“Novel method for soymilk production & its nutritional survey”

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IN

BIOTECHNOLOGY

By

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Certificate

This is to certify that the dissertation report entitled to "**Novel method for soymilk production & its nutritional survey**" submitted by **Ms. Simran Bedi** in the partial fulfillment of the requirement for the award of award of degree of the **Master of Technology Biotechnology**, Thapar University, Patiala is authentic record of my own work during the period of 12 months from July, 2016 to July, 2017 under the supervision of Dr. Jyoti Rani, Assistant Professor (FT), Department of Biotechnology, Thapar University and Prof. (Mrs.) Ravi Kiran, Professor & Former Head, School of Humanities and Social Sciences, Thapar University, Patiala. The report has not been submitted for the award of any degree or certificate in this or any other university or institute

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DECLARATION

I, Simran Bedi (601504010) hereby declare that the work presented in the dissertation entitled **"Novel method for soymilk production & its nutritional survey"** is an authentic record of my own project work which was carried out at Department of Biotechnology, Thapar University, Patiala under the supervision of **Dr. Jyoti Rani**, Assistant Professor (FT), Department of Biotechnology, Thapar University and **Prof. (Mrs.) Ravi Kiran**, Former Head, School of Humanities and Social Sciences, Thapar University, Patiala. The matter presented in this dissertation has not been submitted in part or full to any other university or institute for the award of any degree in India or Abroad.

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ABSTRACT

Standardization of mechanical soymilk extraction method vs enzyme assisted soymilk extraction (cellulase of *Trichoderma viride* source and pectinase enzyme of source *Rhizopus* spp. of Hi-Media at varied concentration, temperature and time combinations) method were studied. Majorly, the effect of soaking time of soybean seeds and boiling methods variation on the milk yield as well on the quality was focused. Two of the methods compared in the study, resulted in exactly double milk yield from enzyme assisted soymilk extraction method (cellulase enzyme was found substantial effective in milk extraction to that milk extraction from pectinase enzyme) to the mechanical soymilk extraction method. Observations for different quality tests for the cellulase enzyme assisted soymilk were pH (6.75), proteins (56mg/ml), fat (12mg/ml), carbohydrates (5.9mg/ml), Solid not fat (SNF:2.1mg/ml), acidity (0.35%), non-reducing sugars (20mg/ml), flavonoids (640mg/ml) and total soluble sugars (5.5%) while for pectinase assisted soymilk observed with pH (6.82), proteins (45mg/ml), fat (13mg/ml), carbohydrates (6.2mg/ml), Solid not fat (SNF:3.1mg/ml), acidity (0.30%), non-reducing sugars (7mg/ml), flavonoids (610mg/ml) and total soluble sugars (5.0 %) and at last the mechanical extracted soymilk had pH (7.2), proteins (26mg/ml), fat (14.2mg/ml), carbohydrates (4.9mg/ml), Solid not fat (SNF:5.2mg/ml), acidity (0.36%), non-reducing sugars (10mg/ml), flavonoids (590mg/ml) and total soluble sugars (6%). From the quality analysis, the cellulase enzyme can be a boon to the soymilk production with higher percentage of proteins, flavonoids with decreased fat, carbohydrates, SNF, acidity, total solids and pH. Beside good nutrition, the enzyme assisted extracted soymilk showed higher sensory score in taste, colour, texture, and flavor on a 9-point hedonic scale to that of mechanical soymilk extraction method (the mean score of overall acceptance of each of formulation from mechanical method (5.80), pectinase (4.87) and cellulase (7.47) showed the significant difference within and between the groups with the F value of 45.793 along with homogeneity of variance with a significant value of 0.557) and also cellulase enzyme assisted soymilk extraction method has shown the significant results within and in between the groups with the LSD value at 5 per cent were protein (0.9), fat(0.2), carbohydrates (0.1), acidity (0.1), flavonoids (0.2), non reducing sugars (0.3) and SNF (0.3) where as mechanical and pectinase enzyme assisted soymilk extraction method has shown non-significant results and also cellulase assisted soymilk extraction method has shown significant positive correlation within and between the quality factors where as the mechanical has shown non-significant correlation within and between the quality factors. Thus, enzyme assisted extraction method which has shown the positive results can be considered as a novel processing method for soymilk extraction with enriched nutritional quality, double milk yield and less of by product (OKARA). As a whole de-hulled soybean can be completely converted to milk.

A health survey on soymilk consumption has been done simultaneously along with laboratory production to find out whether people like or prefer soymilk over the lacteal milk or not at all in Jalandhar area. Various key points studied include to find individual consumption of soymilk in percentage to study the awareness about soymilk and the factors which has to be inherent part of marketing of soymilk. The survey was conducted in Jalandhar city and in Thapar University, Patiala. We found that people are very much aware and prefer soymilk as compare to other milk and we also found that age group (23-30) years, income range of (10-50 thousand) and both the genders prefer soymilk. These factors could be

targeted in future to increase the sale of soymilk and also came to know about different factor to be kept in mind and need improve to increase the marketing of soymilk.

Key words: Novel processing, Soybean, Soymilk, Enzyme assisted soymilk extraction, (cellulase & pectinase), mechanical soymilk extraction method and Soymilk consumption marketing survey (Age group, Income range, gender).

CHAPTER: 1

INTRODUCTION

Apart from 840 million under nourished people, approximately 799 million people belongs to developing countries only and about 24 per cent of the Indian total population i.e. 233.3 million was under nourished as counted in 2005 and this nutritional imbalances problem can only be overcome by the Soybean (*Glycine max*) which has rich amount of protein and fat (Liu *et al*., 2006). It has 45 per cent protein and 20 per cent fat that's high compared to the protein content 13 per cent and 20 per cent of egg and meat, respectively. Soya products are more economic and cheaper than other products containing high-quality protein such as meat, fish, milk and other protein-rich legumes. So, it is also cost effective because of their nutritional content (Oloye *et al.,* 2014).The soybeans are normally green in color but could be brown, yellow or black which grows in pods of length one to four inches with adaptable texture that easily get processed into the variety of other foods. Soybeans are also called as *Edamame* bean when are eaten fresh from pods

Fig 1: Fresh soy bean

1.1. NUTRITIONAL ATTRIBUTES OF LEGUME SEED:

The Soybean majorly known for its high amount of protein that is suitable for all ages, infants and elders as compared to the other foods due to digestibility (92-100) with all the essential amino acids except methionine and iso-flavonoids which are present in high amount up to 1g/kg (Oboh *et al.,* 2006). The other constituents of soybean are like fat (24 per cent), water (6-11 per cent), crude protein (33 per cent), carbohydrates (31 per cent), crude ash (5 per cent) and soluble dietary fiber (14 per cent) along with the fatty acids, nutrients, minerals and anti-nutritional factors (Ensminger *et al.,* 1990).

1.2. ORIGIN:

The soybean plant was an essential crop of ancient China an important part of their livelihood and has been cultivated for almost 13,000 years. In 20th century the Soybeans were introduced into other regions of Asian centuries and then as animal feed in West areas (Smith *et al.*, 1987). From 1970s there was a notable increase in the development of soy foods like sausages, cheese and yogurts and their consumption as well and now 31 U.S states have soya production industries. The main products of soy consumed are:

- **Miso:** used as flavoring agent mainly in Asian cuisine. It is a paste which is fermented containing many minerals (Kiuchi *et al.*, 2011).
- **Tempeh:** Textured solid cake which is famous in Indonesia and a very good source of proteins, minerals and vitamins. It is made by cooking and the de hulling process of soybeans (Steinkraus *et.al.*, 1983).
- **Tofu:** It is a curd of soymilk which is made by the coagulation of proteins with calcium and magnesium salts. So, it is a good source of protein, calcium and iron from which the whey is discarded (Kim *et al.*, 2005).
- **Natto:** Popular in eastern regions of Japan and produced by the fermentation of soybeans with the *Bacillus subtilis var*. It contains powerful smell, strong flavor with a slimy texture.
- **Soya vegetable oil:** It is cooking oil which is mainly extracted from the seeds of soybean. It is also used as base for soy inks and oil paints.

1.3. SOYMILK:

The most important mainly consumed in Asian countries, including Japan, China, Korea, Thailand and also drastically spreading its popularity to Western consumer's of European countries like Australia and the United States (Oloye *et al.*, 2014). Soymilk is nothing but the strained water that has been obtained with dissolve solids during grinding of the boiled and soaked grains. The soaked grains were blanched with sodium bicarbonate followed by processing that was also an inexpensive and convenient source to obtain high quality proteins (Wettstein *et al.*, 1990). So, there was a strong relationship between soyfood consumption and in health promoting effects similar to balanced

nutrient combination that of cow's milk but free of cholesterol, gluten and lactose along with favorable phytochemical compounds and containing phenolic compounds that has anti-oxidant properties and high amount iso-flavones, group of phytoestrogens that lowers the risk of age related and hormonal diseases. Due to increase in consumption of soymilk and its sale, a variety of new products and flavors have been introduced in the market. However, different innovations have been tried to produce "Functional Soy milk" that is to be considered as soymilk with extra bioactive components that enhance the health and lowers the risk of diseases (Banaszkiewicz *et al.,* 2011).

- Soymilk has total solids content of 8-10 per cent, protein content (3.6 per cent), fat (2.0 per cent), carbohydrates (2.9 per cent) and ash (0.5 per cent). It has longer shelf-life as compared to the normal cow's milk. Lactose intolerant patients find soy milk as an excellent substitute to lactose sugar present in cow's milk along with protein casein that may worsen the allergy symptoms, and increase in mucous or problems with the immune system (Arya *et al.,* 2014).
- It also improves the blood lipid profile in human beings that was found absent in dairy milk because of high saturated fat and cholesterol while soy milk fat is mostly unsaturated with zero cholesterol (Ugwu *et al.,* 2005).
- The omega-3 and omegs-6 fatty acids presence with other phyto-antioxidants in soy products protects the blood vessels from lesions and hemorrhage (Velasquez *et al.,* 2007).
- It also promotes weight loss because contain less sugar content as compared to the regular milk having 12 grams and 6 grams in soy milk and along with dietary fiber (Onnings *et al.*,1998).
- Prevents Prostate Cancer as it is a rich source of Phytoestrogen which reduce the production of testosterone and as such reduces the risk of prostate cancer in males and also prevents the Post Menopause Syndromes in women (Woh *et al.,* 2014).

1.4. LIMITATIONS:

Soymilk has a pronounced beany flavour most commonly not accepted by the consumers as it is due to the lipoxygenase enzyme which gets active when the cotyledons broke down and enzymes come in contact with water, oxygen and lipids; these all in

combination gives the beany flavor especially in mechanical method of soymilk extraction .But methods like mechanical method in which excessive heating increases the sensory properties but reduce the nutritional qualities and also produce the large amount of wastage which is called as Okara (Iwuoha *et al.*, 1997). Okara is a soy pulp consisting of insoluble parts of soybean containing 25-28 per cent of protein, carbohydrates (3.8-5.3 per cent) as well as oil and fat (9.3-10.9 per cent) which remains after the filtration of soymilk may sometimes used in the production of Tofu. The other anti-nutrients also limit the consumption such as lectins, phytic acid, indigestible oligosaccharides and the Trypsin inhibitors (TI) (Carthy *et al.,* 1987). So, by modifying the processing methods could be effective way to produce or to improve the health promoting bioactive compounds and also reduced the undesired compounds present in soybeans to support the soymilk product (Yuan *et al*., 2008).

1.5. ENZYME ASSISTED SOYMILK EXTRACTION METHOD:

Enzyme assisted method can be used for soybean softening and also for increasing the porosity of soybean that may increases the milk recovery and also minimize the residue and increases the overall efficiency of extraction (Rosenthal *et al.,* 2009). It is an environment friendly method that lowered the protein damage during extraction. In this method enzymes hydrolyze the structural polysaccharides which form the cell walls of seed. Other conditions also favors the milk extraction like temperature, pH, particle size, agitation rate which only aims to increase the extraction yield. So, here we used different enzymes.

Mainly the reasons to choose the enzyme assisted soymilk extraction method were to reduce the input energy in production of milk. Comparing the yield from mechanical soymilk extraction method to enzyme assisted soymilk extraction method was the major concern along with to improve the quality with reduced loss of Iso-flavones and anti-oxidants which may get destroyed due to exposure to long period of heat. **The objectives of the present study are:**

- Standardization of mechanical soymilk extraction method to the enzyme assisted soymilk extraction method.

- Comparison of yield, quality analysis, sensory analysis of the same with addition of flavoring agents and statically analysis of data and interpretation of results

1.6. MARKETING / NUTRITIONAL SURVEY:

It is a process of planning and executing the conception, pricing, promotion and distribution of goods, ideas, services to create exchanges that satisfy the individual and organizational goals.

The 4Ps of marketing are:

- **Product**: Quality, Features, brand name Packaging, Sizes, Services, Warranties, Returns
- **Price:** The value that is put on the exchange process like List price, Discount allowances, Payment period, Credit terms.
- **Promotion:** Advertising, Personal selling, Sales promotion, Public relations, Direct Marketing.
- **Place:** Channels, Coverage, Locations, Inventory, Transport.

Survey is defined as to view the detail, to inspect and to examine or appraise formally in order to ascertain condition, value etc. So, it focuses on the demand of soymilk in the markets and the objective is to see whether people prefer soymilk over cow milk and also the "secondary data analyses" was done:-

- To find out whether the people are aware of soymilk.
- To find out the reaction (positive/negative) of people regarding soymilk.
- To find out the type (age, gender and income level etc.) of people who are interested to drink soymilk.
- To find out before the making or the marketing of soymilk which factors has to be taken in mind and need to improve to increase its marketing.
- To find out the type (age, gender and income level etc.) of people which should targeted for the marketing and as well as to increase the sale of soymilk

The main tool used for this research was structured questionnaire. I took most of the surveys from Jalandhar and from Thapar University, Patiala students. I have surveyed total 100 people.

CHAPTER: 2

REVIEW OF LITERATURE

2.1. SOYBEAN:

Soybean (*Glycine max*), is the oldest legume belongs to the family of *Fabaceae* and the genus *Glycine* that is the important source of protein and energy occupies an important position among grain legumes (Smith *et al.*, 1987). It is a bushy, erect annual herbaceous plant with the height of 40-100cm and having leafy plant structure in which the number of seeds per pod are 2 to 5. It is a day length frost-sensitive summer crop and the beans takes about 70 to 80 days to fully mature and needs at least 15 degrees Celsius of temperature for the germination along with the phosphorous and potassium fertilizers (Siaram *et al.,* 2003). The shape of soybean fruit is crescent pod and about 3 to7 cm in length having thousand kernel weight of about 115-280g (Banaszkiewicz *et al.,* 2011). Unripe seeds are generally green in colour which becomes brown once they mature. The most desired colour and shape are yellow, green and the spherical respectively (Tindal *et al.,* 2007). About 90 per cent of soybean made of cotyledon and 8 per cent by hulls. The main components which gets accumulated in cotyledons are carbohydrates, fats, proteins and anti- nutritional factors like protease inhibitors, lectins, pectins, lipoxygenase, urease (Liener *et al.,* 2000).

Soybeans on a dry weight basis typically have about 12 to 25 per cent oil and 35 to 50 per cent of protein. The soya protein is a vegetarian source of complete protein can be comparable to the eggs and meat containing all the amino acids essential for the humans and animals. (Smith *et al.,*1989). It also contains both beneficial polyunsaturated fatty acids like linoleic acid (omega-6) and alpha-linolenic acid (omega-3) which reduce all the chronic age related diseases such as cancer and cardiovascular diseases (Parmar *et al.,* 2012). It is free of cholesterol but also reduces the cholesterol during hyper tension. So, soy protein have been developed for use in hospital diets and for the infant food such as rice and wheat based foods (Ugwu *et al.,* 2005).The soybeans one-fifth part also processed for oil consumed as edible product like as cooking oil and utilized by industries to make the products like paints and inks.

The soy flour is used for making the bread, soup thickener, pancakes and pudding as a mix flour. Lecithin emulsifier from soybean used in candy, bread and drugs. There are total 43 varieties of different strains of soybean sauces and pastes in the market.

Fig. 2 Soy and its different products

About 5,000 years the soybean remained as the history of china but now it has been circulated to different parts of the world as a commercial crop which is growing over the 35 countries as the major oil and protein seed (Gracia *et al.,* 1997). In India Madhya Pradesh known as "Soybean State" covers 74 per cent (4.74 million hectares) of total area under soybean which is producing 70 per cent (4.74 million tons) of soybean (Agricultural statistics *et al.,* 2001). The main exporters of soybean are U.S.A (40 per cent), Brazil (33 per cent) and Argentine (11 per cent) and the importer is China with 38 per cent. The FDA (Food and Drug Administration) and EFSA (European Food Safety Authority) currently allows the use of labels stating soya can reduce the risk of heart disease (Adlercreutz *et al.,* 1997). The researcher Arya *et al.,* (2014) explained that the increase in health consciousness among people and also the export value of the de-oiled cake from soya is helping in the expansion of this crop productivity both horizontally and vertically so it is visualized as future energy crop which can be used for the production of bio-diesel to meet the fuel energy requirements in a eco friendly way in some countries. Due to this rapid growth in production areas and production made this crop exposed to biotic and abiotic stresses

2.1.1. Composition of Soybean:

According to the Bellaloui *et al.,* (2008) the soybean seed contains the protein concentration of 341 to 568g/kg of total the total seed weight with a mean of 421g/kg. The amount of oil and saturated fat ranged from 83g/kg to 279g/kg and 100g/kg to

120g/kg respectively. The mean concentration of unsaturated fatty acids for linoleic acid was 540g/kg, for oleic acid was 240g/kg. According to Ensminger *et al.,* (1978) stated that the soybean contains very less amount of starch which observed was 4.66-7.00 per cent with a large amount of hemicelluloses, cellulose and pectins with much amount of protein like lysine, isoleucine, threonine and valine while the amount of sulphuric acid in rapeseed products was less.

Table 1: Basic nutrients in soybean seeds (Van Eys *et al.*, 2004)

Nutrients	Soybean Seeds (Per cent DM)
Crude protein	37.08
Crude ash	4.86
Crude fat	18.38
Crude fiber	5.12
NDF	12.98
ADF	7.22
N-free-extractive	24.00
Starch	4.6

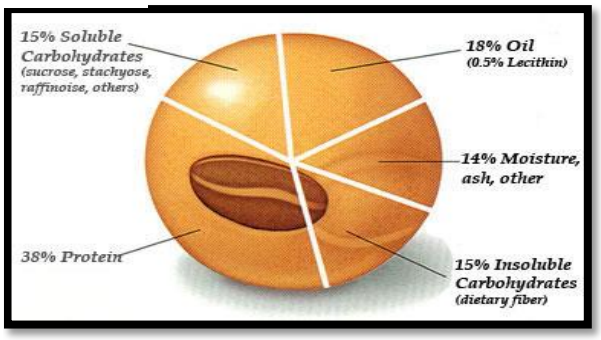


Fig. 3: Composition of Soya

Iancu *et al.,* (2010) also stated that the nutritive value of proteins in soybeans was limited by the sulphur, amino acids and tryptophane. The amino acids in soya protein were the good supplement of grain proteins and also covered all the requirements needed by the animals. The high digestible proteins in soybean reported were lysine and methionine.

Table 2: Content of essential amino acids in soybean products (Ensminger *et al.*, 1978)

Amino acids	**Soybean Seeds (Per cent of Dry matter)**
Arginine	2.45-3.1
Cystine	0.45-0.67
Histidine	1.0-1.22
Isoleucine	1.76-1.98
Leucine	2.2-4.0
Lysine	2.5-2.66
Methionine	0.5-0.67
Phenylalanine	1.6-2.08
Threonine	1.4-1.89
Tryptophan	0.51-2.44
Valine	1.5-2.4

2.2. SOYA PRODUCTS:

In this part of the review of literature the different soy based products, their nutritional quality, processing has been explained along with different products:

2.2.1. Soybean oil:

Serrato *et al.,* (1981) explained that the modern processing plants use solvent liquid transfer for the extraction of soybean oil which involves cleaning, cracking, de-hulling and finally the conditioning of soy flakes before it enters in to the extractor. The extraction is a successive process which includes the countercurrent washing of hexane solvent and then extracted flakes enters in to the sealed conveyor for the de-solventization by the jackets and sparge steam in an enclosed vessel. With the help of rising film evaporators and final vacuum distillation the hexane was removed from oil and later the oil was recovered in atmospheric condensers and from the meal. Li *et al.,* (2006) stated that oil quality should always measured in the terms of crude oil color, free fatty acids and residual hexane absence and the parameters for the determination of meal quality was residual oil, moisture, protein and the meshed size in the finished product. He also defined the method in three steps (1) penetration of solvent in to the soybean solids, (2) solubilization of oil in solvent and then the (3) diffusion of oil into the liquid solvent phase from the matrix of solid. Then, Lander *et al.,* (2001) explained the disadvantage of this method that there was production of carbonic acid due to the reaction of moisture CO_2which then makes the oil rancid.

