M.Sc. PROJECT AND DISSERTATION

ON

DEVELOPMENT OF FUNCTIONALPROBIOTIC BEVERAGE FROM PUMPKIN

DEPARTMENT OF FOOD TECHNOLOGY AND NUTRITION

SCHOOL OF AGRICULTURE

LOVELY PROFESSIONAL UNIVERSITY

INDIA

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PROJECT AND DISSERTATION PLAN PROPOSAL

Of the proposed Research Project for the degree of

MASTER'S OF SCIENCE

IN

NUTRITION AND DIETITICS

Name of the Research Scholar: Gursharan Kaur

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Name of the Supervisor: Er. Poorva

Title of Research**: Preparation of Functional probiotic Beverage from Pumpkin**

Signature of the Research Scholar**:**

Approved by Guide

CERTIFICATE

This is to certify that Gursharan Kaur (Registration $N_{O.}$ 11718387) has personally completed M.Sc. Pre-dissertation entitled 'PREPARATION OF FUNCTIONAL PROBIOTIC BEVERAGE FROM PUMPKIN' under my guidance and supervision. To the best of my knowledge, the present work is the result of hid original investigation and study. No part of per-dissertation has ever been submitted for any other purpose at the university.

The project report is appropriate for the submission and the partial fulfilment of the conditions for evaluation leading to the award of Master of Nutrition and Dietetics.

Date: May, 2018 Signature of Supervisor

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INTRODUCTION

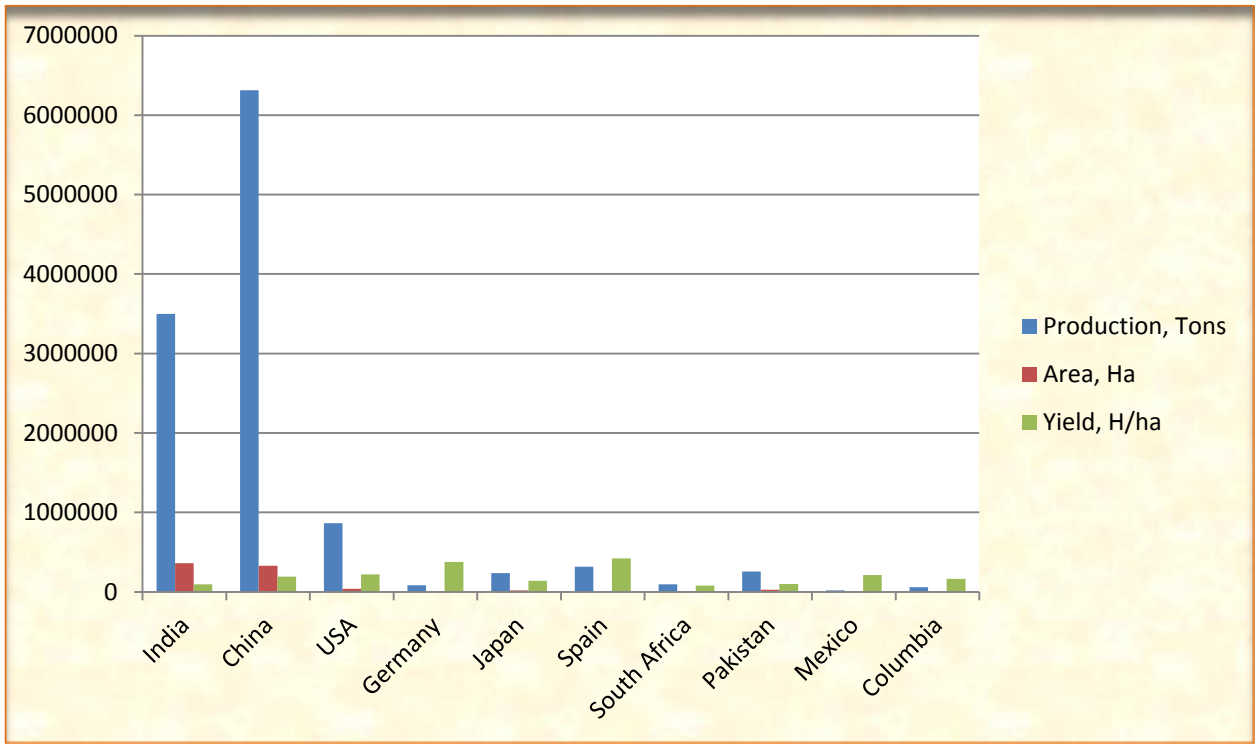
Pumpkin is very nutritional vegetable belong from Cucurbitaceae family6 It is larger in size, weight may vary from 4 to 8 kg and has a thick yellow or orange colour shell but it may also contain dark green, pale green, yellow – orange, grey and white colour pumpkin shell (Anju k Dhiman et al.,2009). Different kinds of varieties of pumpkin are found all around the world. The most Common species of pumpkin i.e. *C.maxima, C.pepo, C.moschata* is found in Worldwide (Mi Young Kim et al., 2012). Pumpkin is also used as to cure the various kinds of diseases in several countries like China, Argentina, India, Mexico, and Brazil. Pumpkin is found to be high in beta Carotene Content, dietary fibres, Vitamins, Minerals and Low in Fat. It Contain various Minerals like Zinc, Phosphorus, Potassium, Selenium, Copper, Manganese, Iron. Pumpkin is very beneficial for certain diseases like Diabetes, Cancer, Malaria, Obesity, Jaundice, Enteritis, and Dyspepsia, Cardiovascular disease, Stomach Disorder and Hydatid disease. Various kinds of products was developed from pumpkin like Snack Food , Bread, Candy , Nuggets, Noodles , Sweets , Biscuits, Flakes , Cake , Bakpao , Sauce , Brownie, Chips , Ice – cream , jam , Kuaci , Dodol , Pickles, and Beverage. Seeds of pumpkin are considered as a Richest Source of Oil. India is considered as a Highest Producer of Pumpkin (Anju k Dhiman et al., 2009). Pumpkin Contain list of Amino Acid such as Aspartic Acid , Serine , Threonine , Proline , Glutamic acid , Alanine , Glycine , Isoleucine , Valine , Phenylalanine , Histidine , Lysine , Cystine , Arginine , Tryptophan , Methionine. And Fatty Acids Like Myristic acid, Palmitic acid , Stearic acid , Arachidic acid , Oleic acid , Vaccenic acid , Gadolic acid , Linoleic acid , alpha linolenic Acid (Sherif E.A Badr et al., 2011) . Flesh was considered as Contain more beta Carotene Content as Compare to Seeds and Rind. All parts of pumpkin like seeds, flowers, leaves were found to be useful.pumpkin is cooked by various method around the world like Baking to get the baked products i.e. bread , brownie ,cake , biscuits, then other methods like boiling, Roasting to get the Snacks i.e. chips, it is also used in powder form. Pumpkins very useful , edible and nutritional vegetable, including this vegetables in our daily diet is very beneficial for our health as it is exhibit various health benefits such as , Anti Cancer Activity, Antihelmintic Activity, Anti ulcer Activity, Anti-fatigue Activity, Anti Viral Activity Anti inflammatory Activity, Anti diabetic Activity , Antihemolytic Activity, Enzymatic Inhibitory Activity etc. pumpkin is categorised in

both the categories in fruit and as well as in vegetables (Consolacion Y. Ragasa et al.,2005 & Hao Wu et al., 2

