“Low calorie vegetable jam: An initiative”

A Dissertation submitted in partial fulfilment of the

requirement for the award of degree of

MASTER OF SCIENCE

IN

BIOTECHNOLOGY

By

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Certificate

This is to certify that the Dissertation report entitled to "**Low calorie vegetable jam: An initiative**" submitted by Mehak Dewan in the partial fulfillment of the requirement for the award of degree of the Master of Science in Biotechnology, Department of Biotechnology, Thapar University, Patiala, is a record of Student's own work carried out by her under my supervision and guidance. The report has not been submitted for the award of any other degree or certificate in this or any other university or institute.

29/8/17

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Candidate's Declaration

I, hereby declare that the work presented in the dissertation entitled "**Low calorie vegetable jam: An initiative**", in partial fulfilment of the requirement for the award of degree of Master of Science, Department of Biotechnology, Thapar University, Patiala, is an authentic record of my own work during the period of six months from January 2017 to July 2017, under the supervision of Dr. Jyoti Rani, Assistant Professor (FT), Department of Biotechnology, Thapar University. The thesis report has not been submitted for award of any other degree or certificate in this or any other University.

Place: Patiala

Date: 29-08-2017

Mehak.

(MEHAK DEWAN)

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Finally, and most importantly, I would like to thank the almighty God, for it is under his grace that we live, learn and flourish.

Place: Patiala
Date: 29-08-2011

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Abstract

The focus of this study was to standardize the process of making low calorie vegetable jam. The fruit jam has already been in the market and established since many years. An initiative has been carried out to produce low calorie vegetable jam using carrot, *Lagernaria siceraria* and beetroot in an appropriate combination with replacement of table sugar with stevia (natural sweetener). Three different formulations of jams like control or apple jam, vegetable jam and sugar free vegetable jam have been designed and their characteristics like tannins, flavonoids, vitamin C, titrable acidity and sensory characteristics have been compared. The apple or control jam had vitamin C content (0.21mg/g), titratable acidity (2.7 per cent), flavonoids (0.42mg/g) and tannins (14.3µg/g). The vegetable jam contained vitamin C content (0.145mg/g), titratable acidity (5.96 per cent), flavonoids (0.45mg/g) and tannins (19.4µg/g). The sugar free vegetable jam had vitamin C (0.102mg/g), titratable acidity (12.16 per cent), flavonoids (0.66 mg/g) and tannins (23.7µg/g). This showed that apple jam had highest vitamin C and lowest titratable acidity, flavonoids and tannins among all the three jams. Vegetable jam was observed to have vitamin C, titratable acidity, tannins and flavonoids higher than apple jam and lower than sugar free vegetable jam. Sugar free vegetable jam contained highest titratable acidity, tannins and flavonoids among all the three jams. Sensory analysis was observed for apple or control jam, vegetable and sugar free vegetable jam. The apple jam was very much liked by people with overall acceptability of 8.6. The vegetable jam was also very much liked by people with overall acceptability of 8.0. The sugar free vegetable jam was moderately liked by people with overall acceptability of 7.1.

Statistical analysis like ANOVA and Pearson's correlation coefficient was also done for all the three jams. By performing ANOVA, F calculated value obtained for pH was 41.78, total soluble solid sugar was 10717.21, moisture content 53.62 while their F critical value obtained was 3.49. F calculated value obtained for titratable acidity was 1537.73, vitamin C 219.49, tannins 2789.62 and flavonoids 2126.76 while their F critical value observed was 4.26. The results proved that F calculated value was greater than F critical value which means results were significant i.e. there was significant difference between mean values of pH, total soluble sugar, moisture content, titratable acidity, tannins and flavonoids of all the three jams. The correlation results were not all found interacted but observed with weak positive correlation between total soluble solids and percent acidity, weak negative correlation between total soluble solids and pH and moderate negative correlation between moisture content and total soluble solids of sugar free vegetable jam.

Keywords: Apple jam, vegetable jam, sugar free vegetable jam, stevia, pectin, bottle gourd, carrot, beetroot, citric acid, total soluble sugar, moisture content, vitamin C, tannins, flavonoids, titratable acidity.

CHAPTER-1

INTRODUCTION:

Due to insufficient storage facility, poor postharvest handling and inadequate marketing, a convincing amount of total fruit production in India goes waste every year. By making chutneys, pickles, jams, sauces and preserves through food processing, industries played a significant role in decreasing loss of fruits and vegetables and are flourishing largely as native food market (S. Singh *et al.,* 2009). Abundance of nutrition causes fruits and vegetables to play a principal role in maintaining good health and preventing several diseases. As a result, these fruits and vegetables can be made available throughout year in the form of processed food like jams (Lal *et al.,* 1986).

The consumption of fruits, vegetables and their products has been increased due to increase in awareness among the consumers. Consuming significant amount of fruits and vegetables helps to prevent diseases like cardiovascular diseases and cancer. The vitamins, fibers, minerals and other substances present in fruits and vegetables provide sufficient nutritive property. For the healthy population, there should be expansion of such products that provide bioactive and nutritional ingredients (Raquel, 2015).

The fruits and vegetables are high quality food products without any preservative. They meet all the quality and safety standards that's why always recommended by consumers. To launch a new product in to the market, the need of sensory analysis is mandatory which will determine the acceptance pattern of product by consumers. The sensory analysis involves acceptance of overall product in terms of taste, flavor, color, texture of the product through senses. (Guiné, R. 2012).

Jam: It is defined as food preserve made by crushing fruits and then cooking the crushed fruits along with water and sugar so that the pectin present in fruit get activated and gel is formed. Jam is semi-solid food or gel made from fruit pulp and sugar. A jam is considered to be good if it has good spreadibility, texture, flavor, color and is without any free liquid. Jams also known as sweet spreads, are normally inclined to hold their shape and are Less firm then jellies. (Ingham, B. H.2008)

Fig: 1 Fruit Jam

The important ingredients for making the jam are pectin, sugar and acid and so called as pectin-acid sugar gels. These ingredients provide structure and body to the jam and help to prevent the growth of microorganism as acid lowers the pH and sugar present will bind to the available water. The jam making begins with cooking crushed pulp of the fruits along with sugar. Until the total soluble sugar content reaches its predetermined level, the mixture needs to be heated and cooked. The accepted total soluble sugars content of jam is 65 per cent. At this concentration temperature will be 8-12°C above the boiling point of water. The amount of sugar added to the recipe depends on the individual fruit sugar and acidity content. The sugar content should be appropriate to the recipe as if it exceeds hard gel will be formed and less sugar produces soft gel. Exact amount of pectin and acid should also be added. External source such as lemon juice can be added if fruit does not contain desired amount of acid. Likewise, if fruit is low in pectin, then commercial pectin can be added. Jam will not set if adequate amount of pectin is not present (Singh, 2014).

The fruits include diverse range of plant foods that vary in nutrients and energy content greatly. They provide dietary fiber which has great laxative property, helps in preventing irregular bowel movements and reduces cholesterol. They improve nitric oxide levels in the blood stream which reduces blood clots, provides antioxidants to body and prevent bad breath. They also supply minerals like Ca, Mg, K and vitamins like vitamin A and β- carotene which are good for eyesight. Citrus fruits also prevent respiratory and urinary disease. They contain number of macronutrients, micronutrients, antioxidants, flavonoids and phytochemicals which are beneficial for health. They also help prevent acne, enhance radiance of skin and also help to promote good hair growth (Slavin, J. L, 2012).

Fig: 2 Vegetable jam

Due to nutrition rich properties of fruits, they can be used for making jams by cooking fruit pulp with extra added sugars. In addition to nutritional properties of fruits, their shelf life can be increased during transportation and commercialization. These processed foods can also be enjoyed throughout the year. Variety of fruits can be used for making jams like sour blackberries, apples, grapes, cranberries, apricots, cherries, peaches, pineapple and strawberries(McGeeH,1997).

Producing vegetable jam has not been researched upon yet. The vegetables jam in the market will help to diversify and provide new product to the consumer especially diabetic consumers. The vegetables can be beneficial for jam production because they contain less sugar than fruits and more fiber, iron, folate, calcium and beta-carotene or so other nutrients. They will help to improve blood pressure skeletal system, digestive and excretory tract .They provide large number vitamins like vitamin B 6, vitamin A, vitamin K as well as also provide carotene from carrots. Vitamin K is usually present in all vegetables and thus maintains bone health.

Fig 3: Vegetables

The vegetables have more water percentage which will keep body hydrated and fat free. Consuming vegetables will expel out toxins and waste from body. They are also good source of iron which is essential as oxygen carrying hemoglobin and also necessary for brain development. Being good source of dietary fiber, they also help to prevent constipation and diarrhea by absorbing extra water from colon and thus, retaining extra water in faecal matter. They also help to reduce weight, improve skin and helps in promoting hair growth (www.scienceofmom.com).

Many vegetables can be used for making vegetable jam like carrot, bottle gourd, cucumber, pumpkin, onion and beetroot. The bottle gourd and carrot have been used in combination to inherit a mixed color and flavor along with pectin. *Lagernaria siceraria* also known as Ghiya or bottle guard from cucurbitaceae family, is an herbaceous plant. Ghiya is best known for its medicinal purposes and is used for treatment of skin diseases, diabetes, jaundice, hypertension and congestive cardiac failure. The pulp of ghiya has many curative properties i.e. can act as diuretic, emetic and purgative. Its analgesic anti- hyperlipidemic, hepatoprotective, anti-inflammatory, antibacterial effects have also been observed.

Fig 4: *Lagernaria siceraria*

Ghiya contains number of amino acids like alanine, valine, tyrosine, proline and cysteine. It also contains choline, carbohydrates, fibers, calcium and minerals like iron and sodium. (Prajapati, R.P.*et al.*, 2010).

Daucus carota, commonly known as carrot, is a root vegetable from apiaceae family was earlier used as medicinal food and then afterwards used as food. The carrots are good for eyesight, vitamins, and polyphenols, carotenoids present in carrots function as

immunoenhancers, anticarcinogens and antioxidants. They also modulate immune response, exert anticarcinogenic activities and reduce inflammatory results.

Anti-diabetic, cholesterol, cardiovascular disease lowering, anti-hypertensive, hepato-protective, reno-protective and wound healing benefits of carrot have also been reported. They tend to clean intestine and thus act as diuretic, antidiarrheal antianemic and an overall tonic for human health. (João Carlos da Silva Dias, 2014).

Fig 5: ***Daucus carota***

Stevia, a sugar substitute also known as sweetener, is obtained from the leaves of plant *Stevia rebaudiana* of asteraceace family. It is commonly called as sugar leaf, sweet leaf or candy leaf due to its sweet nature. Stevia is usually 250-300 times sweeter than sugar (Abdullateef, R. A. *et al.*, 2011).

Fig 6: ***Stevia rebaudiana***

Stevia leaves contain complex mixture of eight glycosides such as steviobioside, isosteviol, dulcoside A, rebaudiosides (A, B, C, D, E, F) steviosides from which rebaudiosides and steviosides are most important for providing sweetness to the plant. It has been observed that stevia has no carcinogenic, mutagenic and teratogenic effects and also no allergies have been observed when stevia is consumed (Pol *et al.,* 2007). Stevia leaf or extracted forms like steviosides, rebaudioside A and steviol glycosides has been used for treating diseases like Diabetes mellitus (DM) which is characterized by insufficient insulin effect and hyperglycemia. Intake of stevia leads to suppression of glucose level and also increases glucose tolerance (Curi *et al*., 1986). Steviol glycosides also lead to enhancement of insulin secretion which will control glucose level in blood. (Jeppesen *et al*., 2000).