2.2.2. Tofu (soybean curd):

According to Verhoeckx *et al.,* (2015) the tofu was processed by the coagulation of soymilk followed by pressing of the resulting curd made by soymilk. It has been recorded in the era of 2,100 years ago of Hoenam King, Han dynasty, China. Then, Kim *et al.,*2005 stated that the chemical composition of soybean was found to be 80-85 per cent of water, protein of 8.5 per cent, fat of 5.5 per cent and 1.5 per cent ash and the protein was about 42-52 per cent showed in solid part. According to Cai *et al.,* (1997) the curdling agent used to make tofu was salt ($CaSO_4$) that produced the soy protein gel which traps soy lipids, water and other constituents within the matrix which forms curd and then pressed in to solid bean cubes formed tofu.

2.2.3. Tempeh:

Steinkraus *et al.,* (1983) explained that traditional fermented soybean food was made by the fermentation of cooked soybeans with *Rhizopus* mould and thus the soybeans formed the compact white cake. In this process the cooking and de hulling takes place followed by the inoculation with different strains like *R. oligosporus, R. oryzae and R. stolonifer* which will lead to solid substrate fermentation. The tempeh is whole soybean product containing high nutritional quantities like vitamins, protein and fatty acids with the different textural quality. Due to the fermentation process and retention there is a strong flavor, firmer texture and more high quantity of protein, dietary fiber and vitamins as compare to the tofu. So, due to all these features it was used as worldwide nutritious of low cost vegetarian food which can also be consumed by all socio-economic groups (Baumann *et al.,*1995).

2.2.4. Miso:

Miso produced in the form of paste produced by fermentation with help of lactic acid and yeast commonly in households of Japan. The local people developed taste but different range of Miso exists can be divided in to two categories: 1. Standard Miso made from soybeans, water, salt, and rice or barley; there were special fortified nutritional Miso contains calcium, Vitamin B1, B2 or B1; 2. low salt Miso which is used as therapeutic food in hospitals (Kiuchi *et al.,* 2011).

2.2.5. Soymilk:

Behrens *et al.,* (2007) explained that the soymilk is an emulsion that contains protein, carbohydrate and also the oil droplets were the water extracts from soy beans. The soymilk was first referred by the Trimble (1896) in United states and the first commercial soymilk was also made by J.A Chard Soy products of New York (Lancu *et al.,* 2010). It has the total solids of 8-10 per cent, 2 per cent of fat, carbohydrate content is 2.9 per cent, ash was 0.5 per cent and protein was 3.6 per cent (Liu *et al.,* 2005). The functional soymilk contains bio active components which also help to enhance the health and also reduces the risk of other diseases but Onuorah *et al.,* (2007) explained that natural anti-nutrients were present like trypsin inhibitor(TI), phytic acids, lectins and indigestible oligosaccharides that limits the excessive consumption of soymilk but

excessive heating during the preparation of soymilk destroys the maximum of anti-nutritional compounds due to which the proteins easily gets digested.

Earlier, the soymilk was only produced by small shops but that milk had a very short shelf life and now, the soymilk produced has longer shelf life due to the new and different technologies exploitation (Byun *et al.,* 1995). However, the shelf life upgraded from months up to years can also be obtained that depends upon the composition of product and the packaging technology. Soymilk is the best substitute of a cow, buffalo and human milk for the infants, lactose intolerant people and allergic people. Even the cow milk was found to be more expensive and unavailable as compare to the soymilk. The only problem with the soymilk is the off flavors and for this Iwuoha *et al.,* (1997) analyzed that the off flavors could be reduced with the use of different techniques involving high temperatures like hydration grinding and also the pH. So, the thermal inactivation of lipoxygenase and the pH of 3.5 or below helped to suppress the off flavors in the legumes. So, the extracted soymilk with pH 2.0 and 6.4 shows inactivation of lipoxygenase with the 100 per cent of protein recovery. In this method filtration was not done only de-hulling was done through hydration and followed by blanching of beans to eliminate the growth of microbes and enzyme inhibitors (Cant *et al.,* 1987).

2.3. COMPARISON OF SOY AND LACTEAL MILK:

Table 3: Comparison of the nutrient content between the soya and lacteal milk (Hajirostamloo *et al.;*2009)

Nutrients per litre of milk	Soymilk	lacteal milk
Fat	4.67g	8.15g
Fatty Acids	0.52	6.07g
Fiber	3.18g	0.00g
Protein	6.73g	7.12g
Carbohydrates	4.43g	11.37g
Water	228.51L	214.69L
Lactose	0g	4.27g
Total solids	10.40g	12.96g
Ash	0.66g	1.75g
Ca(Calcium)	9.80mg	290.36mg
Fe(Iron)	1.42g	0.12g
P(Phosphorous)	120.05g	226.92g
Calories	79 Kcal	150 kcal
Acidity	0.24%	0.21%

The lacteal milk is produced by mammary while soymilk made from soybeans. Soymilk contains plant based proteins and cow milk also has rich nutrients but lacteal milk contains twice of fats and ten times of fatty acids as compare to the soymilk (Abraham *et al.,* (1999). Both milks contain the equal amount of protein, water and fiber. In lacteal milk mineral content except Fe was higher and three hundred times the amount of Ca and twice the amount of P to the soymilk contains ten times which found to have more Fe as compared by Hajirostamloo *et al.,* (2009).

2.4. HEALTH BENEFITS OF SOYMILK:

2.4.1. Anti-cancer effects:

Dong *et al.,* (2011) evidences that the consumption of phytoestrogen was observed to be responsible or cause which protect against breast cancer specially in Asian population in which Genistein and Daidzein were illustrated responsible for the suppression of initiation and proliferation of tumors. Montemayor *et al.,* (2010) suggested that Genistein acts as an inhibitor of tyrosine-kinase activity by the transcriptional regulation of Rho GTPases and of PAK. Another mechanism was given by Guo *et al.,* (2007) in which he found that Isoflavones also inhibits or suppress the angiogenesis of prostate tumor by the suppression of signaling pathway of vascular endothelial growth factor between the tumor and vascular endothelial cells. Su *et al.,* (2005) also demonstrated that the Isoflavones does not create toxicity in normal bladder because of their inhibitory angiogenic effects. So, 30 per cent of people who consume soymilk possess less risk of cancer as compare to the people who does not.

2.4.2. Bone Health:

According to Bolla *et al.,* (2015) reported that the 34millions people have low bone mass and 10millions have osteoporosis where density and calcium of the bones decreases due to the over activity of toosteoclast specially in women after the menopause of first few years in which bones lose the thickness and results the risk of fracture. Wu *et al.,* (2004) demonstrated that intake of soya with Genistein and physical exercise prevents the bone loss and body helped in weight gain too. The intake of soy phytoestrogens acts a natural alternative therapy to the conventional hormone therapy which helped in the prevention of bone loss. Due to high consumption of soy in Asia there was a low risk of

osteoporosis in 50-60 per cent less frequent hip fracture in Asians as compare to the western people.

2.4.3. Hypocholestrolemic effects:

The amphiphilic nature of saponins in soybean interferes with the cholesterol level and with the other mechanisms like interference with the bile acid metabolism (Sautier *et al.,* 1979). Cohn *et al.,* (2010) stated that for saponins only soybean was found the main source in the human diet. Actually, through the possible mechanism saponins interfere with the adsorption of cholesterol form insoluble complexes, formation of micelle and the perturbation of brush bordered membrane because of detergent-like properties. Afrose *et al.,* (2010) demonstrated that in hens laying eggs showed the effect of saponins that reduced the egg cholesterol and serum due to suppression of cholesterol synthesis and cholesterol catabolism was observed in the liver. Zhao *et al.,* 2008 proved that when the hamsters fed with the diet supplemented with saponins by which there was an increase in fecal cholesterol loss and cholesterol adsorption decreased .

2.4.4 Helps in reducing body fat:

The Soy protein was considered as an important source of dietary protein which played an important role in obesity which indicates that regular consumption reduces the body weight and mass which also lowers the plasma cholesterol and triglycerides. It also include the wide spectrum of bio chemical and molecular activities that also effect fatty acid metabolism and homeostasis of cholesterol and main proteins which play important role in obesity were soy saponins, phospholipids, isoflavones and conglycinin (Velasquez *et al.,* 2007)

2.4.5. Pre-menopausal effects:

Messina *et al.,* (2009) explained that phytoestrogens acts as anti estrogens in pre-menopausal women. Similarly, Setchell *et al.,* (2002) studied 40 pre-menopausal women with the intake of soy diet increased the menstrual cycle length by 1.8±0.7 days and also increased the urinary iso-flavonoid excretion as compare to women fed with a control diet. The consumption of isoflavones rich products reduced the Luteinizing hormone (LH) and Follicle-stimulating hormone (FSH) hormone concentration that controls the

development, puberty, maturation and reproduction processes in premenopausal women and as such menstrual cycle was increased by 1.05 days was demonstrated by Hooper *et al.,* (2009).

2.4.6. Post-menopause:

Phytoestrogens acts as estrogen hormone in post menopausal women where the estrogen level becomes low. The 51 premenopausal women who were fed soy proteins containing iso-flavones resulted reduction in the blood pressure, lipoprotein profile improvement and relief from menopausal symptoms (Cassidy *et al.,* 2006). Also, another study in which 40 women were given with the 100mg of iso-flavones caused a decrease in menopausal symptoms and also the decrease in the risk factors for cardiovascular diseases like plasma level and LDL cholesterol level which was all demonstrated by the Washburn *et al.,* (1999).

2.5 SOYMILK PREPARATION:

According to Nelson *et al.,* (1976) soymilk was prepared by the preheating treatment of soybeans which was a traditional method in which the soybean grains about 162g were soaked for 16 hours in cold water at room temperature about 20°C in 500ml of water. The soaked grains were cooked for five minutes in pressure cooker (1.5 Kg/cm^2 at 127°C). The slurry was then cooked and filtered through muslin cloth and soymilk was extracted. Cooked soybeans without pre-soaking were prepared by cooking 100g of dry grains in pressure cooker for 15 minutes in 500ml of water. The modification was first introduced by Embrapa, (1988) in which he stated that the clean and dry soybeans approximate 162g added directly into boiling water of 500ml which contains 0.25 per cent $NaHCO_3$for 3 minutes and then discard the water and cool the whole soybean under cold water. Again, for three minutes re-blanch the soybeans in boiling water of about 1000ml which contains 0.05 per cent of $NaHCO_3$ and the grains were ground in blender. For ten minutes the slurry was cooked and filtered using cotton cloth. The soymilk was extracted and boiled again.

In 1967, the New York State Agricultural Experiment Station, Geneva (NY) and the Cornell University experimented that proved the off flavors of soymilk can be

prevented by the rapid dehydration and grinding of de-hulled beans at temperature above 80°C. The inactivation of lipoxygenase can be done by the quick moist heat treatment before it affects the flavors. So, all soymilk samples till now are pre-heated in the same manner to destroy lipoxygenase before the extraction of soymilk.

Bansal *et al.,* (2014) focused on improving and diversifying the nutrition of soymilk by the germinating of soybeans before the making of soymilk and then the evaluating its quality and potential for the acceptance. She explained that soymilk prepared under 28 hours germination imparts more nutritional quality in terms of increase in protein digestibility, decrease in fat, ash, carbohydrate, ash, total solids, pH and anti-nutrients like total phenolic content. Soymilk developed by the incorporation of short time germination of soybeans have enhanced functional attributes and improved the food.

2.6. CHALLENGES:

2.6.1. Mechanical Method:

Low capital cost method is advantageous but there are certain cones which were associated with it are high operational cost due to high power consumption, excessive heat that affect color and flavor of milk, oil as well as of cake and regular wear and tear of components. The Evans *et.al.*,(1997) explained another method can solve the problem that is solvent extraction by dissolution in liquid solvent which cause the removal of soluble material from an insoluble solid, an effective method for the extraction of oil, milk and cake resulted in to the 95 per cent yield of oil and milk recovery.

2.6.2. Protease inhibitors:

Kakade *et al.,* (1972) explained certain factors and protease inhibitors which were responsible for limiting the nutritive values of soymilk are Trypsin, Chymo trypsin inhibitors and Pectins. In animals, these inhibitors lower nitrogen retention and thus increase the metabolic nitrogen excretion and decrease the performance (Bau *et al.,* 1997).

2.6.3. Lectins (Hemaglutinins):

Kakade *et al.,* (1972) defined that these are the proteins of raw soybean which bind to carbohydrates and thus decreases the growth and increase mortality rate in

animals. In soybean the amount of lectins was stated to be 37 to 323HU/mg of protein. According to Fasina *et al.,* (2004) the content of lectins which were considered as agglutinating lectins disappears after autoclaving.

2.6.4. Beany flavor:

Yuan *et al.*,(2008) stated that soy foods contains the health promoting bioactive compounds like isoflavones, proteins but also some undesirable beany flavor and trypsin inhibitor activity(TIA) and cooking of raw soybeans decrease the protein quality and nutritive value which also cause many diseases like pancreatic hypertrophy. Badenhop *et al.,* (1971) explained that 4-10 per cent residual trypsin inhibitory gave the highest protein nutritive value for the heated soymilk. So, overheating to destroy the 100 per cent trypsin inhibitors may also destroy the tryptophan, lysine and cysteine. The Cysteine retention is very important for protein nutritive value because it is one of the two sulfur amino acids that are the limiting essential amino acids in soy foods (Panizzi *et al.,* 1999). Kwok *et al* (2002) explained the effect of indirect (UHT) heating on soy milk to destroy trypsin inhibitors by passing soymilk through small-diameter stainless tubes, which were heated by an oil bath at selected temperature (143°C) for 60s can inactivate TI to approximately 10 per cent of the original raw soy milk.

2.6.5. Okara:

Noguchi,(1987) explained that the residue left from ground soybeans after extraction of the water extractable fraction used to produce soy milk and tofu termed as okara/draff/tofukasu (Take *et al.,*1980), or soypulp, douzha (Chinese), bejee (Korean) and Tempeh gembus (Indonesian) (Yang, 2016). According to the Khare *et al.*,(1995) about 1.1 kg of fresh okara was produced from every kilogram of soybeans processed for soymilk and also found that 30 per cent of bean solids, 20 per cent of bean protein, and 11 per cent of oil ended up in the okara. The Illinois method, which was given by Liu, (2006) in which no okara liberated as the whole bean was homogenized, but the resulting soymilk unfortunately has undesirable organoleptic properties.

2.7. ENZYME ASSISTED SOYMILK EXTRACTION METHOD:

Rosenthal *et al.,* (2009) stated that the enzymes were used for hydrolyzing the cell wall and lipid body membranes which were made up of structural polysaccharides or proteins. So, in this the disruption of cell wall of cotyledon of soybean with the help of milling operation resulted in to the protein or milk yield. The cellulase, pectinase and protease which are cell wall degrading enzymes increase the yield along with the others factors like temperature, pH, particle size and agitation rate. The enzymatic extraction method has the great potential with suitable enzyme and its operating conditions. The various enzyme combinations are also used for losing the structural integrity and enhance the extraction of various components. He also explained that several factors like incubation time, incubation temperature and enzyme concentration increase the potential of enzymatic method. These enzymes act on soybean cell walls to obtain the product which enhance its nutritional quality by breaking its cell wall polysaccharides in to the digestible saccharides (Hosseini *et al.,* 2003).

2.7.1. Pectinase enzyme:

The pectin has a molecular weight of 50,000 to 150,000 Da is a carbohydrate which is complex and consist of D-galacturonic acid which is also linked by the α,1-4 glycosidic linkages. The spaces between the protein bodies in cotyledons of soya are filled with lipid bodies and cytoplasmic network. The cell wall constitute 86 per cent of carbohydrates which is consists of 20 per cent pectin, 30 per cent cellulose and 50 per cent hemicelluloses (Kalia *et al.,* 2001)

Marshall *et.al.,*(1999) demonstrated that cell wall and the hulls of soybean were used as co product in processing industry as a source of pectin which was extracted using 0.05N hydrochloric acid and then precipitated with alcohol. Singh *et al.,* (2016) also demonstrated the use of pectinase enzyme for the clarification of juice and in improving the characteristics of fruit juices.

2.7.2. Cellulase enzyme:

This enzyme was used to break the cellulose chains of cotyledon in plants and to increase the extraction yield. According to Ghosh *et al.,* (1982) cellulose has the load

bearing network of the primary and secondary cell wall and is a polymers of beta-1,4-D-glucose residues that also joins with other cellulose chains with the hydrogen bonding and vander walls forces. The synthesis of cellulose chains takes place in plasma membrane. The maximum amount of dry weight of cellulose is in dicotyledon and in which the number of glucose molecules are there connected by α,1-4linkages making it a complex structure. Xu *et al.,* (2007) explained that the enzymatic hydrolysis break down the linkages of cellulose and liberates the glucose molecules by means of a bio-conversion process that also includes pre-conditioning of seeds before the soymilk extraction process and this pre-treatment includes the breaking up of seeds and converts the cellulose in to the glucose and also complex lipoprotein molecules to the simple lipids and protein molecules. He used the enzymatic hydrolysis technique to produce the lactic acid from soybean straw in which the optimal conditions for pre treatment using ammonia has been done and then compared it with untreated straw.

Similarly, Aubert *et al.,* (1994) demostrated that soaking increase the hydrolysis of polysaccharides of cell wall and also substituted cellulose which also increase the nutritive quality of fermented foods and cereals. The Galante *et al.,* (2003) explained that the macerating enzymes like cellulase, pectinase and hemi-cellulase were used drastically for the hydrolysis of plant cell wall polysaccharides, for the extraction of protein, stability, quality, and for the clarification of wines.

2.8. MARKET RESEARCH/ NUTRITIONAL SURVEY:

Xu *et al.,* (2011) explained that due to increase in health consciousness awareness the soybean is getting more popular and consumed in the form of textured vegetable protein as a good source of protein. India has high potential for soy and experts also predicted that every year the soya food industry grows by 20 per cent and mainly soymilk whose sales individually rose by 50 per cent in 2001 and 70 per cent in 2005. Dhar *et al.,* (2005) also demonstrated that from the past 2005 years from 1997 to 2002 the price of the soy milk dropped 20 per cent as compared to the other milk to which 16 per cent increase in skim milk, 14 per cent in whole milk, 35 per cent in milk shakes and 21 per cent in flavored milk was recorded. So, this also depicted the drop in differential price between the skim and soymilk from $5.80 to $3.73 per gallon (122 per cent of the skim

milk price). Hence this price difference showed the willingness among the people to pay for the soymilk. On an average, it resulted that the buying ability of premium consumer who bought these types of milk was not affected by the competition between the labeled and unlabelled milk. For the soymilk it drives benefits to the market.

Approximately, 5,000 years ago the archeological evidence showed that in eastern Asia the soybeans were first cultivated and the written record was mentioned dates back in the book of ODES in 1100 B.C in China. Earlier, Chinese people call soybeans with the name TA-TOU which means 'great bean" and the Tofu was developed by the Buddhist monks of china between 25 and 220AD in Han Dynasty. First in 1700's the Europe cultivated it and then in 1800's United States also started cultivating it (Halpern *et al.*,1973).

The Bhatia *et al.,* (2008) explained that that the soybean was introduced in India in 1977 from China, Japan and South East Asia via far eastern tip of India. But today, mainly restricted areas for the production of soybean in India are Madhya Pradesh, Uttar Pradesh, Maharashtra, Gujarat and also areas of Punjab and Himachal Pradesh. The Madhya Pradesh is the main state of India which is cultivating the soybean at a large scale.