REVIEW OF THE LITERATURE

From N Muruganantham et al research it was found that pumpkin is more consumed in India. India is the greatest producer of pumpkin. It is grown in all parts of India, *C.maxima* grown in hills and sub-tropical regions. The main growing season of pumpkin is summer and rainy season but it is also grown during winter in some parts of southern and western India (Anju k Dhiman et al., 2009)

Table No 1. Production of pumpkin in different countries:-



(Anju K. Dhiman et al., 2009)

Pumpkin contains higher content of beta carotene but its beta carotene content may vary from species to species. From Anju k Dhiman et al., 2009 Research, it was found that organically

grown pumpkin contain higher content of beta carotene and Vitamin E on the other side conventionally grown pumpkin contain higher content of Dietary and Ascorbic acid. Pumpkin contain various kinds of essential Amino Acids like Alanine, Arginine, Glycine, Methionine, Serine etc. various kinds of Minerals like Phosphorus, Magnesium, Potassium, Chlorine etc.Various Fatty Acids like Arachidic Acid, Myristic Acid, Eicosenoic Acid, α Linolenic Acidand Tocopherols & Carotenoids as shown in the table No. 2.

Table 2 – Other Chemical Contents of Pumpkin

Amino Acids	**Minerals**	**Fatty Acids**	**Tocopherols & Carotenoids**
Alanine	Calcium	Myristic Acid	α Tocopherol
Arginine	Phosphorus	Palmitic Acid	ɣ Tocopherol
Aspartic acid	Iron	Heptadecanoic Acid	β Carotene
Glutamic Acid	Magnesium	Stearic Acid	β Cryptoxanthin
Glycine	Sodium	Oleic Acid	
Histidine	Zinc	Linoleic Acid	
Isoleucine	Potassium	Arachidic Acid	
Leucine	Sulphur	Eicosenoic Acid	
Lysine	Chlorine	α Linolenic Acid	
Methionine		Behenic Acid	
Phenylalanine			
Proline			
Serine			
Threonine			
Tyrosine			
Valine			

(Mi Young Kim et al., 2012)

2.2 Medicinal properties of pumpkin

Pumpkin Contain a list of Health Benefits. Its Medicinal properties like Antidiabetic Activity, Antioxidant Activity,Antimalarial Activity ,Antifungal, Antiaflatoxigenic , Antioxidant Activity ,Catalytic activity, Larvicidal, Ovicidal, Repellent activity , Anticancer Activity , Antihelmintic Activity etc. as it is described in the table no.3.

Table No.3 Medicinal properties of pumpkin

Species	Region	Part used	Activity	Application	References
C.maxima	World wide	extract from *C.maxima* fruit	Catalytic activity	semiconductorspectroscopy,drug delivery, tissue imaging and cancer therapy	Jia Yu et al.,2016
		Extraction of essential oils from leaves	Antifungal, Antiaflatoxigenic and Antioxidant Activity	Used as traditional medicine against different human diseases.	Priyanka Singh et al.,2010
		Leaf Extract from *C.maxima*	Larvicidal,Ovicidal and repellent activity	Applied as an effective personal protection measure against mosquito bites	Mullai k et al.,2006
		Crude Ethanolic Extract from *C.maxima* Seeds	Antimalarial Activity	Helpful in preventing the development of parasitaemia	Instituto Oswaldo Cruz et al.,2000
		Juice from flesh	Enzymatic Inhibitory Activity i e,Cholinesterase and tyrosinase Inhibitory potential, Antioxidant and Antidiabetic Activity ,Scavenging Activity, Metal Chelating Activity,	Used as a source of Antioxidant agents, functional food and Nutraceuticals	Arumugam Abirami et al.,2014

			Antihemolytic Activity		
		Seed Extract	Antitumour Activity, Antimalarial and Antihelmintic Activity	Used as a traditional intestinal antiparasitic in rural and urban areas	R.C.B Cruz et al.,2006
		Aerial parts	Hepatoprotecti ve Activity	Protect liver damage	Persona Saha et al.,2010
		Flowers	Antimicrobial Activity	Destroy Microbes	N.Muruganant ham et al.,2015
		Leaves	Alpha amylose inhibition Activity and Antioxidant Activity	Useful to manage the glucose induced hyperglycemia	Shahlah Jihad Ahmed Al Shaheen et al.,2013
		Aqueous Extract of Seeds	Alpha Glucosidase Inhibitory Activity and alpha amylase inhibitory Activity	Help to manage diabetes	Devesh kumar khushwaha et al.,2016
		Extract from leaves	Antimicrobial Activity	Destroy Microbes	Sayema khanum et al.,2016
		Flowers	Antimicrobial Activity	Helpful in destroying the microorganisms	Consolacion Y. Ragasa et al., 2005.
		Seeds	Scolicidal Activity	Beneficial in Hydatid disease	Arash Babaei et al.,2017
		Methanol Extract of *C.maxima* from Aerial	Anticancer Activity	Helpful in inhibit the tumour growth including increasing life span	U .K Mazumder et al.,2016

		parts			
		Methanolic extract	Antioxidant Activity	Inhibit oxidative damage	Attarde D L et al.,2010
C.moschata	Worldwide	Extract from seeds	Antihelmintic Activity	Helps in destruction of parasitic intestinal worms	C.Marie Magdeleine et al.,2008
		Polysaccharides from Pumpkin's flesh	Antioxidant Activity	Used in food and medicine industry	Hao Wu ,et al.,2014
		Fruit extract	Anti-fatigue Activity	Help in increasing physical activity	Shih-Yi Wang et al.,2012
		Crude extract	Antioxidant Activity, Scavenging Activity	Inhibit oxidative damage	Azizah et al.,2009
		Moschamindole	Antioxidant Activity, Scavenging Activity, Antimicrobial Activity	Inhibit oxidative and microbial damage	Yashodharan Kumarasamy et al., 2008
C.pepo	Worldwide	Flesh (powder)	hypoglycaemic and hypolipidemic Activity	Beneficial for diabetic patients	Asgary S edigheh et al.,2011
		Rind, flesh and Seeds	Antitumour, Antiviral and Antimicrobial Activity	Inhibit growth of tumour, microbes and virus	Sherif E.A. Badr et al.,2011
		Pumpkin seed snacks	Anti Bladderstone	Reduce the risk of bladder Stone	Fu Caili et al.,2006
		Methanolic extract	Antiobesity Activity	Help in maintaining the weight	Kathryn Wanjiku Nderitu et al.,2017

