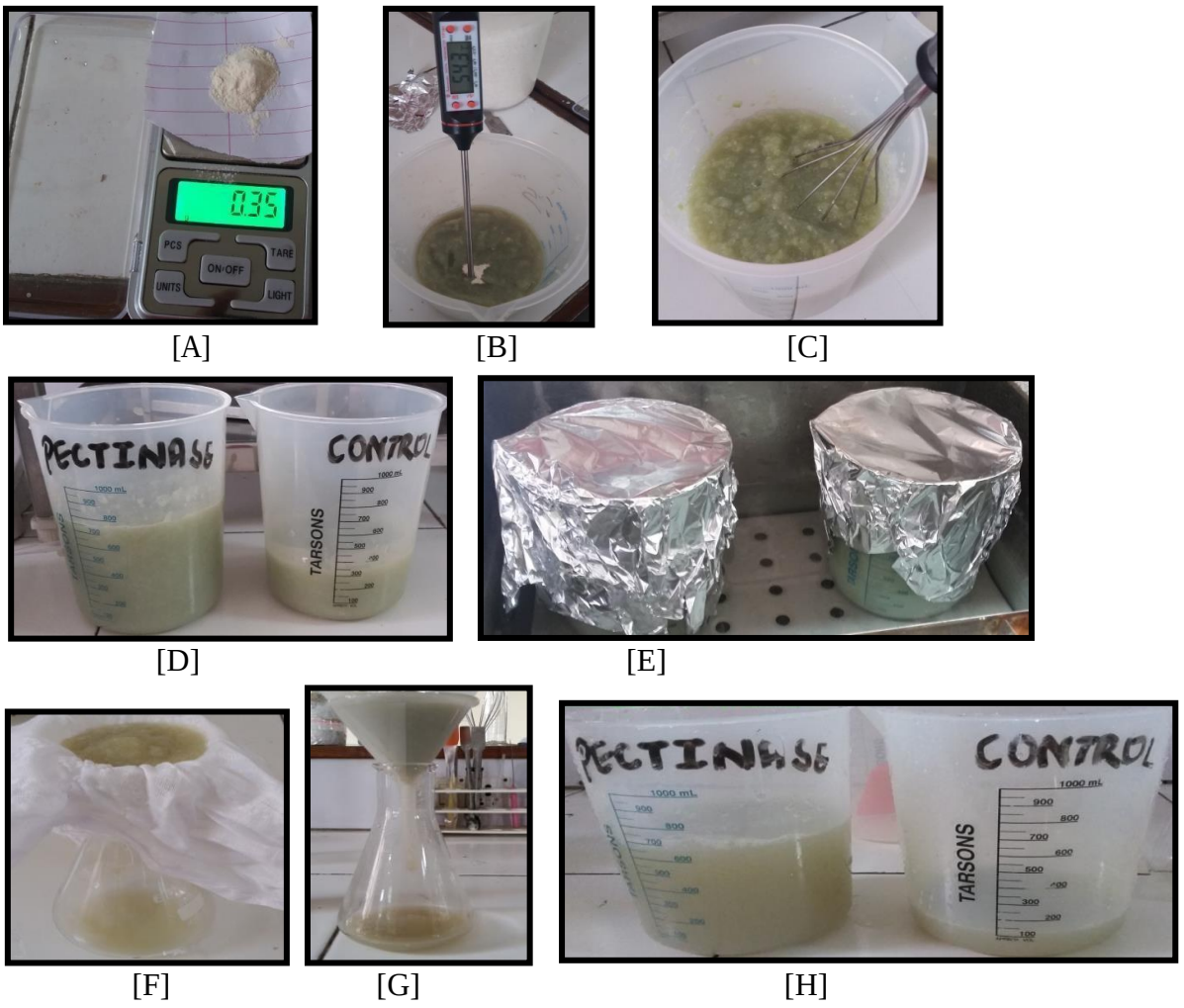
3221 Preparation of control juice:

Once the processing of bottle gourd was done the slurry was kept in water bath for standardized time and then filtered. The juice extraction was done in two steps. First, slurry was passed through muslin cloth and squeezed properly. Then the extracted juice was filtered by using filter paper to get more clear juice. Then sample produced were analyzed for sensory and analytical tests and the data obtained was statistically analyzed against control juice.

3222 Pectinase assisted juice extraction method:

Already made slurry cooled down to room temperature and enzyme was incorporated to it so that enzyme activity may not be destroyed due to high temperature of the slurry. The

beaker with slurry and enzyme was then properly covered with Aluminium foil and placed in the water bath for optimum time. As the solid part converted to juice by means of enzyme is now ready for straining as done similar to the control juice production. Comparison of the pectinase assisted extracted juice and the control extracted juice was done through various analytical parameters.



[A] [B] [C]

[D] [E]

[F] [G] [H]

Fig.13: Pictorial representation of the juice production from pectinase.

[A]: enzyme weight calculated on the basis of the wet weight of the slurry, adding to the cooled slurry [B], Mixing properly [C], Dividing the contents [D], Covering with Aluminium foil and placing in water bath [E], passing through muslin cloth [F], passing through filter paper [G] and, finally getting the juice [H].

For the pectinase assisted juice extraction, certain parameters were first standardized:

1. **Enzyme concentration:** The enzyme concentration was standardized by formulating various samples with varying enzyme concentration. The enzyme concentration tested were- 0, 0.01, 0.015, 0.025, 0.05, 0.1, and, 0.2 per cent. (per cent per 100ml). After this, the percentage yield was calculated for all setups and the enzyme concentration showed maximum juice yield was selected and standardized.

2. **Incubation time:** The incubation time was standardized by formulating various samples with varying time period. The time periods chosen were- 0hr, 1hr, 2hrs, 3hrs, 4hrs and, 5hrs. The percentage yield was calculated for all samples with varying time period and the time period producing highest juice was selected and standardized.
3. **Incubation temperature:** The incubation temperature was standardized by choosing various samples at different temperatures. The temperatures selected were- 35^{o}C, 40^{o}C, 45^{o}C, 50^{o}C, 55^{o}C. Among the samples showed maximum juice yield was selected and standardized.

3223 Spiced version of bottle gourd juice:

The juice extracted through two different methods above was visually perfect but possess a characteristic bottle gourd taste. To mask that taste and formulating more desirable and appealing drink, some spices were added in definite amount. The recipe was formulated using these spices by setting up various trials.

Fig.14: **Spices added in the juice**

Table 14: Recipe of bottle gourd juice with spices

S. No.	Ingredients	Amount added
1	Bottle gourd juice	60 ml
2	Chilled RO water	40 ml
3	Salt	0.5 g
4	Roasted cumin powder	0.5g
5	Black pepper powder	0.5g

3.3 PARAMETERS TEST:

The samples obtained from two different juice extraction methods were subjected to various sensory, analytical tests and data obtained was statistically analyzed.

3.3.1 Sensory analysis:

The obtained juice from different methods juice samples were evaluated for sensory tests for Color, Appearance, Flavor or Aroma, Texture, Taste and Overall acceptability from different subjects. The method given by Larrmond, 1970 was followed. The samples were rated on 9 point Hedonic scale as under:

Table 15: Hedonic 9 point scale

S. No.	Scale	Sensory score
1.	Like extremely	9
2.	Like very much	8
3.	Like moderately	7
4.	Like slightly	6
5.	Neither like nor dislike	5
6.	Dislike slightly	4
7.	Dislike moderately	3
8.	Dislike very much	2
9.	Dislike extremely	1

3.3.2 Analytical Tests:

3.3.2.1 Percentage Yield:

The percentage yield was determined by considering the weight of the slurry before and after pressing and filtration.

$$\% \text{ Yield} = \frac{\text{Weight of the sample - Weight of the filter cake}}{\text{Weight of the sample}} \times 100$$

3.3.2.2 Turbidity:

The turbidity of the juice samples were measured to check the dispersed pulp or fibre and measured by spectrophotometer absorbance at 600nm.