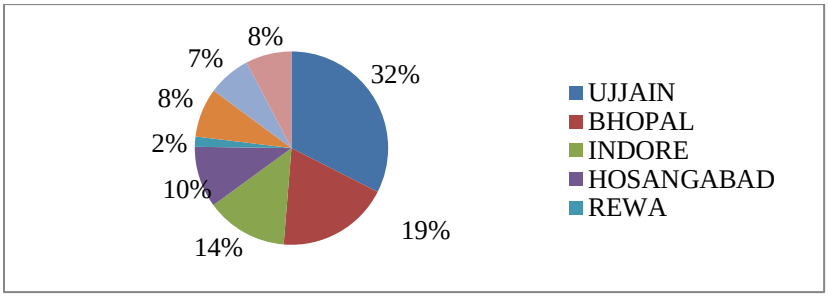


Fig 4: Cultivation of Soybean in different parts of Madhya Pradesh (Bhatia *et al.*, 2008)

The soya protein market has been expanded globally. So, according to him the market is mainly divided into North America, Europe, Latin America, Middle East, Africa and Asia Pacific. Out of all, these markets were led by the North America in 2015 and followed by Europe and Asia pacific. The favorable growth of protein and milk was in Latin America, which allowed the others part of the globe to look for it and to start

manufacturing of soy milk. The almond and soy milk production industry covers a high level of market share concentration and due to this revenue generated more than 80 per cent in 2016 (Oliveira *et al.*, 2016).

Joshi *et al.*, (2003) explained that in India the soy milk attracted a growth of 29 per cent from 2003 to 2007 in an annual growth of 6.6 per cent. The research firm predicted that the conservative growth of soy-based products estimated out to the total value of the Soy Foods and Beverages market by $3 billion by 2012. It was also demonstrated that India is the 5th largest producer of soybean in world with the production of 4,809 TMT (Thousand Metric Tons) of soybeans and with average consumption in India is 4,812 TMT of soybeans. Soybean output has more than doubled in the last 10 years. It is expected to reach a level of 8.6 million tons in 2007-08, making available around 6.2 MMT (Million Metric Tons) of Soybean Meal with 4 MMT for exports (Achi *et al.*, 2005).

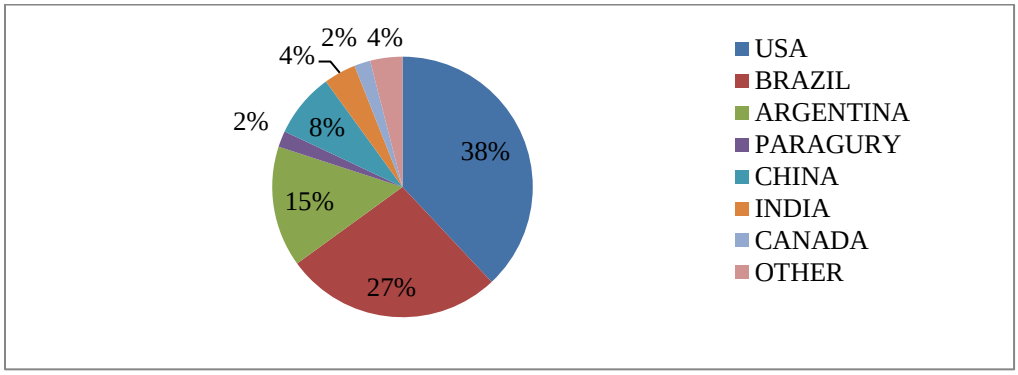


Fig 5: Top producers of Soybean and Soymilk (Oliveira *et al.*, 2016).

Approximately 65 per cent of the total soy meal produced and exported by India and has turned out to be one of the largest exporter of soy meal usually to the Asian countries. There is no other country which produces cheaper soymeal than India, which also contains high protein content of around 48 per cent. Only India supplies non-GM (genetically modified) soy meal whereas the U.S., Argentina and Brazil manufactures and supplies GM soya meal (Joshi *et al.*, 2003).

CHAPTER : 3

MATERIAL AND METHODS

3.1. PRE-REQUISITES FOR THE STUDY:

3.1.1. Collection of soybean samples:

The Soybeans dried seeds were obtained from the market of Madhya Pradesh which is also known as soya state of India during the 2016-2017. Different packets of soybean were ordered from the local vendors of Madhya Pradesh at regular intervals from November, 2016 to March, 2017 and brought for research and experiment purpose to the Department of Biotechnology, TU, Patiala.

3.1.2. Sodium Bicarbonate:

It was used in the boiling water for the blanching of Soybean to make the water alkaline which makes the soybean soft and then it easily gets de-hulled and grinded. It was used of Hi-media brand and manufactured in November, 2015.

3.1.3. Pectinase Enzyme:

The Pectinase enzyme was used for the extraction of the milk from the soybean. It was of Hi-media brand and the enzyme activity was 8000-12000U/g source of *Rhizopus sp*. It was made available by the Department of Biotechnology, TU, Patiala.

3.1.4. Cellulase Enzyme:

The Cellulase enzyme was used for the extraction of the milk from the soybean. It was of Hi-media brand and the enzyme activity was 10.0U/mg of source *Trichoderma viride* and was made available by the Department of Biotechnology, TU, Patiala.

3.1.5. Grinder:

The Kalsi grinder was used for the grinding of boiled soybean for the extraction of soymilk in both enzyme assisted and mechanical method. This type of grinder was made available by the Department of Biotechnology, TU, Patiala.

3.1.6. Muslin Cloth:

It was used in the extraction of the soymilk. Basically it was used for the squeezing and to remove the left okara byproduct. Filtration of soymilk was done with

the help of muslin cloth which was made available by the Department of Biotechnology, TU, Patiala having the pore size varied from 0.7mm to 0.22mm.

3.1.7. Pressure cooker:

It was of Prestine Company was used for the cooking of soybeans for 10 minutes at 120 degree Celsius at the pressure of 1 bar.

3.1.8. Auto clave:

It was also used for the standardization of the heating time or the boiling procedure of soybean for the extraction of milk Equitron Company is situated in STEP of Thapar University, Patiala.

3.1.9. Aluminum foil:

The Hindalco fresh wrap aluminum foil was used for the wrapping the milk samples to avoid the oxidation of the milk.

3.1.10. Water bath:

The Serological water bath of Equitron Company with temperature range of 0 to 100 degree Celsius was used for the extraction of milk with the help of enzymes. The standardization of enzyme assisted extraction method the activity of cellulase and pectinase was tested along with the trials for the extraction of milk at different temperatures was performed.

3.1.11. Refrigerator and Deep freezers:

The LG refrigerator was used for the storage of the enzymes and milk.

3.1.12. pH meter:

The pH meter of Omega Company was used measuring the pH of the milk samples which was made available by the Department of Biotechnology, TU, Patiala.

3.1.13. Spectrophotometer:

Hitachi spectrophotometer of model no U-2900/2910 of 2015 manufactured was used for measuring the wavelength of the different samples for flavonoid and for non-reducing sugars test.

3.1.14. Kjeldahl Flask:

This flask was used for the protein estimation of different samples of soymilk and was made available by the Department of Biotechnology, TU, Patiala.

3.1.15. Gerber Instrument:

This was used for the fat estimation of soymilk samples and is situated in STEP of Thapar University, Patiala.

Fig 6: a) Pressure cooker b) Gerber instrument c) Autoclave

3.2. EXPERIMENTAL PLANNING:

3.2.1. Processing of Soymilk by Mechanical Method:

For the processing of soymilk initially the 40g of soyabean cleaned and the beans should be dry, sound and free of mould and extraneous matter. The beans were washed with clean water to remove dirt and were soaked in approximately 300ml of water was boiled. After this, the boiled soybeans with extra added boiled water were grinded until the soft slurry was made and the filtration of the slurry was done using muslin cloth from which the soymilk was extracted in a beaker and the okara was left on a muslin cloth after several grindings and straining. Then the pasteurization of soymilk was done in which we heated the milk until the temperature reaches to 80^{o}C for few seconds and then immediately we put the beaker containing hot milk in to the chilled iced water to make it free from microbes and to store it for the long time.

3.2.1.1. Standardization of Mechanical soymilk extraction method:

In this we standardized the soaking time of beans at different temperatures and the cooking conditions like Autoclaving, boiling and cooking in pressure cooker after the de-hulling of soybeans in the mechanical process mentioned in the table 4.

TABLE 4: Soaking of soybeans at different temperatures-time combinations

Sr.no	Temperature (^{0}C)	Time (hrs)
1.	45	6
2.	50	5
3.	55	4
4.	60	3

3.2.2. Standardization of enzyme assisted soymilk extraction method using cellulase enzyme:

Cellulase enzyme was used for the extraction of soymilk. Initially, pre-weighed 4g of soybeans were cleaned, soaked for 5 hours and then de-hulled beans were cooked in the pressure cooker for 10mins and poured in to the beaker. Then for standardization of enzyme assisted soymilk extraction method different concentrations of enzymes in triplicates were mixed with the boiled soyabeans like 0.05 per cent, 0.08 per cent, 0.1 per cent, 0.2 per cent and 0.3 per cent of cellulase enzyme on the basis of wet weight of soybean and kept in water bath at different temperatures (37^{0}C, 40^{0}C, 45^{0}C, 50^{0}C, 55^{0}C, 60^{0}C, and 65^{0}C) at different incubation time like 2hrs, 3hrs, 4hrs, 5hrs and 24hrs. After this the beakers were removed from water bath and squeezed through muslin cloth and milk was extracted, yield calculated. The extracted milk was stored at 0-6^{0}C in the bottles and covered with foil. All the steps were performed under sterile condition that is laminar. The samples obtained were taken for quality analysis.

3.2.3. Standardization of enzyme assisted soymilk extraction method using pectinase enzyme:

In this process pectinase enzyme was used for the standardization of enzyme assisted soymilk extraction method. So, after the pre-weighing of soybean grains, they were soaked in water overnight, boiled for 1 hr in water, de-hulled and pressure cooked in pressure cooker and after that transferred to beakers with different concentration of pectinase enzyme 0.05 per cent, 0.08 per cent, 0.1 per cent, 0.2 per cent and 0.3 per cent on the basis of the wet weight of soybeans and kept in water bath at different temperatures (37^{0}C, 40^{0}C, 45^{0}C, 50^{0}C, 55^{0}C, 60^{0}C, and 65^{0}C) at different incubation time like 2hrs, 3hrs, 4hrs, 5hrs and 24hrs then beakers were removed from water bath and filtered through muslin cloth from which the milk was extracted and stored at 0-6^{o}C in

the vacuum tight bottles covered with foil. All these processes were performed under asepsis conditions. The samples obtained were taken for quality analysis.

3.3. TESTS FOR THE QUALITY ANALYSIS:

The milk samples obtained from mechanical soymilk extraction method as well as the enzyme assisted soymilk extraction methods were analyzed.

3.3.1. Protein test of different milk samples:

This method was performed by the Wiles *et al.*, (1997) for the protein determination of milk. In this method the 5ml of milk sample was taken and 10ml of concentrated sulphuric acid was added to it along with racemic mixture in a digestion flask. Then, digestion flask was placed in inclined position with fume ejection system and kept on burner on high setting. Digestion may take 30minutes to 1hour until white fumes appear in flask and then the sample was heated again for 15 minutes until the digest was cleared (clear with light blue-green color) and then continuously boiling was done for 1-1.5 hr at maximum setting. At end of digestion, digested material was clear and free of undigested material. Cool acid digestion with liquid or liquid with few small crystals to room temperature Large amount of crystallization before addition of water indicates too little residual H_2SO_4 at end of digestion and can result in low test values.) After this the digested material was cooled at room temperature and the 150-200ml H_2O was added to flask and swirled to mix. After cooling the distillation process was done in which the condenser water was turned on and then 50ml of H_3BO_3 solution was added with indicator to graduated 500ml of Erlenmeyer titration flask and then flask was placed under condenser tip so that tip is well below H_3BO_3 solution surface. Then 75ml of 50 per cent NaOH was added down sidewall of Kjeldahl flask with no agitation. The flask to distillation bulb on condenser was connected and then flask was swirled to mix contents thoroughly and was heated until all NH_3 has been distilled and the liquid was drained from condenser tip. Distillation heater was turned off and then H_3BO_3 receiving solution was titrated with standard 0.1000N HCL solution to first trace of pink. Lighted stir plate was visualized at the end point and recorded used mL of HCL.

Calculations:

Calculate results as follows:

$$\text{Nitrogen, \%} = \frac{1.4007 \times (\text{mL HCL, sample} - \text{mL HCL, blank}) \times \text{normality HCL}}{\text{g sample}}$$

For the determination of protein the nitrogen per cent was multiplied by the factor 6.38, to calculate per cent protein.

3.3.2. Fat estimation of different milk samples:

The fat estimation of soymilk was performed by the Gerber's method which was also performed by the Lucas *et al.,* (1978) in which the 10.75ml of milk was mixed with 10ml sulphuric acid and 1ml of iso-amyl alcohol and closed with a lock stopper, shaked until homogeneous, inverting it for complete mixture of the acid in butyrometer with Sulphuric acid for the dissolution of protein and released the fat. The tubes were centrifuged at 2000rpm and then the clarified fat was read into the calibrated part of the tube and is measured as a percentage of the fat content of the milk sample. The tubes were put in centrifuge, so as to conform to radial symmetry, and as evenly spaced as possible, in order to protect bearings of the centrifuge. After this the butyrometer tubes were removed and placed in water bath for 5min at 65±2°C. The percentage of fat after adjusting the height in the tube was noted as necessary by movements of the lock stopper with the key.

The scale reading corresponding to the lowest point of the fat meniscus and the surface of separation of the fat and acid was noted. When readings were being taken the butyrometer was holded with the graduated portion vertical, the point being read was kept in level with the eye, and then butyrometer was noted to the nearest half of the smallest scale division.

3.3.3. Carbohydrate test of the soymilk samples

This test was performed by Hartman *et al.*,(2007) for the testing of milk. The 3 beakers and the 3 Erlenmeyer flasks were labeled and then we poured the milk samples into their respective beakers

Heat the beakers until temperature reached 55°C

⇩

Few drops of acetic acid added in each beaker

⇩

Mass of casein protein formed and removed

⇩

Calcium carbonate was added in to the remaining liquid

⇩

Boiled and the remaining precipitates of protein removed

⇩

100ml of ethanol solution added, filtered, cooled down and measured

Fig 7: Filtration of Sample

3.3.4. Flavonoid test for the different samples of milk:

Determination of total flavonoid content was performed by Kalita *et al.*, 2013. In this method the total flavonoid content was measured with the aluminum chloride colorimetric assay. 1ml of aliquots and 1ml standard quercetin solution (100, 200, 400, 600, 800, 1000 µg/ml) was positioned into test tubes and 4ml of distilled water and 0.3 ml of 5 per cent sodium nitrite solution was added into each. After 5 minutes, 0.3 ml of 10 per cent aluminum chloride was added. At 6thminute, 2 ml of 1 M sodium hydroxide was added. Finally, volume was made up to 10 ml with distilled water and mix well. Orange yellowish color was developed. The absorbance was measured at 510 nm spectrophotometer using UV-visible Jasco *V-630*instrument. The blank was performed using distilled water. Quercetin was used as standard. The samples were performed in triplicates. The calibration curve was plotted using standard quercetin. The data of total

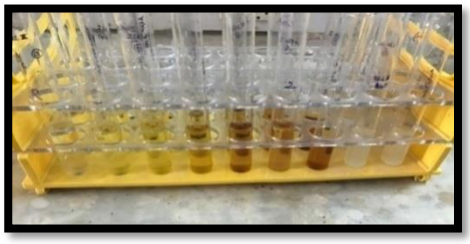


Fig 8: Flavonoid test samples

flavonoids of polyherbal formulation were expressed as mg of quercetin equivalents/ 100 g of dry mass.

3.3.5. Acidity test of the different samples:

In this test the burettes were filled with the N/10 solution and the 10ml of milk was transferred in to the flask and also equal quantity of distilled water and then we added the 3-4 drops of phenolphthalein indicator which was stirred with the glass rod. The initial reading of alkali in burette was note down. Titration was done with the N/10 NAOH and completed it with in the 20 seconds and the readings were note down.

Test burette with N/10 solution was filled

⇩

10 ml of milk and equal quantity of water was poured

⇩

Few drops of phenopthalein was added

⇩

Initial reading of alkali in burette was noted down

⇩

Titration with sodium hydroxide until the color changes to ink for 20 seconds

Calculations:

$$\% \text{ Lactic acid} = \frac{\text{No of ml. of 0.1NaOH solutions required for neutralization} \times 0.009}{\text{Weight of sample}} \times 100$$

(Weight of sample = Volume of milk x specific gravity)

3.3.6. Non-reducing sugars test for the different samples of milk:

This test was also performed by the Somersall *et al.,* (2007) performed this for the estimation of non-reducing sugars in milk. For this the DNS reagent was prepared by dissolving 1g of 3,4-dinitrosalicyclic acid in 100 ml H_2O in 200ml of beaker and then while stirring the 1g of NaOH and 0.25g sodium sulphite was added in to the beaker. The 19.2gm of potassium sodium tartrate tetrahydrate was also added and the mixture was stored for 20-30mins at below 20°C.

3.3.6.1. Preparation of standard curve and sample

Glucose standard solution was prepared in 0.05M Acetate buffer (pH 4,8) ranging from 6-4 µmol/ml and then 1ml was added in to the tubes . In the blanks and as well in the tubes containing samples the 1ml of 0,05 M Acetate buffer (pH 4,8) was added. After the 3ml of DNS reagent was added in each test tube and mixed well which were then placed in water bath at boiling temperature for 5 minutes. The tubes were cooled down and the absorbance at 510nm was measured.

Fig 9: Sample and DNS in the Test Tubes

3.4. SENSORY ANALYSIS:

The prepared soymilk samples were evaluated by sensory analysis for colour, appearance, flavour 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 5: 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

3.5. STATISTICAL ANALYSIS:

The experimental data obtained was analyzed for ANOVA from milk samples produced by mechanical soymilk extraction method, enzyme assisted extraction method with cellulase and pectinase enzyme.

3.6. MARKETING OBJECTIVES, HYPOTHESIS AND THE METHODOLOGY:

The present study uses a survey method to understand the perception of different stakeholders towards Soya milk. A survey was conducted to collect the data from students. A sample of 100 respondents was collected from Jalandhar city and some responses were taken from Thapar University, Patiala.

3.6.1. Broad objective:

- Whether people prefer, aware of soymilk or not

3.6.1.1. Specific objectives:

- To find out whether people like to drink soya milk or not.
- To find out whether people prefer soymilk over cow's milk.
- To find out the reaction (positive/negative) of people regarding soya milk.
- To find out the type (age, gender and income level etc.) of people who are interested to drink soya milk.
- To find out which factors have to be considered before the making of soymilk and need to be improved to increase its marketing.
- To find out the type (age, gender and income level etc.) of people which should targeted for the marketing and as well as to increase the sale of soymilk

3.6.2. Hypothesis:

- Null Hypothesis: "People don't prefer and are not aware of soymilk in Jalandhar and in Thapar University, Patiala.
- Alternative Hypothesis: "People prefer and are aware of soymilk in Jalandhar in Thapar University, Patiala.

3.7. RESEARCH METHODOLOGY:

The study is based on primary data. Primary data have been collected through survey. The main tool used for this research is a self-structured questionnaire. The respondents were chosen from Jalandhar city and Thapar University, Patiala. Data were

also collected from some of the customers of soya milk. A total of 100 respondents constituted the sample.

3.7.1: Research Design:

The present research is exploratory research. Our primary research was to collect the raw data. Collecting raw data from various sources was required to process, tabulate, edit for the purpose of the study. After processing the data, statistical tools were applied to have meaningful results. Besides, some graphical presentations such as pie chart, bar chart are also used. A schedule of our working process and period is given below.

3.7.2. Ethical considerations:

In our marketing research project, the utmost confidentiality has been maintained to protect the information of our respondents and gathered the questionnaire from every respondent serially rather haphazardly. Moreover, the individual personal information of the respondents was not focused. In addition, we let them know our purpose of conducting this research and ensured them that identity will not be reveal to anyone. Furthermore, as the marketing research has been done and were well prepared for how to conduct an in-depth survey. So we have applied the all the procedures.

3.7.3. Scheduling:

TABLE 6: Scheduling of the survey

No.	Process	Description
1.	Pre research study and design process	Questionnaire prepared and tested in the field level
2.	Survey	Total 100 questionnaires are conducted
3.	Data processing	Crosschecking and data processing of survey conducted with key information.
4.	Data analysis, report writing and Submission of report	Data processed and analyzed

3.7.4. Editing and the cleaning of the data:

After collecting the data from the survey, it had to be edited. Editing is the process of checking the data for necessary corrections. We found some errors in responses, some of the questions were not filled up properly and left incomplete. We did not want to have any missing values. Finally there were 100 complete questionnaires which were taken for further analysis.

3.7.5. Methods for obtaining data for SPSS:

After getting all the questionnaires filled up, each of the question was coded along with the variables with SPSS. Here, all of the options in each of the questions are entered to make a database. This SPSS database then became the source of further analysis. With the help of the data sheets then it was analyzed further by using various methods like frequency, cross tabulation, ANOVA, Correlation and One-sample T-test.