		Fruit and seed	Hepatoprotective Activity,Anti hyperglycemic ,Anti ulcer,Anti inflammatory, Anti Cancer Activity	Exhibit property of reducing Cancer, ulcer etc.	Perez Guteirrez et al.,2016
		Methanolic Water Extract	Scavenging Activity		Savitree Mongkolsilp et al.,2004
		Pulp	Antiulcer Activity	Treat Jaundice, Enteritis, Dyspepsia, Stomach Disorder	Sentu Sarkar et al.,2008
		Juice	Antioxidant Activity	Inhibit oxidative damage	Gabriella Gazzani et al.,1998
		Leaves	Soluble Activity	Provide enzymes and coenzymes	Iheanacho et al.,2009

2.2.1*Catalytic Activity*

Gold nanoparticles (AuNPs) were extracted from extract of *C.maxima*. These particles are exhibit applications in semiconductors, spectroscopy, drug delivery, tissue imaging and cancer therapy. For evaluating the catalytic activity of Gold nanoparticles (AuNPs), the reduction of 4-Nitrophenol (4-NP) to 4-Aminophenol (4-AP) by NaBH4 was selected as a probe reaction. The result of this experiment was concluded that the Gold nanoparticles (AuNPs) exhibit the good Catalytic Activity for the reduction of 4-nitrophenol to 4-aminophenol by excess NaBH4. (Jia Yu et al., 2016)

2.2.2 *Antimicrobial, Anti-fungal and Antiaflatoxigenic Activity*

The essential oils obtained from *C.maxima* by the method of hydro distillation and their chemical evaluation by GC and GC–MS analysis showed the Anti-fungal and Antiaflatoxigenic Activity (Priyanka Singh et al., 2010) .This oils are recommended as

antimicrobial for increasing the shelf life of the food by regulating their fungal infestation, aflatoxin production and lipid peroxidation. For antimicrobial activity and Anti-fungal Activity, the compound ethyl acetate fractions of *C. maxima* flowers was analyzed against strains i e., *S. typhi, E. coli, E. faecalis* and *B. cereus* and two fungal strains i e. *C. lunata and C.albicans*.The One Another test of ethyl acetate extract of *C.maxima* was performed against eleven human pathogenic bacteria including both gram positive and negative bacteria and three fungi by disc diffusion method and the result of this experiment was concluded that the ethyl acetate extract of *C.maxima* contain good Antimicrobial Activity against all the human pathogens. The extract of n-hexane compound from *C.maxima* showed medium to Maximum Antibacterial Activity against all the human pathogens. The n-hexane also show very good Anti-microbial Activity against gram positive bacteria i.e. *Bacillus Subtilis* and gram negative bacteria i.e. *Staphylococcus* (Sayema khanum et al., 2016). An Average zone of inhibition of 8-14mm also showed by Chloroform extract of *C.maxima* against all the human pathogens (Sayema khanum et al., 2016).The Extracts of leaves from *C.pepo* exhibit Antimicrobial Activity, it was evaluated against certain bacteria i.e. *Providenciastuartii, Pseudomonas aeruginosa, K. pneumoniae, Escherichia coli, Enterobacteraerogene and Enterobactercloacae* (Perez Gutierrez RM et al., 2016).

The Antimicrobial Activity of *C.pepo* was confirmed by S.E.A. Badr et al., 2009. The rind, flesh, seed oil extract of pumpkin was analyzed to confirm the Antimicrobial Activity by using the agar disc method. For the examination of the Antimicrobial Activity two Gram positive bacteria i.e. *B. Subtilis, B. cereus* and one yeast i.e. *S. Cerevisiae* was used.(Sherif E A Badr et al ., 2009) .The extraction of Moschamindole from seeds of *C.moschata* was found to be exhibit Antimicrobial Activity. For Antimicrobial Activity, Moschamindole inhibited the growth of three pathogenic bacteria species, namely *Proteus mirabilis, Streptococcus epidermis* and *Lactobacillus plantarum* (Yashodharan Kumarasamy et al., 2008).

2.2.3 *Antioxidant Activity*

To determine the antioxidant activity of essential oil of *C. maxima* was spotted separately on TLC plate. Formation of yellow spot due to bleaching of purple colour of DPPH reagent was recorded as positive antioxidant activity of essential oil. Free radical scavenging activity of essential oil of *C. maxima* was measured by recording the extent of bleaching of the purple-

colour DPPH solution to yellow (Priyanka Singh et al., 2010).The leaves of *C.maxima* also exhibit property of Antioxidant Activity .Its antioxidant activity proved by comparing the IC_{50} of methanol extract of *C.maxima* with other three standard compounds (BHA, BHT and Vitamin C)(Shahlah jihad Ahmed Al Shaheen et al., 2013). Complete Analysis of *Cucurbita moschata* polysaccharides concluded that it has good Antioxidant Activity, high reducing power, positive radical and it can be used as a natural antioxidant in the food or medicine industry (Hao Wu et al., 2014).The methanolic seed extract of *C.pepo* also exhibit Antioxidant Activity, it was determined by using free radical DPPH (2, 2-diphenyl-1-picrylhydryzyl) scavenging and soybean lipoxygenase [LOX] inhibition (Rosa Martha Perez Gutierrez et al., 2016).

C.maxima contains various kinds of phytoconstituents like triterpenoids, flavonoids, coumarins, saponines, cucurbitacins, carotenoid, vitamins, minerals and amino acid. These phytoconstituents are responsible for higher Antioxidant Activity of *C.maxima* as compared to Antioxidant Activity of Petroleum ether, Chloroform extract and Methanolic extract of pericarp of *C.maxima* (Attarde D L et al., 2010)

By Azizah et al., 2009 Antioxidant Activity of *C.moschata* was analysed. For Antioxidant Activity, Beta-carotene and lycopene were determined using HPLC and total phenolic measured by Folin-Ciocalteu method .The extraction of Moschamindole from seeds of *C.moschata* was found to be exhibit Antioxidant Activity. The Antioxidant Activity of *C.moschata* was evaluated by DPPH assay which was based on proton radical scavenging action. By Yashodharan Kumarasamy et al., 2008 it was concluded that the Moschamindole was considered as a natural Antioxidant. Its Antioxidant property was found to be due to presence of phenolic moiety in the structure of Moschamindole.