Objectives:

- Standardization of ingridients and procedure for production of vegetable jam.
- Replacement of sugar with stevia to produce low calorie vegetable jam and quality and sensory analysis of same in comparison to fruit jam and vegetable jam.

CHAPTER-II

REVIEW OF LITERATURE

2.1 JAM:

The concentrated fruit pulp with sugar or jam produced by boiling fruit pulp with an acid, pectin and sugar is an intermediate moisture fruit product (Baker *et al*., 2005). The fundamental principle of jam making is to increase the solid content due to water evaporation, conversion of sucrose in to invert sugar and formation of gel on cooling. All the ingredients present in jam affect texture, structure and sensory attributes. Sugar in jam, in addition to providing sweetness to jam also maintains physical, chemical and microbiological stability of the product. Sugar also reduces water activity in jam as it binds with the water available in the product due to which spoilage of jam by micro-organisms can be prevented. If the concentration of these ingredients is varied then change in texture and gel structure of jam may be inevitable (Ingham, B. H. 2008).

2.1.1 Vegetable jam:

The use of vegetables in jam may help to launch new product in to the market. The sweetness that comes from good quality vegetables is more balancing and nourishing for our bodies. The vegetable jam could be helpful in stabilizing blood sugar from refined sweeteners and fruits. The vegetables contain all the necessary ingredients that are required for making jam. In my study vegetables like bottle gourd, carrot and beet root were used for making jam as they possessed many health benefits.

2.1.2 Health benefits of Bottle gourd:

- known as „Ghiya", it is traditionally used for treatment of jaundice, piles, insanity, ulcer, diabetes, skin diseases, hypertension and congestive cardiac failure.
- it has hepatoprotective, antioxidant, antihyperglycemic, cytotoxic, and antihyperlipidemic activity.
- it also has antidepressant, antistress, analgesic and adaptogenic property.
- it also helps in controlling body weight as it is rich in dietary fiber which aids to cure constipation.
- it is low in fat and has fewer calories and thus best for obese people.
- due to ample of water in Ghiya, its consumption will provide cooling effect and thus act as sedative.

- its high water content is also useful for treating urinary disorders.
- it is helpful for preventing insomnia and thus helps to treat sleeping problems.(Kumar, A. *et al*, 2012).

2.1.3 Health benefits of Carrot:

- Vitamin K present in carrots help to prevent bleeding whereas vitamin C present is used for fighting infections.
- antioxidants and vitamin K present in carrots protects the skin, prevents acne, blemishes, dry skin.
- they also help to clean teeth and brush food particles and plaques. Carrots activate saliva which being alkaline neutralizes the acidic bacteria
- they also help to clean the body by removing toxins and also decrease fat content in liver.
- being rich in beta carotene, carrots prevent cell damage and slow down aging of cells.
- they also act as immune-boosters by activating white blood cells.
- Carotenoids present in carrots are beneficial for diabetic patients by lowering the blood sugar levels (João Carlos da Silva Dias, 2014).

2.1.4 Beetroot as colorant:

Food coloring or color additive is a pigment that imparts color to the food when added. Food colours are used in both domestic and commercial cooking. Food colors are added so that the color that is lost during processing or storage can be regained or to give desired color to the food. Color additive is added to make food more informative and appetizing. Also they provide color to colorless to make them fun foods. The aim of food coloring is also to identify the food on sight like flavors (http://foodscintech.blogspot.in). Food coloring can be imparted through artificial or natural coloring. Artificial food colours produced from petroleum were not pure. Although artificial food colours have some advantages over natural colours like deepest color, stable and are cheapest. But they have adverse effects like they cause tumour of kidneys or urinary bladder, hypersensitivity, can cause skin rashes, nausea, vomiting, high blood pressure, breathing problems and damage unborn babies (www.turmeriq.com). Due to harmful effects of artificial colors, natural colorants have been exploited as they are safe, not harmful to environment and to human health, like beetroot are commonly used for coloring jams and jellies, sauces, ice-cream, candies and sauces. It is used for making wine. Beetroot

is a taproot portion of beet plant, which is red in color. Betanin and vulgaxaxtin are the two main components responsible for red color of the beetroot (http://www.food-info.net).

2.2 HEALTH BENEFITS OF VEGETABLE JAM:

Numerous health benefits have been documented and proved scientifically. The vegetables are enriched with vitamin C, tannins, flavonoids, antioxidants, potassium, folate, minerals and amino acids. Each of these components has its own benefits. (Slavin, J. L, 2012).

Vitamin C also known as ascorbic acid is present in higher percentage in bottle gourd and carrot. It is water soluble and very essential nutrient for human health. It is renowned as antioxidant and further regenerates other antioxidants in body. Being an antioxidant, it scavenges the free radicals that are present in the body. It is also important for the synthesis of collagen, L-carnitine and also leads to the conversion of dopamine in to norepinephrine (Li, Y.*et al*., 2007). Vitamin C helps to prevent cancer and thus limit the formation of carcinogens like nitrosamines (Hecht, S. S. 1997). Also being an antioxidant, it also reduces the oxidative damage, a cause of cancer (Li, Y.*et al.,* 2007). It helps to prevent cardiovascular diseases (Chen, GC.*et al*., 2013). It also helps to prevent common cold and wound healing (Naidu, K. A.2003).

Flavonoids are polyphenolic compounds that are widely present in vegetables and are very important for human health. Flavonoids have number of biological activities, like anti-inflammatory, antiviral, anticarcinogenic and antiproliferative activities. They have antioxidant properties which help to prevent human diseases like cardiovascular and cancer diseases and other disorders like allergies, ulcers and viral and bacterial infections. Flavonoids also have pharmacological properties like anti-allergic, anti-diabetic, ant-oxidative and anti-neoplastic activities (Yao, L. H.2004).

Tannins are a group of polyphenolic compounds**.** They contain number of hydroxyl groups that can interact with alkaloids, proteins to form precipitation. Tannins have antioxidant activity, improve cardiovascular health, treat cancer, help to treat diarrhea, and immunity (Chung, K. T. *et al.,* 1998).

Folate helps in forming red blood cells. Pregnant women should consume adequate amount of folate and this reduces the risk of neural tube defects (https://www.choosemyplate.gov).

Potassium is widely present in vegetables and thus helps to keep the body calm inside by relaxing the blood vessels and keeps the blood pressure low (https://www.choosemyplate.gov)

2.3 MAIN INGREDIENTS IN JAM MAKING AND THEIR ROLE:

The ingredients essential for making jam:-

- Pectin
- Sugar
- Acid
- Heat

2.3.1 Pectin and its structural components:

Pectin is a complex plant hetero-polysaccharide present in the primary cells of terrestrial plant which is responsible for the structure and firmness of the plant. Pectin is responsible for gelling property and this property is dependent upon degree of methoxylation (DM).

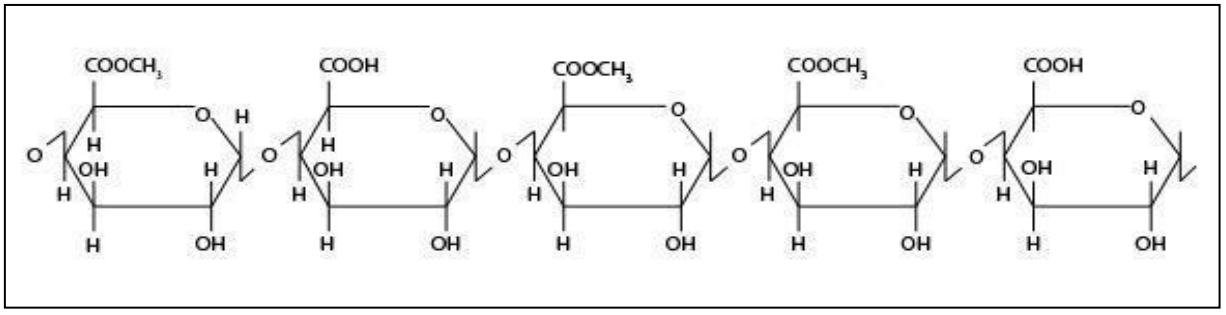


Fig 7: Structure of pectin

The pectic substances have backbone of galacturonic acid sub-units connected by α (1-4) glycosidic linkages as shown in fig 7. In galacturonic acid, the carboxyl groups are partly esterified by methyl groups and fully or partly neutralized by ammonium, sodium or potassium ions. Pectin is made from a number of polysaccharides like homogalacturonan (HG), xylogalacturonan (XGA), rhamnogalacturonan I and II (RG) as shown in fig 8.

Homogalacturonan (HG):

Backbone of HG is made of α-D- galacturonate units linked by α- (1, 4) glyosidic bonds. It is a linear structure so, called as smooth region of pectin. Carboxyl group of D-galactouronic acid can be acetylated or methyl esterified.

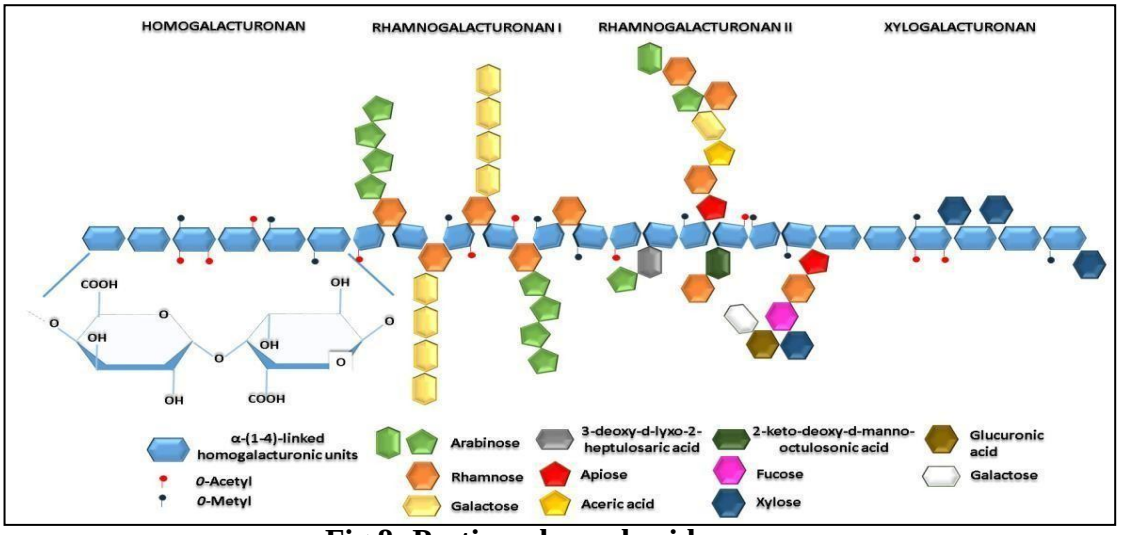


Fig 8: Pectin polysaccharide

Xylogalacturonan (XGA):

It is α-(1, 4) linked D–GA chain which is substituted by β-D-xylose at C_3 position.