3.7.6. Developing the Data Analysis:

- **Coding:** Each question was coded to a computer readable form for using in the SPSS. Then the data were entered from the questionnaires. This was time consuming and needed much skill.
- **Data entry**: After entering all the responses from each question, the data entering part was done. Then we proceeded forward for the final job, which was analysis.
- **Data Analysis:** By using SPSS, suitable commands were given, the software automatically gave us the required result. Along with these a number of tables, charts were also added. Then we proceeded for the data conversion and interpretation part.
- **Data conversion**: All the SPSS result sheets had to be converted into managerially understandable form. We converted them to word documents, made tables, graphs and wrote the analysis with

3.8. JUSTIFICATION OF THE QUESTIONS IN THE SURVEY:

The survey questionnaire had intended to reveal two different aspects of the respondents. For the first part, every respondent had to fill three questions to provide their demographic information. The second part of the questionnaire was intended to disclose

whether the respondents are aware of soya milk. Then they had to answer details about the soya milk, from where they want to purchase, how much quantity or packet size they wanted etc. Are they really aware of their health, if they aware of health what should prefer dairy milk or soya milk etc. then there were marketing questions.

CHAPTER: 4

RESULTS AND DISCUSSIONS

Under the present study an attempt has been done to standardize the method of soy milk production via two different methods and comparison of Mechanical soymilk extraction method to the Enzyme assisted soymilk extraction method using two enzymes pectinase and cellulase enzyme. Once the method has been standardized the experimental milk samples were analyzed for milk yield from different methods, byproducts percentage, the quality and sensory tests for the obtained samples. The experimental data obtained from all the parameters has been statistically analyzed by ANOVA and correlation.

The survey was done on soymilk to know which age group, income range and gender prefer soymilk more over the lacteal milk available in the market and also studied that before the sale of soymilk which factors has to be kept in mind and need improval to increase the marketing of soymilk over cow's milk. Both the hypothesis has been tested. So, the results has been expressed in the tables, growth curves, and the standard graphs of the flavonoid content, non-reducing sugars, protein, fat, carbohydrates and SNF concentration in soymilk and the cross tabs of the survey have been discussed below under the heading and the sub heading.

4.1. STANDARDIZATION OF ENZYME ASSISTED SOYMILK EXTRACTION METHOD USING CELLULASE ENZYME:

Fig 10: Cellulase enzyme assisted Milk extraction

Fig 11: Cellulase enzyme assisted Okara extraction.

Considering the time and temperature as the dependent factors for enzyme activity. The two factors have been used at different concentration of enzymes at different time temperatures for different timings followed by the analysis on the basis of production of milk and okara. The trials were repeated thrice and then means, standard variation has been shown in tables 7.

4.1.1. Production of milk at different concentration of cellulase enzyme:

Using different concentration of cellulase enzyme (percentage weight of the soaked soybeans), a macerating enzyme helps in the hydrolysis of cell wall by converting the cellulose in to glucose. It was observed that average values for the production of milk after thrice attempts of trials 17.26ml, 18.16ml, 19.16ml, 20.2ml and 18.03ml at 0.05 per cent, 0.08 per cent, 0.10 per cent, 0.20 per cent and 0.30 per cent, respectively. Table 7 showed that maximum production of the milk was observed at the 0.20 per cent concentration of cellulase enzyme with a mean value of 20.2 with a standard variation of 0.06. Similar quantity of milk was obtained either 0.30 per cent or 0.20 per cent of cellulase enzyme. As a result the 0.20 per cent concentration of enzyme was chosen for producing the milk as the production rate observed were same. Comparing to the mechanical soymilk extraction method the production of milk 0.20 per cent concentration of cellulase enzyme was more as shown in fig. no 12.

Table 7: Production of milk at different concentration of cellulase enzyme.

Sr no.	Cellulase enzyme conc. (% of wet weight of soya)	Production of milk (ml)
1.	0.05	17.26±0.17
2.	0.08	18.16±0.08
3.	0.10	19.16±0.08
4.	0.20	20.2±0.06
5.	0.30	19.67±0.08

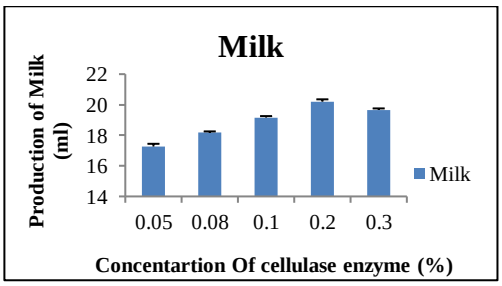


Fig 12: Milk produced

4.1.2. Production of Okara at different concentration of cellulase enzyme: After extraction of soymilk the left over solid matter i.e. okara was observed with the mean values 2.52g, 2.73g, 2.53g, 1.32g and 2.23g at concentration of 0.05 per cent, 0.08 per cent, 0.10 per cent, 0.20 per cent and 0.30 per cent, respectively as shown in 8. As the fig

no 13 explained that the least production of okara was noted concentration of 0.20 per cent of cellulase enzyme with the mean value of 1.32 with the standard variation of 0.05. Comparing to the production of okara in enzyme assisted soymilk extraction method it was very less as compare to the okara produced by the mechanical method. So that may be inferred that the full conversion of cellulose has taken place with the help of cellulase

Table 8: Production of okara at different concentration of cellulase enzyme

Sr no.	Cellulase enzyme conc. (% of wet weight of soya)	Production of Okara (g)
1	0.05	2.52±0.15
2	0.08	2.73±0.03
3	0.10	2.53±0.03
4	0.20	1.32±0.05
5	0.30	2.23±0.08

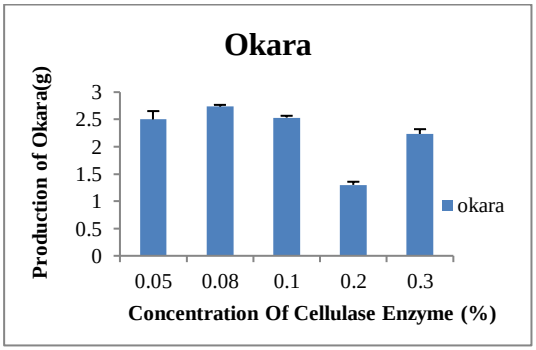


Fig 13: Okara Produced

4.1.3. Production of milk at 0.2 per cent concentration of cellulase enzyme with variation in incubation time: It was observed that varying the incubation time for the production of milk such as 2hrs, 3hrs, 4hrs, 5hrs and 24hrs the production of milk observed was 14.43ml, 18.12ml, 20.11ml, 19.43ml and 14.43ml, respectively as shown in the table number 9. From the table the maximum production of milk observed was at 4 hrs of incubation time in water bath at optimum temperature while after 5hrs of incubation time there was a slight change in texture and color of the milk. After 24hrs of incubation the curdling observed in milk. From the results the quantity and quality of milk was produced from cellulase activity on soya maximum for 4 hrs as shown in the fig. 14.

Table 9: Production of milk with variation in incubation time

Sr.no	Time (Hrs)	Production of milk (ml)
1	2	14.43±0.33
2	3	18.12±0.05
3	4	20.11±0.05
4	5	19.43±0.30
5	24	14.43±0.28

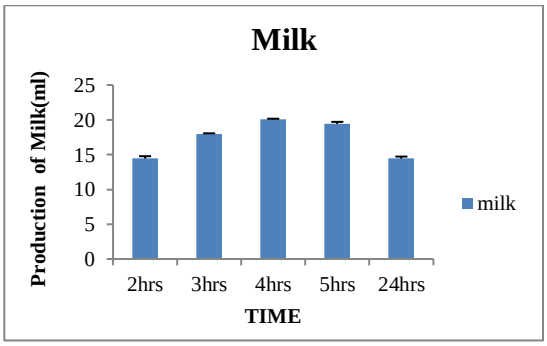


Fig. 14: Production of Milk

4.1.4. Production of Okara at 0.2 per cent concentration of cellulase enzyme with variation in incubation time: Results shown in the table 10 explained that variation in incubation time amount of okara produced was also varied like at 2hrs, 3hrs, 4hrs, 5hrs and 24hrs the okara was 3.16g, 2.53g, 1.70g, 1.93g and 2.30g, respectively. The maximum okara produced was after 2hrs and the minimum was after 4hrs. So, it may be inferred that the optimum time for the production of minimum okara with maximum milk percentage was at 0.2 per cent cellulase enzyme concentration after incubation time for 4hrs.

Table 10: Production of Okara with variation in incubation time

Sr.no	Time (Hrs)	Production of okara (g)
1	2	3.16±0.08
2	3	2.53±0.03
3	4	1.70±0.25
4	5	1.93±0.26
5	24	2.30±0.05

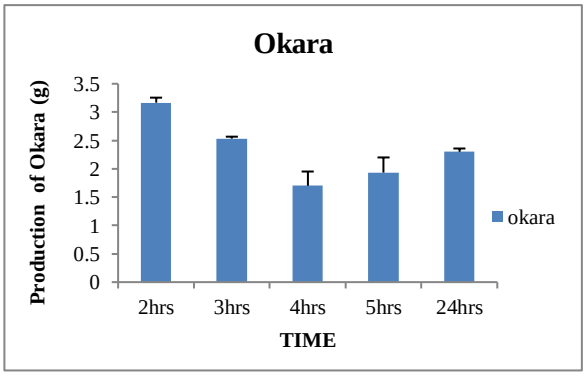


Fig 15: Production of Okara

4.1.5. Production of milk with 0.2 per cent cellulase enzyme concentration after 4hrs of incubation with variation in temperature: The trials were performed at different temperatures for 4 hrs and results postulated that at 37^{0}C, 40^{0}C, 45^{0}C, 50^{0}C, 55^{0}C and 60^{0}C the milk production was 14.76ml, 15.13ml, 20.1ml, 18.9ml, 17.86ml respectively.

The minimum milk obtained was at 37^0C that means the cellulase has not shown any activity at this temperature. The maximum activity by cellulase enzyme causing maximum production of milk was observed at temperature of 45^0C. At 60^0C the texture and the colour of the milk changed like the browning of milk has taken place due to high temperature. So, the optimum temperature for high percentage of quantity and quality in soymilk was observed at 45^0C as shown in the table number 11 and fig. no 16.

Table 11: Production of milk at different temperature with the Cellulase enzyme

Sr.no	Temperature (^{0}C)	Production of milk(ml)
1	37	14.76±0.14
2	40	15.13±0.06
3	45	20.1±0.05
4	50	18.96±0.03
5	55	17.86±0.08
6	60	16.96±0.03

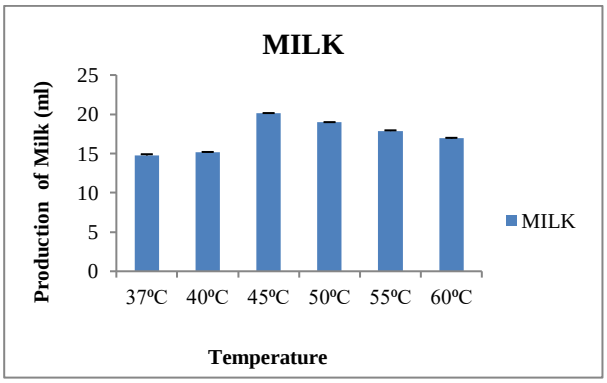


Fig 16: Production of Milk

4.1.6. Production of Okara at 0.2 per cent cellulase enzyme after 4hrs of incubation with varied temperature:

Table 12: Production of okara at different temperature with Cellulase enzyme

Sr no	Temperature (^{0}C)	Production of Okara (g)
1	37	3.63±0.08
2	40	2.70±0.05
3	45	1.90±0.05
4	50	2.72±0.15
5	55	2.63±0.03
6	60	2.3±0.05

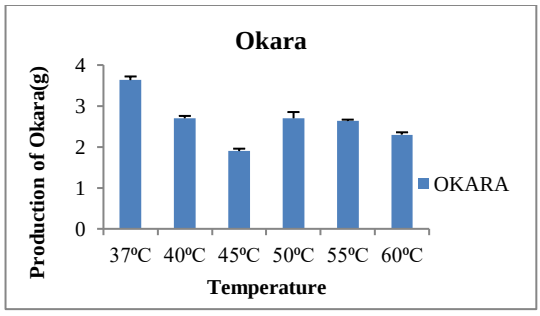


Fig 17: Production of Okara

The okara produced at different temperatures 37^0C, 40^0C, 45^0C, 50^0C, 55^0C and 60^0C were 3.63g, 2.70g, 1.90g, 2.72g and 2.63g respectively as depicted in the table

number 12. The minimum okara was produced at 45^0C and maximum at 37^0C as shown in the fig 17. So, this shows at cellulase works at optimum temperature of 45^0C.

4.2. STANDARDIZATION OF ENZYME ASSISTED SOYMILK EXTRACTION METHOD USING PECTINASE ENZYME:

Here, the pectinase enzyme was used to dissolve the pectin content in the soybean in the form insoluble carbohydrates**.** The different concentrations of pectinase enzyme used at different temperatures with different time combinations for the standardization of the method and showed below in the form of tables and graphs.

Fig 18: Pectinase enzyme assisted Milk

Fig 19: Pectinase enzyme assisted okara

4.2.1. Production of milk at different concentration of Pectinase enzyme: From the table number it was observed that at different concentration of 0.05 per cent, 0.08 per cent , 0.10 per cent, 0.20 per cent and 0.30 per cent per cent the produced milk was 18.4ml, 18.03ml, 17.06ml, 17.0ml and 17.10ml, respectively. So, the maximum production of milk was observed at 0.05 per cent and at 0.08 per cent of pectinase enzyme concentration The production of milk was also nearly equal to 0.05 per cent but the concentration of 0.05 per cent was preferred more as the small amount of enzyme was producing equal amount of milk as compare to other concentrations of the enzymes. The milk produced with the pectinase enzyme was also more as compare to the milk produced by mechanical method but the only lacking point of this milk was the texture compared to the milk obtained with the cellulase and by mechanical method**.**

Table 13: Milk produced at different concentration of pectinase enzyme

Sr.no.	Pectinase enzyme conc. (% of wet weight of soya)	Production of milk(ml)
1	0.05	18.4±0.20
2	0.08	16.03±0.08
3	0.10	17.06±0.12
4	0.20	17.01±0.11
5	0.30	17.10±0.05

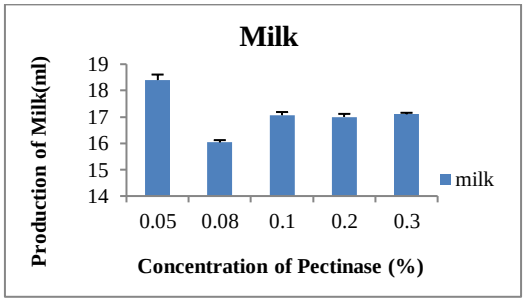


Fig 20: Production of Milk

4.2.2. Production of Okara at different concentration of Pectinase enzyme: As shown in the table number 14that at different concentrations i.e. 0.05 per cent, 0.08 per cent, 0.10 per cent, 0.20 per cent and 0.30 per cent of pectinase enzyme the produced okara was 2.40g, 2.76g, 3.01g, 3.03g and 2.96g. as shown in the fig 21 the minimum okara was produced at 0.05 per cent

Table 14: Production of Okara at different concentration of Pectinase enzyme.

Sr.no.	Pectinase enzyme conc. (% of wet weight of soya)	Okara produced (g)
1	0.05	2.40±0.03
2	0.08	2.76±0.03
3	0.10	3.01±0.05
4	0.20	3.03±0.08
5	0.30	2.96±0.12

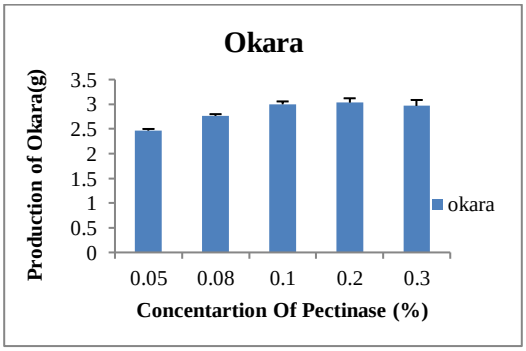


Fig 21: Production of Okara

4.2.3. Production of milk at 0.05 per cent concentration of pectinase enzyme with variation in incubation time: From the below table 15 the produced milk after 2hrs, 3hrs, 4hrs and 5 hrs was 15.46ml, 16.23ml, 18.03ml, 18.60ml and 15.06ml respectively. So, this from the fig 22 it was analyzed that the optimum time for the production of milk at 0.05 per cent concentration for 5hrs in water bath was found best. After 24hrs, the

curdling took place and in 2hrs the pectinase has not shown any activity in the soybean slurry

Table 15: Production of milk with variation in incubation time

Sr.no	Time (Hrs)	Production of Milk (ml)
1	2	15.46±0.12
2	3	16.23±0.08
3	4	18.03±0.08
4	5	18.60±0.05
5	24	15.06±0.08

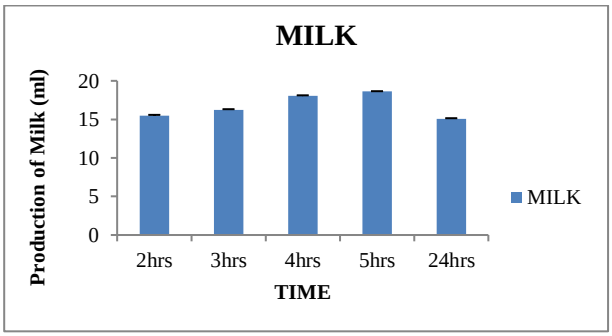


Fig 23: Production of Milk

4.2.4. Production of Okara at 0.05 per cent concentration of pectinase enzyme with variation in incubation time:

Table 16: Production of okara with variation in incubation time

Sr.no.	Time (Hrs)	Production of Okara (g)
1	2	3.16±0.08
2	3	2.76±0.06
3	4	2.73±0.03
4	5	2.46±0.03
5	24	2.83±0.03

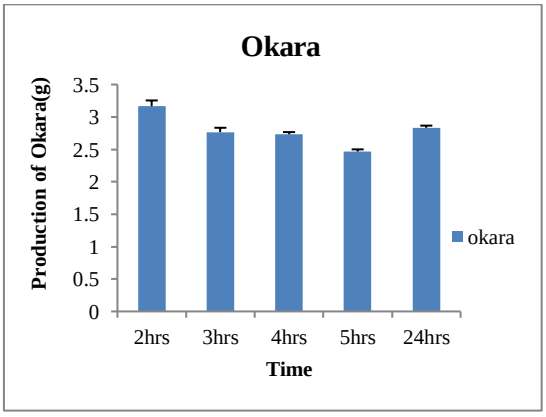


Fig 24: Production of Okara

As shown below in the table number 16 the amount of okara resulted was different at different incubation time like at 2hrs, 3hrs, 4hrs and 5hrs the okara was 3.16g, 2.76g, 2.73g, 2.46g and 2.83g produced, respectively. It may be postulated that after 2hrs of pectinase enzyme activity the maximum okara was produced against 5hrs when it was minimum. So, the optimum time for the production of milk was found to be 5hrs using pectinase enzyme for the production of soymilk as shown in the fig. no 23.

4.2.5. Production of milk at 0.05 per cent Pectinase enzyme concentration of after 5 hrs at different temperatures: From the table number 17 the milk produced at different temperatures 37^{0}C, 40^{0}C, 45^{0}C, 50^{0}C, 55^{0}C and 60^{0}C was 18.01ml, 16.90ml, 15.06ml, 15.42ml, 15.23ml and 16.03ml, respectively. From the fig. 25 it was observed that at temperature of 37^{0}C the maximum production of milk was there and minimum at the temperature of 45^{0}C. So, the optimum temperature for the production of milk with the help of pectinase enzyme was 37^{0}C in the water bath for 5 hrs.