2.2.4 *Larvicidal, Ovicidal and Repellent Activity*

The leaf extracts of *C.maxima* showed the larvicidal and ovicidal properties and they can also be applied as a protection barrier against mosquito bite. Its larvicidal, ovicidal and repellent activities are proved by the comparison of *C.maxima* with other solvents. (Mullai, K et al., 2006).

2.2.5 *Antimalarial Activity*

The crude ethanolic extract of *C.maxima* was considered helpful in the prevention of the development of the parasitaemia in *p.berghei* infected mice. The Antimalarial activity of *C.maxima* was analysis by 4 d suppressive test. The treatment of infected mouse with the use of crude ethanolic extract and with pyrmethamine showed a protection against malaria. (Claudia Zuany Amorim et al., 1991).

2.2.6*Antihelmintic*

The experiment was conducted to confirm the Antihelmintic Activity of *C.moschata* by evaluating the in vitro effect of three seed extracts i.e. aquatic, methanolic and dichloromethane against the parasitic nematode of small ruminants *Haemonchuscontortus* and the result of this experiment concluded that *C.moschata* exhibited larval development inhibition at all concentrations (C. Marie-Magdeleine et al .,2009).

2.2.7 *Antifatigue Activity*

Antifatigue Activity of *C.moschata* was analyzed by various physiological activities like exhaustive swimming time, forelimb grip strength. The muscular and hepatic glycogen was also evaluated after 14-day supplementation of *C.moschata* extract. From this experiment, it was concluded that supplementation with *C.moschata* extract increased grip strength , increased 5% body weight loaded swimming time, blood glucose, muscular and hepatic glycogen levels and decreased the level of plasma lactate, ammonia, creatine kinase activity after a 15-min swimming test (Shih-Yi Wang et al .,2012). *C.pepo* also exhibit Antiobesity Activity, it was analyzed orally by Kathryn WanjikuNderitu et al 2017 .For Antiobesity Activity, the methanolic leaf extracts was bioscreened in progesterone induced obese mice at 200mg/kg/bw and 400mg/kg/bw and at these concentrations methanolic leaf extract show significant effect on body mass index. This methanolic leaf extract contain various photochemical like saponins, flavonoids, alkaloids, and steroids and due to presence of these contents it was taken as an Antiobesity product. (Kathryn WanjikuNderitu et al., 2017).

2.2.8 *AntidiabeticActivity*

The Antidiabetic Activity of *C pepo* was analyzed by evaluating the hypoglycaemic and hypolipidemic effects of different doses of pumpkin powder in male diabetic rats .In the result, insulin was found to be decreased in diabetic rat but other components were found to be

in normal range. High dose of pumpkin in normal rat decrease content of cholesterol but low dose decrease the amount of glucose, triglycerides, low density lipoprotein and C-reactive protein as compared to diabetic group(Asgary Sedigheh et al.,2011). The other species of pumpkin i.e. .*C.maxima* also exhibit Antidiabetic Activity. The presence of flavonoids compounds of *C.maxima* suggested their Antidiabetic Activity.

2.2.9*Scolicidal Activity*

Hydatid disease is a parasitic infestation by a tapeworm of the genus *Echinococcus*. The evaluation of scolicidal activity of Cucurbita maxima seed's methanolic extract against protoscolices of hydatid cyst had confirmed their scolicidal activity and stated that no hydatid cyst formation observed in mice treated with *C. maxima* seeds methanolic extract (Arash Babaei et al.,2018).

2.2.10*Anti-inflammatory Activity*

The steroid extraction, 3β-hydroxycholest-7-en-24-one from *C.pepo* seeds was analysed to confirm the Anti-inflammatory activity. The result of this experiment was concluded that 3β-hydroxycholest-7-en-24 had a capacity to work as a anti inflammatory agent. (Perez Gutierrez RM et al., 2016).

2.2.11 *Anticancer Activity*

Alcoholic extract of *Cucurbita pepo* also exhibit Anticancer Activity. It was also exhibit a significant dose-dependent inhibitory effect against HeLa cell growth (Perez Gutierrez RM et al., 2016).In Sherif et al., 2009 research, Antitumour activity of *C.pepo* was examined against the tumour cell lines of liver carcinoma and breast carcinoma. These all extracts exhibit potentate tumour activities, potential cytotoxicity against liver carcinoma and breast carcinoma (Sherif et al., 2009).

2.2.12 *Anti Bladder Stone Activity*

By Fu Caili et al 2006 it was found that pumpkin seed snacks exhibit significant Anti bladder stone Activity. Intakes of pumpkin seed snacks inhibit Crystal formation and reduce the risk of bladder stone disease. Regular intake of this in optimum amount i.e. 60 mg/kg remarkably

reduced risk of bladder Stone disease. Pumpkin seed oil also helps to reduced bladder pressure, urethral pressure and increase the bladder compliance (Fu Caili et al., 2006).

2.2.13 *Enzymatic Inhibitory Activity*

C.maxima exhibit Enzymatic Inhibitory Activity, It was analyzed by Arumugam Abirami et al., 2014.Diabetes disease increased rapidly during the populations, it is a disease characterized by increased sugar level in blood. The α-glucosidase inhibition activities of *C. maxima* (Red) and *C. maxima* (White) were 72.83% and 71.88%. That was why it is suggested as a therapeutic diet for diabetic patient.

C.maxima also contains Tyrosine inhibitory Activity, Tyrosine helps in the formation of Melanin. *C.pepo* was supposed to inhibit the Tyrosine Activity. It can be used to treat the hyper pigmentation which occurs due to high production of melanocytes, oxidation of foods. (Arumugam Abiramiet al., 2014)

The methanolic leaf extract of *C.maxima* exhibit amylose inhibition Activity. It showed α-amylase inhibition activity that varied from 21.49% to 97.01% .(Shahlah Jihad Ahmed Al-Shaheen et al ., 2013).

2.2.14 *Scavenging Activity*

Arumugam Abirami et al., 2014 analysed the Scavenging Activity of *C.maxima*. The juice obtained from *C.maxima* showing Scavenging Activity by reducing DPPH to the yellow-colour diphenylpicrylhydrazine.

The Superoxide anion radical scavenging activity was analyzed by Arumugam Abirami et al., 2014. Formation of Superoxide anion free radical results in oxidative destruction in biomolecules. In the comparison between *C.hystrix*, *C.maxima*(white) and *C.maxima* (red), *C.maxima* (red) exhibit high Scavenging Activity These extract play its important role in protecting the food like proteins, DNA(Arumugam Abirami et al., 2014).