3.3.2.3 The pH:

The pH of the juice samples was measured by using digital pH meter having glass electrode potentiometer of Cyberscan Company which was calibrated and standardized by using buffers of pH 4.0, 7.0 and, 9.0 values. The pH was measured at 25^{o}C according to AOAC (1995).

3.3.2.4 Total Soluble Sugars (TSS):

The TSS of the juice samples was measured by using Abbe hand Refractometer at 20^{o}C. The TSS of the samples was expressed as ^{o}Brix. The range of refractometer used was 0-32 ^{o}Brix as per the method described by Ranganna, 1991.

3.3.2.5 Per cent Acidity:

The acidity of the juice samples was estimated by titration method. The samples were titrated against 0.1N NaOH solution. Phenolphthalein was used as an indicator. The per cent acidity is expressed as per cent citric acid present in the sample as the method described by Ranganna, 1997.The samples were prepared by diluting 1ml of sample with 9ml of distilled water. First the burette was filled with freshly prepared 0.1N NaOH solution. The initial reading of the burette was noted down. The prepared sample was added into a flask and 3-4 drops of phenolphthalein indicator was added to it. Then the sample was titrated against the alkali and the end point is colorless to pink color which sustains for a few seconds then the final reading is noted down. The volume of 0.1N NaOH used is calculated by subtracting the final and initial burette reading.

$$\% \text{ Acidity} = \frac{10 \times \text{Normality of NaOH} \times \text{Volume of NaOH used} \times \text{Equivalent weight of NaOH}}{\text{Volume of sample} \times 1000} \times 1000$$

3.3.2.6 Ascorbic acid content:

The vitamin C content of the juice samples were measured by titration method. The samples were titrated against the iodine solution.1 per cent starch solution (freshly prepared) is used as an indicator. The results were compared against the titer value of the standard i.e. pure ascorbic acid. The **x** is the vitamin C content in the sample. The results were expressed as mg/100ml as given by Ranganna, 1986.

$$\frac{\text{Titer value of standard (ml)}}{\text{0.25g of vitamin C}} = \frac{\text{Titer value of sample (ml)}}{\text{x}}$$

3.3.2.7 Tannins content:

The tannins content of the juice samples was determined by plotting a standard curve of tannic acid. The tannic acid solution should be freshly prepared and concentration was 1mg/ml. The dilutions of tannic acid were prepared from the stock solution. From the stock, following dilutions were prepared: 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0mg/ml. The sample was prepared by adding 10ml of juice sample, 0.5ml of Folin Denis reagent, 1ml of sodium carbonate solution (freshly prepared) and 10ml of distilled water. Vortex all and

measured the absorbance of all standards and the samples at 720nm using UV spectrophotometer. The results were expressed in mg/100ml as given by Saxena *et al.,* 2013.

Fig.15: Freshly prepared FD reagent and tannic acid solution

3.3.2.8 Flavonoids content:

The flavonoids content of the juice samples was determined by plotting a standard curve of Quercetin. 100 µg/ml stock solution of quercetin was prepared. From this stock the following dilutions were prepared: 6.25, 12.5, 25, 50, 80 and 100µg/ml. The sample was prepared by adding 0.5ml of juice sample, 1.5ml methanol, 0.1ml 10 per cent Aluminum chloride, 0.1ml 1 per cent 1M potassium acetate solution and 2.8ml distilled water. Then, vortexed all of the samples and the dilutions were filtered. Absorbance was measured at 415nm with the help of UV spectrophotometer. The results were expressed as µg/ml as given by Bag *et al.,* 2015.

3.3.3 Statistical analysis:

The experimental data was subjected to various statistical tests. The analysis of data was performed at 5 per cent significance level. Pearson correlation was also calculated with statistical software. (www.socialsciencestatistic.com)

CHAPTER – IV

RESULTS AND DISCUSSION

Under the present study, an attempt has been made to standardize the method of bottle gourd juice production via two different methods and comparison of juices produced by these methods. Control bottle gourd juice extraction method and the enzyme assisted bottle gourd juice extraction method using enzymes pectinase were compared. Once the method has been standardized, the experimental juice samples were analyzed for percentage yield, Total Soluble Sugars, Vitamin C content, Tannins content, Flavonoids Content, per cent acidity, pH and turbidity. Then sensory analysis was done. The samples were analyzed thrice for each parameter. The experimental data obtained from all the parameters has been statistically analyzed by ANOVA and correlation. Here in this chapter the results have been discussed with the help of Tables and graphs wherever possible. The work has been done in Science and Technology Entrepreneur's Park (STEP), Thapar University, Patiala and the results are discussed under following headings and sub-headings.

4.1 Standardization of various parameters for enzyme assisted bottle gourd juice extraction method:

4.1.1 Enzyme Concentration: The enzyme concentration standardization was done to get the higher juice yield at minimum concentration of pectinase enzyme on the basis of weight. The concentration of pectinase enzyme was formulated at 0, 0.01, 0.015, 0.025, 0.05, 0.1, and 0.2 per cent by weight of the bottle gourd pulp. Then the per cent yield was calculated. The enzyme concentration which is giving maximum yield was standardized.

Table 16: **Enzyme concentrations and bottle gourd juice yield**

Sr. No	Samples	Enzyme Concentration (%)	Per cent Yield
1	S1	0	81.21±1.44
2	S2	0.01	84.36±1.79
3	S3	0.015	90.67±0.88
4	S4	0.025	91.43±1.56
5	S5	0.05	95.74±1.98
6	S6	0.1	95.81±0.75
7	S7	0.2	95.97±1.12

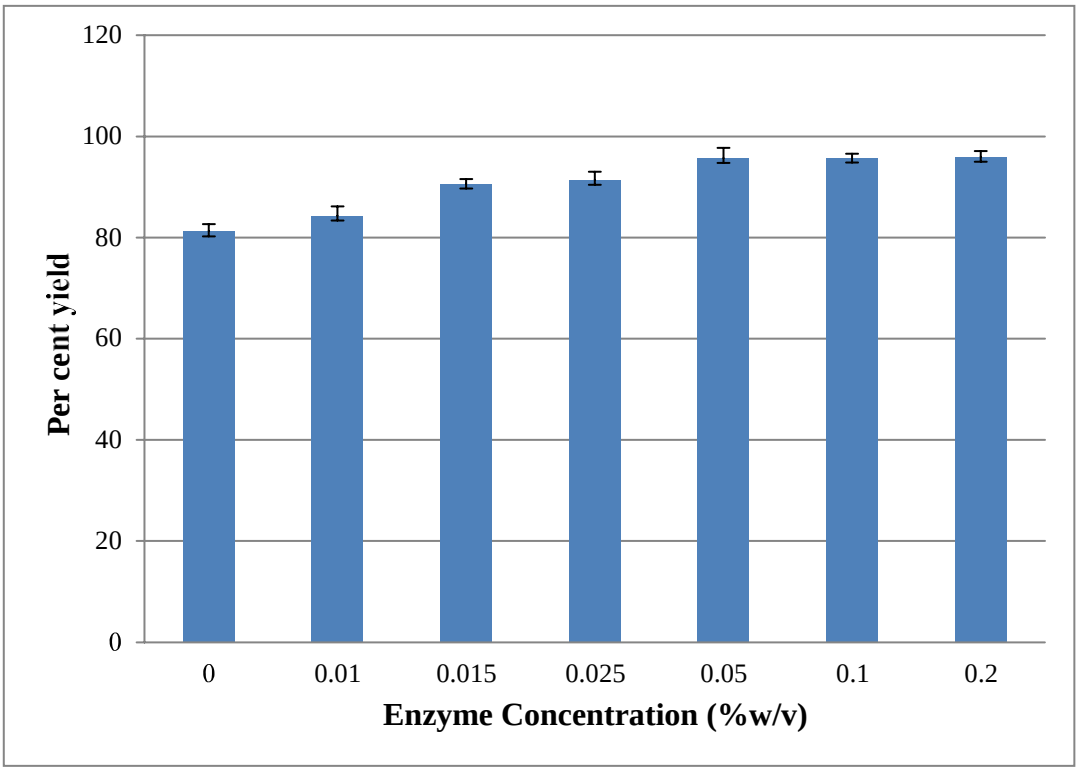


Fig.16: Enzyme concentration correlation to bottlegourd juice yield

As shown in the Table and graph the juice yield increased till 0.05 per cent concentration of enzyme and then became constant even after with the increase in concentration negligible changes were observed. Thus, 0.05 per cent was considered as standardized pectinase enzyme concentration for further studies. The residue left after filtration i.e. filter cake produced at varied enzymes concentration weight were as shown in the Table 17 below:

