

Development of functional probiotic beverage from carrot juice

Dissertation 1 Report

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CERTIFICATE



This is to certify that has personally completed M.Sc. dissertation 1 entitled under my guidance and supervision. To the best of my knowledge, the present work is the result of hid original investigation and study. Part of dissertation-1 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.

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DECLARATION

I hereby declare that the work presented in the **dissertation 1** report entitled '**Development of functional probiotic beverage from carrot juice**' is my own and original. The work has been carried out by me at school of Agriculture, Lovely Professional University, Phagwara, Punjab, India; under the guidance of Er, Poorva Assistant Professor (Food Technology) at school of Agriculture, Lovely Professional University, Phagwara, Punjab, India, for the award of the degree of Master of Science in Nutrition and Dietetics.

Date: 14/05/2018

I certified that the above statement made by the student is correct to the best of my knowledge and belief.

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INTRODUCTION

Carrots juice has a high nutritional value, it is important dietary source of carotenoids such as alpha and beta-carotene, zeaxanthin, lutein and lycopene. Beta carotene, one of the most biologically active carotenoids, act as provitamin A. The preservation of carrot juice is due to the low acidity which provide ideal environment for the growth of many spoilage and spore forming bacteria. Blanching of carrots in acid is also improve color of carrot juice. Carrot are one of the major sources of vegetables used to human nutrition, it is the rich in beta carotene, ascorbic acid, tocopherol and classified by the vitaminized food. In this study is to determine and combination of carrot juices contraction and fermentation with probiotics production of probiotics and carrot juices was fermentation with streptococcus and bifidobacteria. According to the National Institute of Health a lack of vitamin A is one of the main preventable causes of blindness in children carrot juices are contain vitamin. They constitute about 10 % of the vegetables consumed in Sweden (National Food Administration, 2002) Carrots are a good source of carbohydrates and minerals like Ca, p, Fe and Mg. Gopalan et al. The carrot juice are obtained by hydraulic press and their effects on the most important quality parameters of carrot juice, such as beta-carotene, reducing the sugar, pectin, vitamin C viscosity, pH and acidity. Besides being one of the richest vegetable sources of viscous soluble dietary fibre, they also contain antioxidants, in particular, carotenoids, as well as vitamins C and E, phenolic compounds and phytate (Alasalvar et al. 2001). Their soluble dietary fibre is known to lower plasma cholesterol & medicinal properties alkaline elements which purify and revitalized the blood and it also has a reputation a vegetables that helps to maintain the good eyesight, it contain much water which will help in healthy hydration of the body system in transport of nutrients, elements of waste in body temperature regulation, and protein content is % 1.067 which will serve as a collagen for supporting tissue. the fermentation of carrot juice with probiotics and the microorganism like lactic acid, bifidobacteria, lactobacillus Acidophilus In this study composition of probiotic increase in level of protein content and reduction in level of carbohydrate than the fresh carrot juice and 100g of carrot contains 6to15mg carotenoids, B-carotene 2to10mg. The presence of these carotenoids and other antioxidant protect the types of cancer and cardiovascular diseases,

high blood pressure, Osteoporosis, cataract heart disease etc.the purpose of present carrot juice as a raw material for production of probiotic by lactic acid bacteria.

PROBLEM BACKGROUND

Probiotics are good for digestive system, gastrointestinal tract, and immune system and on the other hand, carrot juice help in improving muscle strength, immune system, reduce fat, build lean body mass and enhance performance.

In order to combine the properties of both these components, Selective probiotic micro-organisms will be inoculated in carrot juice so as to develop a healthy, functional carrot juice which would enhance the nutritional status of the individual

REVIEW OF THE LITERATURE

From N Muruganatham et al research it was found that carrot is more consumed in India. India is the greatest producer of carrot. 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)

Carrot 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 Acid and Tocopherols & Carotenoids as shown in the table No. 2.