Scavenging Activity of *C.pepo* was evaluated by Savitree Mongkolsilp et al., 2004 on DPPH radicals. In the result of this experiment it was stated that crude methanolic water extract of *C.pepo* exhibit IC_{50} 23.12 mg/mL value for Scavenging Activity and it is considered as good Antioxidant Source. (Savitree Mongkolsilp et al., 2004)

By Azizah et al., 2009 Scavenging Activity of *C.moschata* was analysed and high Scavenging Activity was found in cooked pumpkin. The extraction of Moschamindole from seeds of *C.moschata* was found to be exhibit Free Radical scavenging Activity (Azizah et al., 2009).

2.2.15 *Metal chelating Activity*

The metal chelating activity of *C.maxima* was analyzed by Arumugam Abirami et al., 2014. The values obtained in result were reported as EDTA equivalents. The formation of violet colour takes place when Ferrozine reacted with ferrous ion and the formation of this colour can be inhibited by the use of chelating agents. The Metal chelating values obtained from juices of *C.maxima* (white) and *C.maxima* (red) was ranging between 7.02–7.73 mg EDTA/g. These juices by displaying its metal chelating effect help in protecting the food from oxidative destruction. (Arumugam Abirami et al., 2014)

2.2.16 *Antihemolytic Activity*

Antihemolytic Activity of *C.maxima* was analyzed by Arumugam Abirami et al., 2014.For Antihemolytic Activity, protecting capability of *C.maxima* (white) and *C.maxima* (red) on RBCs against oxidative damage by AAPH method was analyzed. Result of this experiment was concluded that the *C.maxima* (red) contain higher Hemolytic Inhibition value (93.60%) as compared to *C.maxima* white (92.03%).(Arumugam Abirami et al.,2014)

2.2.17 *Antiulcer Activity*

C.pepo pulp is high in Vitamin C Content and it was also used to treat liver disorder like jaundice, enteritis, dyspepsia, stomach disorder. (Sentu Sarkar et al., 2008). By Sentu Sarkar et al., 2008 it was experimented that albino rat who was treated with aspirin showed a decrease in alkaline phosphate Activity ,mucosal thickness and increase in Ulcer Index (UI) But the albino rat who was treated with fruit pulp extract of *C.pepo* showed a significant increase in Alkaline phosphate Activity and decrease in mucosal thickness, ulcer index . From this experiment it was concluded that the *C.pepo* exhibit gastroduodenal protective and Anti ulcer properties. (Sentu Sarkar et al., 2008)

2.3 Products made from pumpkin

2.3.1 Ready to Eat (RTE) Snack Food – Ready to Eat (RTE) Snack Food was prepared by Norfezah Md Nor et al .,2013 by addingβ-carotene, vitamin A precursor , desirable flavour , natural sweeteners to Pumpkin flour. This Snack Food was developed on the idea of increasing Consumers Consumption to ready to eat food due to their convenience, value, attractive appearance, taste, texture, and lifestyle.(Norfezah Md Nor et al 2013)

2.3.2 Wheat Bread From Pumpkin –Wheat Bread From Pumpkin was developed by Tatjana Rakcejeva et al 2011 and Compare it with wheat bread sample (without Pumpkin) by measuring its various components like moisture content , vitamin C content , carotenoid content , total fat content , degree of bread liking, bread baking loss and dry off. In the result of this experiment, it was found that the wheat bread sample with dried pumpkins additive was higher in carotenoid Content and reducing sugars as compared to a control wheat bread sample. (Tatjana Rakcejeva et al 2011).

2.3.3 Bakery Products from Pumpkin *(C.pepo)* - By Nyam KL et al 2013 it was found that the fibres obtained from Seeds and Rind of the *C.pepo* can be used in the development of Bakery Products. For the development of Bread, fibres extracted from pumpkin seed and rind was substituted with0%, 5%,10% in dough and various properties of this product was analysed.(Nyam KL et al 2013)

2.3.4 Fibre Rich Food from Pumpkin *(C.moschata)* –production of Fibre rich food from pumpkin (C. *Moschata*) was carried out by Ana M.Rojas et al 2009. The mesocarp tissues obtained from the pumpkin was considered as a Fibre Rich Food and can be used for the development of functional food product in future (Ana M.Rojas et al 2009).

2.3.5 Candy (*C.moschata)* –Development of Candy from pumpkin was carried out by Sabeera Muzzaffar et al 2016 .The preparation of candy was carried out by following the basic method given by Lal, Sidappa, Tandon, G. C. (2010). Various Contents of prepared Candy was analysed like Moisture Content, Ash Content, Pectin Content, Fiber Content and also other physicochemical characteristics , Microbial Analysis and Sensory Analysis. It was found that by the production of Candy from pumpkin Vitamin C and carotene content of pumpkin can be retained and increase the shelf life of vegetables by maintaining its antioxidant potential. (Sabeera Muzzaffar et al 2016).

2.3.6 Nuggets –Nuggets are low-quality meat cutlet, high in protein content but have low beta-carotene, pro-vitamin A and pumpkin have higher Content of beta-carotene and pro-vitamin A. By the fortification of these two products highly nutritional Nuggets was prepared by Aniswatul Khamidah et al 2013.

2.3.7 Pumpkin Flour- pumpkin Flour is very useful product, from pumpkin flour production of various type of products was carried out like noodles, biscuits, cake, ice cream, pasta, sweet etc. From this point of view pumpkin was considered as intermediate product.

2.3.8 Noodles –Noodles are very familiar food around the world and easy to serve food. Four types of Noodles like Wet noodles, Fresh Noodles, Dry noodles, Instant noodles were developed. Pumpkin pasta noodles was found to be high in beta carotene Content and by fortification with 10% soy flour, soy flour noodles was developed. This soy flour Noodles was found to be high in protein Content. (Aniswatul Khamidah et al 2013). By Ju Hwan Park et al., 2015 Pumpkin was substituted into model system of noodles for the development of a healthy and value added product. (Ju Hwan Park et al., 2015)

2.3.9 Sweets (manisan) wet and dry– Two types of sweets dry and wet were developed from pumpkin. Dry Sweets had more shelf life of about 6-9 months as compared to wet sweet, which is about 5-7 days. From wet sweet production of other products like syrup, jelly, dodol and by immersion next stage which is dry sweet was also obtained. (Aniswatul Khamidah et al 2013)

2.3.10 Biscuits – Biscuits was considered as a thin pastry and crisp, with low water content. 4 types of Biscuits were introduced, hard biscuits, Crackers, Cookies and Wafer(Aniswatul Khamidah et al 2013). By kulkarni et al., 2013 Pumpkin biscuits was developed by using pumpkin powder in place of wheat flour. The biscuits prepared by this method had more carotene Content and yellow colour. (Kulkarni et al., 2013)

2.3.11 Flake –By the fortifications of Pumpkin, development of flakes like pasta, flour was carried out by Aniswatul Khamidah et al., 2013. Flakes were considered as a ready to eat

breakfast. For the development of pasta , pumpkin was fortified with cereals in the ratio of 1:1 (corn flour: pumpkin flour) with a water content of 5,3%, ash of 1,1%, protein 14,7%, fat 24, 7%, 9,5% crude fibre and 54,1% carbohydrate. By Aniswatul Khamidah et al., 2013 pumpkin pasta was prepared by the addition of various other ingredients like egg yolks, egg white, sugar, wheat flour, butter, milk powder, baking powder.