Table 17: Enzyme concentrations correlation to filter cake weight

S. No.	Sample	Enzyme Concentration (%)	Weight of filter cake (in grams)
1	S1	0	18.79±0.66
2	S2	0.01	15.64±0.21
3	S3	0.015	9.33±0.45
4	S4	0.025	8.57±0.77
5	S5	0.05	4.26±0.13
6	S6	0.1	4.19±0.16
7	S7	0.2	4.03±0.78

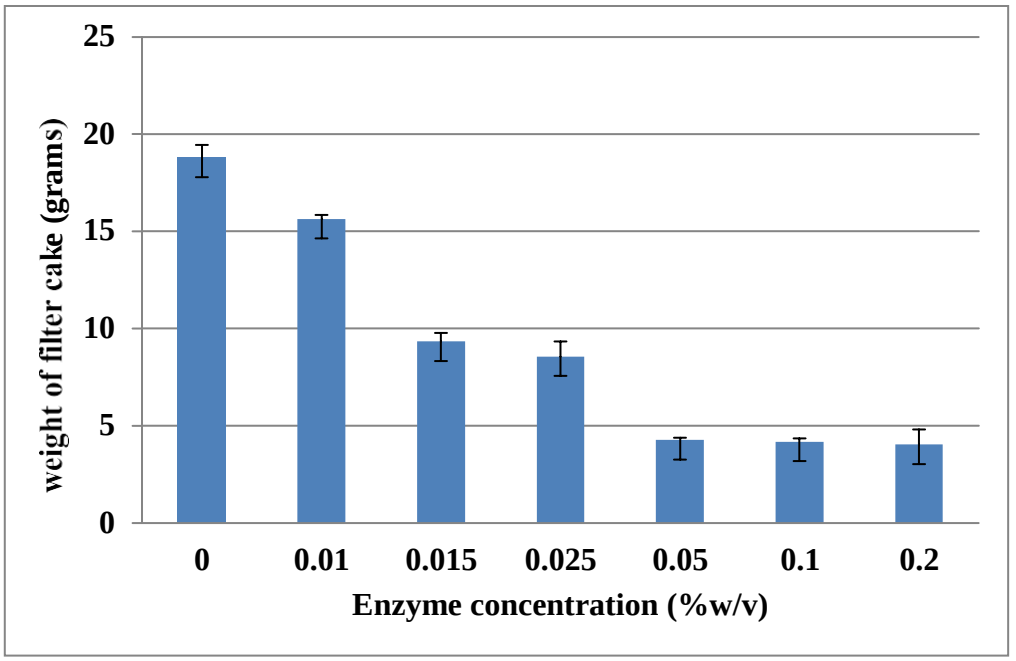


Fig.17: Graphic representation of Table 17 data showing relation between enzyme concentration and filter cake weight

According to the Table and graph 17, the weight of filter cake observed minimum at 0.05 per cent concentration of pectinase enzyme and after that very slight variations in the filter cake weight were observed. Enzyme concentration of 0.05 per cent was finally taken into consideration for the further studies..

4.1.2 Incubation time:

The incubation time was initially kept on trial basis at 0, 2, 3, 4 and 5 hours for the pectinase enzyme activity. Once, the percentage yield was recorded higher at a particular the time period that is giving maximum juice yield was selected and carried on further for the next experiments.

Table 18: Incubation time correlation to the yield of juice at varied timings

S. No.	Sample	Incubation time (hour)	Per cent Yield
1	Control	0	83.61±0.31
2	S1	1	87.01±0.58
3	S2	2	94.21±0.24
4	S3	3	95.25±0.62
5	S4	4	96.77±0.88
6	S5	5	96.81±0.74

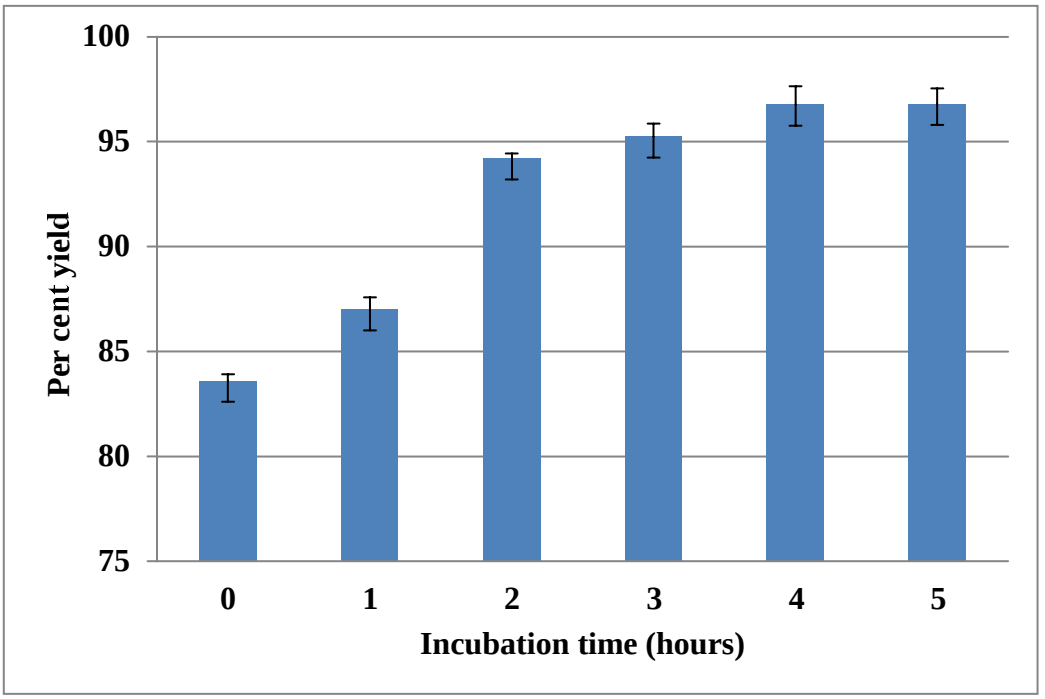


Fig.18: **Graphic representation of Table 18 data showing relation between incubation time and per cent yield**

The Table and graph 18 explains themselves that the juice yield was increased till 4th hour and then static observations at 5th hour can be seen. The incubation time of 4 hrs was found best to get higher juice yield. The weight of the filter cake produced at varied incubation times was also analyzed and the results as given below:

Table 19: Incubation time correlation to the weight of filter cake at varied timings

S. No.	Sample	Incubation time (in hours.)	Weight of filter cake (in grams)
1	Control	0	16.93±0.14
2	S1	1	12.99±0.36
3	S2	2	5.79±0.71
4	S3	3	4.75±0.42
5	S4	4	3.23±0.37
6	S5	5	3.19±0.66

According to the results, the filter cake was found minimum after 4hours or incubation of the slurry and after that very slight reduction in the filter cake weight was observed. Incubation time for 4 hours was found highly efficient.