Table 17: Production of milk at different Temperatures

Sr.no	Temperature (^{0}C)	Production of Milk (ml)
1	37	18.01±0.14
2	40	16.90±0.05
3	45	15.06± 0.06
4	50	15.42±0.05
5	55	15.23± 0.08
6	60	16.03±0.08

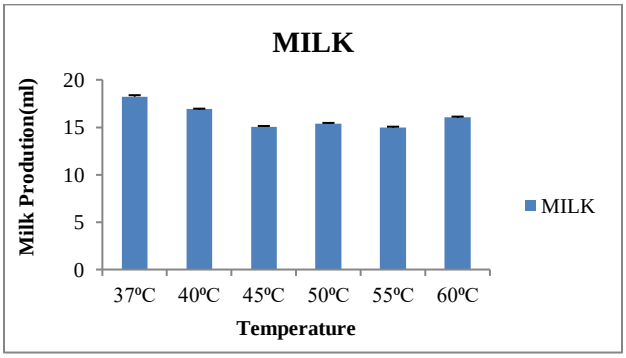


Fig 25: Production of Milk

4.2.6. Production of okara at 0.05 per cent Pectinase enzyme concentration of after 5 hrs at different temperatures: From the table 18 it was observed that the minimum production of okara was at the temperature of 37^{0}C which was 2.92g and the maximum was at the temperature of 40^{0}C of 3.03g. Hence, the optimum temperature for the activity of pectinase and for the minimum production of okara was 37^{0}C.

Table 18: Production of okara at different temperatures

Sr.no	Temperature (^{0}C)	Production of Okara(g)
1	37^{0}C	2.92±0.05
2	40^{0}C	3.03±0.08
3	45^{0}C	2.43±0.14
4	50^{0}C	2.46±0.03
5	55^{0}C	2.93±0.03

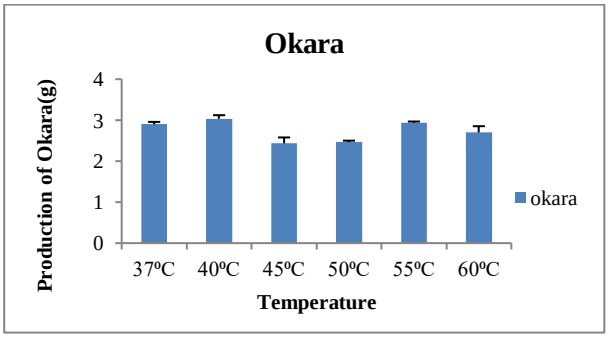


Fig 26: Production of Okara

4.3 STANDARDIZATION OF MECHANICAL SOYMILK EXTRACTION METHOD.

4.3.1 Different boiling conditions before the grinding of soybeans for soymilk: Before grinding of soybean the cooking of soyabeans in pressure cooker for about 10mins was found the most suitable method as compare to the boiling. As cooking of soyabeans in pressure cooker was time efficient and also the production of milk was more and in autoclave due to high temperature and more timing it changed the color, flavor and produced the brown color of milk.

Fig 27: Milk samples after Different boiling conditions

Initially the soybeans were soaked at different temperatures at different soaking time before boiling for the production of milk as shown in the table (19). So, the best result was 20ml of milk produced at 55^0C after 4hrs of soaking time and at 60^0C the browning of soymilk was observed due to which the color changed.

4.4 QUALITY TESTS:

4.4.1. Protein content in each 10ml of milk sample:

The protein content of different samples of milk was detected by the kjeldahl method. After analysis it was observed that the maximum content of protein (0.56g per 10ml of milk) was observed in the milk produced by the enzyme cellulase. As acknowledged earlier that the after the production of milk through mechanical method the left over residue amount contains the 20 per cent bean solid i.e. and 30 per cent of bean proteins which contains more of cellulose and less of pectin. So, the enzyme helps in the hydrolysis of the structural polysaccharides and also hydrolyzes the proteins which form

The cell and lipid body of soybean which was also pondered by the Rosenthal *et al.*, 2000. The main degrading enzymes were cellulase and pectinase but the cellulose per cent found to be more amount in the soybean that is why the cellulase was producing slightly more protein content as compared to the enzyme pectinase.

Table 19: Soybeans dipped at different temperature and time before the boiling for the production of milk

Sr.no	Temperature (^{0}C)	Time (Hrs)	Production of milk (ml per 4g of wet soya)
1.	45	6	16
2.	50	5	17
3.	55	4	20
4.	60	3	20

Table 20: Protein content in milk produced by different methods

Sr.no	Method	Protein content (g per 10ml of milk)
1.	Mechanically	0.26
2.	Enzyme assisted (Pectinase)	0.45
3.	Enzyme assisted (Cellulase)	0.56

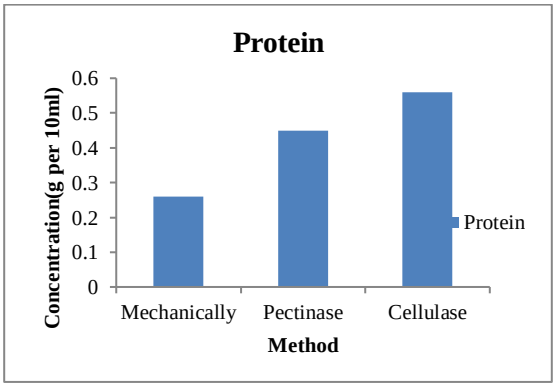


Fig 27: protein content

4.4.2. Fat content in each 10ml of milk sample:

The fat content was observed by the Gerber method. It was detected that the fat content produced by the mechanical method was 0.14 g compared to the enzyme assisted soymilk extraction through pectinase was 0.13g and cellulase was 0.12g. Again as shown in table 21 and proved in fig number 28 that in terms of fat content there was not a huge

difference between the milks produced by the different methods but the maximum fat content was shown by the mechanical method and the least was by the cellulase enzymes assisted method. So, this shows that in case of fat content the cellulase has done the reaction but in less amount.

Table 21: Fat content in milk produced by different methods

Sr.no	Method	Fat content (g per10ml of milk)
1.	Mechanical	0.142
2.	Enzyme assisted (Pectinase)	0.13
3.	Enzyme assisted (Cellulase)	0.12

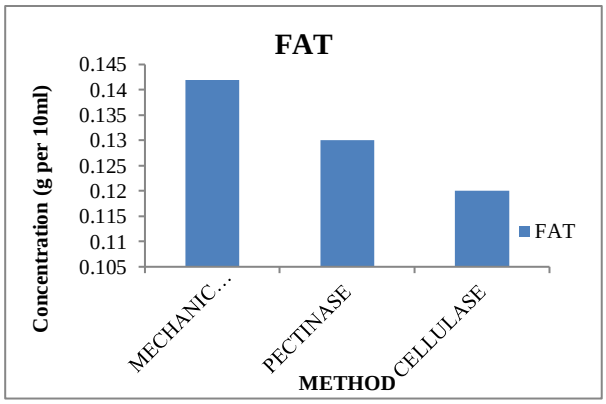


Fig 29: Fat content

4.4.3. Carbohydrates content in each 10ml of milk sample:

The carbohydrate content was observed in all the milk samples produced by different methods and it was observed that the amount of carbohydrate content in the milk produced by the mechanical method was less as compare to others. The maximum carbohydrates of 0.062g per 10ml of milk was in the milk produced by the pectinase enzyme assisted soymilk extraction method, by the mechanical method it was 0.14g per 10ml of milk which was least among all and by cellulase enzyme assisted soymilk extraction method was 0.59g per 10ml of milk. As both enzymes which are usually present in the form of insoluble carbohydrates in the left over residue after milk extraction as in mechanical method was composed of pectin cellulose. So, both the enzymes break down this insoluble carbohydrate and increase its content.

Table 22 Carbohydrates content in milk produced by different methods

No	Method	Carbohydrates content (g per 10ml of milk)
1.	Mechanical	0.049
2.	Enzyme assisted (Pectinase)	0.062
3.	Enzyme assisted (Cellulase)	0.059

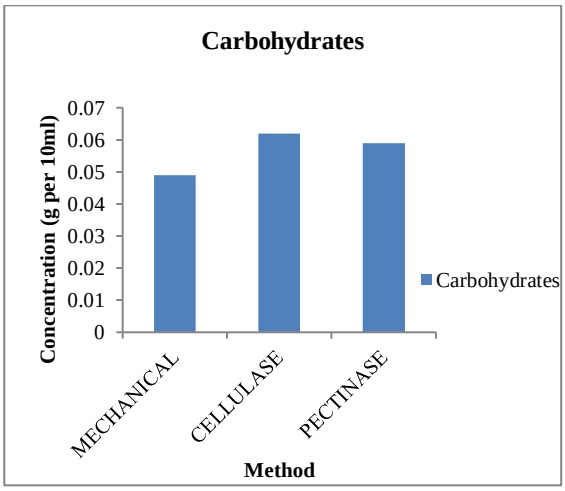


Fig. 30 Carbohydrate content

4.4.4. Acidity in each 10ml of milk sample:

It was observed that percentage acidity in milk produced through mechanical soymilk extraction method (0.36 per cent) has more acidity as compared to the milk produced by the enzymes cellulase (0.35 per cent) and pectinase (0.30 per cent).The least acidity observed was in the milk produced by the enzyme pectinase and cellulase may showed some acidity due to the increase in non reducing sugars as shown in the table 23 and proved in fig. number 30.

Table 23: Acidity content in milk produced by different methods

Sr.no	Method	Acidity (% per 10ml of milk)
1.	Mechanical	0.36
2.	Enzyme assisted (Pectinase)	0.30
3.	Enzyme assisted (Cellulase)	0.35

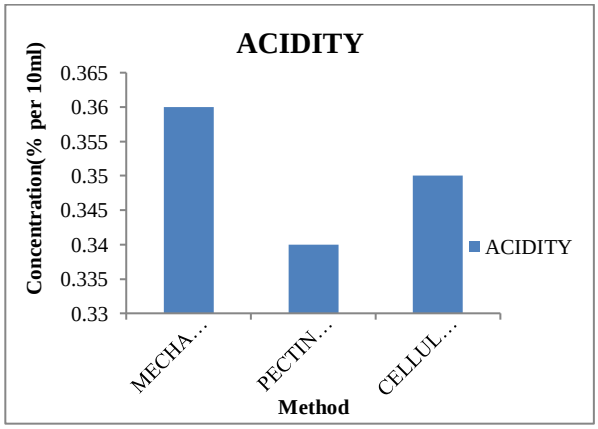


Fig 31: Acidity in Soymilk

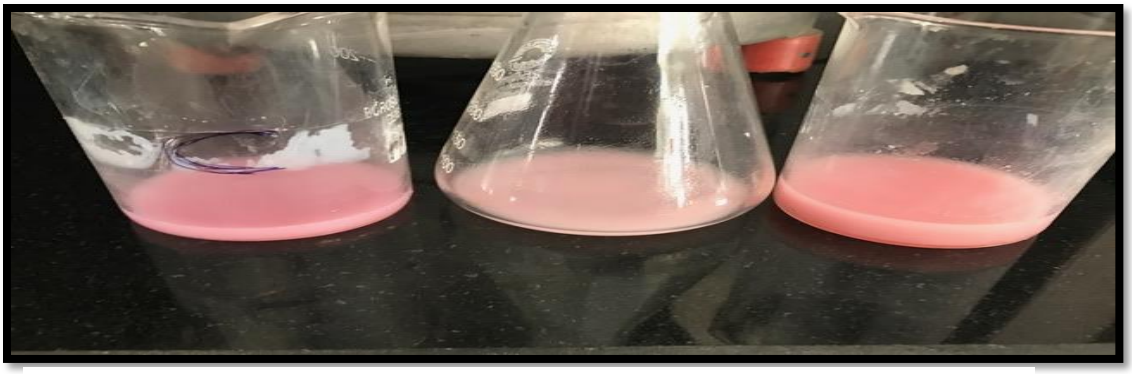


Fig 32: Acidity Test of different samples

4.4.5. Flavonoid content in each ml of milk sample:

The standard curve of the Flavonoid was also expressed as 'g' per ml of soymilk and observed that enzyme assisted soymilk showed the highest total flavonoid content as compared to others. The concentration was calculated from the slope (y=0.054x-0.026) with the R2 value of 0.971. the concentration was observed maximum in the milk produced by the cellulase which was 0.64g per ml of milk and less in case of mechanical method which was 0.59g per ml of milk as shown in the table 24 and fig. number 33.

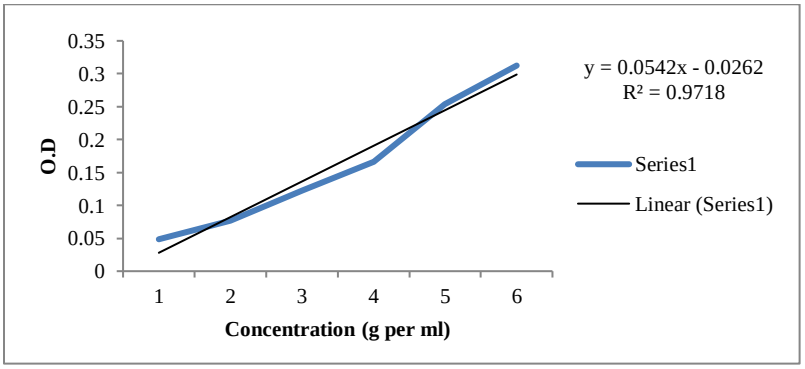


Fig 33: Standard curve for the Flavonoid Content

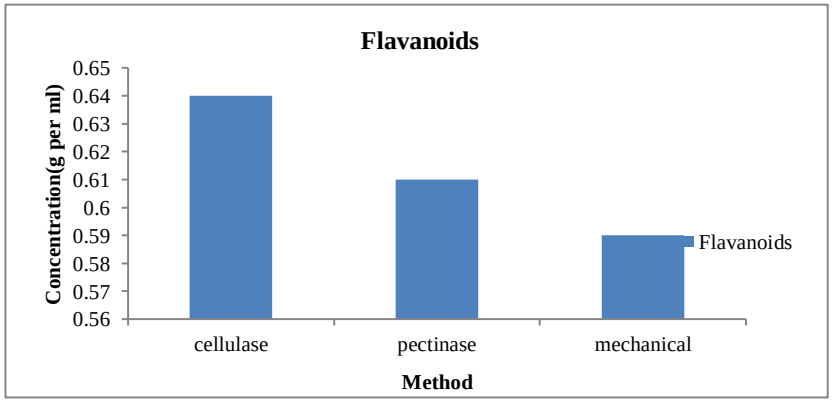


Fig 34: Flavonoid Content

4.4.6. SNF content in each 10ml of milk sample:

It was observed that SNF was observed minimum (0.021g) in the milk produced by the enzyme cellulase as it may have converted the left over bean solid after milk extraction by the mechanical method while the maximum (0.052g) was found in the milk produced by the mechanically method as also proved in table number 25 and figure35. number 35.

Table 24: Flavonoid content in each 10ml of milk sample

Sr.no	Method	SNF (g per 10ml of milk)
1.	Mechanical	0.052
2.	Enzyme assisted (Pectinase)	0.031
3.	Enzyme assisted (Cellulase)	0.021

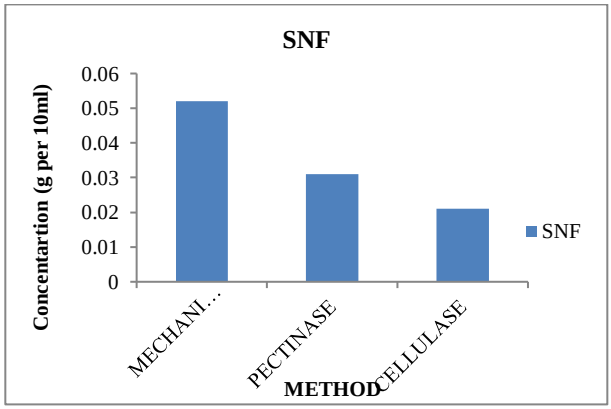


Fig 35: SNF Content

Table 25: SNF content in each 10ml of milk sample

Sr.no	Method	Absorbance (510nm)	Flavonoid content (g/ml of milk)
1	Mechanical	0.006±0.02	0.59
2	Enzyme assisted (Pectinase)	0.007±0.01	0.61
3	Enzyme assisted (Cellulase)	0.009±0.04	0.64

4.4.7 Non-reducing sugars content in each 10ml of milk sample:

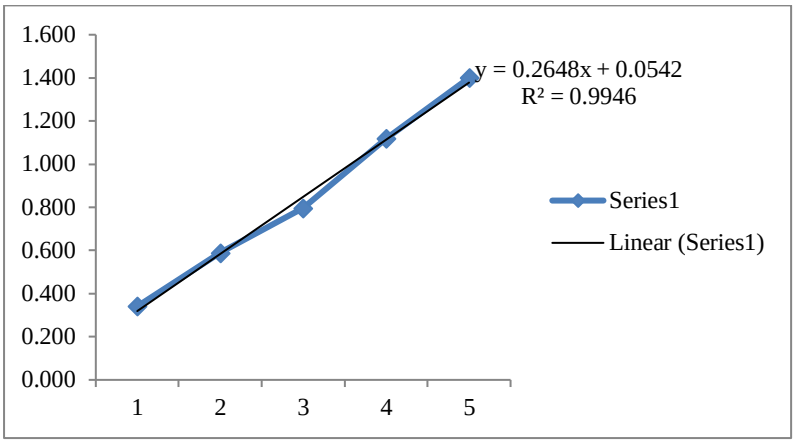


Fig 36: Standard curve for the Non-reducing sugar content

The non-reducing sugars content was assessed in all the samples of soymilk with the DNS method. Again, the standard of the non-reducing sugars was expressed as 'g' for non-reducing sugars per ml of milk and then the concentration calculated from the slope (y=0.264x+0.054) with the R2 value of 0.994 which has shown the results of 0.02g/ml of cellulase enzyme assisted soymilk extraction method, 0.007g/ml by the pectinase enzyme assisted soymilk extraction method and the 0.001 g/ml by the mechanical method. So, the maximum of non-reducing sugars observed were in the milk obtained by the cellulase enzyme as compare to other methods. As cellulase enzyme converts the cellulose of soy in to the glucose molecules by breaking the 1-4 linkages which then convert in to the non-reducing sugars.

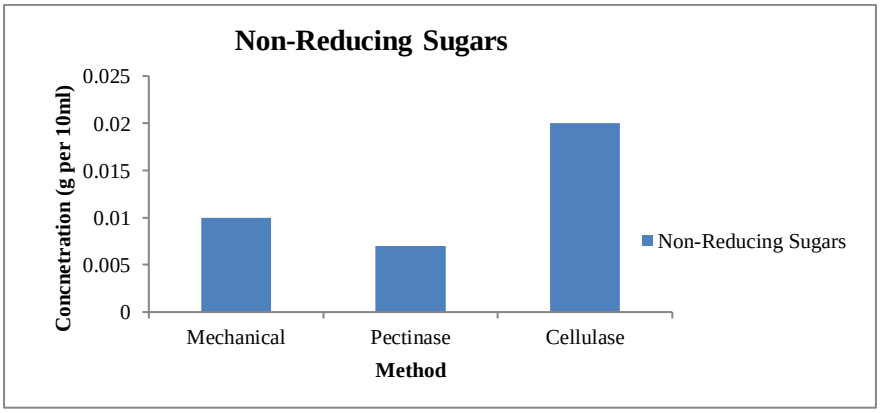


Fig 37: Non Reducing Sugar Content

4.5. SENSORY EVALUATION OF THE SELECTED FORMULATION MADE FROM THE SOYMILK OBTAINED FROM DIFFERENT METHODS:

The 9-point Hedonic scale was used to assess the different parameters. The parameters selected for the further sensory analysis (appearance, color, flavor or aroma, texture, taste, overall acceptability and percentage acceptability.

Table 26: Non Reducing Sugar content in different samples of milk

Sr.no	Method	Absorbance(510nm)	Non Reducing Sugars (g/ml)
1	Mechanical	0.057±0.01	0.01
2	Enzyme assisted (Pectinase)	0.056±0.03	0.007
3	Enzyme assisted (Cellulase)	0.061±0.01	0.02

Table 27: Sensory evaluation of different samples of milk

Code	Appearance	Color	Flavor	Texture	Taste	Overall Acceptability	Per centage Acceptability
SM	6.30±0.12	5.42±0.15	5.20±0.15	5.31±0.14	6.15±0.15	5.80±0.14	60.7%
SP	4.80±0.17	4.30±0.16	4.35±0.16	5.70±0.14	6.65±0.13	4.87±0.12	57.6%
SC	7.22±0.13	6.85±0.15	7.55±0.16	8.10±0.22	8.05±0.21	7.47±0.12	75.6%

Foot notes: SM : mechanically formulated soymilk SP: Pectinase formulated SC: Cellulase formulated

4.5.1. Appearance: The data regarding the appearance score of the different sample have been tabulated in table (27) the mean value with the standard deviation of the appearance of the milk were ranged from 6.30 to 8.10. So, according to the hedonic scale the 6.30 was designated as slightly liked and 8.10 as very much liked by the group of people. The highest mean value was shown by the SC (7.22±0.13) sample and the lowest

was shown by the sample SP (4.80±0.17). The SC has highest appearance score due to its good and thick texture like milk and the SP shows very less score as pectinase made the milk more clear like juice not like milk

4.5.2. Color: All the samples have their own color. The data regarding the color was given in the table (27).The highest mean value was shown by the SC (6.85±0.15) sample and the lowest was shown by the sample SP (5.30±0.16). In this case the perception of the different individuals was near about same so, the standard deviation was not also not high. It was difficult to rate between the SM and SC because color of both the milks was quite similar but in case of SP the color of the milk was clear like a juice which was least accepted. The color ranged from 5.30 to 6.85. All the samples were stored under refrigerator for 3 days. After 3 days the color of milk were changed due to the curdling.