2.3.12 Cake –Cake is a bakery product and by Aniswatul Khamidah et al., 2013 it was prepared from Flour. For the development of Cake, the process involve steps like preparation of the dough, mixing, and roasting at a temperature from 163-204°C.Bakpaofrom pumpkin was also prepared by Aniswatul Khamidah et al., 2013. By Yun et al., 1999, Pumpkin Rice Cake was prepared by using different amounts of Pumpkin and Cake Showed high Sensory Scores. (Yun et al., 1999).

2.3.13 Sauce - In front of high prices of Tomato sauce, Pumpkin Sauce was considered as a good alternative source. Pumpkin Sauce is bright golden orange in colour and has high content of β carotene (Aniswatul Khamidah et al., 2013). The sweet pumpkin-doenjang sauce was also prepared by Chang, Kyung-Ho et at., 2012 by adding thickening agent, amount of stock and doenjang was considered as independent variables. The prepared sauce was analysed by using the response surface methodology, based on the central Composition design. (Chang, Kyung et al., 2012). The soy Sauce from pumpkin was also prepared by QI Feng-yuan et al., 2012. This soy Sauce Contain many kind of Health benefits.

2.3.14 Brownies–Brownies is baked cake based product, dark brown in colour and has a distinctive.For the preparation of Brownie, flour pumpkin and pumpkin pasta was mixed with other the ingredients like eggs, sugar, cocoa, flour, margarine, etc.(Aniswatul Khamidah et al., 2013). By Devon Needlerand Michelle Wellman Brownie was prepared by adding pumpkin and it contain certain Health benefits like it lower the risk of Cancer and heart disease.

2.3.15Jelly candy–Jelly is a clear transparent Candy prepared by mixing pasta pumpkin with the gel-forming material like carrageenan, agar-agar powder, sugar, glucose syrup and citric acid. (Aniswatul Khamidah et al., 2013)

2.3.16 Crackers (Kerupuk) - Crackers is a dry food, higher in starch Content. Different Stages of Preparation include making dough, steaming, drying and frying and ingredients like pumpkin crackers 250 gram, 200 gram tapioca flour, and wheat flour 50 and 0,011ug/g beta-carotene content. (Aniswatul Khamidah et al., 2013)

2.3.17Chips–Chips is a fry product, pumpkin Chips was prepared by vacuum frying technology due to higher water Content of Pumpkin at 70-85°C. The Chips obtained from this technique was loss 85 % water and Vitamin Content but Capable to retain Minerals. The main aim to use Vacuum machine for frying is to remove moisture content completely. (Aniswatul Khamidah et al., 2013).

2.3.18 Ice – cream – Ice cream mainly Contain milk fat globule, air, small crystals of ice, and the water. Pumpkin Ice cream was prepared by adding pumpkin pasta with ice materials like milk, ice cream powder, emulsifier and stabilizer. (Aniswatul Khamidah et al., 2013)

2.3.19 Jam–Jam is a semi solid product prepared by adding 45 % part of fruit with 55 % sugar. Pumpkin jam was prepared by adding pumpkin strands with thickeners, citric acid, margarine and sugar. (Aniswatul Khamidah et al., 2013). By Anju K Dhiman et al., 2009 jam was prepared by adding pumpkin puree to other ingredients like carrageenan, agar and gelatine. (Anju k Dhiman et al., 2009).

2.3.20 Kuaci – For the preparation of kuaci from pumpkin, pumpkin seeds was soaked in a solution of chlorine for 1 minute, after washed and soaked in lime solution, dry seeds properly in Sun or oven, and then boiled these seeds in solution of salt until cooked. By Aniswatul Khamidah et al., 2013 it was stated that pumpkin Seeds has more advantages than a watermelon seeds. (Aniswatul Khamidah et al., 2013)

2.3.21 Dodol–For the preparation of Dodol, pumpkin pasta was added with glutinous flour, brown sugar and coconut milk. During the process of making Dodol from pumpkin, firstly

Pumpkin pulp cooked with sugar, then mixed with other ingredients like glutinous flour, ,coconut milk. (Aniswatul Khamidah et al., 2013)

2.3.22 Oil –Seeds of pumpkin is considered as a good source of Oil. Seeds substituted3.1% of total pumpkin fruit weight , higher in sulphur Containing amino acid , Zn, P, K, Se, Mn, Mg , Fe, Cu. Pumpkin seed Contain higher Content of palmitic, stearic, oleic , linoleic acids, glutamic acid, arginine acid , aspartic acid and phytosterols.(Anju K Dhiman et al ., 2009)