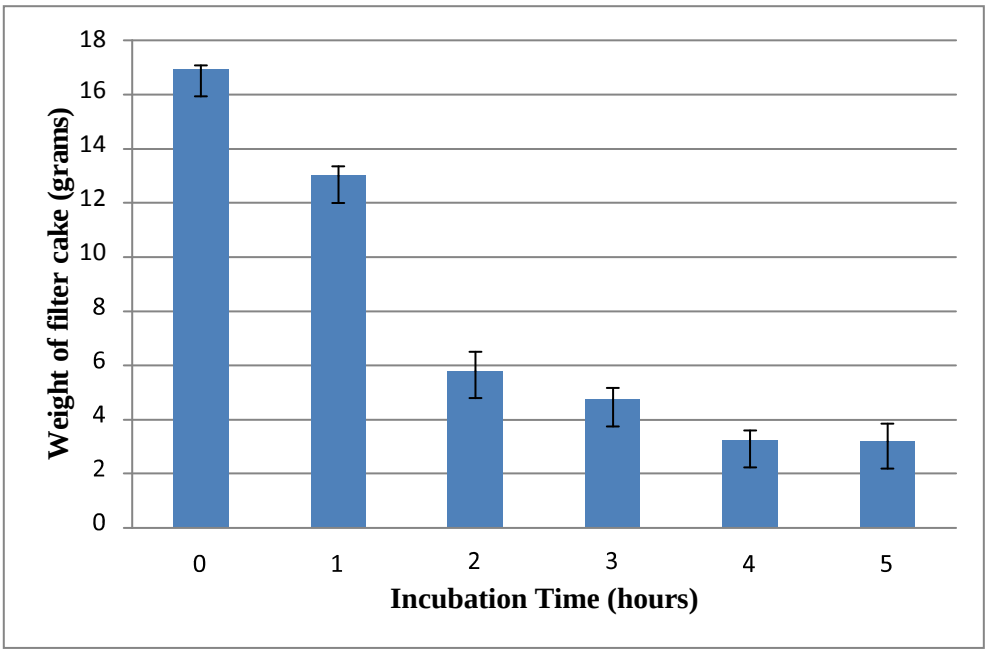


Fig.19: Graphic representation of Table 19 data showing relation between incubation time and filter cake weight

4.1.3 Incubation temperature: The incubation temperature was standardized at 35ºC, 40ºC, 45ºC, 50ºC and 55ºC. After this, the percentage yield was calculated and the temperature that is giving maximum juice yield was selected and standardized.

Table 20: Incubation temperature correlation to yield of juice at varied concentrations

S. No.	Samples	Incubation temperature (ºC)	Per cent Yield
1	S1	35	71.46±0.51
2	S2	40	82.22±1.42
3	S3	45	96.02±1.61
4	S4	50	93.42±2.11
5	S5	55	90.57±0.92

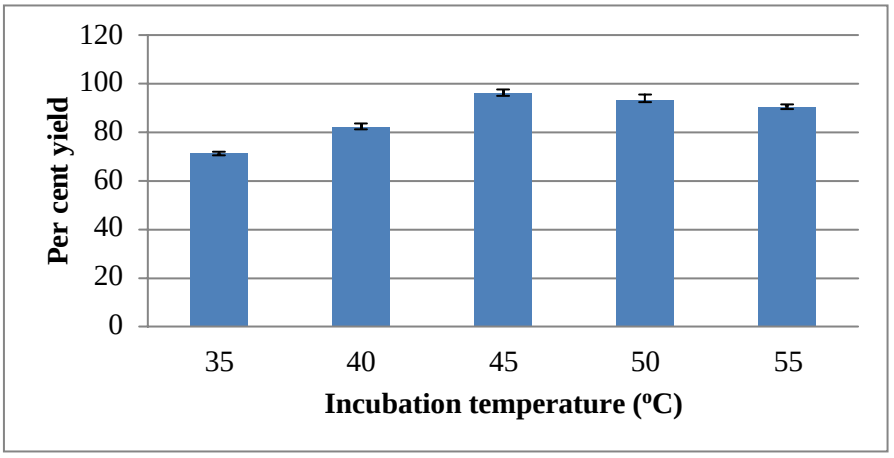


Fig.20: Graph showing correlation between incubation temperature and yield

According to the results, the yield was observed increasing till 45°C and after that slight variations in the juice yield were observed. Thus, the incubation temperature of 45°C was found best. The filter cake weight produced at different incubation temperatures was also analyzed and the results were:

Table 21: Incubation temperature effect on the weight of filter cake.

S. No.	Samples	Incubation temperature (°C)	Weight of filter cake (in grams)
1	S1	35	28.54±0.22
2	S2	40	17.78±1.71
3	S3	45	4.43±1.24
4	S4	50	6.98±0.94
5	S5	55	8.33±1.56

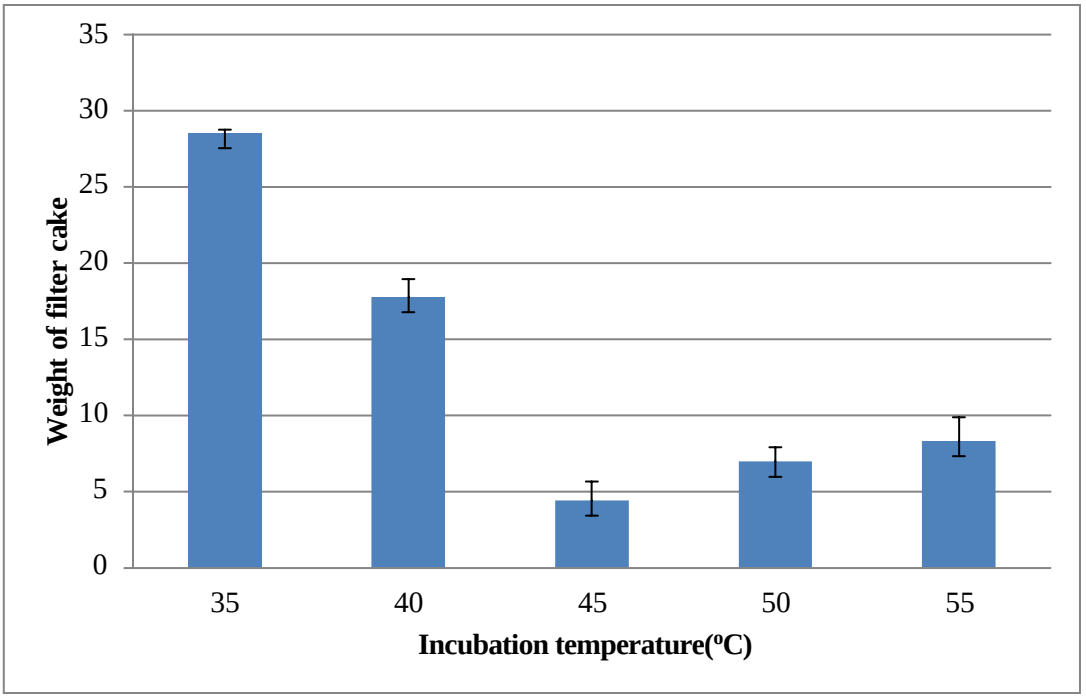


Fig.21: Graph showing correlation between incubation temperature affect weights of filter cake

According to the results, the weight of filter cake was minimum at 45°C and after that minimal changes were seen. The incubation temperature of 45°C was found best for the juice yield.

4.1.4 Blanching period:

Blanching is pre-processing of the bottlegourd pulp before actual imparted to juice extraction to a very high temperature through steam followed by immediate cooling. To

produce juice from bottle gourd it was first required to be blanched. Blanching is essential to inactivate peroxidase enzyme which causes browning of fruits and vegetables. If this enzyme was not inactivated the bottlegourd juice was found to turn bitter and brown due to oxidation.