Rhamnogalacturonan I:

D-galacturonic acid residues in backbone are interrupted by α-1, 2-linked L-rhamnose residues to which galactan and arabinan chains can be attached at 0-4 position.

Rhamnogalacturonan II:

A Cluster of complex side chains are also attached on to O-2 or O-3position in D-GA backbone. (Sharma, B.R *et al.*, 2006)

2.3.2 Functional groups:

Esterification of galacturonic acid with acetic acid or methanol is essential characteristic of pectic substances. Percentage of carboxyl groups esterified with methanol is called degree of methylation (DM). DM is inversely proportional to solubility. Based on DM, pectin is classified in to two categories:-

High methoxy (HM) pectin:

HM has more than 50 percent of carboxyl group methylated. It has ability to form gel in presence of high sugar content and low pH value. It does not contain acidic groups that can gel or precipitate with calcium ions. Hydrogen bonding and hydroscopic interactions are important are for aggregation of pectin molecules. Pectin molecules in neutral or slightly acidic dispersions contains unesterified carboxyl group. There is conversion

of carboxyl ions to unionized carboxyl acid groups when acid is added. Due to decrease in negative charge, there is less interaction of pectin and water molecules and also repulsion between pectin molecule decreases. Hydration of pectin decreases with addition of sugar. Thus, pectin is no longer in dispersed state after cooling less hydrated pectin forms the gel.

Low methoxy (LM) pectin:

If the degree of methylation found less than 50 percent then they are called low methoxy pectin. They have ability to form gels in the presence of bivalent salts, normally $Ca2^{+}$ ions and require low sugar content and wide ph range. The gelation of LM pectin is mainly dependent on "egg box"""" model shown in fig 9. According to egg box model, junction zones are created side by side linkages of galacturonan. Gal-A monomer sequence is linked intermolecular via ionic/ electrostatic bonding of carboxyl group to adjacent chains. The junction zones that are created usually consist of dimers of 21 helical symmetry. A number of oxygen atoms are involved in bonding like oxygen atom of hydroxyl group, ring oxygen atom and bridging oxygen atom of sugar units. At least, seven consecutive carboxyl should be in the inner side of participating chain for the bond to be stable. Although, sugar is not needed for LM pectin to gel but if small amount of sugar is added it will lead to formation of more firm gel and there will be decrease in syneresis. Requirement of calcium decreases for formation of gel if sugar is added. But if high concentration of sugar is added, gel will not form properly (Tyagi, V *et al*, 2015).

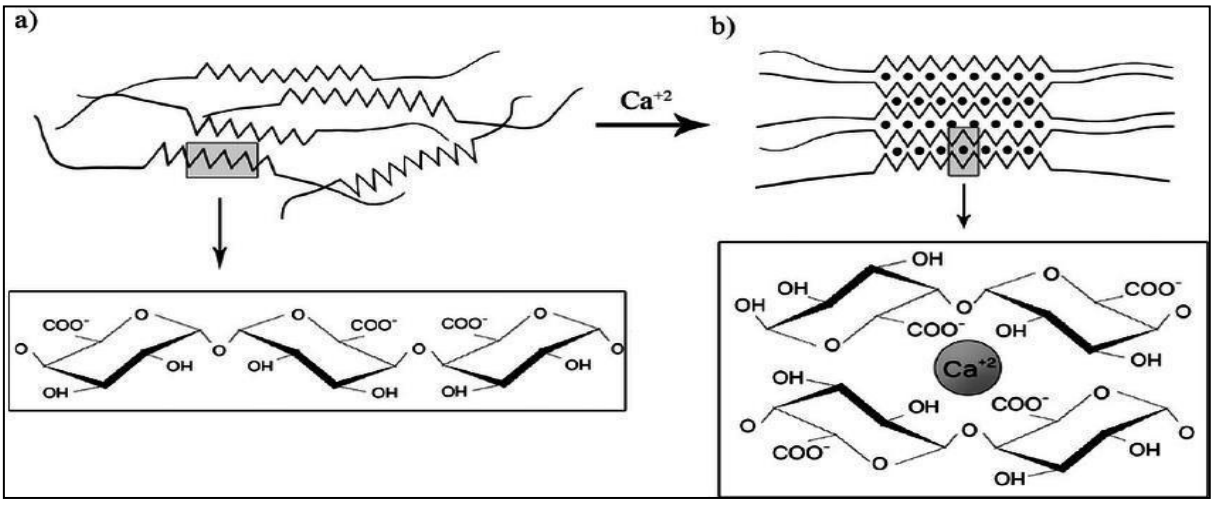
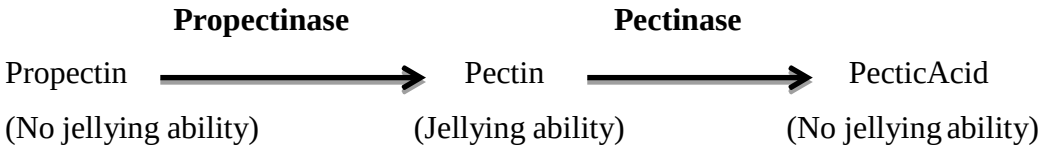


Fig 9: Ca $^{2+}$ induced gelation of pectin

2.3.3 Pectin and its effect in jam making:

In fruits and vegetables, pectin is present in abundant amount. Pectin is responsible for setting/gelling characteristics of jam. Pectic substances present in middle lamella of plant tissues act as glue which holds plant tissue in place. These pectic substances have gelling

ability and are converted from propectin to pectic substances. The conversion of propectin to pectin is catalyzed by enzyme propectinase already in plant. When the fruit matures propectin is converted in to pectin, therefore there is increased content of pectin. When the fruit gets further matured, pectin is converted to pectic acid which is catalyzed by enzyme pectinase. Among all the pectic substances, only pectin has the gelling ability so, has much interest in food industry (Arthey, D *et al*., 1995).



Pectin is an indigestible fiber, released from fruit when boiled. In order to form a gel network, these pectin molecules need to interact with each other via intermolecular interactions. But pectin molecules repulse when they are dissolved in water for two reasons: First of all they have negative charge and thus repel each other. Secondly, they are hydrophilic which means they stick to water molecules than each other. Therefore, in order to form gel it is important to stop pectin to bind water and start binding to each other. The jam generally sets at 104˚C and also known as setting point of gel. Once the setting point reached, jam is allowed to cool and water is trapped in gel network.

2.3.4 Role of sugar:

Sugar is necessary ingredient in jam making. It disturbs equilibrium between water and pectin and thus, acts as dehydrating agent. Microorganism‟s (yeasts, mold, and bacteria) that harm or cause spoilage can survive only in the presence of water. All of the microorganisms (yeasts, mold, and bacteria) that cause spoilage need water to survive and proliferate. These microorganisms can‟t survive when water molecules bound with sugar.

2.3.5 Role of acid:

When water molecules bind with sugar, pectin is left alone. Because pectin has negative charge and acid has positive charge, so in order to neutralize pectin, acid was added to jam mixture. Acid increases the concentration of positive ions in water. Neutralizing effect will be observed when positively charged acid interact with negatively charged pectin. Pectin molecules interact with each other to form gel once the neutralization is achieved.

2.3.6 Role of heat:

In order to remove excess water, mixture of jam is cooked and water is removed by evaporation. Once the water is evaporated gel reached to its set point. And at this point pectin molecules stick to each other to form 3-D structure (http://www.seriouseats.com).

2.4 SUGAR FREE VEGETABLE JAM:

Dietary risk factors are responsible for increase in incidence of diseases. Among them, one of the most responsible risk factor for disease is sugar consumption, due to its high calorie content. Sugar may also cause insulin resistance, tooth decay and obesity (Alizadeh, M. *et al*., 2014). So we have to produce low calorie vegetable jam so that it can be consumed by diabetic patient also. By simply lowering the sugar content of jam, acceptable structure, texture and quality will not be obtained. Sugar besides providing sweetness, is also responsible for gelling in the jam. The concentration of sugar needed to form gel is directly dependent on concentration of pectin present in vegetable or commercial pectin added to recipe. In order to form sugar free vegetable jam, sugar substitutes are needed. Jam with less or no sugar requires commercial pectin to obtain perfect texture (MORRIS, W. C., 2004).

2.4.1 Types of sugar substitute:

Sugar substitute is a sugar like product that provides sweetness to the product but has less energy. Some substitutes are produced by nature and others are produced synthetically. Manmade sweeteners that were produced synthetically are called as „artificial sweetener". There are number of artificial chemical sweeteners in the market like neotame, aspartame, acesulfame potassium, advantsme, saccharin and sucralose. But these artificial sweeteners have side effects and commonly reported include headaches, dizziness, fatigue and mood swings. So natural substitutes like stevia are better to use because they help to control weight, are cheap, have long shelf life, prevent plaque formation and eventually tooth decay and can be used to make food for diabetic patient (Morris, W. C. 2004).

2.4.2 Stevia:

Stevia, a natural sweetener obtained from the leaves of plant *Stevia rebaudiana*, has 250-300 times more sweetness than normal sugar. It is low calorie compound and is helpful for making products for diabetic patient. Its leaves contain eight sweet glycosides, including isosteviol, steviosides, rebaudiosides (A, B, C, D, E, F), steviobioside and dulcoside-A. The steviosides and rebaudioside-A are the major metabolites responsible for sweetness of stevia. Its glycosides are metabolized by microbes present in digestive tract. The rebaudioside A is metabolized in to steviosides by microbes present in colon. Steviosides are further converted

in to glucose and steviol. The glucose molecule that has been released is utilized by bacteria and not absorbed by blood. In stevia metabolism, steviol is the final product (Koyama *et al.*, 2003). Stevia doesn't affect the pH, acidity, vitamin C content. But there was decrease in TSS content due to replacement of sugar with stevia. (Alizadeh, M. 2014)

2.4.3 Uses of Stevia:

- Does not affect blood sugar level and as safe for diabetic patient.
- does not have any neuroglial or renal side effects like other sweeteners possess.
- glycosides in stevia can increase the urination, eliminate sodium from body and relax the blood vessels.
- it has antibacterial and antifungal properties and thus can be used in mouthwash and toothpastes.
- due to presence of antioxidants, stevia is used for treating cancer. (Goyal, S.K.*etal.,* 2010).

2.4.4 Jam production with stevia and pectin:

Special pectin products have been launched in to the market for low calorie jam. The pectin that was used for making sugar free jam is called as low methoxy pectin (LM). For LMP, calcium salt was needed to make gel. Calcium salt was prepared by mixing calcium chloride with water. In order to make sugar free jam, stevia was first mixed with LMP. Then the fruit pulp was boiled and stevia-pectin mixture was added and further boiling was given. At last calcium solution was added and stirring was done (www.extension.oregonstate.edu).

2.5 DETERMINATION OF THE END POINT OF A JAM:

1. Cold Plate Method: Small plate in the freezer should be placed for chilling for around 10 minutes. Spoon hot jam onto the cold plate and let it rest for 30 seconds. Tilt the plate to one side, if mixture is runny and thin then more cooking is needed. If jam moves slightly then set point of jam has been obtained (www.portlandpreserve.com).

2. Drop Test: Small drop of boiled jam is put on a spoon after cooling it slightly, drop into a glass of cold water. If the drop falls in a single piece until it reaches the bottom of the glass the end point has reached. If it disperses in the water it requires boiling for more time (www.practicalaction.org).