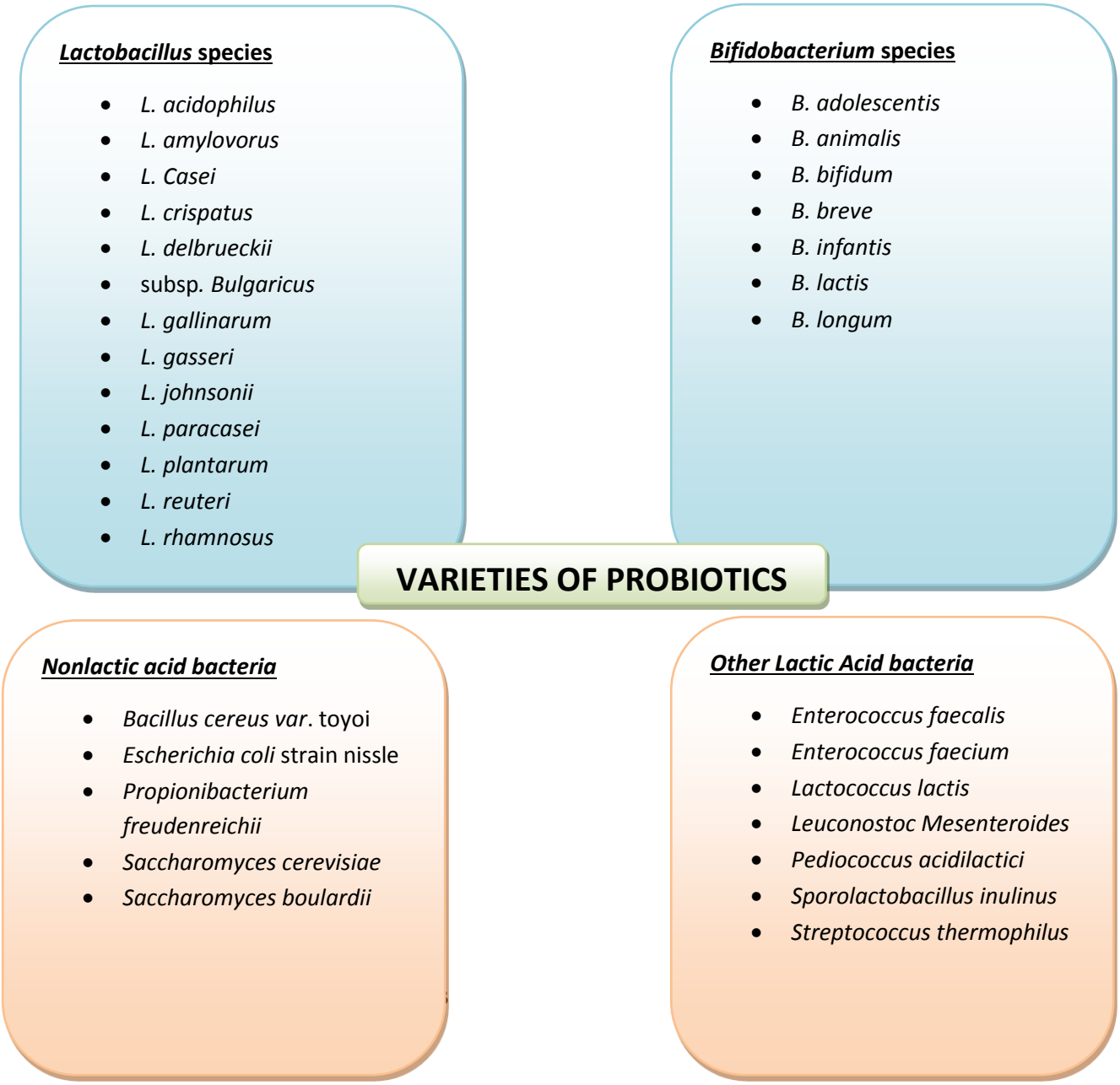
2.3.23 Pickles (*C.moschata*) and Halwa– Pumpkin pickles and halwa was prepared by Anju K Dhiman et al., 2009.For the development of Pickles firstly Vegetables was washed in aqueous solution containing sodium sulphate or sodium chloride, an inorganic salt such as polyphosphate, calcium chloride or calcium carbonate and a sorbitan-poly oxy ethylene sorbitan or sucrose fatty acid ester. The halwa prepared from pumpkin has good shelf life.(Anju K Dhiman et al., 2009)

2.3.24 Beverage- By H Jing, pumpkin beverage was prepared by using the formula of pumpkin juice 20.000%, vanillin 0.005%, sodium cyclamate 0.005%, citric acid, 140%, benzoic acid 0.015%, composite gum (agar∶ xanthan gum= 1∶ 1) 0.120%. From the research of Kong Jin Li Xin Zheng Cai Pengfei et al., 2005, Pumpkin beverage was prepared by the use of orthogonal design and optimized stabilizers. The pumpkin beverage prepared by this method, had unique taste. By LI Hong-Gao pumpkin beverage was prepared by firstly mixing the pumpkin with lucidum, sugar, stabilizer and then by fermented with intermixture of Lactobacillus bulgaricus and Streptococcus thermophilus.

2.4 Probiotics

During late 19thcentury microbiologist found a microflora in the gastrointestinal tract in the healthy individual and in diseased individual and stated that the microflora found in healthy individual was different from diseased individual. These microflora which are found in gastrointestinal tract are termed as probiotics**.** Probiotics means ‘for life ‘, these microorganisms consider beneficial for human and animals health.

Table 4 – Varieties of Probiotics



2.4.1 Desirable probiotic properties

In order to a potential probiotic strain following properties are expected:

- Acid and bile tolerance.
- Bile salt hydrolase activity.
- Antimicrobial activity against pathogenic microorganisms.
- Adhesion to mucosal and epithelial surfaces (Mercenier et al., 2008).

- Antimutagenic and Antigarcinogenic properties.

2.4.2 Health benefits of probiotics

In 1908, Metchnikoff proposed the beneficial effects of the probiotics microorganisms on human health, he found that Bulgarian are healthy and lived long life due to the consumption of fermented milk products which contain *Lactobacillus* spp. More than 400 bacterial species found in human intestinal tract. Probiotics helps to protect the host from various kinds of intestinal diseases by replacing the harmful bacteria with harmless bacteria. The most commonly used microorganism in probiotic product is lactic acid bacteria Probiotics had various kind of effect on our health like reduction in risk factor of colon, control of irritable bowel syndrome etc. As it is shown it diagrams.

- It produces inhibitory substances like organic acids, hydrogen peroxide, bacteriocin etc. In our body.
- It inhibits the pathogens to adhering with intestinal epithelial surfaces by blocking the adhesion sites.
- Probiotics inhibit the pathogen's nutrients consumption from our body
- It stimulates our specific and non specific immunity.
- Probiotics used to treat the Diarrhoea.
- Probiotics also used to treat the allergy and cancer.

2.4.3 Probiotics and food products:

The range of food products containing probiotic strains is wide and still growing. The main products existing in market are dairy based ones including fermented milks, cheese, ice cream, buttermilk, milk powder, and yoghurt, the latter accounting for the largest share of sales (Stanton et al., 2001). The factors that must be addressed in evaluating the effectiveness of the incorporation of probiotic strains into such products are, beside safety, the compatibility of the product with micro-organism and the maintenance of its viability through The factors that must be addressed in evaluating the effectiveness of the incorporation of probiotic strains into such products are, beside safety, the compatibility of the product with micro-organism and the

maintenance of its viability through food processing, packaging, and storage conditions (Kechagia et al., 2013).

2.4.4 Probiotic foods in market:

- Miso
- Cheese
- Kefir-kefir has been reputable fermented dairy product with many health benefits
- Sauerkraut
- Pickles
- Chocolates
- Tofu
- Tempeh- a fermented soybean with nutty taste food from Indonesia.