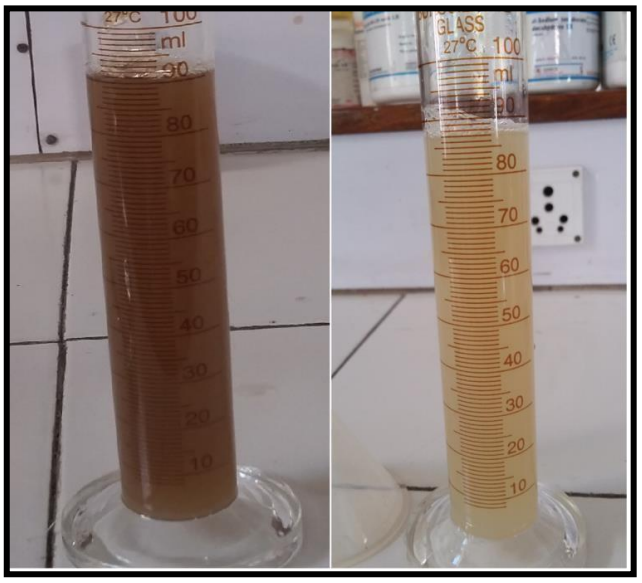
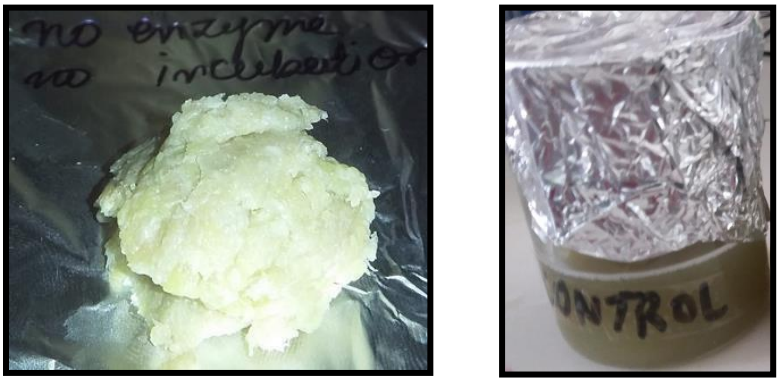


Fig.22: Bottlegourd juice without blanching vs. blanched.

The bottle gourd was steam blanched for 15mins prior to crushing. Steam blanching was observed better than the regular water dipped blanching as there was no loss of nutrients in water.

4.2 Quality characteristics of bottle gourd juice extracted:

4.2.1 Control Juice: This juice did not undergo any enzymatic pre-treatment. After washing, peeling, and cutting of the bottle gourd it was kept in water bath but without any enzyme and then pressed for juicing. The juice thus obtained have low yield. The filter cake obtained after pressing the slurry is bright, greenish and glossy. The pH of the juice was nearly neutral and the Total Soluble Solids were also observed less in quantity. The quality characteristics of this juice are represented in the Table 22.



[A] [B]

Fig.23: **Filter cake [A] and Control juice [B]**

Table 22: Characteristics observed in control juice

S. No.	Characteristics	Values
1	Per cent Yield	79.4
2	Total Soluble Solids	4.2
3	pH	6.66
4	Per cent Acidity	1.1
5	Turbidity	1.0397
6	Vitamin C	0.1871mg/ml
7	Tannins	7.3 µg/ml
8	Flavonoids	0.0364 mg/ml

4.2.2 Pectinase Juice:

The juice prepared by treating the bottlegourd with pectinase enzyme had significantly higher yield than the control juice. The filter cake obtained after pressing the pulp was dark green in color and dry. The Total Soluble Solids were increased after enzyme treatment. The pH was also decreased slightly as the enzyme convert pectin to the reducing sugars which may develop the acidity and indirectly reduce the pH too. The turbidity was significantly reduced may be because the pectin is dispersed fibre which was hydrolyzed by the enzyme and thus, juice was visually more appealing than the control juice. The taste of the juice was observed with mild characteristic bottle gourd flavor.

[A] [B]

Fig.24: Enzyme treated juice [A], filter cake [B]

The quality characteristics of enzyme assisted extracted bottlegourd juice as represented in the Table 23.

Table 23: Characteristics of pectinase treated juice.

S. No.	Characteristics	Values
1	Per cent Yield	89.5
2	Total Soluble Solids	6.2
3	pH	5.2
4	Per cent Acidity	1
5	Turbidity	0.0977
6	Vitamin C	0.5153 mg/ml
7	Tannins	8.9875 µg/ml
8	Flavonoids	0.556 mg/ml

4.2.3 Spiced version of Pectinase assisted extracted bottlegourd juice:

The juice was prepared from blanched bottlegourd processed slurry and treating that with pectinase enzyme was observed with significantly higher juice yield with characteristic flavor and aroma of bottle gourd which is not desirable and appealing for many subjects. To mask that characteristic taste, various spices were added to it along with water dilution (percentage of the to the total juice need to be written down) a drink was formulated. The parameters like Total Soluble Solids and per cent acidity was higher than the control juice.

Fig.25: Spiced version of enzyme assisted juice.

The quality characteristics enzyme assisted extracted bottlegourd juice as represented are:

Table24: Parameters observation for spiced juice made from enzyme assisted extracted bottlegourd juice.

S. No.	Characteristics	Value
1	Per cent Yield	89.5
2	Total Soluble Solids	8.1
3	pH	5.0
4	Per cent Acidity	1.22
5	Turbidity	0.0977
6	Vitamin C	0.4005 mg/ml
7	Tannins	12.95 µg/ml
8	Flavonoids	0.536 mg/ml

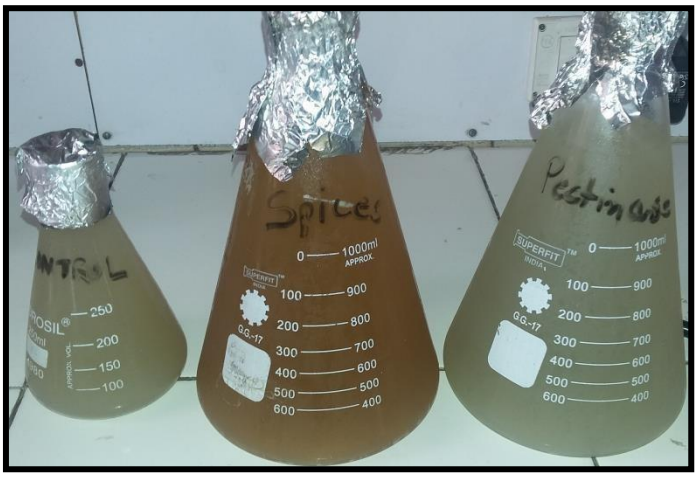


Fig.26: All three types of bottle gourd juice: control juice, pectinase assisted juice and spiced version of it

After standardizing the juice processing and production process; and after optimizing the recipe, various analytical, sensory and statistical tests were performed on these three juice to compare them.

4.3 SENSORY ANALYSIS:

For the sensory analysis of bottle gourd, Hedonic 9 point scale was used. Different parameters like appearance, color, flavor or aroma, texture, taste and overall acceptability were analyzed. The mean value along with the standard deviation is shown in the Table 25.

Fig27: Coded samples for tasting.

Table 25: Ratings according to Hedonic 9 point scale

A- Control Juice, B- Pectinase treated juice, C-Spiced version of B

Sample Code*	Appearance	Color	Flavor	Texture	Taste	Overall Acceptability	Percentage Acceptability
A	7.66±1.71	7.57±1.5	5.43±1.53	7.81±1.17	5.28±1.49	6.73±1.01	67
B	7.86±1.55	7.62±1.72	5.76±1.64	7.76±1.37	5.48±1.33	6.9±1.11	69
C	8.67±0.73	8.62±0.67	8.76±0.62	8.66±0.79	8.95±0.22	8.76±0.48	87.6

4.3.1 Appearance:

The data regarding the appearance score of the different sample has been tabulated. The mean value with the standard deviation of the appearance of the juice was ranged from 7.66 to 8.67. So, according to the hedonic scale the 7.66 was designated as liked moderately and 8.10 as liked very much by the members of panel. The highest mean value was shown by the sample C (8.67±0.73) and the lowest was shown by the sample A (7.66±1.71). The sample C has highest appearance score because it was more appealable as a drink and the sample A showed fewer score as it was turbid.