4.5.3. Flavor or Aroma: The highest mean score 7.55±0.16 was recorded for SC sample of soy milk and the least value of 5.20±0.15 was obtained for the SM. The beany flavor was maximum in SM and least in SC and also in SP.

4.5.4. Texture: The viscosity or the texture of SC was observed best among all the milks. The highest and lowest value of the texture was recorded as 5.70 and 8.10 respectively. The SC has the thick texture like milk but SP has a clear juice like texture.

4.5.5. Taste: According to the hedonic scale the SC has the maximum mean value of 8.05 and SM has least value of 6.15 but in this case the SP has the mean value of 6.65 which is slightly liked by the people. The least mean value of SM was due to bitter taste with beany flavour which is less in SC. After the storage of 3 days the taste of all samples became bitter.

4.5.6. Overall Acceptability: In table (21) the mean score for the overall acceptability of all the milks have been tabulated. Highest score for overall acceptability was observed for the SC (7.47) followed by the SM (5.80) and the least mean score was shown by SP (4.87). The least value of SP was due to its clear appearance and texture which was more like juice not milk.

4.5.7. Percentage of overall Acceptability: The percentage of overall acceptability was calculated on the basis of sensory analysis shown in table 21. SC has shown the maximum percentage of the overall acceptability as 75.6% because the colour, appearance, taste, texture of SC was perfect as compare to mechanical based . According to the percentage acceptability the order of liking of the milk was SC > SM > SP. After the analysis so it was found that SC was the best milk.

4.6. STATISTICAL ANALYSIS OF THE SOYMILK OBTAINED FROM DIFFERENT METHODS:

The different soymilk samples were analyzed in triplicates and also statically analyzed by the cpsi1 software in which we analyzed the different quality factors for all the milk samples and the results are shown in table number 28.

Table 28: Statistical Analysis of different samples of milk

Sr.No	Type of Formulation ⇨	Mechanical	Pectinase	Cellulase
Protein	**Mean**	0.27	0.45	0.55
	LSD (5%)	NS	0.568	0.972
Fat	**Mean**	0.14	0.13	0.12
	LSD (5%)	0.110	NS	0.221
Carbohydrates	**Mean**	0.04	0.06	0.05
	LSD (5%)	0.102	0.121	0.115
Acidity	**Mean**	0.35	0.36	0.35
	LSD (5%)	0.12	NS	0.152
SNF	**Mean**	0.052	0.031	0.022
	LSD (5%)	NS	0.110	0.221
Flavonoids	**Mean**	0.58	0.60	0.63
	LSD (5%)	NS	0.156	0.221
Non-Reducing Sugars	**Mean**	0.057	0.056	0.062
	LSD (5%)	NS	0.11	0.332

4.6.1. Protein: From the table 28 it was analyzed that the mean value of triplicates of protein by mechanically soymilk extraction method was 0.27, pectinase assisted soymilk extraction method (0.45) and for cellulase assisted soymilk extraction method (0.55). The LSD value at 0.05 showed that the mechanical soymilk extraction method has shown non-significant results in protein analysis and cellulase assisted soymilk extraction method has shown the best and significant results. So, this may be concluded that the mechanical soymilk extraction method may not be a significant sample for the good components of protein and the protein has shown positive correlation with the flavonoids with significant value of 0.23 in cellulase soymilk extraction method and in mechanical method the protein has shown non significant correlation with the other quality factors of it.

4.6.2. Fat: From the table 29 the mean values of triplicates of protein by mechanically soymilk extraction method was 0.14, pectinase assisted soymilk extraction method (0.13) and for cellulase assisted soymilk extraction method (0.12). The LSD value at 0.05 showed that the Pectinase soymilk extraction method has shown non-significant results in fat analysis and also cellulase soymilk extraction method has shown significant negative correlation of fat with the proteins and positive with carbohydrates. In mechanical it has shown the positive significant correlation with the carbohydrates. So, this may be concluded that Pectinase assisted soymilk extraction method may not significant sample for the good components and analysis of fat.

4.6.3. Carbohydrates: The mean value of the triplicates of each formulation was mechanically soymilk extraction method was 0.04, pectinase assisted soymilk extraction method (0.06) and for cellulase assisted soymilk extraction method (0.05). The LSD value at 0.05 showed that for carbohydrates the Pectinase assisted soymilk extraction method has shown good results but cellulase enzyme extraction method and mechanical method has shown significant positive correlation of fat and carbohydrates as shown in table number 27.

4.6.4. Acidity: From the table 28 it was analyzed that the mean values of the triplicates of acidity were found to be mechanically soymilk extraction method was 0.35, pectinase assisted soymilk extraction method (0.36) and for cellulase assisted soymilk extraction

method (0.35). The LSD value at 0.05 showed that the pectinase assisted soymilk extraction method has shown the non significant results. So, this may be concluded that Pectinase assisted soymilk extraction method may not be the significant sample for the acidity and cellulase extraction method and mechanical method both has shown non-significant correlation with the other quality factors .

4.6.5. SNF: The mean value for the SNF of triplicates by mechanically soymilk extraction method was 0.052, Pectinase assisted soymilk extraction method (0.031) and for cellulase assisted soymilk extraction method (0.022). The LSD value at 0.05 value showed that the mechanical soymilk extraction method has shown non-significant results in SNF analysis and cellulase assisted soymilk extraction method has shown the best results and also positive significant correlation with the non reducing sugars. In mechanical method it has shown the non significant correlation with the other quality factors. So, this may be concluded that the mechanical soymilk extraction method may not be a significant sample for the good components of SNF as also shown in table 27.

4.6.6. Flavonoids: From table number 27 it was analyzed that the mean values of the flavonoid of triplicates of each formulation were by mechanically soymilk extraction method was 0.58, Pectinase assisted soymilk extraction method (0.60) and for cellulase assisted soymilk extraction method (0.63). The LSD value at 0.05 value showed that the mechanical soymilk extraction method has shown non-significant results in flavonoids analysis and cellulase assisted soymilk extraction method has shown the best results and also positive correlation of flavonoids with the protein in cellulase enzyme soymilk extraction method.

4.6.7. Non-Reducing sugars: The mean value for the non reducing sugars of triplicates by mechanically soymilk extraction method was 0.057, Pectinase assisted soymilk extraction method (0.056) and for cellulase assisted soymilk extraction method (0.062). The LSD value at 0.05 value showed that the mechanical soymilk extraction method has shown non-significant results in SNF analysis and cellulase assisted soymilk extraction method has shown the best results with the significant positive correlation of non reducing sugars with the SNF in cellulase enzyme soymilk extraction. So, this may be

concluded that the mechanical soymilk extraction method may not be a significant sample for the good components of non-reducing sugars as also shown in table 27.

4.6.8. Sensory analysis: For sensory analysis the one way anova in SPSS software was used and it was found that the enzyme assisted extracted soymilk showed higher sensory mean score of overall acceptance of each of formulation was from mechanical method (5.80), pectinase (4.87) and cellulase (7.47) which showed the significant difference within and between the groups with the F value of 45.793 along with homogeneity of variance with a significant value of 0.557. So. it may concluded that the enzyme assisted extracted soymilk showed higher sensory score to that of mechanical soymilk extraction method.

4.7. PEARSON'S CORRELATION BETWEEN THE DEMOGRAPHIC AND PRIORITY QUESTIONS:

Table 29 Correlation between the demographic and priority questions

Priority ➡ / Demographic ⬇	Health consciousness	Price	Advertisement	Nutrition	Taste	Fat Content	Easily Available
Gender	-.286**	0.553**	0 .073	-0.128	-135	-0.146	-0.011
Age	-.474**	0.026	-0.567**	0.522**	-325**	0.445**	0.087
Income	-0.133	-0.569**	-0.433**	0.423**	-312**	0.367**	-0.039

.**. Correlation is significant at the 0.01 level (2-tailed).

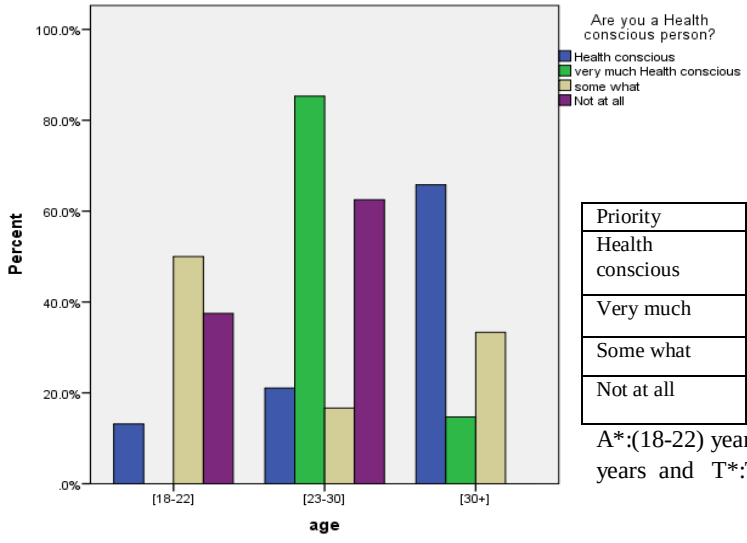
This correlation was done to examine the relationship of gender, age and income with the other factors like health consciousness, price, advertisement, nutrition, taste, fatcontent and easy availability. So, as to see that which gender, age group and range of income would prefer more soy milk in future and also to check the hypothesis.

This table 29 shows that there is a significant negative correlation of the gender with the health consciousness and positive with the price. In case of age there is significant negative correlation of age with the health consciousness, advertisement, and of taste that means people belong to the higher age like 30+ age are more health conscious as compared to the middle and lower age as the value is -0.4 and people belonging to the higher age group are also less interested in advertisements as the value is -0.5.Income shows a negative significant relation with the price, advertisements and taste that means people who earn more than 1lakh take price, advertisement and taste as their least priority, as compared to others and for them nutrition and fat content are at higher priority as compare to others because there is a positive significant relation of income with the nutrition and fat content. All of the significant correlations suggest acceptance of alternative hypothesis and others suggest acceptance of null hypothesis.

4.8 CROSS TABULATION AND THE BAR CHART BETWEEN THE PRIORITY AND DEMOGRAPHIC FUNCTIONS:

This was done to see the number of respondents in each of the factor of every priority question on the basis of age, gender and income.

Cross tab 1. Age and the Health consciousness:



Priority	A*	B*	C*	T*
Health conscious	4	19	19	38
Very much	7	30	15	52
Some what	5	2	0	7
Not at all	1	2	0	3

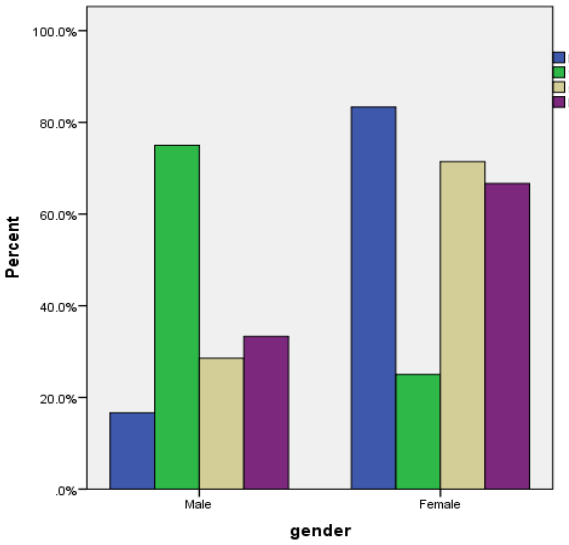
A*:(18-22) years, B*:(23-30) years, C*:(30+) years and T*:Total

From this Cross tab we analyzed that more people are very much health conscious and the age group of 18-22 age are less health conscious but of 23-30 age group especially gym freaks are very much health conscious which is also a negative

impact. The age groups belong to more than 30 and above are health conscious and of aging they care for their health. So, the targeted population for the consumption of soymilk should be 23-30 and more than 30 age.

Cross tab 2. Gender and the Health Consciousness:

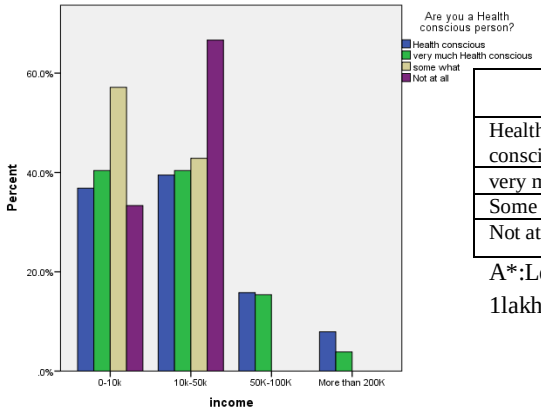
From this it was analyzed that more females are health conscious and some are trying to be conscious. In case of males, they are very much health conscious. Thus, in this case both genders should be targeted for the consumption of soy milk.



Priority	M*	F*	T*
Health conscious	6	32	38
Very much	37	15	52
Some what	2	5	7
Not at all	1	2	3

M*: Male, F*: Female and T*:Total

Cross tab 3. Income and the Health Consciousness:



	A*	B*	C*	D*	T*
Health conscious	14	15	6	3	38
very much	21	21	8	2	52
Some what	4	3	0	0	7
Not at all	1	2	0	0	3

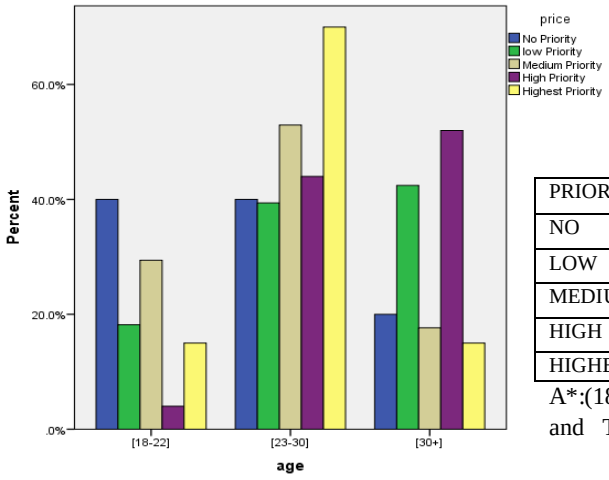
A*:Less than 10thousand, B*:10-50thousand, C*:Less than 1lakh, D*:more than 2lakh and T*:Total

The people who are earning income in the range of less than 10 thousand and in between 10-50 thousand are very much health conscious and some of them are just

conscious about their health but not that much. And people belong to income range of more than 2 lakhs are health conscious, but not very much. So, from this it was analyzed that people who belong to the income range of less than 10 thousand and in between 10-50 thousand should be targeted for the consumption of soymilk.

Cross tab 4. Age and the Price:

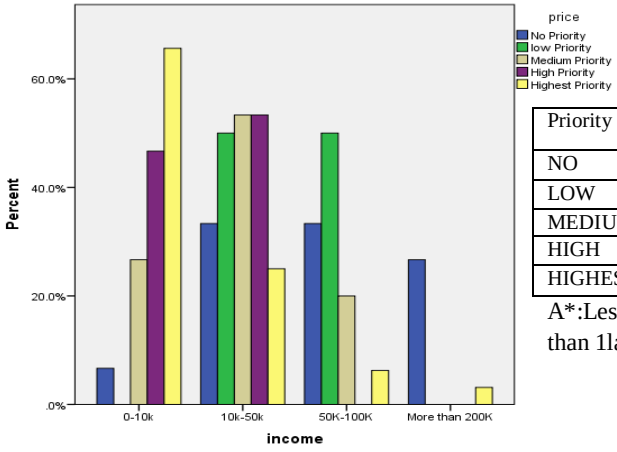
From this cross tab it can be analyzed that people who belong to more than 30 years age group and half of the 23-30 age group take the price at least priority. So, this age group would prefer the soy milk in terms of price and even if the price would increase.



PRIORITY	A*	B*	C*	T*
NO	2	2	1	5
LOW	6	13	14	33
MEDIUM	5	9	3	17
HIGH	1	11	13	25
HIGHEST	3	14	3	20

A*:(18-22) years, B*:(23-30) years, C*:(30+) years and T*:Total

Cross tab 5. Income and the Price:

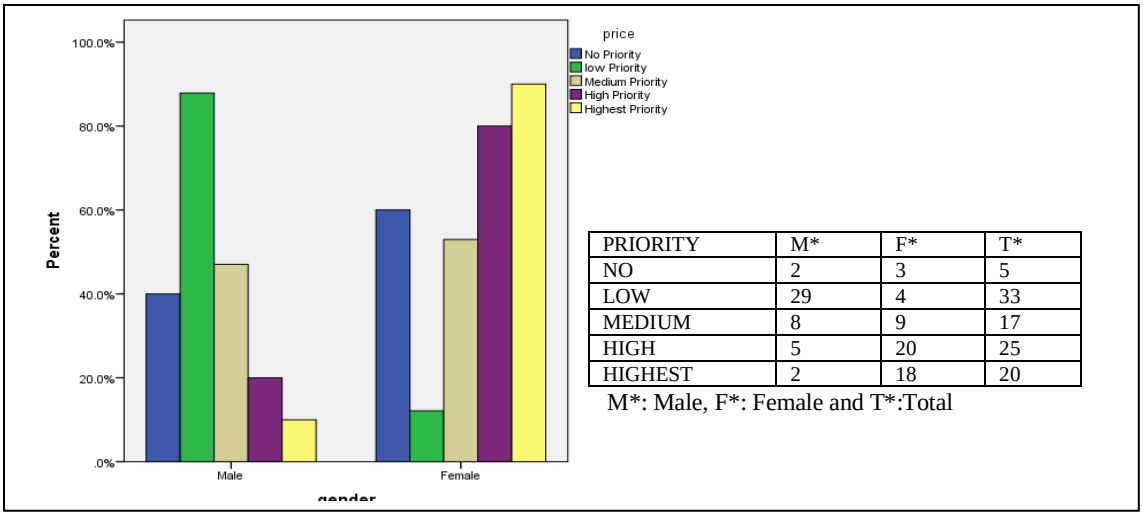


Priority	A*	B*	C*	D*	T*
NO	1	5	5	4	15
LOW	0	4	4	0	8
MEDIUM	4	8	3	0	15
HIGH	14	16	0	0	30
HIGHEST	21	8	2	1	32

A*:Less than 10thousand, B*:10-50thousand, C*:Less than 1lakh, D*:more than 2lakh and T*:Total

In this cross tab we analyzed that the price is at high priority for most of the people specially people which belong to the income range of less than 10 thousand and in between 10-50 thousand. So, if the price of soy milk increases, we should target more people in the income range less than 1 lakh, more than 2 lakhs and 10-50 thousand.