STATEMENT PROBLEM

Probiotics have positive effect on digestive system, immune system like it enhances our specific and non-species immunity, it helps in the degradation of toxic receptors, it reduces the risk factor of colon cancer etc. And on the other hand pumpkin are good for diabetic patients, it acts as antioxidants, inflammatory substances. In order to combine the properties of these products, selective probiotics microorganism will be inoculated in pumpkin so as to develop a healthy, functional pumpkin beverage which would enhance the nutritional status of the individual.

OBJECTIVES

- To check the Growth profiling of probiotic bacteria,
- To formulate of functional pumpkin beverage using selective probiotic bacteria,
- To analyse the Nutritional Properties of functional pumpkin beverage,
- To analyse Sensory evaluation and the estimation of shelf life of functional pumpkin beverage.

MATERIALS AND METHODS

4.1 Chemicals

Chemicals like Hydrochloric Acid, Glucose, Potato Dextrose Agar (PDA), Sodium Chloride, Sodium Hydroxide, Phenolphthalein, Ethanol, Petroleum Ether, Sulphuric Acid, Anhydrous sodium sulphate, Copper, MRS Cysteine Medium (M369) etc. will be used for the preparation of functional pumpkin beverage

4.2 Plastic Ware and Glasswares

Storage bottles, micro-centrifuge tubes, Petri dishes, Measuring cylinders, beakers, conical flasks, glass bottles, test tubes, Micropipettes, syringes , syringe filters , vials, glass pipettes, aluminium foil etc. will be used during the preparation of functional pumpkin beverage.

4.3 Growth medium, microorganisms and culture conditions

MRS-Cysteine Medium (M369) will use as growth medium for probiotic strains. Growth medium will be prepared as per manufacturer instructions followed by sterilization by autoclaving at 15 psi for 15 min prior to use. Pumpkin will be buy from local Market, Jalandhar and used as substrate for fermentation.

4.4 Equipments

The equipments like Bio-safety cabinet, Electronic balance, pH meter, Autoclave , Incubator shaker, Centrifuge, Microwave, Vortex , UV/Visible Spectrophotometer, Digital oven, Magnetic stirrer , Mixer grinder , Water bath and Refrigerator etc. will be used to check the growth profile of selective probiotic strain.

4.5 Preparation of MRS-Cysteine medium

MRS broth will be prepared as per manufacturer's instructions and autoclave at 121°C, 15 psi for 15 min. After autoclaving, medium will cooled down to the room temperature and then 1 % (v/v) of filter sterilized (0.22 µm) Cysteine HCl will aseptically added to the prepared MRS medium and mixed carefully to avoid any gas bubble formation.

4.6 Growth profile of probiotic strains in MRS-Cysteine medium

MRS-Cysteine medium will be used for the revival of lyophilized probiotic cultures. In order to understand the growth pattern of probiotic bacteria in MRS-Cysteine medium, static fermentation will be carried out for 24 h at 37 °C.

4.7 Sensory evaluation of fermented product

The functional pumpkin beverage will be evaluated for their sensory characteristics namely appearance, aroma, flavour, taste, texture and overall acceptability. The evaluators will ask to record their observation on a hedonic scale based on 0-3 scale.

0 - Bad

1 - Good

2 - Very Good

3 - Excellent

4.8 Physico-chemical analysis functional pumpkin beverage

Functional pumpkin beverage will be analyzed for various Physico-chemical parameters like moisture content, total soluble solids, pH, titrable acidity, ash content, fat, protein, carbohydrates and energy value.

4.8.1 Determination of pH level

The pH of the pumpkin juice will be measured by withpH meter.

4.8.2 Determination of colour and viscosity

The colour of the pumpkin juice will be observed by with spectrophotometer and viscosity with viscometer (Jing Zhao et al., 2014).

4.8.3 Moisture content determination

The moisture content of the functional pumpkin beverage will be determined according to the Association of Official Analytical Chemists method (AOAC, 1995).For this, the sample (250 ml) will placed in an oven at 105 ºC for 3 h. Reading will taken at a constant weight. The moisture content then expressed as the percentage (%) of the dry weight of the sample (Olugbuyiro and Oseh, 2011).

Moisture content %= W_2-W_1/W×100

Where W_1 and W_2=weights of Petri plates along with the sample before and after drying respectively and W=weight of sample.

4.8.4 Titrable acidity

The titrable Heat the acidity will determined by measuring the produced lactic acid by titrating 20 ml sample with 0.1 N NaOH to pH 8.2 in the presence of phenolphthalein as an indicator. The used amount of NaOH (milliliter) will multiplied by two, and titratable acidity will thus obtained in Soxhlet-Henkel degrees (ºSH) while the volumetric productivity expressed in g $l^{-1}h^{-1}$, calculated by multiplying the ºSH by 0.225 and dividing by the fermenting time. (Bulatovic et al., 2012, Varga, 2006).

4.8.5 Total soluble solids

Total soluble solids will calculated by using hand Refractrometer

4.8.6 Fat content

The fat content will be determined by Soxhlet Method.

4.8.7 Protein content

Protein content was estimated by Kjedahl Method and the reagents used in this method like sulphuric acid, % NaOH solution, Kjedahl catalyst, Boric Acid etc.

4.8.8 Ash Content

Dry Ashing will carry out by incineration of food samples at a very high temperature (525 ^{0}C) in a muffle furnace. Ash is equivalent to the mineral content of the food sample. Accurately weighed samples 3g were taken in a tarred silica dish and ignited over a low flame to char organic matter. After complete charring, the dishes were placed in a muffle furnace and heated at 550 ^{0}C for 3-4 h, till greyish to off white colour ash was obtained. The silica dish containing ash was cooled in Desiccator and weighed. Percentage of total ash calculated as follows:-

$$\textbf{Ash (\%)} = \frac{\textbf{Weight of ash}}{\textbf{Weight of sample}} \times 100$$

4.8.9 Carbohydrate content

Total carbohydrates (%) = 100 - moisture (%) - protein content (%) - crude fat (%) - ash (%) (AOAC method, 2000)

4.8.10 Determination of Carbohydrates

The Carbohydrates like sucrose, glucose and fructose will determine byhigh-performance liquid chromatography (HPLC) system (P. Semjonovs et al., 2013).

4.8.11 Energy content

The energy content was measured in calorific value according to the system of Atwater, namely: kcal = (3.36 × % protein fresh weight) + (3.60 × % total carbohydrate fresh weight) + (8.37 × % fat).

EXPECTED OUTCOME

Preparation of functional probiotic pumpkin beverage using selective probiotic organism to develop a healthy beverage which would enhance the nutritional value of individual.

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