4.3.2 Color:

The color of the drink has a great impact on the preference of the consumers. The highest mean value was shown by the sample C (8.62±0.67) and the lowest was shown by the sample A (7.57±1.5). It was difficult to rate between the sample A and sample B because color of both the juice samples was quite similar but in case of sample C the color of the juice was very appealable due to the addition of spices so it was highly accepted. The mean value with the standard deviation of the color of the juice was ranged from 7.57 to 8.62. So, according to the hedonic scale the 7.57 was designated as liked moderately and 8.62 as liked very much by the members of panel.

4.3.3. Flavor or aroma:

The flavor and aroma of the drink is the major factor for the acceptability of juice by the consumers. The highest mean value was shown by the sample C (8.76±0.62) as spice addition led to enhancement of flavor as well as aroma. The lowest was shown by the sample A (5.43±1.53) as it has characteristic bottle gourd flavor. The mean value with the standard deviation of the color of the juice was ranged from 5.43 to 8.76. So, according to the hedonic scale the 5.43 was designated as neither like nor dislike and 8.76 as liked very much by the members of panel.

4.3.4. Texture:

The highest mean value was shown by the sample C (8.66±0.79) and the lowest was shown by the sample A (7.81±1.17). The mean value with the standard deviation of the color of the juice was ranged from 7.81 to 8.66. So, according to the hedonic scale the 7.81 was designated as liked moderately and 8.66 as liked very much by the members of panel.

4.3.5. Taste:

All other parameters are important but the most important factor in deciding the acceptability but the most influencing factor is taste. The highest mean value was shown by the sample C (8.95±0.22) as the addition of spices led to enhancement in the taste and made it very delicious drink. In this case the perception of the all members was near about same so,

the standard deviation was not high. The lowest was shown by the sample A (5.28±1.33). The mean value with the standard deviation of the color of the juice was ranged from 8.95 to 5.28. So, according to the hedonic scale the 5.28 was designated as neither like nor dislike and 8.95 as almost like extremely by the members of panel.

4.3.6 Overall acceptability:

The Table showed the mean score for the overall acceptability of all the juice samples. Highest score for overall acceptability was observed for the sample C (8.76) followed by the B (6.9) and the least mean score was shown by sample A (6.73). The sample C scored maximum in all the sensory parameters- appearance, color, flavor or aroma, texture and the taste.

4.3.7 Percentage of overall acceptability:

The percentage of overall acceptability was calculated on the basis of sensory analysis shown in the Table 25. Sample C has shown the maximum percentage of the overall acceptability 87.6per cent. The least accepted sample was sample A with 67 per cent as this sample was not given any treatment. According to the percentage acceptability the order of liking of the juice samples was C>B>A. After the analysis it was found that C was the best juice sample. Thus a successful drink was formulated which can be commercialized.

4.4. ANALYTICAL ANALYSIS:

4.4.1. Per cent Acidity:

It was observed that percentage acidity in the control juice sample is 1.1 per cent which is slightly more acidic than the juice treated with pectinase which is 1per cent. The addition of spices enhanced the acidity of the spiced version of pectinase treated juice. The acidity of this juice is 1.23per cent. The per cent acidity increased and the similar work has been shown by Vijayanand P. *et al.*, (2010) in his work in litchi juice.

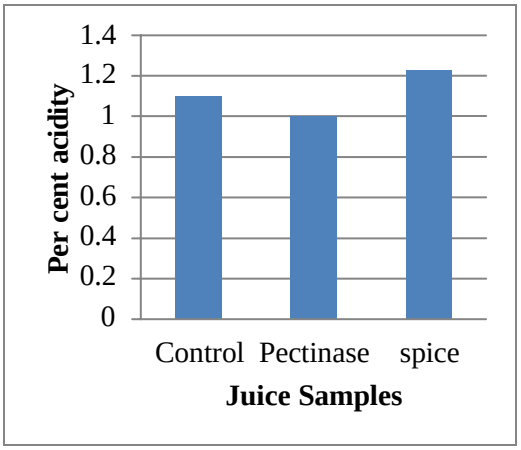


Fig.28: Per cent acidity of juice samples

Table 26: Per cent Acidity of juice samples

Sample	per cent acidity
Control	1.1
Pectinase	1
spice	1.23

4.4.2. Vitamin C:

The vitamin C content of the juices was determined by titration method and is represented in the following graph and the Table. The Vitamin C content is highest in the spiced version of the pectinase treated bottle gourd juice. It is 0.516 mg/ml or 516 mg/100ml. This quantity is quite good. The vitamin C content increased and the similar work has been shown by Vijayanand P. *et al.*, (2010) in his work in litchi juice.

Table 27: Vitamin C content of juice samples

Sample	Vitamin C (mg/ml)
Control	0.187±0.09
Pectinase	0.491±0.06
Spice	0.516±0.02

Fig29: starting and end point of titration

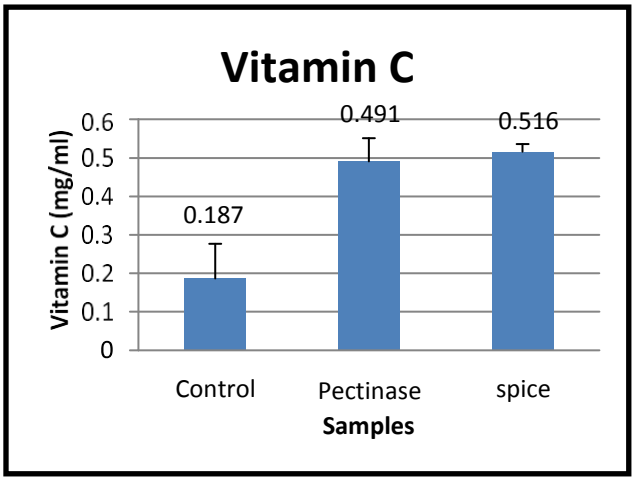


Fig.30: Vitamin C content of juice samples

4.4.3. Tannins: The tannin content of the juices was determined by plotting a standard curve of tannic acid against the samples and is represented in the figure 31. The tannins content is highest in the spiced version of the pectinase treated bottle gourd juice. It is 12.9µg/ml. The tannins content of pectinase treated juice is 9µg/ml which is higher than the control juice which is 7.37µg/ml. The R^2 is 0.9611 and the equation is y=0.1162x-0.0455.The tannins content of spiced version of the pectinase treated bottle gourd juice is higher because of the presence of various spices. Spices are rich in tannins and they leach it with time into the juice. The tannins content increased and the similar work has been shown by Vijayanand P. *et al.*, (2010) in his work in litchi juice.