Table 1 – Other Chemical Contents of Carrot Juice

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

Threonine			
Tyrosine			
Valine			

(Mi Young Kim et al., 2012)

2.2 Medicinal properties of carro

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

Table No.2 Medicinal properties of carrot

Species	Region	Part used	Activity	Application	References
<i>C.maxima</i>	World Wide	extract from <i>C.maxima</i> fruit	Catalytic activity	semiconductorspectroscopy, drug delivery, tissue imaging and cancer therapy	Jia Yu et al., 2016
		Extraction of essential oils from leaves	Antifungal, Antiaflatoxicogenic and Antioxidant Activity	Used as traditional medicine against different human diseases.	Priyanka Singh et al., 2010
		Leaf Extract from <i>C.maxima</i>	Larvicidal, Ovicidal and repellent activity	Applied as an effective personal protection measure against mosquito bites	Mullai k et al., 2006
		Crude Ethanolic Extract from <i>C.maxima</i> 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, Antihemolytic Activity	Used as a source of Antioxidant agents, functional food and Nutraceuticals	Arumugam Abirami et al.,2014
		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	Hepatoprotective Activity	Protect liver damage	Persona Saha et al.,2010
		Flowers	Antimicrobial Activity	Destroy Microbes	N.Muruganatham 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	Help to manage diabetes	Devesh kumar khushwaha et al.,2016

			Activity		
		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 <i>C.maxima</i> from Aerial parts	Anticancer Activity	Helpful in inhibit the tumour growth including increasing life span	U .K Mazumder et al.,2016
		Methanolic extract	Antioxidant Activity	Inhibit oxidative damage	Attarde D L et al.,2010
<i>C.moschata</i>	Worldwide	Extract from seeds	Anthelmintic 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
		Moschaminole	Antioxidant Activity, Scavenging Activity, Antimicrobial Activity	Inhibit oxidative and microbial damage	Yashodharan Kumarasamy et al., 2008
<i>C.pepo</i>	Worldw	Flesh	hypoglycaemic	Beneficial for diabetic	Asgary S

	ide	(powder)	and hypolipidemic Activity	patients	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

2.2.1Catalytic Activity

Gold nanoparticles (AuNPs) were extracted from extract of *C.maxima*. These particles are exhibit extensive 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 impact of concentration of AuNPs on the speed of catalytic reduction was evaluated by use of different quantities of AuNPs colloids but after the addition of gold colloids, reduction was ascertained by recording. When the size of gold nanoparticles decreases, there was an increase in the number of low-coordinated Au atoms which promote the absorption of the reactants on the catalyst surface and facilitates the reduction .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 andantiaflatoxigenic 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. During antifungal assay, the concentration of *C.maxima* oil had highly control from day-2 to day-10 according to ANOVA and turkey’s comparison tests. 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 Analysis of ethyl acetate fraction of *C.maxima*by disc diffusion method for Antimicrobial Activity showed the zone of inhibition for 10 mg/ml as 5 mm, 5 mm, 0 mm and 0 mm, for 20 mg/ml as 12 mm, 13 mm, 11 mm and 13 mm, for 30 mg/ml showing 20 mm, 16 mm, 18 mm and 23 mm and for 40 mg/ml as 29 mm, 25 mm, 28 mm and 30 mm, against *S.typhi*, *E. coli*, *E. faecalis* and *B. cereus* when compared with standard drug Chloramphenicol showing 17 mm, 18 mm, 23 mm and 21 mm zone of inhibition. For Anti-fungal Activity, the results are showed the zone of inhibition for 10 mg/ml as 0 mm and 0 mm, for 20 mg/ml as 16 mm and 13 mm, for 30