Cross tab 6. Gender and the Price:

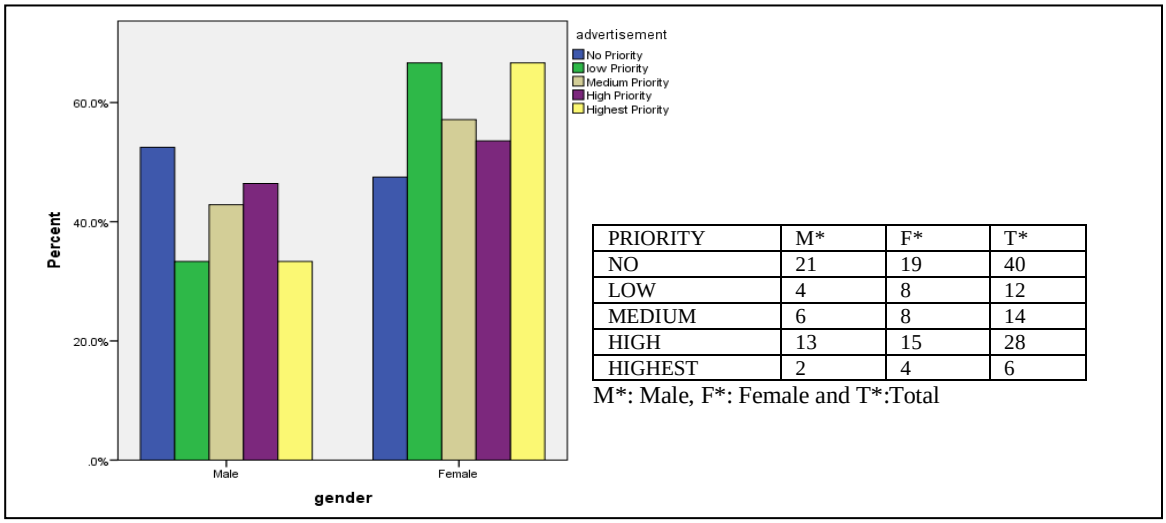


PRIORITY	M*	F*	T*
NO	2	3	5
LOW	29	4	33
MEDIUM	8	9	17
HIGH	5	20	25
HIGHEST	2	18	20

M*: Male, F*: Female and T*:Total

From this cross tab we analyzed that if the price increases we should target the males more as compared to the females because for males the price is at least priority and for females it is at high priority.

Cross tab 7. Gender and the Advertisement:

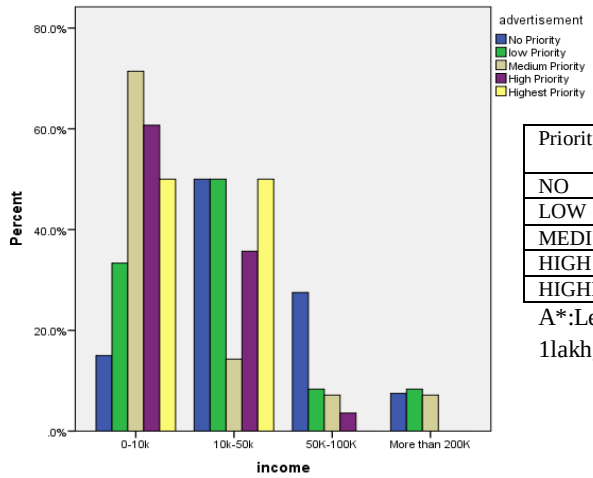


PRIORITY	M*	F*	T*
NO	21	19	40
LOW	4	8	12
MEDIUM	6	8	14
HIGH	13	15	28
HIGHEST	2	4	6

M*: Male, F*: Female and T*:Total

From this it could be analyzed that both male and female gives equal and high priority to the advertisement. So, we should target both the genders for the advertisements and advertisements should be used accordingly so that even an illiterate would get to know about the advantages of soymilk and buy it.

Cross tab 8 Income and the Advertisement:

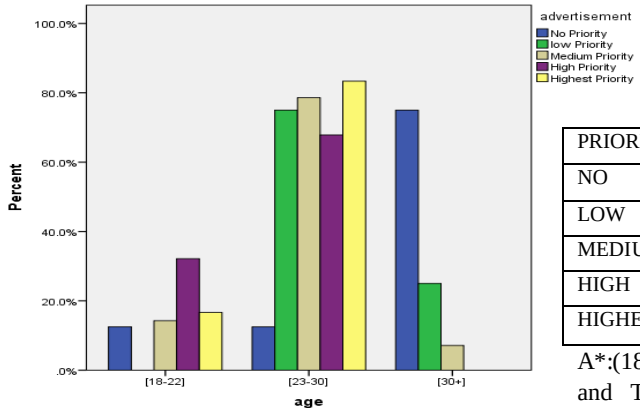


Priority	A*	B*	C*	D*	T*
NO	6	20	11	3	40
LOW	4	6	1	1	12
MEDIUM	10	2	1	1	14
HIGH	17	10	1	0	28
HIGHEST	3	3	0	0	6

A*:Less than 10thousand, B*:10-50thousand, C*:Less than 1lakh, D*:more than 2lakh and T*:Total

From this crosstab it is understood that people who belong to the income range of less than 10 thousand and in 10-50 thousand category consider the advertisements at a high priority. People who earn high income and are in the range of less than 1 lakhs and more than 2 lakhs take the advertisements at their least priority. Thus, advertisements should be made accordingly so that it targets mainly less than 10 thousand and 10-50 thousand mainly. Lower income group categories are more lured by advertisements.

Cross tab 9 Age and the Advertisements

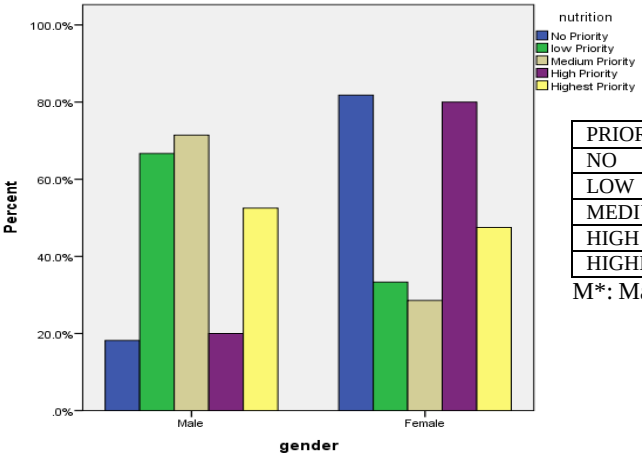


PRIORITY	A*	B*	C*	T*
NO	5	5	30	40
LOW	0	9	3	12
MEDIUM	2	11	1	14
HIGH	9	19	0	28
HIGHEST	1	5	0	6

A*:(18-22) years, B*: (23-30) years, C*:(30+) years and T*:Total

From this cross tab it was analyzed that the age group of 18-22 and 23-30 rate advertisement as high priority. So, targeted population should be this only and advertisements should be done accordingly to increase the sale of soymilk.

Cross tab 10 Gender and the Nutrition:

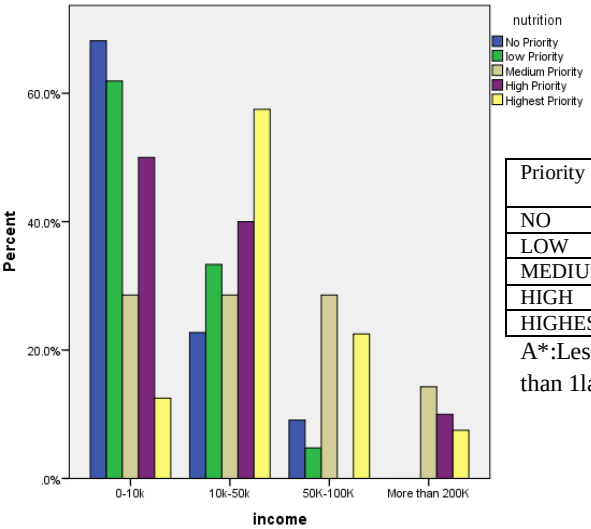


PRIORITY	M*	F*	T*
NO	4	18	22
LOW	14	7	21
MEDIUM	5	2	7
HIGH	2	8	10
HIGHEST	21	19	40

M*: Male, F*: Female and T*:Total

From this cross tab it could be inferred that for both male and female nutrition is at highest priority. So, if they will get to know that nutritional quality of soymilk is high as compared to other milks they will definitely buy and consume it more.

Cross tab 11. Income and the Nutrition:

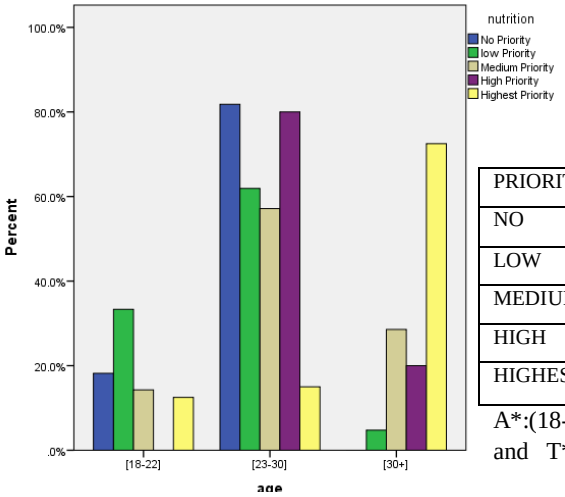


Priority	A*	B*	C*	D*	T*
NO	15	5	2	0	22
LOW	13	7	1	0	21
MEDIUM	2	2	2	1	7
HIGH	5	4	0	1	10
HIGHEST	5	23	9	3	40

A*:Less than 10thousand, B*:10-50thousand, C*:Less than 1lakh, D*:more than 2lakh and T*:Total

From this cross tab it is inferred that people who belong to the income range of 10-50 thousand take nutrition at a high priority. So, they should be targeted more as compared to others.

Cross tab 12. Age and the Nutrition:

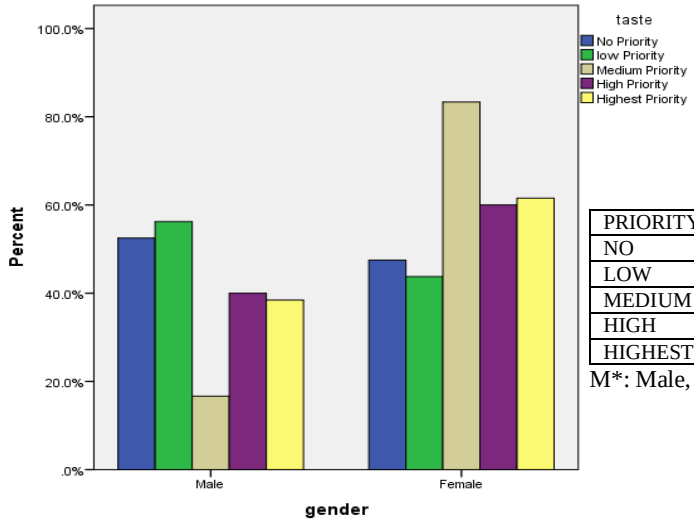


PRIORITY	A*	B*	C*	T*
NO	4	18	0	22
LOW	7	13	1	21
MEDIUM	1	4	2	7
HIGH	0	8	2	10
HIGHEST	5	6	29	40

A*:(18-22) years, B*:(23-30) years, C*:(30+) years and T*:Total

We analyzed that the nutrition is at highest priority for the people who belong to the age group of more than 30age. So, they would prefer soymilk more if the nutritional quality of the soya milk as compared to other milk varieties increases.

Cross tab 13. Gender and the Taste:

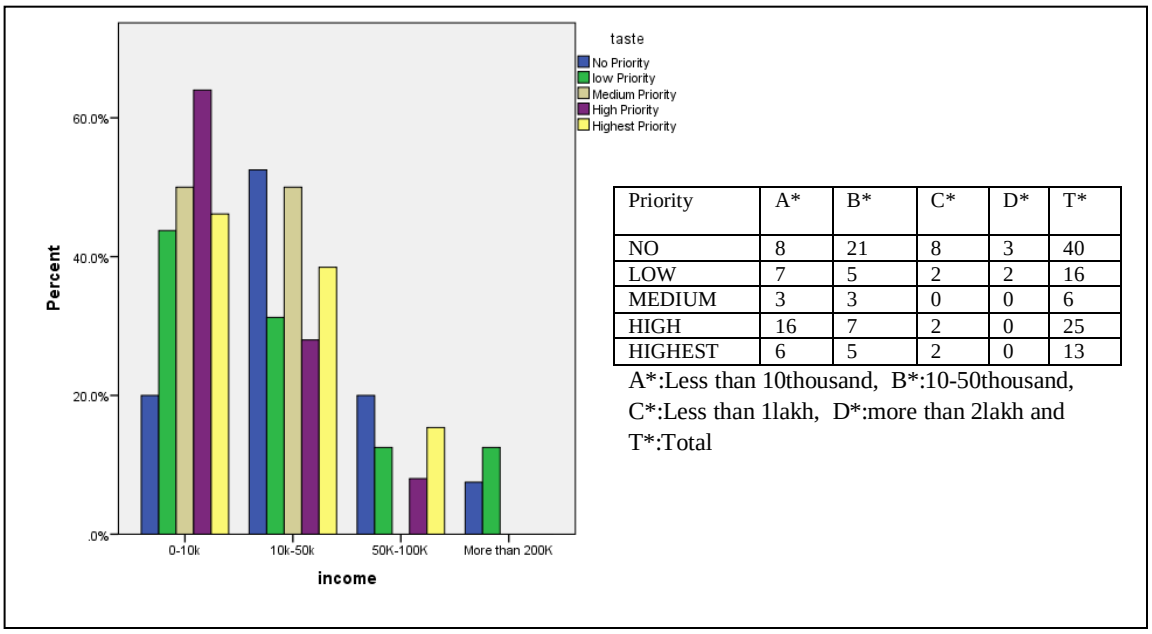


PRIORITY	M*	F*	T*
NO	21	19	40
LOW	9	7	16
MEDIUM	1	5	6
HIGH	10	15	25
HIGHEST	5	8	13

M*: Male, F*: Female and T*:Total

From this cross tab It was analyzed that the taste is at higher priority for both of the genders. So, Beany flavor should be in less amount and different flavors should also be available. So, that people who take the taste at high priority also consume soy milk.

Cross tab 14. Income and Taste

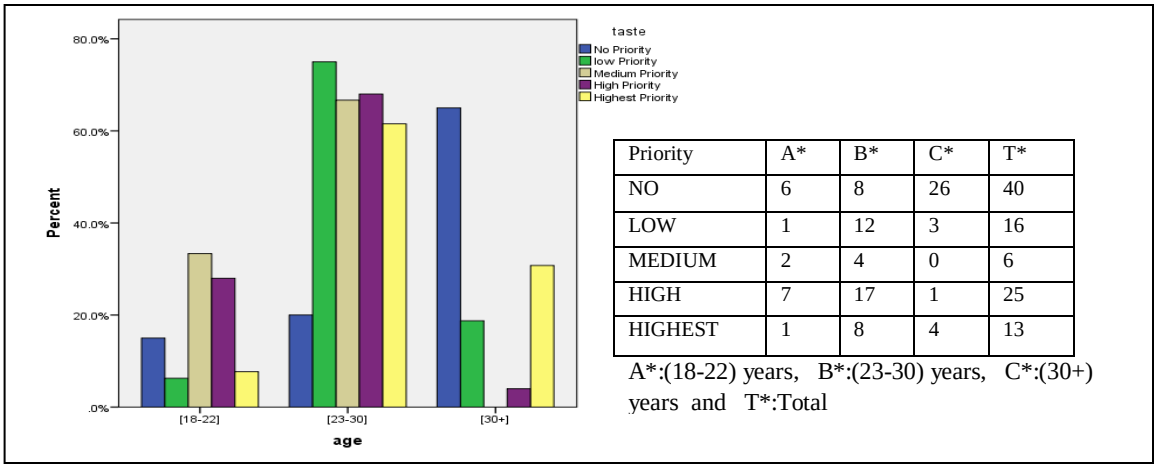


Priority	A*	B*	C*	D*	T*
NO	8	21	8	3	40
LOW	7	5	2	2	16
MEDIUM	3	3	0	0	6
HIGH	16	7	2	0	25
HIGHEST	6	5	2	0	13

A*:Less than 10thousand, B*:10-50thousand, C*:Less than 1lakh, D*:more than 2lakh and T*:Total

From the above table it was analyzed that the taste is at least priority for the people who belong to the income range of less than 10 thousand and at high priority for the people belong to the income range of 10-50 thousand. So, if the different flavors would be added targeted population should be accordingly.

Cross tab 15. Age and the Taste:

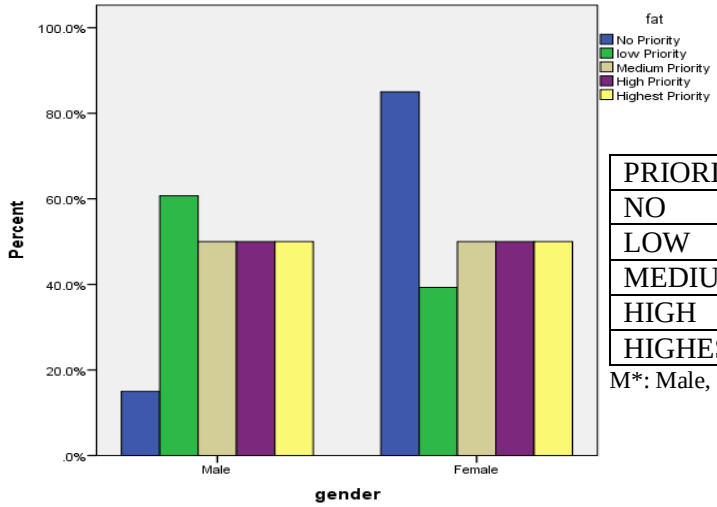


Priority	A*	B*	C*	T*
NO	6	8	26	40
LOW	1	12	3	16
MEDIUM	2	4	0	6
HIGH	7	17	1	25
HIGHEST	1	8	4	13

A*:(18-22) years, B*:(23-30) years, C*:(30+) years and T*:Total

From this cross tab it was analyzed that taste is at high priority for the age group of 23-30 age and 18-22age. So, targeted population should be these age groups and flavors should be added accordingly.

Cross tab 16. Gender and Fat:

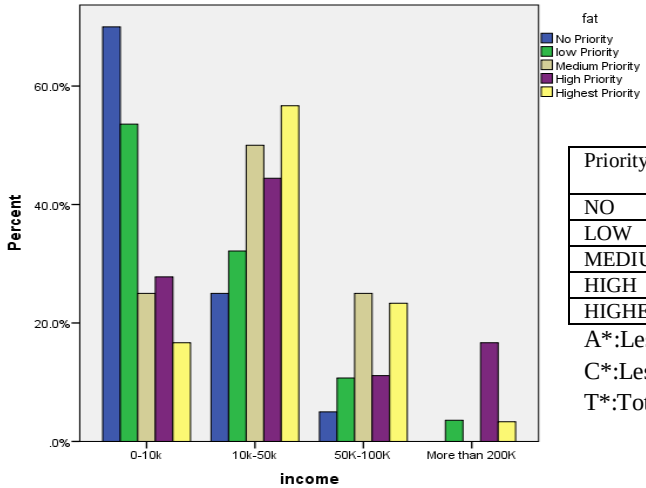


PRIORITY	M*	F*	T*
NO	3	17	20
LOW	17	11	28
MEDIUM	2	2	4
HIGH	9	9	18
HIGHEST	15	15	30

M*: Male, F*: Female and T*:Total

From this cross tab it was analyzed that the fat is at highest priority for both of the genders. So, both the genders should be targeted and made aware that in soymilk the fat content is low as compared to the cow milk to induce them to consume it more.

Cross tab 17. Income and the Fat:

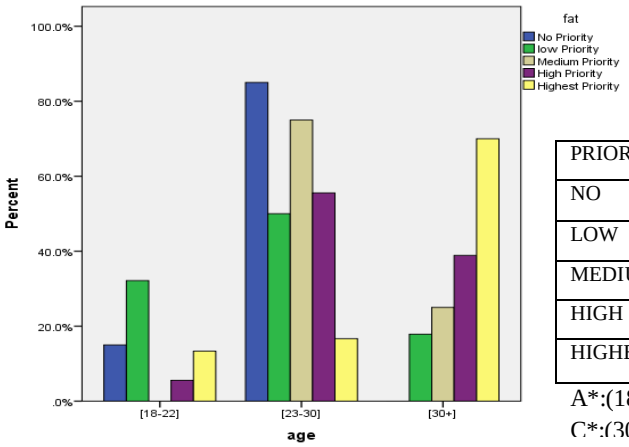


Priority	A*	B*	C*	D*	T*
NO	14	5	1	0	20
LOW	15	9	3	1	28
MEDIUM	1	2	1	0	4
HIGH	5	8	2	3	18
HIGHEST	5	17	7	1	30

A*:Less than 10thousand, B*:10-50thousand, C*:Less than 1lakh, D*:more than 2lakh and T*:Total

From this cross tab it was analyzed that the people who belong to the age group of 10-50 thousand take the fat at low priority as compared to the others. So, this group should be targeted and also make them aware about the low fat content in a soy milk.