3. Sheet or spoon test: Dip a cool metal spoon into the boiling jelly mixture. Raise the spoon out of the steam about twelve inches above the pan. Turn the spoon, so the liquid runs off the side. The jelly is done when the syrup forms two drops that flow together and form a sheet that hangs off the edge of the spoon (www.portlandpreserve.com).

2.6 ROUTINE ANALYSIS OF JAM:

Once the jam was produced, it was analyzed on daily basis for three basic parameters. These parameters include the determination of total soulable solids, pH and moisture content of jam.

- **TSS (Total soluble sugars) –** Total soluble sugar of normal vegetable sugar jam should be between 65-68 °brix. If TSS of mixture is 70°Brix – crystallization may occur, 68 °Brix– good texture of jam was observed, 65 °Brix– legal minimum, 62 °Brix– weak gel will form, 60 °Brix– no gel formation, viscous liquid was observed. Therefore, the gel sets best at 65°brix of TSS.
- **The pH -** pH for normal jam recorded usually between 3-3.2. If pH is 3.6 – no gel will be formed, 3.4 – weak gel will form, 3.2– good firm gel will form, 2.8-weak gel and there will be no gel formation at ph 2.4.
- **Moisture content-** The moisture of jam should be usually between 31-34Percent of moisture content (www.iufost.org).

2.7 FAILURE OF GEL:

- Undesired consistency: disturbed proportion among sugar, pectin, acid and raw material.
- Less quantity of pectin and this could be either as it was not adequately mixed with sugar or added at the beginning of the heat treatment.
- Total soluble solids have to reach the final desired Brix degree.
- The pH values should be above 3.0, but when the pH value is less than 3, this may lead to jam bleeding.

As above described reviews, from different authors has contributed to preparation fruit or mixed fruit jam. A concerted attempt has been done to find out the literature on the vegetable jam but unable to get the encrypted evidences except in the videos, food recipes etc. Hence, an initial attempt has been carried out to prepare a purely vegetable and sugar free jam from the natural sources purely.

CHAPTER-III

MATERIALS AND METHODS:

3.1 Pre-requisites required for study:

3.1.1 Collection of fruit and vegetable samples:

The fruit like apple and vegetables like bottle gourd, carrot, and beetroot were bought from local market of Patiala. The fruit and vegetables were not completely ripped. These fruits and vegetables were brought for research and experiment purpose in the laboratory of Department of Biotechnology, TU, Patiala.

3.1.2 Sugar:

Sugar has been used to provide sweetness to the apple jam. Granulated sugar has been bought from local market of Patiala.

3.1.3 Citric acid:

Citric acid of Hi Media brand was used and was made available by Department of Biotechnology, TU, Patiala. It provides perfect gel structure that absorbs fruit juice and other liquids in form of hydrocolloid.

3.1.4 Sugar substitute: *All Natural* – iStevia

It was purchased from local market of Patiala. Stevia has been used to produce diabetic jam by replacing sugar in vegetable jam. It is 200-300 times sweeter than normal and is calorie free thus can be used for making diabetic products. The date of manufacture was February 27, 2017 and batch number was 010114121.

3.1.5 Pectin:

Pectin of Pract brand was used and was made available by DBT, TU, Patiala. It is low methoxy pectin with degree of methoxylation of 6%. It was used in pure vegetable jam preparation.

3.1.6 Divalent Calcium ions:

Calcium ions were provided by adding anhydrous calcium chloride of Hi Media and this was made available by Department of Biotechnology, TU Patiala. It was required for the formation of gel as low methoxy pectin can gel in presence of calcium.

Fig 10: a) Pectin b) Calcium chloride

3.1.7 Aluminum foil:

Aluminum foil of "Freshwrapp" company was used. It was used to cover the jam samples in order to avoid contamination during storage.

3.1.8 Blender:

Hand blender of company Glen, model number A4450, was used to make the puree of vegetables and fruits.

3.1.9 Steamer:

Steamer was used to blanch the fruit and vegetables to soften them. Steamer was provided by STEP, Thapar University, Patiala.

3.1.10 Weighing balance:

Weighing balance of least count 0.01g was used to weigh the ingredients that were used for making jam. It was provided by STEP.

3.1.11 Refractometer:

Hand refractometer of three different ranges was used to measure TSS content of jam and was made available by STEP.

3.1.12 The pH meter:

The pH meter was used to measure the pH of jam samples and was provided by STEP, Thapar University, Patiala.

Fig11: a) Hand blender b) Refractometer c) pH meter d) Thermometer e) Hot air oven

3.1.13 Digital Thermometer:

It was used to determine the temperature of jam i.e.to check the endpoint. It was made available by DBT, TU, Patiala.

3.1.14 Hot air oven:

Oven was used to dry the jam sample in order to determine the moisture content of jam. It was provided by STEP.

3.1.15 Refrigerator:

It was used for storage of jam samples and was provided by STEP,TU, Patiala.

3.2 EXPERIMENTAL PLANNING:

3.2.1 Processing of fruit and vegetables:

Apples, bottle gourds, carrots and beetroots were washed under running tap water in order to remove surface microflora, soil and dust particles. All the fruits and vegetables were peeled with peeler. The apples, bottle gourds and carrots were cut in to very small pieces while the beetroot was grated with grater. The pieces of apple were steam blanched for 10 minutes while vegetables were blanched for 20 minutes. The hand blender was washed with tap water and dried. Then pieces of fruit and vegetables were put in to the jar of blender along with 100ml of purified water. Blender was run and puree of apple and vegetables was obtained as shown in fig 12.

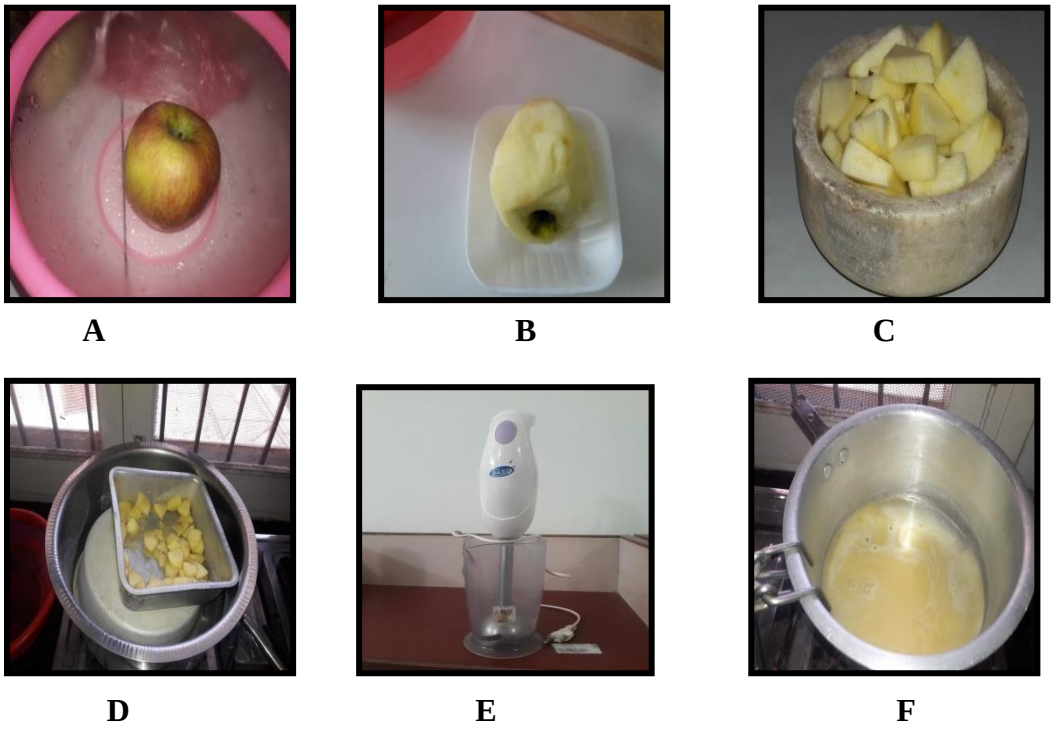


Fig12: Pictorial representation of processing of apple in laboratory A) Fresh apple fruit B) Peeled apple C) Pieces of apple D) Blanching E) Blending apple with blender F)Puree

3.2.2 PROCESS STANDARDIZATION OF APPLE AND VEGETABLE JAM:

3.2.2.1 Preparation of control jam (Apple jam):

After processing apples, puree of apples was added in to pan along with purified water and grated beetroot. After boiling this mixture for 2-3 minutes, sugar was added with continuous stirring. Whole mix was boiled for 10 minutes. Total soluble sugar was checked between the intervals and when the total soluble sugar reaches 65° Brix, end point of jam

supposed to be reached. At this point, jam started getting thick and leaves the corner of pan. Citric acid was added and one stir was given and flame was switched off. The cooled jam was tested for analytical, sensory tests and data was also statistically analyzed. Standardized recipe for the control sample is given in table 1:-

Table 1: Ingredients for Apple jam

Ingredients	**Quantity in grams**
Apple	100
Beetroot	5
Sugar	50
Citric acid	0.6
Water	200 ml

3222 Preparation of vegetable jam:

After processing 50g ghiya and 50g carrot, the puree of vegetables and grated beetroot was heated in the pan with small amount of water. After this, 50g sugar was added in to mixture and the whole mixture was boiled for 10-15 minutes with continuous stirring as shown in fig 13. Total soluble sugar was noted in between and once total soluble sugar reaches 62° Brix, 0.65g of citric acid was added and mixed properly and flame was switched off. Cooled jam was tested for analytical, sensory tests and data was statistically analyzed. Standardized recipe for the vegetable jam is given in table2:

Table 2: **Ingredients for vegetable jam**

Ingredients	**Quantity in grams**
Bottle gourd	50
Carrot	50
Beetroot	5
Sugar	50
Citric acid	0.65
Water	200ml

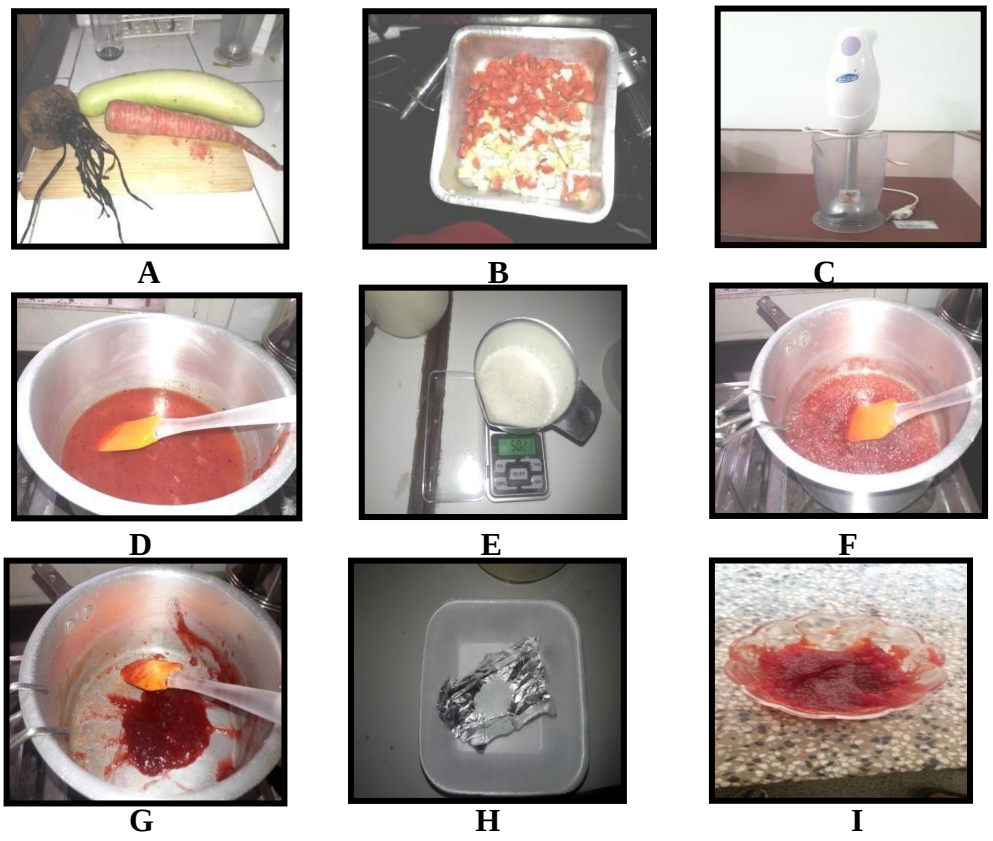


Fig13: Pictorial representation of preparation of vegetable jam: A) Fresh vegetables, B) Blanching of chopped vegetables, C) Blending of vegetables, D) Boiling of puree, E) Weighing of sugar, F) Boiling of jam mixture, G) Set point of jam, H) Weighing of citric acid, I) Final jam product.