mg/ml as 19 mm and 20 mm and for 4e0 mg/ml as 22 mm and 30 mm against *C. lunata*, and *C.albicans* when compared with standard drug Fluconazole showing 21 mm and 19 mm of inhibition (N Muruganantham et al.,2016).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 Analysis of sterols extract,ethyl-5 α -cholesta-7,22,25-trien-3 β -ol by agar cup method showed its Antimicrobial Activity and the result of this experiment was concluded that the spinasterolis slightly active against the fungi *A. Niger* and *C. Albicans* with activity index (AI) of 0.3 at a concentration of 30 μ g.It is active against the gram-positive bacterium *B. Subtilis* and gram-negative bacterium *P. aeruginosa* with AI of 0.2 but it was inactive against the gram-positive bacterium *E. coli*, the gram-negative bacterium *S.aureus*, and the fungus *T. Mentagrophytes* (Consolacion Y. Ragasa et al., 2005).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* and the result revealed that this extract of *C.pepo* leaves showed the best MBC spectrum with the values below to 1024 μ g/ml recorded on 58.62% of the studied microorganisms (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 carrot 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* with the value of 0.001, 0.01 and 0.001 mg/mL (Yashodharan Kumarasamy et al., 2008)

2.2.3 Antioxidant Activity

To determine the antioxidant activity of essential oil of *C. maxima* 5(1:10 dilution in methanol) was spotted separately on TLC plate and developed in ethyl acetate and methanol (1:1). The plate was sprayed with 0.2% DPPH solution in methanol (2,2-diphenyl-1-picrylhydrazil) and left at room temperature for 30 min. 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).

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 (PECM), Chloroform extract (CECM) and Methanolic extract (MECM) of pericarp of *C. maxima* (MECM). By Attarde D L et al., 2010 it was concluded that Methanolic extract (MECM) of pericarp of *C. maxima* contain higher Antioxidant Activity as compared to Petroleum ether (PECM), Chloroform extract (CECM). (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. In result, an increase in both beta-carotene (2 to 4 minutes) and lycopene (17 to 40 minutes) content of pumpkin after cooking for 2, 4 and 6 minutes. For Phenolic content there was a loss from 18 to 54%. The extraction of Moschamindole from seeds of *C. moschata* was found to 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. These solvents are benzene, ethyl acetate, petroleum ether and methanol and the mosquito was *Culexquinquefasciatus*. *C.maxima* shows the *LC*₅₀ values of 123.02, 75.91, 117.73 and 171.64 ppm. The complete mortality was observed at 600 ppm for *C. Maxima*. During skin repellent test, *C.maxima* exerted the complete protection time of 78 to 215 minutes (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. This test is considered as the classical method for the primary screening of drugs with potential Antimalarial Activity. The treatment of infected mouse with the use of crude ethanolic extract and with pyrimethamine showed a protection against malaria.(Claudia Zuany Amorim et al., 1991).

2.2.6Antihelminthic

The experiment was conducted to confirm the Antihelminthic Activity of *C.moschata* by evaluating the in vitro effect of three seed extracts i.e. aquatic, methanolic and dichloromethaneagainst the parasitic nematode of small ruminants *Haemonchuscontortus*. These seed extracts were tested in vitro on four developmental stages of *H. contortus* using egg hatch assay (EHA), larval development assay (LDA), L3 migration inhibition (LMI) assay and adult worm motility (AWM) assay 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, as well as levels of plasma lactate, ammonia, glucose, and creatine kinase after an acute swimming exercise. 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 experiment, total 35 rats was categorized into 7 groups Group 1: Normal control; Group 2: Diabetic control; Group 3: Diabetics administered with low doses of pumpkin powder (1 g/kg); Group 4: Diabetics administered with high doses of pumpkin powder (2 g/kg), and Group 5: Diabetics administered with glibenclamide (0.6 mg/kg), as positive control. The rats were made diabetic by injected with alloxan (120 mg/kg body weight (BW)) and then treated for 4 weeks .In the result, insulin was found to be decreased in diabetic rat but other components i.e. . Glucose, cholesterol, triglycerides, low density lipoprotein (LDL) and C-reactive protein (CRP) 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, LDL and CRP 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). Necropsy evidences had showed that methanolic extract of *C. maxima* seeds had a preventive effect on formation of hydatid cyst in mice. There was no hydatid cyst formation takes place in mice treated with methanolic extract of *C. maxima* seeds (50 mg/ml in drinking water for 6 months) and Albendazole (150 mg/kg body weight/day for 10 days).