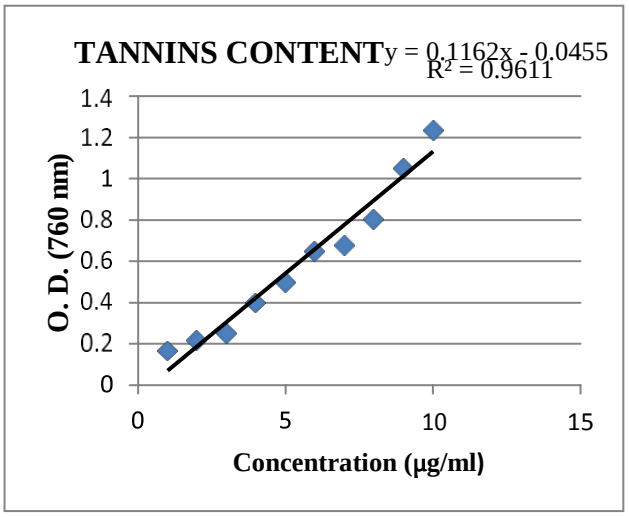


Fig.31: Standard curve of tannic acid

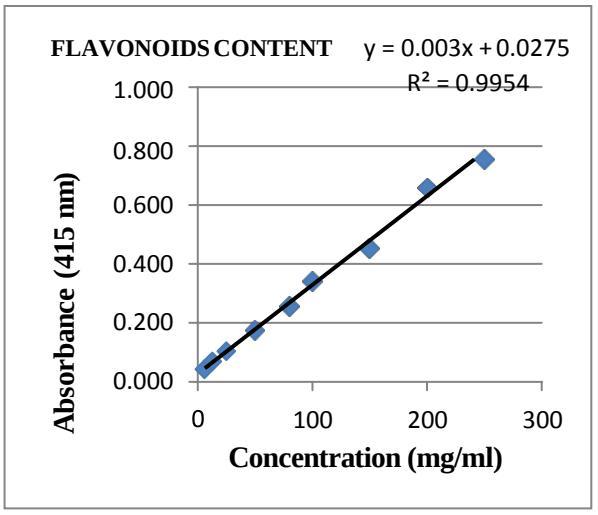


Fig32: Standard curve of quercetin

4.4.4 Flavonoids:

The flavonoids content of the juices was determined by plotting a standard curve of quercetin against the samples and is represented in the figure 32. The flavonoids content in the pectinase treated bottle gourd juice is 0.53mg/ml or 530mg/100ml. The flavonoids content of spiced version of pectinase treated juice is highest and is 0.56mg/ml or 560 mg/100ml and in control juice it is 0.36mg/ml or 360 mg/100ml. The R^2 is 0.9954 and the equation is y=0.003x- 0.0275. The flavonoids content increased and the similar work has been shown by Vijayanand P. *et al.,* (2010) in his work in litchi juice.

4.5. STATISTICAL ANALYSIS:

4.5.1 Analysis Of Variance (ANOVA)

To check whether there is any statistically significant difference between the means of all above parameters discussed earlier, ANOVA was performed. ANOVA is used to analyze the difference within and among the group means and their associated procedures. It is used to check variation among and between the groups. ANOVA was performed on all the parameters used in the study.

- **The pH:** Experimental data of the pH recorded is given in the following Table 29 & 30 ANOVA was performed on the above data. The software had given the F ratio value along with the P value (probability). This F ratio has to be compared with the f critical value which was found from the Table of F ratio, for this degree of freedom must be known.

The F value given by the software is 21.72212. The F critical value from the Table is 4.26. The p value is 0.000359. The result is significant at $p<0.05$. As the F ratio is greater than F critical, the result is **significant** at given probability value

Table 28: pH of all the trails of all samples

Trails	Control	Pectinase	Spiced
1	6.81	5.64	5.08
2	6.87	5.45	4.88
3	6.51	5.39	4.98
4	6.86	5.26	4.94
5	6.84	4.98	5.02
6	6.75	5.01	5.11
7	6.86	5.26	4.81
8	6.68	5.27	4.92
9	5.78	5.2	5.16
10	6.65	5.17	4.99
11	6.68	4.82	5.02
12	6.67	5.11	5.22
13	6.64	5.02	4.97
Mean	6.66	5.2	5

.

Table 29: Summary of data for pH

Data	Control	Pectinase	Spiced	Total
N	4	4	4	12
$\sum N$	25.99	20.87	20.07	66.93
Mean	6.4975	5.2175	5.0175	5.5775
$\sum X^2$	169.5833	109.2129	100.7329	379.5291
Standard Deviation	0.4876	0.3285	0.1028	0.7524

Table 30: Results of ANOVA for pH

Source	SS (Simple Square)	Df (degree of freedom)	MS (Mean Square)
Among group	5.1584	8	2.5792
Within group	1.0686	9	0.1187
Total	6.227	11	-

- **Total Soluble Solids (TSS):** Experimental data of the TSS recorded is given in the Table 31.

Table 31: TSS of all the trails of all samples

Trails	Control	Pectinase	Spiced
1	4.1	6.7	8.5
2	4.0	4.9	8.3
3	4.0	6.6	8.1
4	4.2	5.9	8.9
5	4.4	6.2	7.55
6	4.1	6.5	7.2
7	4.3	6.45	8.4
8	4.4	6.24	8.1
9	4.7	6.57	8.0
10	4.0	6.49	8.1
11	4.4	5.7	7.6
12	4.4	5.8	8.1
13	4.0	6.0	8.2
Mean	4.2	6.2	8.1

ANOVA was performed on the above data. The software had given the F ratio value along with the p value (probability). This F ratio has to be compared with the F critical value which was found from the Table of F ratio, for this degree of freedom must be known.

Table 32: Summary of data for TSS

Data	Control	Pectinase	Spiced	Total
N	9	13	6	28
$\sum$N	38.2	80.05	48.55	166.8
Mean	4.2444	6.1577	8.0917	5.9571
$\sum X^2$	162.56	495.9151	394.8025	1053.2776
Standard Deviation	0.2297	0.4993	0.6248	1.4861

Table 33: Results of ANOVA for TSS

Source	SS (Simple Square)	Df (degree of freedom)	MS (Mean Square)
Among group	54.26	2	27.13

Within group	5.3661	25	0.2146
Total	59.6262	27	-

The F value given by the software is 126.39456 The F critical value from the Table is 3.39. The p value is <0.00001. The result is significant at p<0.05. As the F ratio is greater than F critical, the result is **significant** at given probability value.

- **Per cent Yield:** Experimental data of the per cent yield recorded is given in Table 34.

Table 34: Per cent Yield of all the trails of all samples

Trails	Control	Pectinase
1	76.64	97.81
2	81.01	98.29
3	83.91	97.07
4	71.46	96.024
5	84.69	95.69
6	81.21	95.45
7	72.66	95.1
8	83.61	93.95
9	78.22	94.67
10	78.34	94.4
11	82.24	93.85
12	79.29	96.06
13	81.54	95.67
Mean	79.4	89.5

ANOVA was performed on the above data. The software had given the F ratio value along with the p value (probability). This F ratio has to be compared with the F critical value which was found from the Table of F ratio, for this degree of freedom must be known.

Table 35: Summary of data for per cent Yield

Data	Control	Pectinase	Total
N	8	13	21
$\sum$N	635.19	1243.734	1878.924
Mean	79.3988	95.6718	89.4726
$\sum X^2$	50621.2973	119012.0727	169633.37

Standard Deviation	5.1825	1.3461	8.7213

Table 36: Results of ANOVA for per cent Yield

Source	SS (Simple Square)	Df (degree of freedom)	MS (Mean Square)
Among group	1311.4581	1	1311.4581
Within group	209.7501	19	11.0395
Total	1521.2082	20	-

The F value given by the software is 118.79713. The F critical value from the Table is 4.3807.The p value is 0.000359. The result is significant at $p<0.05$. As the F ratio is greater than F critical, the result is **significant** at given probability value.

- **Turbidity:** Experimental data of the turbidity recorded is given in the Table 37.

Table 37: **Turbidity of all the trails of all samples**

Trails	Control	Pectinase
1	0.864	0.0249
2	0.617	0.0245
3	1.164	0.0282
4	1.421	0.170
5	0.941	0.164
6	1.231	0.094
7	0.856	0.201
8	1.018	0.019
9	1.225	0.116
10	0.854	0.087
11	0.915	0.118
12	1.147	0.126
13	1.247	0.097
Mean	1.0397	0.0977

ANOVA was performed on the above data. The software had given the F ratio value along with the p value (probability). This F ratio has to be compared with the F critical value which was found from the Table of F ratio, for this degree of freedom must be known.

Table38: Summary of data for Turbidity

Data	Control	Pectinase	Total
N	6	13	19
∑N	6.238	1.2696	7.5076

Mean	1.0397	0.0977	0.3951
$\sum X^2$	6.9022	0.1676	7.0698
Standard Deviation	0.2887	0.0603	0.4775

Table 39: Results of ANOVA for Turbidity

Source	**SS (Simple Square)**	**Df (degree of freedom)**	**MS (Mean Square)**
Among group	3.6429	1	3.6429
Within group	0.4604	17	0.0271
Total	4.1033	18	-

The F value given by the software is 134.51912. The F critical value from the Table is 4.513. The p value is <0.00001. The result is significant at p<0.05. As the F ratio is greater than F critical, the result is **significant** at given probability value.