3223 Preparation of sugar free vegetable jam:

After processing 50g bottle gourd, 50g carrot and 5g beetroot, the puree was put in to the pan. Five sachette of stevia along with 0.8g of low methoxy pectin was dissolved in 20ml of about to boil water. This solution was added in to the vegetable puree and was boiled in order to dissolve low methoxy pectin and stevia. Standardized recipe for low calorie vegetable jam is given in table 3:

Table 3: Ingredients for sugar free vegetable jam

Ingredients	**Quantity in grams**
Bottle gourd	50
Carrot	50
Beetroot	5
Pectin	0.8
Stevia	5 sachette
Calcium solution	1 tsp
Citric acid	0.6
Water	200ml

After boiling solution for some time, 1teaspoon of calcium solution was added which was made by mixing 62ml water with 1.42g of calcium chloride. Then the whole jam mixture was cooked till set. Cooled jam was tested for analytical, sensory tests and data was statistically analyzed.

3.3 PARAMETERS TESTS:

Analytical, sensory and statistical analysis of apple jam, vegetable jam and sugar free vegetable jam was done.

3.3.1 Sensory analysis:

The prepared jam samples were evaluated by sensory analysis for colour, appearance, flavor or aroma, texture, taste and overall acceptability from different subjects. This method was used according to the Larrmond, 1970. The samples were rated on 9 point Hedonic scale as under:

Table 4: Hedonic scale

Sr.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 Moisture content:

The moisture content of jam samples was determined using oven drying method (AOAC, 2000). In order to determine the moisture content of jam, 3-5 grams of sample was spread on to the dried and pre-weighed empty dish. Dish containing sample was dried for three hours at 105°C or until sample dried. Once the sample gets dried, it was cooled, kept in desiccator and then dish containing sample was reweighed and the calculations and given as under:

Calculations for Moisture Content:-

$$\text{Percent Moisture} = \frac{Wi - Wf}{Wi}$$

Wi= weight of sample before drying,

Wf= weight of sample after drying

3.3.2.2 Total Soluble Sugar (TSS):

The hand refractometer (Abee refractometer) of three different ranges like 0-32° Brix, 33- 62° Brix and 63-90° Brix were used for measuring total soluble sugar of three different jams (Ranganna, 1991). Total soluble sugar was usually measured at 20°C. The observations were expressed in ° Brix.

3.3.2.3 The pH:

The pH of all the three different jams was measured using glass electrode potentiometer, also known as digital pH meter of Cyber Scan (AOAC, 2000). The pH meter was calibrated using three different buffer solutions of pH 4.0, 7.0, 9.0.

3.3.2.4 Acidity:

The acidity of jam samples was estimated by titrating it with 0.1N NaOH solution using phenolphthalein indicator. It is expressed in percentage of citric acid (Ranganna, 1997). The 0.1N NaOH solution was prepared and filled in to the burette. Then 10ml sample (1ml sample+9ml water) was added in flask along with 3-4 drops of phenolphthalein indicator. Initial reading of burette was noted and titration was done until the solution in flask changes from colorless to pink. The final reading of burette was noted. The volume of NaOH used was calculated by subtracting initial reading from final reading.

$$\text{Acidity}(\%) = \frac{\text{Dilution factor} \times \text{Normality of NaOH} \times \text{volume of NaOH} \times \text{eq. weight of NaOH} \times 1000}{\text{Volume of sample} \times 1000}$$

3.3.2.5 Vitamin C:

The Vitamin C content of jam samples was measured by titrating it against Iodine solution (Ranganna, 1986). Starch solution was used as an indicator and was added into flask containing jam sample. The initial reading of burette was taken and titration was done until color of jam sample changes to purple-blue color. Once the color changes, final reading of burette was taken. The results are expressed as mg/100ml of jam. The results were compared against the standard of pure ascorbic acid using following formula:-

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

3.3.2.6 Tannins:

The tannins content of jam samples was calculated from the standard curve of tannic acid (Saxena, *et al*., 2013). First the stock solution of 1mg/ml of tannic acid was prepared. From this stock solution of tannic acid, dilutions of 0.1, 0.2, 0.3, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1mg/ml were prepared. In to these dilutions, 0.5ml FD reagent, 1ml sodium bicarbonate and 10ml distilled water was added. From these dilutions, standard curve was plotted. Sample was prepared by adding 10ml jam, 0.5ml FD reagent, 1ml sodium bicarbonate solution and 10ml distilled water and the whole mixture was mixed well. Absorbance of all the dilutions of tannic acid and sample was noted at 720nm in spectrophotometer.

Fig 14: Tannic acid and FD reagent

3.3.2.7 Flavonoids:

The flavonoids content of jam samples was measured using spectrophotometer (Bag, *et al*., 2015). The stock solution of 100µg/ml of Quercetin was prepared first. From this stock solution of Quercetin, dilutions of 6.25, 12.5, 25, 50, 80 and 100 µg/ml were prepared. Into these dilutions, 1.5ml methanol, 0.1ml aluminum chloride, 0.1ml potassium acetate and 2.8ml distilled water was added. From these dilutions, standard curve was made. Sample was prepared by mixing 0.5ml jam, 1.5ml methanol, 0.1ml aluminum chloride, 0.1ml potassium acetate and 2.8ml distilled water. All the dilutions and sample were mixed well and were filtered using filter paper. Absorbance of all the dilutions and sample was noted at 415 nm.

3.3.3 STATISTICAL TESTS:

ANOVA and Pearson‟s correlation coefficient was used for comparing different parameters of all the three jam samples with the help of online tool named as “Social Science Statistics”.

CHAPTER- IV

RESULTS AND DISCUSSION:

In this study, an attempt has been made to standardize the method or optimize the production of sugar free vegetable jam over the well-established fruit jam. During the production of sugar free vegetable jam, all the parameters like the pH, total soluble sugars, moisture percentage, vitamin C, tannins and flavonoids content were studied and recorded. Results of the investigation on physical, phsyico-chemical properties of sugar free vegetable jam have been discussed with tables, graphs, standard graphs of titratable acidy, tannins content, flavonoids content and vitamin C concentration in comparison to the fruit jam.

4.1 STANDARDIZATION OF JAM (RECIPE):

After varying each factor the sample was sensory analyzed and only that recipe was selected which was found best.

4.1.1 Recipe standardization of Control or Apple jam: The following factor of jam has been considered as given below:

4.1.1.1 Sugar:

1. Initially the apple jam was prepared by adding 60g of sugar in 100g of apple pulp. But this amount of sugar led to very thick, glossy, tough and hard jam. Gelling characteristics and structure was poor. The jam observed was extremely sweet which was overtaking other flavors of jam. Total soulable sugar content of 81° Brix was observed for 60g of sugar. Appearance and texture of jam observed was very poor as shown in fig 15.

Fig 15: Apple jam with TSS 81° Brix

2. Then, second attempt made was using 55g of sugar in 100g of apple pulp. Total soluble sugar observed was 74° Brix. This jam was still tough and hard. It was not having appealable texture and observed very sweet in taste. Appearance and taste of the jam was better than above jam but not perfect.

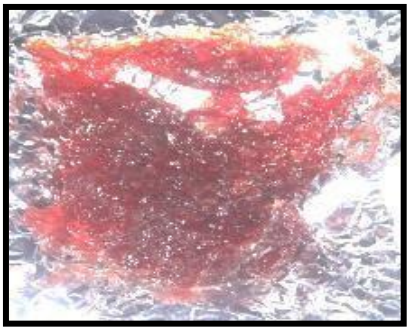


Fig 16: Apple Jam with TSS 74 ° Brix

3. Final recipe was made by using 50g of sugar in 100g of apple pulp and refractometer observed reading was 65° Brix which is perfect for good quality jam. This percentage of sugar leads to perfect texture, appearance and structure of jam. This jam was having perfect gelling characteristics and desired sweetness. So, this amount of sugar was standardized for the recipe.

. **Fig 17: Apple jam with TSS 65° Brix**

4.1.1.2 Citric acid:

1. In the first recipe of apple jam, 0.2g of citric acid was added. This amount of citric acid leads to high pH of jam i.e. 4.5. The jam made was not having perfect jellying characteristic. The jam was also runny in nature and was not thick.

Fig18: Apple jam with pH 4.5

2. In the second recipe, 0.5g of citric acid was added in 100g of fruit. This amount of acid leads to 3.8 pH. Jam made was better than above made but still was not having perfect texture and structure.

3. In the third recipe, 0.6g of citric acid was added (Jam, Jellies & Marmalade). This amount of citric acid provides perfect texture and thickness to jam. This jam was having perfect jellying characteristic. It was having good appearance, texture and taste. So, this amount of acid was standardized for the recipe**.**

Fig 19: Apple jam with pH 3.34

4.1.1.3 Beetroot:

1. In the first recipe, 3g of beetroot was added to the 100g of fruit. But the jam was light in shade and was not appealing.

2. In the final recipe, 5g of beetroot was added and this leads to more appealing and vibrant jam. Beetroot does not possess its own flavor to the jam as it was added in very small amount. It only enhances the color of jam. So, this amount of beetroot was found best for the perfect texture.

4.1.2 Standardization of vegetable jam: In order to standardize vegetable jam, bottle gourd and carrot were added in different proportions and 3 different recipes were tried

1. Ratio of 25:75: In this recipe, 25g of bottle gourd and 75g of carrot was added along with 50g of sugar and 0.6g of citric acid. This leads to total soulable sugar of 65° Brix and 3.6 pH. This jam was more like puree and was fibrous in nature. It was not visually appealing and also not good in taste.

Fig 20: Vegetable jam with 25:75

2. Ratio of 50:50: The jam prepared with 50:50 proportion of bottle and carrot was observed best among all three jams. It has recorded pH of 3.42 and Total soluble sugar content of 64° Brix. It was found best in taste, texture, appearance, flavor and structure. It did not possess any characteristic taste of either carrot or bottle gourd. It was also observed with perfect jellying nature. So this proportion of bottle gourd and carrot was finalized for vegetable.