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 (HEPG2) and breast carcinoma (MCF7). These all extracts exhibit potentate tumour activities, potential cytotoxicity against liver carcinoma (HEPG2) and breast carcinoma (MCF7). (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 intake 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 contain Tyrosine inhibitory Activity, Tyrosine helps in the formation of Melanin .Melanin is a pigments which is responsible for enzymatic browning of fruits and vegetables and Over activity of this enzyme leads to hyper pigmentation of the skin. *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 ScavengingActivity

Arumugam Abirami et al., 2014 analysed the Scavenging Activity of *C.maxima*. The DPPH is dark purple colour, stable organic nitrogen centred free radical but it appeared colourless when it reacts with antioxidants to form non-radicals. The Scavenging Activity of *C.maxima* was analyzed by DPPH assay which was based on the principle that a hydrogen donor is an antioxidant and the results was expressed as a Trolox equivalent. The juice obtained from *C.maxima* showing Scavenging Activity by reducing DPPH to the yellow-colour diphenylpicrylhydrazine. The Scavenging Activity of *C.maxima* (red) was compared with *C. maxima* (White) and *C. Hystrix* and it was concluded that the *C.maxima* (red) higher Scavenging value as compared to *C.maxima* (White) and *C. Hystrix*.

The Superoxide anion radical scavenging activity was analyzed by Arumugam Abirami et al., 2014. Formation of Superoxide anion free radical like hydroxyl radical and hydrogen peroxide, results in oxidative destruction in biomolecules, such as DNA, lipids and proteins. 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 IC₅₀ 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. The free radical scavenging activity was analysed by DPPH assay(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 EDTAequivalents. 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. Oxidative damage leads to generation of a peroxy radical that attacks the erythrocyte to induce the chain oxidation of lipids and proteins, disturbs the membrane organization and eventually leads to hemolysis. 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 (AP) Activity ,mucosal thickness, 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 carrot

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 adding β -carotene, vitamin A precursor , desirable flavour , natural sweeteners to carrot bevrage. 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 carrot –Wheat Bread From carrot was developed by Tatjana Rakcejeva et al 2011 and Compare it with wheat bread sample (without carrot) by measuring its various components like moisture content , vitamin C content , carotenoid content , reducing sugars , colour changes , 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 carrot (*C.pepo*) - By Nyam KL et al 2013 it was found that the fibres obtained from Seeds and Rind of the *C.pepocan* be used in the development of Bakery Products. For the development of Bread, fibres extracted from carrot seed and rind was

substituted with 0%, 5%, 10% in dough and various properties of this product was analysed like appearance, aroma, flavour, texture, overall acceptability. Also the physical properties dough expansion, loaf volume, crumb colour, bread texture. (Nyam KL et al 2013)

2.3.4 Fibre Rich Food from carrot (*C.moschata*) –production of Fibre rich food from carrot (*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 carrot 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 carrot Vitamin C and carotene content of carrot 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 carrot 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 Carrot Flour- carrot Flour is very useful product, from carrot flour production of various type of products was carried out like noodles, biscuits, cake, ice cream, pasta, sweet etc. From this point of view carrot 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 carrot. 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 carrot biscuits was developed by using carrot powder in place of wheat flour at different levels i.e.0, 2.5, 5.0 7.5 and 10% (w/w) in the standard formulation. 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 Carrot, 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, carrot 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 carrot pasta was prepared by the addition of various other ingredients like egg yolks, egg white, sugar, wheat flour, butter, milk powder, baking powder and ovalet.

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 carrot was also prepared by Aniswatul Khamidah et al., 2013. By Yun et al., 1999, Carrot 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, Carrot Sauce was considered as a good alternative source. Carrot Sauce is bright golden orange in colour and has high content of β carotene (Aniswatul Khamidah et al., 2013). The sweet carrot-doenjang sauce was also prepared by Chang, Kyung-Ho et al., 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). Pumpkin sauce was also prepared by LIU Zhenget al 2011. In the result it was stated that using 500 g pure water per 100 g raw pumpkin, adding 12 g sugar, 5 g xylitol, 0.3 g citric acid, 0.3 g sodium alginate for Sauce preparation give best carrot sauce in respect with consistency, unique taste etc. (LIU Zheng et al 2011). The soy Sauce from carrot 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 carrot and carrot 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.