Cross tab 18. Age and the Fat:

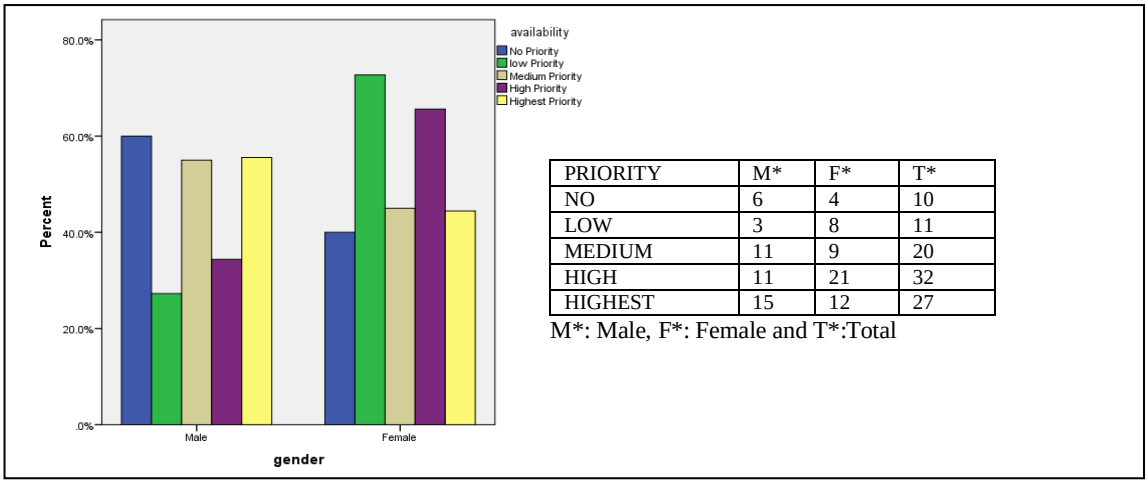


PRIORITY	A*	B*	C*	T*
NO	3	17	0	20
LOW	9	14	5	28
MEDIUM	0	3	1	4
HIGH	1	10	7	18
HIGHEST	4	5	21	30

A*:(18-22) years, B*:(23-30) years, C*:(30+) years and T*:Total

From this cross tab it was analyzed that fat is at highest priority for the age group of more than 30 years age. So, this age group should targeted more and taught about the benefits of soymilk in the context of lower fat and how it would be beneficial for them if they consume it.

Cross tab 19. Gender and the Availability:

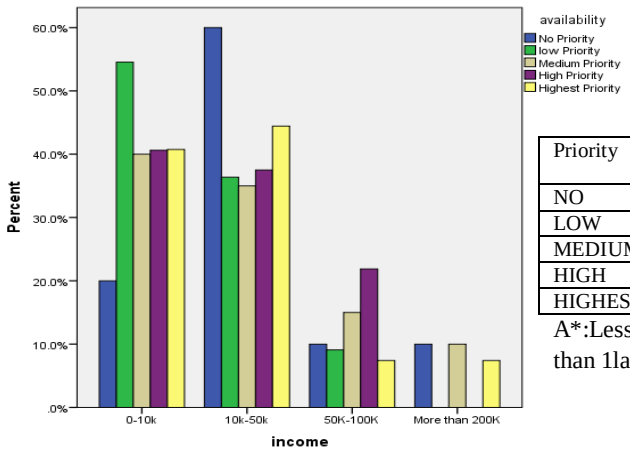


PRIORITY	M*	F*	T*
NO	6	4	10
LOW	3	8	11
MEDIUM	11	9	20
HIGH	11	21	32
HIGHEST	15	12	27

M*: Male, F*: Female and T*:Total

From this cross tab it was analyzed that availability is at high priority for the males and at highest for females. So, both the genders should be targeted for deciding the area for the sale of soya milk.

Cross tab 20. Income and the Availability:

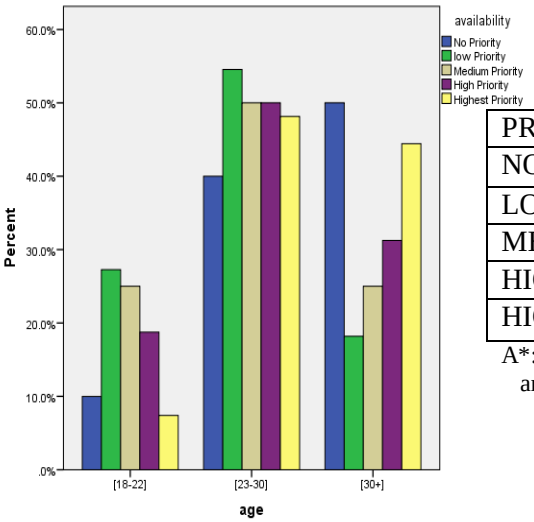


Priority	A*	B*	C*	D*	T*
NO	2	6	1	1	10
LOW	6	4	1	0	11
MEDIUM	8	7	3	2	20
HIGH	13	12	7	0	32
HIGHEST	11	12	2	2	21

A*:Less than 10thousand, B*:10-50thousand, C*:Less than 1lakh, D*:more than 2lakh and T*:Total

From this cross tab it was analyzed that availability was at high priority for all the people belong to all the income ranges. So, for this soymilk should be made available accordingly like in malls, stores and complexes so that everyone can consume it easily.

Cross tab 21: Age and the Availability:



PRIORITY	A*	B*	C*	T*
NO	1	4	5	10
LOW	3	6	2	11
MEDIUM	5	10	5	20
HIGH	6	16	10	32
HIGHEST	2	13	12	27

A*:(18-22) years, B*:(23-30) years, C*:(30+) years and T*:Total

From this table it is suggested that availability in terms of age was also at high priority for all the age groups, so everybody should be targeted for the availability of soymilk in the market.

4.9. ONE WAY ANOVA FOR SHOWING THE RELATION BETWEEN DEMOGRAPHIC AND PRIORITY FEATURES:

One way ANOVA is done to see the significant mean difference between and within the groups of three or more independent variables statistically. So One way ANOVA was done for Income, Age and Gender to see whether there was a significant difference in mean on the basis of Income, Age and Gender.

Table 30: **One way ANOVA for priority features by income**

		Sum of Squares	df	Mean Square	F	Sig.
Price	Between groups	63.785	3	21.262	15.598	.000***
	Within groups	130.855	96	1.363		
	Total	194.640	99			
Advertisement	Between groups	1.039	1	1.039	.520	.473
	Within groups	195.921	98	1.999		
	Total	196.960	99			
Nutrition	Between groups	4.438	1	4.438	1.621	.206
	Within groups	268.312	98	2.738		
	Total	272.750	99			
Taste	Between groups	4.271	1	4.271	1.832	.179
	Within groups	228.479	98	2.331		
	Total	232.750	99			
Fat	Between groups	5.232	1	5.232	2.138	.147
	Within groups	239.768	98	2.447		
	Total	245.000	99			
Availability	Between groups	.020	1	.020	.012	.913
	Within groups	160.730	98	1.640		
	Total	160.750	99			

***significant at .01 per cent; ** significant at 1 per cent; * significant at 5 per cent

This table 30 is showing that there is no significant difference in means of different income groups with respect, Advertisement, Nutrition, Taste, Fat and availability. However there is there is a significant difference in means of different income groups with respect to price. Thus, the alternate hypothesis is rejected and for income groups Price, Advertisement, Nutrition, Taste and Fat do not matter. However, there is similarity of perception of different income levels with respect to price.

Table 31 : One way ANOVA of the priority functions by age

		Sum of Squares	df	Mean Square	F	Sig.
Price	Between groups	46.867	1	46.867	43.210	.000***
	Within groups	106.293	98	1.085		
	Total	153.160	99			
Advertisement	Between groups	40.508	3	13.503	8.285	.000***
	Within groups	156.452	96	1.630		
	Total	196.960	99			
Nutrition	Between groups	61.782	3	20.594	9.371	.000***
	Within groups	210.968	96	2.198		
	Total	272.750	99			
Taste	Between groups	25.412	3	8.471	3.922	.011*
	Within groups	207.338	96	2.160		
	Total	232.750	99			
Fat	Between groups	43.345	3	14.448	6.878	.000***
	Within groups	201.655	96	2.101		
	Total	245.000	99			
Availability	Between groups	.503	3	.168	.100	.960
	Within groups	160.247	96	1.669		
	Total	160.750	99			

***significant at .01 per cent; ** significant at 1 per cent; * significant at 5 per cent

This table 31 is showing that there is a significant difference in means of different age groups with respect to Price, Advertisement, Nutrition, Taste and Fat. However there is no significant difference in means of different income groups with respect to availability. Thus, the alternate hypothesis is accepted and for age groups Price, Advertisement, Nutrition, Taste and Fat do matter. However, there is similarity of perception of different income levels with respect to availability.

Table 32 : One way ANOVA of the priority functions by Gender

		Sum of Squares	df	Mean Square	F	Sig.
Price	Between groups	75.04	2	54.241	12.142	.000***
	Within groups	169.01	97	1.562		
	Total	244.05	99			
Advertisement	Between groups	91.795	2	45.897	42.334	.000***
	Within groups	105.165	97	1.084		
	Total	196.960	99			
Nutrition	Between groups	114.766	2	57.383	35.232	.000***
	Within groups	157.984	97	1.629		
	Total	272.750	99			
Taste	Between groups	43.437	2	21.718	11.128	.000***
	Within groups	189.313	97	1.952		
	Total	232.750	99			
Fat	Between groups	74.059	2	37.029	21.012	.000***
	Within groups	170.941	97	1.762		
	Total	245.000	99			
Availability	Between groups	1.456	2	.728	.443	.643
	Within groups	159.294	97	1.642		
	Total	160.750	99			

***significant at .01 per cent; ** significant at 1 per cent; * significant at 5 per cent

This table 32 is showing that there is a significant difference in means of genders with respect to Price, Advertisement, Nutrition, Taste and Fat. However there is no significant difference in means of gender with respect to availability. Thus, the alternate hypothesis is accepted and for income groups Price, Advertisement, Nutrition, Taste and Fat do matter. However, there is similarity of perception of different income levels with respect to availability.

4.10: SHOWING OTHERS FACTORS OF SOYMILK OF THE SURVEY:

Fig 37:

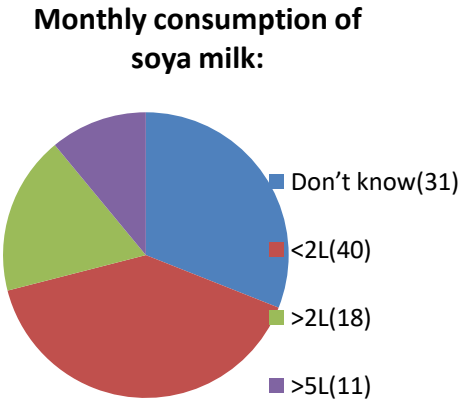


Fig 38:

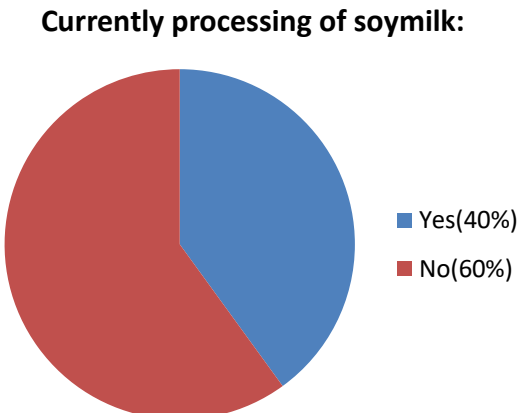


Fig 37: From this graph it was analyzed that more people consume soymilk less than 2L but there are also many people who consume soymilk more than 2L and 5L.So targeted population should be the people who consume soymilk more and who consume less should be educated about the benefits of soymilk and how it is healthier than other milks.

Fig 38: From this graph it was analyzed that more people do not know about the processing of the soymilk. So, before the marketing of soymilk people should be educated about the advantages and disadvantages of the processing of soymilk and also they should be educated about that how the processing is affecting their health and causing the harmful diseases also.

Fig 39:

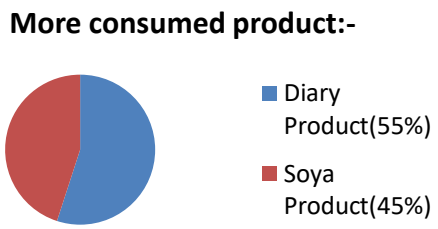


Fig 40:

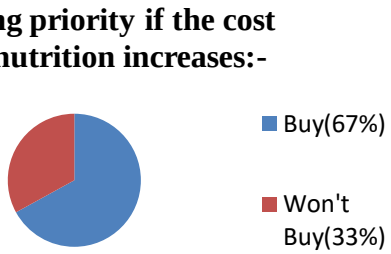


Fig 39: From this graph it was analyzed that both diary and soya products are equally consumed but to increase the consumption of soya products people should be educated about the benefits of soy products over the diary products.

Fig 40: From this graph it was analyzed that for most people cost is at less priority if the nutrition gets enhanced. So, we should try to find out the different methods to increase the nutritional content of soymilk to increase the sale of soymilk.

Fig 41:

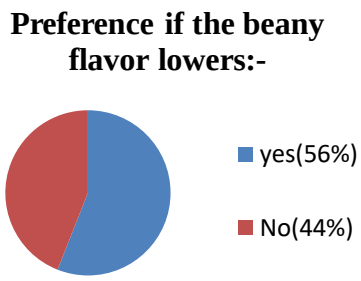


Fig 42:

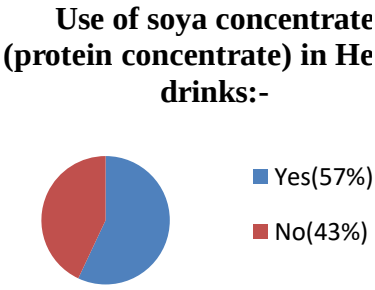


Fig 41: If the beany flavor lowers then more people would consume soymilk. So, for this we should find the techniques or the way for reducing the beany flavor and adding more flavors of soy milk to increase its consumption.

Fig 42: From this graph it was analyzed that most of the people know the use of protein concentrate in soymilk, but there are also some people who don't. So, we should educate them for same so they also start consuming it.

Fig 43:

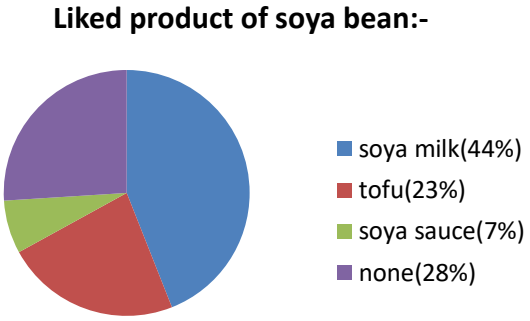


Fig 43 From this graph it was analyzed that more people prefer soy milk as compare to other soya products. So, we should target to manufacture mainly soymilk and its different flavors to increase the sale of soy products in market.

4.10.1: T-TEST for the testing of beany flavor:

One-Sample Statistics:

Table 33: Mean and Std. deviation of the beany flavor.

	N	Mean	Std. Deviation	Std. Error Mean
In case of taste if the beany flavor lowers would you prefer it?	100	1.2200	.49889	.04989

As reflected through table 33 mean is 1.22 and Standard deviation is .49

One sample test: Test value=1.22

Table 34: One sample test

	t	df	Sig.(2tailed)	Mean Difference	95% Confidence Interval of the Difference	
In case of taste if the beany flavor lowers would you prefer it?	4.410	99	.000	0.22000	upper	lower
					0.1210	0.3190

As the calculated value is higher than table value (1.22), hence t- test is significant. From this t-test it was analyzed that the people would recommend soy milk if the beany flavor lowers and more flavors would be added.

CHAPTER 5

CONCLUSION

Enzyme assisted soymilk extraction in comparison to mechanical method of soymilk extraction has been studied. Initially the trials were performed for both the methods to standardize the process for mechanical as well as for enzyme assisted soymilk extraction. Mechanical method extracted soymilk was compared to enzyme assisted soymilk extraction method in terms of milk quantity and quality along with a beverage innovated from both methods milk as a base and sensory analyzed. In mechanical method of soymilk extraction the soaking and boiling conditions of soybean were standardized while two different enzymes (cellulase and pectinase at different concentration at different temperature and time combinations) were explored to compare in between groups and also between mechanical and enzymatic method too. All the soymilk extracted samples from different enzymes as well as from mechanical methods were analyzed for approximate percentage of protein, moisture, pH, total solids, carbohydrates, fat, SNF, acidity, non-reducing sugars, flavonoids and total soluble solid and sensory attributes used. The soymilk extracted from enzymes was observed to be exactly double in yield from mechanical method especially from cellulase apart from pectinase without such effects. cellulase enzyme assisted soymilk were pH (6.75), proteins (56mg/ml), fat (12mg/ml), carbohydrates (5.9mg/ml), Solid not fat (SNF:2.1mg/ml), acidity (0.35 per cent), non-reducing sugars (20mg/ml), flavonoids (640mg/ml) and total soluble sugars (5.5 per cent) while for pectinase assisted soymilk observed with pH (6.82), proteins (45mg/ml), fat (13mg/ml), carbohydrates (6.2mg/ml), Solid not fat (SNF:3.1mg/ml), acidity (0.30 per cent), non-reducing sugars (7mg/ml), flavonoids (610mg/ml) and total soluble sugars (5.0 per cent) and at last the mechanical extracted soymilk had pH (7.2), proteins (26mg/ml), fat (14.2mg/ml), carbohydrates (4.9mg/ml), Solid not fat (SNF:5.2mg/ml), acidity (0.36 per cent), non-reducing sugars (10mg/ml), flavonoids (590mg/ml) and total soluble sugars (6 per cent).

From the quality analysis, the cellulase enzyme can be a boon to the soymilk production with higher percentage of proteins, flavonoids with decreased fat,

carbohydrates, SNF, acidity, total solids and pH and also statistically the cellulase enzyme assisted soymilk extraction method has shown significant results as compare mechanical method and pectinase enzyme assisted soymilk extraction method. Beside good nutrition, the enzyme assisted extracted soymilk showed higher sensory score in taste, colour, texture, and flavor on a 9-point hedonic scale to that of mechanical soymilk extraction method (the mean score of overall acceptance of each of formulation from mechanical method (5.80), pectinase (4.87) and cellulase (7.47) showed the significant difference within and between the groups with the F value of 45.793 along with homogeneity of variance with a significant value of 0.557). The Cellulase enzyme assisted soymilk extraction method has shown the significant results within and between the groups with the LSD value at 5 per cent were protein (0.9), fat(0.2), carbohydrates (0.1), acidity (0.1), flavonoids(0.2), non reducing sugars(0.3) and SNF(0.3) where as mechanical and pectinase enzyme assisted soymilk extraction method has shown non-significant results and cellulase assisted soymilk extraction method has shown significant positive correlation within and between the quality factors where as the mechanical has shown non-significant correlation within and between the quality factors. Thus, enzyme assisted extraction method can be considered as a novel processing method for soymilk extraction with enriched nutritional quality, double milk yield and less of by product (OKARA). As a whole de-hulled soybean can be completely converted to milk.

A health survey on soymilk consumption has been done simultaneously along with laboratory production to find out whether people like or prefer soymilk over the lacteal milk or not at all in Jalandhar area. Various key points studied include to find individual consumption of soymilk in percentage to study the awareness about soymilk and the factors which has to be inherent part of marketing of soymilk. The survey was conducted in Jalandhar city and in Thapar University, Patiala. We found that people are very much aware and prefer soymilk as compare to other milk and we also found that age group (23-30) years, income range of (10-50 thousand) and both the genders prefer soymilk. These factors could be targeted in future to increase the sale of soymilk and also came to know about different factor to be kept in mind and need improve to increase the marketing of soymilk.

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5	Submitted to British Institute of Technology and E-commerce Student Paper	<%1
6	Jiang, Susu, Weixi Cai, and Baojun Xu. "Food Quality Improvement of Soy Milk Made from Short-Time Germinated Soybeans", Foods, 2013. Publication	<%1
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8	Pakistan Student Paper	<%1
9	Yuan, Shaohong, Sam K. C. Chang, Zhisheng Liu, and Baojun Xu. "Elimination of Trypsin Inhibitor Activity and Beany Flavor in Soy Milk by Consecutive Blanching and Ultrahigh-Temperature (UHT) Processing", Journal of Agricultural and Food Chemistry, 2008. Publication	<%1
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21	O'Toole, Desmond K.. "Characteristics and Use of Okara, the Soybean Residue from Soy Milk ProductionA Review", Journal of Agricultural and Food Chemistry, 1999. Publication	<%1
22	Submitted to North Dakota State University Student Paper	<%1
23	YONG LI. "LIFE CYCLE ASSESSMENT OF SOYBEAN OIL PRODUCTION", Journal of Food Process Engineering, 8/2006 Publication	<%1
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