Fig 21: Vegetable jam with 50:50

3. Ratio of 75:25: The jam was prepared by adding 75g of bottle gourd and 25g of carrot. Total soluble sugar of this jam was 64°Brix and pH was 3.57. In this, bottle gourd possessed its characteristic taste and also this jam was not visually appealing.

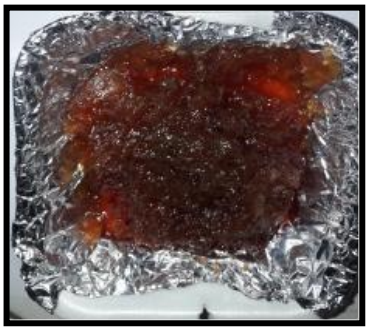


Fig 22: Vegetable jam with 75:25

4.1.3 Standardization of sugar free vegetable jam: Standardization of two factors has been done for sugar free jam:

4.1.3.1 Stevia: One sachette of stevia is equal to 2 teaspoon or 8 g of sugar (written on sachette of stevia). According this calculation, 6 sachette of stevia were needed.

1. In the first recipe, 6 sachette of stevia was added in 100g of jam. But this leads to very sweet jam and after taste of stevia was also felt.

2. In the second recipe, 5 sachette of stevia were added. This provides perfect sweetness and texture to the jam. So this amount of stevia was standardized for sugar free jam.

4.1.3.2. Selection of gelling agent: Sugar provides body or gel like structure to jam. But in sugar free vegetable jam, sugar was replaced by stevia, so some jellying compound was needed like gelatin or pectin for perfect texture.

1. Gelatin: The jam made by adding gelatin was not good in texture and also it altered the pH of jam to 3.8. Also gelatin is non veg source, so it was not preferred by all.

Fig23: Vegetable jam with gelatin

2. Pectin: It was reported by "Jams, Jellies and Marmalade" article that 0.8g of pectin is needed for 100g of jam. So, according to this paper 0.8g pectin was added along with stevia for sugar free vegetable jam Pectin provided good texture and body to the jam. Also pectin is vegetarian source so, can be consumed all. Also it was reported by "Low Sugar Jams and Jellies"[4] that sugar free jams require Low methoxy pectin. And in order to use LM pectin, dicalcium salts are needed. No trial was conducted because good results were obtained in the first trial.

Fig 24: Vegetable Jam with pectin.

4.2 OBSERVATIONS FOR THE QUALITY CHARACTERISTICS OF JAM:

4.2.1 Apple jam:

The apple jam made as control sample was vibrant red in color. It was having perfect gelling nature and good body. It was visually more appealing than other two jams. It was also having good spreadibility. The parameters and their values that were checked for apple jam are listed in table 5. Apple jam prepared has all the parameters in perfect range that are needed for good gelling and body characteristics which means it has pH 3.34 and the pH near 3.2-3.4 is reported to be best for gelling of jam (http://www.iufost.org). The total soluble sugars in the range of 62-65° Brix was also reported as good for gelling. The total soulable sugar obtained for apple jam was 64.6° Brix, moisture of 12.27 per cent, titratable aci-

Fig 25: Apple jam

dity of 2.62 per cent, vitamin C of 0.21 mg/g, tannins of 14.37 µg/mg and flavonoids of 0.415mg/g was observed.

Table 5: Quality Characteristics of Apple jam

S.No	Characteristics	Value
1	pH	3.34
2	Total soluble sugars	64.6° Brix
3	Moisture content	12.27%
4	Titrable acidity	2.62 %
5	Vitamin C	0.21 mg/g
6	Tannins	14.37 µg/mg
7	Flavonoids	0.415 mg/g

4.2.2 Vegetable jam:

It was formulated including bottle gourd, carrot and beetroot to provide dark red appealing color to the final jam. It was observed with good appearance, aroma and good gelling characteristics. The vegetable jam was also having well spreadibility. It has total soluble sugar of 62.4° Brix which is perfect for good gels. The pH of 3.58 for vegetable jam was reported which is nearer to the range needed for gelling of jam. (http://www.iufost.org). Moisture content of 6.48 per cent, titratable acidity of 5.85 per cent, vitamin C of 0.14 mg/g, tannins of19.37 µg/mg and flavonoids of 0.43 mg/g was observed. The parameters checked in the prepared jam with values are listed in table 6.

Fig 26: Vegetable jam

Table 6: Quality Characteristics of Vegetable jam

S.No	Characteristics	Values
1	pH	3.58
2	Total soluble sugars	62.4° Brix
3	Moisture content	6.48%
4	Titrable acidity	5.85%
5	Vitamin C	0.14 mg/g
6	Tannins	19.37 µg/mg
7	Flavonoids	0.43 mg/g

4.2.3 Sugar free vegetable jam:

The sugar free vegetable jam showed light red color. Its gelling characteristics were not so good as compared to apple and vegetable jam. It was having good spreadibility but lacking good texture. It was prepared by adding low methoxy pectin, stevia powder, and dicalcium ions. Total soluble sugar content of 21.3°Brix was observed. Moisture content of sugar free vegetable jam was less than that of apple jam. The pH of this jam was reported to be 3.42 which may be due to addition of divalent Ca ions, stevia which is further basic in nature. Moisture content of 7.73 per cent, titratable acidity of 12.27 per cent, vitamin C of 0.10 mg/g, tannins of 23. 72 µg/g and flavonoids of 0.65 mg/g was observed for this jam.

Fig 27: Sugar free vegetable jam

Table 7: Quality characteristics of Sugar free vegetable jam

S.No	Characteristics	Value
1	pH	3.42
2	Total Soluble Sugars	21.3° Brix
3	Moisture content	7.73%
4	Titrable acidity	12.27 %
5	Vitamin C	0.10 mg/g
6	Tannins	23.72 µg/g
7	Flavonoids	0.65 mg/g

4.3 SENSORY EVALUATION OF SELECTED FORMULATIONS:

Different parameters of the control, vegetable and sugar free vegetable jam were evaluated by 9-point Hedonic scale. The data analysis has been shown in table 8.

Table 8: Sensory analysis of various jams

Code	Appearance	Color	Flavor	Texture	Taste	Overall Acceptability	Percentage Acceptability
A	8.66±0.73	8.8±0.40	8.5±0.9	8.6±0.58	8.5±1.1	8.6±0.6	86
B	8.19±0.87	8.23±0.62	7.9±1.0	8.0±0.62	8.0±0.9	8.0±0.6	80
C	7.619±0.80	7.90±0.83	7.7±1.0	7.6±0.97	8.1±0.8	7.1±0.7	71

Foot notes*: A = Apple jam, B= Vegetable jam, C= Sugar free vegetable jam

4.3.1 Appearance:

The appearance score of different jam samples have been reported in table 8. The mean value with standard deviation of appearance all the jams ranged from 7.619 to 8.66. According to the 9.0 Hedonic scale, 7.61 was designated as moderately liked and 8.66 as very much liked by people. The highest mean value of appearance was shown by sample A (8.66±0.73) and lowest value was shown by sample C (7.619±0.80). Sample B was also very much liked by people as it was new to them and was also appealable. Sample A has scored highest appearance score due its vibrant color, shiny nature and good gelling nature. Appearance of sample C was also liked by people but little less than that of sample A because it was having little less gelling characteristic. The standard deviation showed that variation may be the individuals tasted for first time and reviews varied personally.

4.3.2 Colour:

All the jam samples have their own distinct color. The color score for different jam samples have been reported in table 8. The mean value with standard deviation of colour of all the jams was ranged from 7.9±0.83 to 8.8 ±0.40. Highest mean value of color was shown by sample A (8.8 ±0.40) because sugar present in the sample enhances the color and lowest value was shown by sample C (7. 9±0.83) because sugar was not present in this sample. In this case color of jam samples were perceived as same, so less standard deviation was observed. However there was little difference in color of jam sample B and A. So, the color of sample A and B was very much liked by people whereas color of sample C was moderately liked by people because it was one shade lighter than A and B.

4.3.3 Flavour or Aroma:

The flavour score was listed in table 8. The highest mean score 8.5±0.9 was recorded for A sample and the least value of 7.7±0.9 was obtained for sample C. Sample B was also very much liked by people as it was giving unique flavour of bottle gourd and carrot with stevia. The variation in jam flavour observed may be due to individual acceptance.

4.3.4 Texture:

Texture or body score of jam ranges from 8.6 to 7.6. The texture of apple jam has highest score of 8.6 while that of sugar free vegetable jam was 7.6. Sample A and B have similar structure but C has completely different body. The texture of sample A was jelly like while sample C has less jelly or fragile texture which breaks during spreading. Sample B has also jelly or fragile texture but little less than A.

4.3.5 Taste:

The taste score ranged from 8.5±1.1 to 8.1±0.8 as shown in table 8. Sample A had maximum mean value of 8.5 while sample C has least value of 8.1 according to the hedonic scale. In this case, the perception of the different individuals was not same, so standard deviation was quite high. All the samples were reported sweeter in taste but sample A recorded maximum sweetness. Sample A has been very much liked by people whereas sample C was moderately liked by people because it was not giving complete jam like taste but having a new taste to their taste buds. Taste of sample B was also very much liked by people.

4.3.6 Overall Acceptability:

Overall acceptability scores of all the jams have been tabulated in table 8. Highest score for overall acceptability was observed for the sample A (8.6) followed by the sample B (8.0) and the least mean score was shown by sample C (7.1). The least value of sample C was may be because it was offering later sweeter taste from stevia but appealable than A and B due to intense brick red color. But sample C was very much beneficial for diabetic patients who cannot consume sugar jam. Vegetable jam was also beneficial because it may provide more fiber which was beneficial for controlling diabetes and obesity.

4.3.7 Percentage of overall Acceptability:

The percentage of overall acceptability was calculated on the basis of sensory analysis table 8. Sample A has shown the maximum percentage overall acceptability of 86 per cent because the colour, appearance, taste, texture calculated on the basis of sensory analysis was more as compared to sample B and sample C. According to the percentage acceptability the order of liking of the jam was followed in the sample as A > B> C. Once, the analysis done, it can be inferred that sample A was best jam in physical characteristics but sample C was more nutritious and more beneficial for the diabetic patients as well as the normal individual too.

4.4 ANALYTICAL TESTS:

4.4.1 Acidity:

It was observed that the titratable acidity in control jam, vegetable jam and sugar free vegetable jam were 2.7 per cent, 5.96 per cent 12.16 per cent values respectively as shown in the table 9 and fig 8. The titratable acidity of apple jam observed was lowest i.e. 2.7 per cent while sugar free jam had highest acidity of 12.16 per cent. The sugar free vegetable was having highest per cent acidity due to increase in reducing sugar content due stevia usage.