2.3.15 Jelly candy—Jelly is a clear transparent Candy prepared by mixing pasta carrot 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 carrot crackers 250 gram, 200 gram tapioca flour, wheat flour 50 g and 0,011ug/g beta-carotene content. (Aniswatul Khamidah et al., 2013)

2.3.17 Chips—Chips is a fry product, carrot Chips was prepared by vacuum frying technology due to higher water Content of carrot 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).

By Parastoo Yasaie Mehrjardi et al., 2012 carrot Chips was prepared by Vacuum Frying at 84.53°C, pressure of 40 mbar for 18 minutes. (Parastoo Yasaie Mehrjardi et al., 2012)

2.3.18 Ice – cream – Ice cream mainly Contain milk fat globule, air, small crystals of ice, and the water. Carrot 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. Carrot jam was prepared by adding carrot 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).

2.4 Probiotics

Probiotics are live microorganisms that when administered in adequate amounts confer a health benefit on the host (UNFAO/WHO 2001).Probiotics are commonly isolated from human and animal intestinal tracts. Dead bacteria and end product of bacterial growth also may transmit some health benefits but they are not considered as probiotics because they are not alive when administered.

Over the past two decades consumption of probiotic drink is increasing due to the awareness of people about the health benefits of these drinks (Ostlie et al., 2005). Probiotics can be consumed in any form but mainly they are marketed as capsules, powders, enriched yogurts, yogurt-like products and milks.

The history of probiotics

The origin of cultured dairy products dates back to the dawn of civilization; they are mentioned in the Bible and the sacred books of Hinduism. Climatic conditions for sure favoured the development of many of the traditional soured milk or cultured dairy products such as kefir, koumiss, leben and dahi. These products, many of which are still widely consumed, had often been used therapeutically before the existence of bacteria was recognized. At the beginning of the

20th century the main functions of gut flora were completely unknown. Ilya Ilyich Metchnikoff, the Nobel prize winner in Medicine in 1908, at the Pasteur Institute linked the health and longevity to ingestion of bacteria present in yoghurt. He believed that the constitution of the human body presented several disharmonies inherited from primitive mammals, such as body hair, wisdom teeth, stomach, vermiform appendix, caecum, and large intestine. In 1907, he postulated that the bacteria involved in yoghurt fermentation, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, suppress the putrefactive-type fermentations of the intestinal flora and that consumption of these yoghurts played a role in maintaining health. Indeed, he attributed the long life of Bulgarian peasants to their intake of yoghurt containing *Lactobacillus* species. In particular, he reported that the large intestine, useful to mammals in managing rough food composed of bulky vegetables, is useless in humans. Moreover, it is the site of dangerous intestinal putrefaction processes which can be opposed by introducing lactobacilli into the body, displacing toxin-producing bacteria, promoting health, and prolonging life. (A. Hosono et al. 1992)

Probiotic Microorganisms

The probiotic potential of different bacterial strains, even within the same species, differs.

Different strains of the same species are always unique, and may have differing areas of adherence (site-specific), specific immunological effects, and actions on a healthy vs. an inflamed mucosal milieu may be distinct from each other.

According to Shah and Chow: the most popular strains are represented by the following genera:

Lactobacillus, *Streptococcus*, and *Bifidobacterium*. (Shah et al. 2007).

Probiotics Microorganism

Microorganism	Strain	Company Product	References
Bifidobacterium Adolescentis	ATCC 15703	Chr-Hansen	V. Krishna kumar (2001)
Bifidobacterium Animalis	BB-12	Chr-Hansen	V. Krishna kumar (2001)

Bifidobacterium bifidum	Bb-11	Chr-Hansen	V.Krishna kumar (2001)
Bacillus lactis	DR10		V.Krishna kumar (2001)
Lactobacillus Acidophilus	LA-1/LA-5 NCFM DD5-1	Danisco (Howaru™)	V.Krishna kumar (2001)
Lactobacillus Bulgaricus	SBT-2062	Chr.Hansen	V.Krishna kumar (2001)

V. Krishnakumar et al. (2001)

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 Anticarcinogenic properties.
- Antagonistic activity against pathogens such as *Helicobacter pylori*, *Salmonella* sp., *Listeria monocytogenes* and *Clostridium difficile* (Saarela et al., 2000).