- **Per cent acidity:** ANOVA was performed on the data. Tool had given the F ratio value along with the p value (probability). This F ratio has to be compared with the F critical value which was found from the Table of F ratio, for this degree of freedom must be known.

Table 40: Summary of data for per cent acidity

Data	**Control**	**Pectinase**	**Spiced**	**Total**
N	6	6	6	18
$\sum N$	6.6	6	7.32	19.92
Mean	1.1	1	1.22	1.1067
$\sum X^2$	7.28	6.04	8.9308	22.2508
Standard Deviation	0.0632	0.0894	0.0089	0.1101

Table 41: Results of ANOVA for per cent acidity

Source	**SS (Simple Square)**	**Df (degree of freedom)**	**MS (Mean Square)**
Among group	0.1456	2	0.0728
Within group	0.0604	15	0.004
Total	0.206	17	-

The F value given by the software is 18.0794. The F critical value from the Table is 3.68. The p value is 0.000101. The result is significant at $p<0.05$. As the F ratio is greater than F critical, the result is **significant** at given probability value.

- **Vitamin C:**

ANOVA was performed on the data. The software had given the F ratio value along with the p value (probability). This F ratio has to be compared with the F critical value which was found from the Table of F ratio, for this degree of freedom must be known.

Table 42: Summary of data for Vitamin C

Data	Control	Pectinase	Spiced	Total
N	8	8	8	24
$\sum N$	1.497	4.122	3.204	8.823
Mean	0.1871	0.5153	0.4005	0.3676
$\sum X^2$	0.2801	2.124	1.2832	3.6873
Standard Deviation	0.0008	0.0036	0.0016	0.1389

Table 43: Results of ANOVA for Vitamin C

Source	SS (Simple Square)	Df (degree of freedom)	MS (Mean Square)
Among group	0.4436	2	0.2218
Within group	0.0001	21	0
Total	0.4437	23	-

The F value given by the software is 40726.98689. The F critical value from the Table is 3.47. The p value is < 0.00001. The result is significant at $p<0.05$. As the F ratio is greater than F critical, the result is **significant** at given probability value.

- **Tannins:**

ANOVA was performed on above data. The software had given the F ratio value along with the p value (probability). This F ratio has to be compared with the F critical value which was found from the Table of F ratio, for this degree of freedom must be known.

Table 44: Summary of data for Tannins

Data	Control	Pectinase	Spiced	Total
N	4	4	4	12
$\sum N$	29.2	35.95	51.8	116.95
Mean	7.3	8.9875	12.95	9.7458

$\sum X^2$	213.1626	323.1725	670.86	1207.1951
Standard Deviation	0.0294	0.1548	0.1291	2.4757

Table 45: Results of ANOVA for Tannins.

Source	**SS (Simple Square)**	**Df (degree of freedom)**	**MS (Mean Square)**
Among group	67.2954	2	33.6477
Within group	0.1245	9	0.0138
Total	67.4199	11	-

The F value given by the software is 2432.85298. The F critical value from the Table is 4.26. The p value is <0.00001. The result is significant at $p<0.05$. As the F ratio is greater than F critical, the result is **significant** at given probability value.

- **Flavonoids:** ANOVA was performed on the data. The software had given the F ratio value along with the p value (probability). This F ratio has to be compared with the F critical value which was found from the Table of F ratio, for this degree of freedom must be known.

Table 46: Summary of data for Flavonoids

Data	**Control**	**Pectinase**	**Spiced**	**Total**
N	5	5	5	15
$\sum N$	1.82	2.78	2.68	7.28
Mean	0.0364	0.556	0.536	0.4853
$\sum X^2$	0.6626	1.5462	1.4368	3.6456
Standard Deviation	0.0055	0.0114	0.0089	0.0896

Table 47: Results of ANOVA for Flavonoids

Source	**SS (Simple Square)**	**Df (degree of freedom)**	**MS (Mean Square)**
Among group	0.1114	2	0.0557
Within group	0.001	12	0.0001
Total	0.1124	14	-

The F value given by the software is 696.33333. The F critical value from the Table is 3.89. The p value is <0.00001. The result is significant at $p<0.05$. As the F ratio is greater than F critical, the result is **significant** at given probability value.

4.5.2 PEARSON CORRELATION:

The Pearson correlation coefficient is a statistical function which is used to measure the strength of a linear association between any two variables. The R value ranges from 1 to -1, where R=1 means there is a perfect positive correlation between those two variables and R=-1 signifies a perfect negative correlation. R=0 means there is no relation between the given variables. Nearer the value to 0, weaker the relationship is.

Table 48: Correlation between parameters

S. No.	X-variable	Y-variable	R values	R^2 value	Correlation
1	TSS	per cent Acidity	-0.6799	0.4623	Moderate negative correlation
2	pH	TSS	-0.5557	0.3088	Moderate negative correlation
3	pH	per cent Acidity	0.3641	0.1326	Technically positive correlation

There is no correlation between the rest all of the parameters. There was no trend, neither positive nor negative, between them. The result explained that these were the parameters which were linearly correlated within and between the groups.

V. CONCLUSION:-

The juice extraction method was first optimized by standardization of certain parameters. Enzyme concentration was standardized at 0.05 per cent w/v of the slurry. The incubation temperature was standardized at 45^{o}C. Incubation time was standardized for four hours. Bottle gourd must be blanched to prevent the darkening of the juice, thus, the blanching period was standardized for 15minutes.

From the above study, it was clearly indicated that using pectin in juice extraction method can significantly enhance the yield. In the control juice the final juice yield was 79.4per cent. When pectinase was used, the juice yield increased and it became 89.5per cent. Thus it was clear that using pectinase can enhance the yield by 10per cent.

The juice extraction with the aid of pectinase added the nutritional qualities like increase in vitamin C content, tannins and flavonoids content. Physio-chemical properties of all three juices: control, pectinase assisted and spiced version, showed that they had tannins content (7.37 µg/ml, 9 µg/ml and 12.9 µg/ml); per cent acidity (1.1 per cent, 1 per cent and

1.23 per cent); Vitamin C content (0.187 mg/ml, 0.491 mg/ml and 0.516 mg/ml) and flavonoids content (360 mg/ml, 530 mg/ml and 560 mg/ml).

The organoleptic properties were also enhanced as TSS increases. Sensory analysis was done of all three juice samples with Hedonic 9 point scale. Control juice scored 6.7 that means this juice sample was liked slightly by the panel. Pectinase assisted bottle gourd juice scored 6.9 which means this juice sample was liked moderately by the panel and finally our formulated spiced version drink scored 8.76 which means this juice sample was liked very much by the panel.

The statistical analysis of the juice samples was done. Two statistical tests were performed: ANOVA and Pearson correlation. ANOVA showed that all the obtained results had significant difference between them. The significance level was 0.05per cent. Relationship between the data sets was found by performing Pearson correlation and it was found that certain parameters did showed relationship. The Total soluble solids and percent acidity are having negative correlation. TSS and pH were also negatively correlated whereas the pH and per cent acidity were positively correlated.

ANNEXURE-I

LIST OF FIGURES

ANNEXURE-II
LIST OF TABLES

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1	Roopan, Selvaraj Mohana, V. Devi Rajeswari, V. N. Kalpana, and G. Elango. "Biotechnology and pharmacological evaluation of Indian vegetable crop Lagenaria siceraria: an overview", Applied Microbiology and Biotechnology, 2015. Publicat ion	%1
2	jakraya.com Internet Source	%1
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4	Irfan Ahmad. "Nutritional and Medicinal Potential of Lagenaria siceraria", International Journal of Vegetable Science, 04/2011 Publicat ion	%1
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