Table 9: Per cent acidity in control, vegetable and sugar free vegetable jam

Sr. No.	Samples	Per cent acidity (%)
1	Control jam	2.7
2	Vegetable jam	5.96
3	Sugar free vegetable jam	12.16

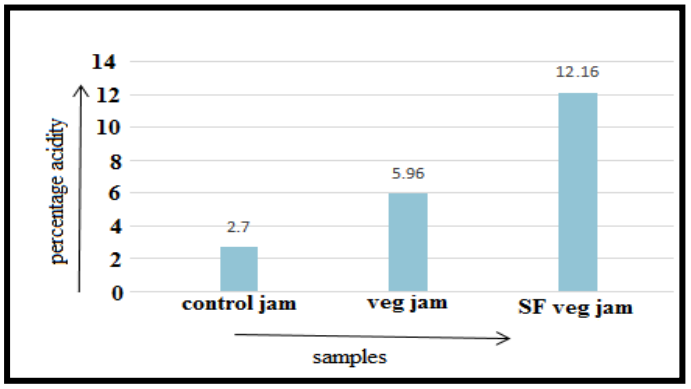


Figure 28: Per cent acidity in control, vegetable and sugar free vegetable jam

4.4.2 Vitamin C:

The vitamin C content in all the three jam samples has been reported in table10 and in fig 29. Apple jam has highest concentration of vitamin C of 0.21 mg/g. The sugar free vegetable jam has lowest concentration of vitamin C of 0.102 mg/g. It may be due to high tannin content in sugar free vegetable jam.

Table 10: Vitamin C content of control, vegetable and sugar free vegetable jam

Sr. No	Samples	Vitamin C (mg/g)
1	Apple jam	0.21
2	Vegetable jam	0.145
3	Sugar free vege table jam	0.102

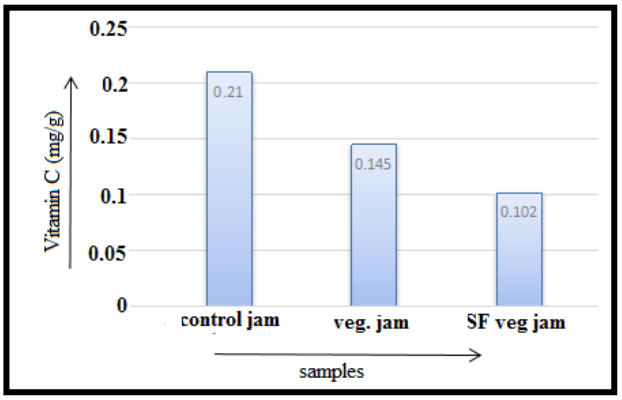


Fig 29: Vitamin C of control, vegetable and sugar free vegetable jam

4.4.3 Tannins:

The tannins content of all the three samples of jams has been calculated by plotting against the standard curve made from concentrations of tannic acid. Tannins content has been reported in table 11. The apple jam was recorded with the lowest tannins content i.e.14.3 while sugar free vegetable jam was having the highest tannins content with 23.7. The reason for high tannins content in the sugar free jam may be because of high antioxidant acidity as antioxidant property was related to tannin content of bottle gourds and carrots to some extent. As the antioxidants were not recorded but the antioxidant activity was contributed by tannins too. From the graph linear equation i.e. y = 0.1162x+0.455 was calculated which represents the relationship between the concentration and absorbance of samples. Using this equation, we can calculate the value of x (concentration) as we have value of y (absorbance). R^2 of tannins graph was 0.9611that was near to 1.0 which mean there was better fit of the regression line i.e. the closer the line passes through all of the points.

Table 11: Tannins content in control, vegetable and sugar free vegetable jam

Sr. No	Samples	Tannins (µg/g)
1	Apple jam	14.3
2	Vegetable jam	19.4
3	Sugar free vegetable jam	23.7

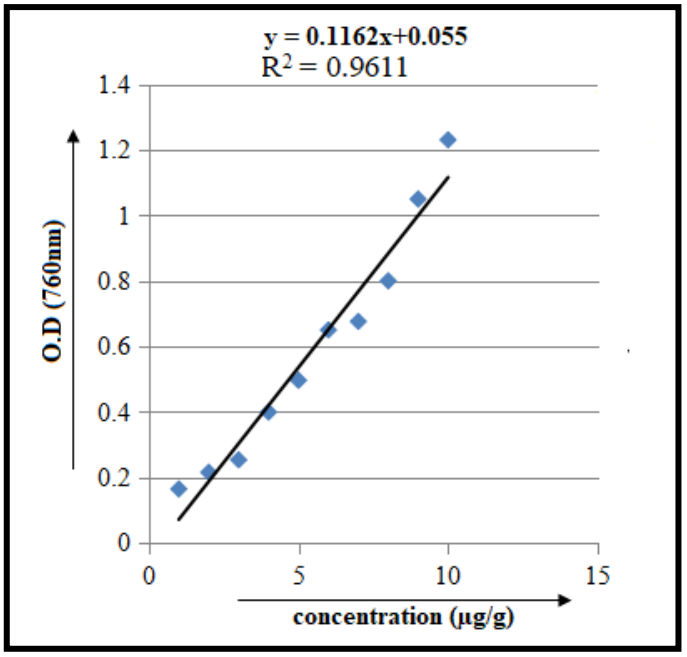


Fig 30: Tannins content in control, vegetable and sugar free vegetable jam

4.4.4 Flavonoids:

The flavonoids content in all three jams has been calculated by plotting against the standard curve prepared from different concentrations of quercetin. From the standard curve, it has been concluded that sugar free vegetable jam was having highest flavonoid content i.e. 0.66mg/g while control jam has lowest flavonoid content of usually 0.42 mg/g. indirectly this revealed that sugar free jam contained some alkaloids which are a part of group of flavonoids that may be coming from beet root (http://undergroundhealthreporter.com). From the graph linear equation i.e. y = 0.003x+0.027 was calculated which represents the relationship between the concentration and absorbance of samples. Using this equation, we can calculate the value of x (concentration) as we have value of y (absorbance). R^2 of flavonoids graph is 0.9954 which is near to 1 which means there is better the fit of the regression line i.e. the closer the line passes through all of the points.

Table 12: Flavonoids content of control, vegetable and sugar free vegetable jam

Sr. No	Samples	Flavonoid (mg/g)
1	Apple jam	0.42
2	Vegetable jam	0.45
3	Sugar free veg table	0.66

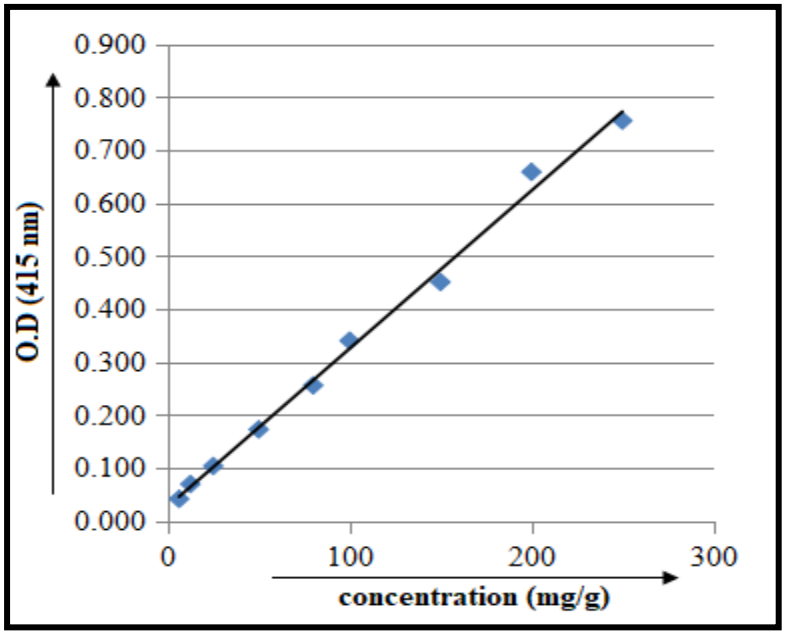


Fig 31: Flavonoids content of control, vegetable and sugar free vegetable jam.

4.5 STATISTICAL ANALYSIS:

ANOVA (Analysis of Variance) was used to check the efficacy of the data obtained and its significance in relation to different factors and to analyse the variation in the means of above parameters. Pearson correlation coefficient was also calculated to check whether there is positive or negative correlation between the factors.

4.5.1 ANOVA:

4.5.1.1 The pH:

Experimental data for pH in the samples of control, vegetable and sugar free vegetable jam has been discussed below in table 13:

Table13: The pH readings observed for three samples of jam

Trials	Control jam (Apple jam)	Vegetable jam	Sugar free vegetable jam
1	3.34	3.6	3.6
2	3.4	3.58	3.4
3	3.3	3.62	3.4
4	3.31	3.52	3.49
5	3.36	3.5	3.41
6	3.31	3.61	3.59
7	3.4	3.63	3.43
8	3.31	3.58	3.40
9	3.3	3.57	3.4
10	3.4	3.62	3.42
Mean	**3.34**	**3.58**	**3.42**

ANOVA results were shown in table 14.

Table14: Summary of ANOVA obtained on the pH data

Data	Apple Jam	Vegetable jam	Sugar free vegetable jam	Total
N	10	7	6	23
ΣN	33.43	25.06	21.23	79.72
Mean	3.34	3.58	3.42	3.46
ΣX^2	111.77	89.73	75.15	276.65
Standard Deviation	0.043	0.050	0.081	0.12

Table 15: Results of ANOVA obtained on the pH data

Data	SS (Simple square)	DF (Degree of freedom)	MS (Mean Square)
Among group	0.27	2	0.13
Within group	0.06	20	0.003
Total	0.33	22	-

The F value calculated was 41.78357 while the F value from the table i.e. Fcritical value was 3.49 P at value <0.0001. The result was significant at $p < 0.5$ as the obtained F ratio value was larger than Fcritical value. This shows that the pH variation in the mean values within the group and among group was significantly varied. Hence the pH factor varied in the all the three jam samples due to significant difference between the formulations as well as the composition of the different jam samples.

4.5.1.2 Total soluble sugars:

ANOVA applied results from the jam samples of control, vegetable and sugar free vegetable jam has been discussed below in table 16.

Table 16: Total soluble sugar readings observed for three samples of jam

Trials	Apple jam	Vegetable jam	Sugar free vegetable jam
1	64	62	22
2	65	62	22
3	65	63	21
4	65	63	21
5	64	62	22
6	65	62	20
7	65	63	22
8	64	63	20
9	65	62	21
10	64	62	21.2
Mean	**64.6**	**62.42**	**21.33**

ANOVA results were shown in table 17.

Table 17: Summary of ANOVA on Total soluble sugars data

Data	Apple Jam	Vegetable jam	Sugar free vegetable jam	Total
N	10	7	6	23
ΣN	646	437	128	1211
Mean	64.6	62.42	21.33	52.65
ΣX^2	41734	27283	2734	71751
Standard Deviation	0.51	0.53	0.81	19.05

Table 18: Results of ANOVA on Total soluble sugars data

Data	SS(Simple square)	DF(Degree of freedom)	MS(Mean Square)
Among group	7981.17	2	3990.88
Within group	7.44	20	0.37
Total	7989.21	22	

The F value calculated was 10717.21005 while the F value from the table i.e. F critical was 3.49 at P value <0.0001. The result was significant at p<0.5as the obtained F ratio value was larger than Fcritical value. These results revealed that the total soluble sugars variation within the formulations and between the formulations observed with significant difference which is acceptable.

4.5.1.3 Moisture content:

Experimental data of all the 3 different jams have been discussed in table 19.