2.4.2 Mechanisms of probiotic activity

Exact mechanism of their action is not yet known but they act by the following:

- Production of bacteriocin and short chain fatty acid.
- Lowering of gut pH.
- Nutrient competition to stimulation of mucosal barrier function and immunomodulation.
- Induce phagocytosis and IgA secretion.

- Modify T-cell response (Guarner and Malagelada, 2003, McNaught and MacFie, 2001, Isolauri et al., 2001)

2.4.3 Health benefits of probiotic bacteria

The microorganisms present in the colon act as a barrier to pathogenic microorganisms but these microorganisms diminish their integrity due to stress, illness, antibiotic treatment, changes in diet, or physiological alterations in the gut. Consumption of probiotics maintains the balance of microorganisms in the gut and prevents the risk of many diseases such as gastrointestinal disorders including gastrointestinal infections, inflammatory bowel diseases, and even cancer by reinforcing the body's natural defence mechanisms (Macfarlane and Cummings, 1999, Saarela et al., 2000). Due to their health benefits probiotic bacteria have been increasingly included in yoghurts and fermented milks during the past two decades.

2.4.3.1 Probiotics as Diarrhoea treatment

Rotavirus is the most common cause of severe diarrhoea in the infants and young children. *Bifidobacteria* (which constitute the predominant intestinal flora of breastfed infants), as well as other lactic-acid-producing organisms such as *Streptococcus thermophilus* were found to have a protective effect against acute diarrhoeal disease (Saavedra et al., 1994).

S. thermophilus, *L. bulgaricus*, *L. acidophilus*, and *B. bifidum* were also found to be effective against enterotoxigenic *E. coli* a causative agent to traveller diarrhoea (Black et al., 1989).

Probiotics microorganisms such as *L. rhamnosus*, *L. casei*, and the yeast *S. boulardii* was also found to reduce the risk of antibiotic associated diarrhoea caused by *Clostridium difficile*.

2.4.3.2 Probiotics for lactose intolerance

Lactose intolerance is the inability to digest and absorb lactose (the sugar in milk) that results in gastrointestinal symptoms such as abdominal discomfort, flatulence and diarrhoea when milk or food products containing milk are consumed. It is basically due to the deficiency of beta galactosidase enzyme which is required for the hydrolysis of lactose. Probiotic bacteria such as *S. thermophilus* and *L. delbrueckii* sp. *Bulgaricus* have high beta galactosidase activity and improve the metabolism of lactose in the lactose intolerant people (Kechagia et al., 2012).

2.4.3.3 Probiotics as allergy treatment

Probiotic bacteria also found to be effective against food allergen and atopic dermatitis. *B. lactis* and *L. rhamnosus* GG were found to be effective in decreasing the eczema severity in the infants. *L. rhamnosus* GG has been found successful in preventing the occurrence of atopic eczema in high risk infants, when supplied prenatally to selected mothers who had at least one first degree relative with atopic eczema, allergic rhinitis, or asthma.

2.4.3.4 Probiotic bacteria against cancer

Probiotic bacteria reduce the risk of cancer by decreasing the levels of carcinogenetic enzymes produced by colonic flora through normalization of intestinal permeability and microflora balance as well as production of Antimutagenic organic acids and enhancement of the host's immune system (Hirayama and Rafter, 1999).

PROBLEM STATEMENT

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 carrot beverage which would enhance the nutritional status of the individual.

OBJECTIVES

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

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 carrot juice.

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. Carrot 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 be cooled down to the room temperature and then 1 % (v/v) of filter sterilized (0.22 µm) Cysteine HCl will be 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.