Table 19: Moisture content of from the three samples of jams

Trials	Apple jam	Vegetable jam	Sugar free vegetable jam
1	12	6.1	7
2	10	7.1	8
3	13	5.9	8.2
4	10	5.6	7.9
5	14	6.9	7.23
6	10.2	6.8	8.1
7	13.6	7.0	7.7
8	14	6.48	7.5
9	11.9	6.47	7.3
10	14	6.48	7.7
Mean	**12.27**	**6.48**	**7.73**

ANOVA results obtained were displayed in Table 20.

Table 20: Summary of ANOVA on the moisture content

Data	Apple Jam	Vegetable jam	Sugar free vegetable jam	Total
N	10	7	6	23
ΣN	122.7	45.4	46.43	214.53
Mean	12.27	6.48	7.73	9.32
ΣX^2	1531.61	296.64	360.53	2188.78
Stand.Deviation	1.70	0.60	0.49	2.92

Table 21: Results of moisture content reading by ANOVA

Data	SS(Simple square)	DF(Degree of freedom)	MS (Mean Square)
Among group	158.26	2	79.13
Within group	29.51	20	1.47
Total	187.77	22	-

The Fcalculated value was 53.6283 while the F value from the table i.e. Fcritical was 3.49 at p<0.0001. The result was significant at p<0.5 as the obtained F ratio value was larger than critical F value ratio, the result was significant at level of probability. These results

revealed moisture content variation within the formulations and between the formulations with significant difference which is acceptable.

4.5.1.4 Percentage acidity:

ANOVA was performed on the acidity readings of control jam, vegetable jam and sugar free vegetable jam.

Table 22: Per cent acidity of all the 3 jams

S No.	Apple jam	Vegetable jam	Sugar free vegetable jam
1.	2.61	6.4	12.8
2.	2.63	4.8	11.02
3.	2.60	6.2	13.4
4.	2.64	6.0	11.86
Mean	2.62	5.85	12.27

Table 23: Summary of AVOVA on percentage acidity data

Data	**Apple Jam**	**Vegetable Jam**	**Sugar free vegetable jam**	**Total**
N	4	4	4	12
ΣN	10.5	23.4	49.1	83
Mean	2.62	5.85	12.27	6.91
ΣX^2	27.7	136.94	603.07	767.72
Standard Deviation	0.22	0.12	0.35	4.19

Table 24: Results of ANOVA on percentage acidity

Data	**SS (Simple square)**	**DF (Degree of freedom)**	**MS (Mean Square)**
Among group	193.07	2	96.53
Within group	0.56	9	0.062
Total	193.63	11	-

The F value calculated was 1537.73894 while the F value from the table i.e. Fcritical was 4.26 at P value <0.001. The result was significant at p<0.5 as the obtained F ratio value was larger than Fcritical. These results revealed the percentage acidity variation within the formulations and between the formulations observed with significant difference which is acceptable.

4.5.1.5 Vitamin C:

Experimental data of vitamin C of all the 3 jams have been discussed in table 25.

Table 25: Vitamin C content of all the 3 jams

S No.	Apple jam	Vegetable jam	Sugar free vegetable jam
1.	0.19	0.15	0.11
2.	0.24	0.15	0.80
3.	0.20	0.14	0.90
4.	0.21	0.12	0.12
Mean	**0.21**	**0.14**	**0.10**

ANOVA has been performed for all the jams‟ samples and results were discussed in table 26.

Table 26: Summary of ANOVA on Vitamin C content

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Data	**Apple Jam**	**Vegetable jam**	**Sugar free vegetable jam**	**Total**
N	4	4	4	12
ΣN	0.86	0.57	0.411	1.84
Mean	0.21	0.14	0.10	0.15
ΣX^2	0.18	0.08	0.04	0.31
Standard Deviation	0.012	0.0018	0.002	0.04

Table27: Results of vitamin C content by ANOVA

Data	**SS (Simple Square)**	**DF (Degree of freedom)**	**MS (Mean Square)**
Among group	0.02	2	0.0129
Within group	0.0005	9	0.0001
Total	0.026	11	-

The F value calculated was 219.49078 while the F value from the table i.e. Fcritical was 4.26 at p <0.0001. The result is significant at p<0.5 as the obtained F ratio value was larger than Fcritical. These results revealed the vitamin C variation within the formulations and between the formulations observed with significant difference which is acceptable.

4.5.1.6 Tannins:

Experimental data of tannins content of all the 3 jams have been discussed in table 28.

Table 28: Tannins content of all the 3 jams

S No.	Apple jam	Vegetable jam	Sugar free vegetable jam
1.	15.88	21.26	21.36
2.	14.68	18.38	24.50
3.	13.90	19.25	24.40
4.	13.42	18.59	24.66
Mean	**14.37**	**19.37**	**23.72**

ANOVA was performed on the tannins content of control jam, vegetable jam and sugar free vegetable jam.

Table 29: Summary of tannins data

Data	Apple Jam	Vegetable jam	Sugar free vegetable jam	Total
N	4	4	4	12
ΣN	57.5	77.5	94.9	229.9
Mean	14.37	19.37	23.72	19.15
ΣX^2	826.71	1501.65	2251.55	4579.9
Standard Deviation	0.221	0.17	0.12	3.99

Table 30: Results of tannins content by ANOVA

Data	SS (Simple Square)	DF (Degree of freedom)	MS (Mean Square)
Among group	175.12	2	87.56
Within group	0.28	9	0.0314
Total	175.40	11	-

The F value calculated was 2789.62832 while the F value from the table i.e. Fcritical was 4.26 at <0.0001. The result is significant at p<0.5 as the obtained F ratio value was larger than F critical value. These results revealed the tannins content variation within the formulations and between the formulations observed with significant difference which is acceptable.

4.5.1.7 Flavonoids content:

Experimental data of flavonoids content all the 3 jams have been discussed in table 31.

Table 31: Flavonoids content of all the 3 jams

S No.	Apple jam	Vegetable jam	Sugar free vegetable jam
1.	0.51	0.48	0.70
2.	0.45	0.43	0.66
3.	0.35	0.39	0.74
4.	0.33	0.42	0.50
Mean	**0.41.**	**0.43**	**0.65**

Table 32: Summary of flavonoids content data

Data	Apple Jam	Vegetable jam	Sugar free vegetable jam	Total
N	4	4	4	12
ΣN	1.66	1.75	2.6	6.01
Mean	0.41	0.43	0.65	0.5008
ΣX^2	0.68	0.76	1.69	3.14
Standard Deviation	0.012	0.027	0.02	0.11

Table 33: Results of flavonoids content by ANOVA

Data	SS (Simple Square)	DF (Degree of freedom)	MS (Mean square)
Among group	0.13	2	0.067
Within group	0.004	9	0.0005
Total	0.13	11	

F value calculated was 126.76963 while the F value from the table i.e. Fcritical was 4.26 at <0.0001. The result is significant at p<0.5 as the obtained F ratio value was larger than F critical value. These results revealed the flavonoids content variation within the formulations and between the formulations observed with significant difference which is acceptable.

4.5.2 Correlation of jams

Pearson correlation coefficient was used to measure the strength of linear association between two variables, where there will be positive correlation if value of r=1 and if value of r= -1, then there will be negative correlation.

Table 34: Correlation between parameters of jams

S. No.	X-Variable	Y-variable	R values	R^2 value	Correlation
1	TSS	pH	-0.1392	0.1392	Weak negative correlation
2	Moisture	TSS	-0.6618	0.438	Moderate negative correlation
3	TSS	% Acidity	0.194	0.0376	Weak positive correlation

In the table 30, the correlation was between the mean value of TSS, moisture content, pH and percentage acidity of sugar free vegetable jam. The correlation results were not all found interacted but observed with weak positive correlation between total soluble solids and percent acidity, weak negative correlation between total soluble solids and pH and moderate negative correlation between moisture content and total soluble solids of sugar free vegetable jam.

CHAPTER-V

Conclusion:

The investigation was based primarily to develop a vegetable jam and further making it sugar free. Various components used were beet root to provide appealable color, stevia to replace sugar and pectin to provide body and spreadable smooth texture. It was concluded that control jam (apple jam) was incomparable to vegetable jam and low calorie vegetable jam. But if considered without comparing it was moderately liked by people. It may be very good and helpful for a diabetic patient. As vegetables possess high amount of fiber that may help in more excretion of waste. More excretion of waste helps to reduce blood sugar level.

Standardization of different ingredients of jam was done before actual production of jam. Amount of sugar, citric acid and beetroot were standardized in apple jam. The best formulation that was chosen for making apple jam includes 100g fruit, 50g sugar, 5g of beetroot and 0.6g citric acid. In case of vegetable jam, ratio of two different vegetables, amount of pectin, stevia, calcium ions were standardized. The best recipe was standardized at 50g carrot, 50g bottle gourd, 0.8g pectin, 5 sachette stevia, 1 teaspoon calcium solution and 6g of citric acid.

Physico chemical analysis of all three jams has been done. In the apple jam, total soluble sugar observed was (64.6° Brix), pH (3.34), moisture content (12.27 per cent), titrable acidity (2.62 per cent), vitamin C (0.21 mg/g), tannins (14.37µg/g) and flavonoids (0.41 mg/g). In the vegetable jam, total soluble sugar analyzed was (62.42° Brix), pH (3.58), moisture content (6.48 per cent), titrable acidity (5.85 per cent), vitamin C (0.14 mg/g), tannins (19.37µg/g) and flavonoids content (0.4 mg/g). In sugar free vegetable jam total soluble sugar observed was (21.3°Brix), pH (3.42), moisture content (7.7 per cent), titrable acidity (12.27 per cent), vitamin C (0.10 mg/g), tannins (23.72µg/g) and flavonoids (0.65 mg/g). While sugar free vegetable jam has highest titrable acidity, tannin content and flavonoid content. So from the quality analysis, sugar free vegetable is boon to diabetic patients as it helps in lowering the blood sugar level and also is highly nutritious.

After the sensory analysis, it was found that the sample A was very much liked by people and was best in all the cases like taste, texture, flavour, colour, appearance and overall acceptability. Sample A showed the highest overall acceptability of 86 per cent. Sample B was also very much liked by people and was having 80 per cent overall acceptability. However, sample C was moderately liked by people and has 71 per cent of overall acceptability.

Statistical analysis has also been done for all the three jams like ANOVA and Pearson

correlation coefficient. By performing ANOVA it has been analyzed that F calculated value for pH was 41.78 while the F critical value was 3.49 P at value <0.0001, F calculated for total soluble solids was 10717.21 while F critical was 3.49 at P value <0.0001, F value calculated for moisture content was 53.62 while Fcritical was 3.49 at p<0.0001, F value calculated for titrable acidity was 1537.73 while F critical was 4.26 at P value <0.001, F value calculated for vitamin C was 219.49 while Fcritical was 4.26 at p <0.0001, F value calculated for tannins was 2789.62, Fcritical was 4.26 at <0.0001, F value calculated for flavonoids was 126.76, Fcritical was 4.26 at <0.0001. As the F calculated was larger than then F critical value, so this means results are significant at p<0.5. The correlation results were not all found interacted but observed with weak positive correlation between total soluble solids and percent acidity, weak negative correlation between total soluble solids and pH and moderate negative correlation between moisture content and total soluble solids of sugar free vegetable jam.Thus a commercial product can be made as it has all the properties that are important for any product that can be launched in to the market.

ANNEXURE-1

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ANNEXURE-II

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