Procedure

Carrot Seed culture will be prepared by inoculating lyophilized culture (1%) of probiotic in 70 ml MRS-Cysteine broth in 100 ml schott bottle and then incubated statically at 37°C for 24 h.

1. 10% (v/v) of primary seed culture then transferred into 70 ml secondary seed culture (MRS-Cysteine broth) and cultivated for 24 h at 37°C in static condition.
2. Secondary seed culture (10%, v/v) again transferred to 70 ml MRS-Cysteine broth and incubated at 37°C for 24 h and designated as tertiary seed culture. After each 2 h of incubation, whole cell culture fluid (2 ml) will be withdrawn to monitor the growth profile of probiotic bacteria. Optical density will be measured at 600 nm.

4.7 Sensory evaluation of fermented product

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

0 - Bad

1 - Good

2 - Very Good

3 - Excellent

4.8 Physico-chemical analysis functional carrot juice

Functional carrot juice 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 carrot juice will be measured by with pH meter.

4.8.2 Determination of colour and viscosity

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

4.8.3 Moisture content determination

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

$$\text{Moisture content \%} = \frac{W_2 - W_1}{W} \times 100$$

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 acidity will be 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 be multiplied by two, and titrable acidity will thus be obtained in Soxhlet-Henkel degrees (°SH) while the volumetric productivity expressed in $\text{g l}^{-1}\text{h}^{-1}$, calculated by multiplying the °SH by 0.225 and dividing by the fermenting time. (Bulatovic et al., 2012, Varga, 2006).

4.8.5 Total soluble solids

Total soluble solids were calculated by using hand Refractometer.

4.8.6 Fat content

The fat content will be determined by Soxhlet Method.

Reagents

- Petroleum ether

Method

1. Place the bottle and lid in the incubator at 105° C overnight to ensure that weight of bottle is stable.
2. Weigh about 3- 5 g of sample to paper filter and wrap.
3. Take the sample into extraction thimble and transfer into Soxhlet.
4. Fill petroleum ether about 250 ml into the bottle and take it on the heating mantle.
5. Connect the Soxhlet apparatus and turn on the water to cool them and then switch on the heating mantle.
6. Heat the sample about 14 h (heat rate of 150 drop/ min).
7. Evaporate the solvent by using the Vacuum condenser.
8. Incubate the bottle at 80 – 90 ° C until solvent is completely evaporated and bottle is completely dry.
9. After drying, transfer the bottle with partially covered lid to the Desiccator to cool. Reweigh the bottle and its dried content.

4.8.7 Protein content

Protein content was estimated by Kjeldahl Method

Reagents

- Kjeldahl catalyst: Mix 9 part of potassium sulphate with 1 part of copper sulphate.
- Sulphuric acid
- 40 % NaOH solution

- 0.2 N HCL solution
- 4 % Boric Acid
- Indicator solution : Mix 100 ml of 0.1 methyl red (in ethanol) with 200 ml of 0.2 % bromocresol green (in 95 % ethanol)

Method

1. Place sample (0.5 – 1.0) in digestion flask.
2. Add 5g Kjeldahl catalyst and 200 ml of conc. Sulphuric Acid.
3. Prepare a tube containing the above chemical except sample as blank. Place flask in inclined position and heat gently until frothing ceases. Boil briskly until solution clears.
4. Cool and add 60 ml of distilled water cautiously.
5. Immediately Connect flask to digestion bulb on condenser and with tip of condenser immersed in standard acid and 5-7 drops of mix indicator in receiver. Rotate flask to mix Content thoroughly, and then heat until all ammonia is distilled.
6. Remove receiver, wash tip of condenser and titrate excess standard acid distilled with standard sodium hydroxide.

4.8.8 Ash Content

Dry Ashing was carried out by incineration of food samples at a very high temperature (525 °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 °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:-

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

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 by high-performance liquid chromatography (HPLC) system (Jing Zhao et al., 2014).

4.8.11 Energy content

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

EXPECTED OUTCOME

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

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