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IN VITRO EXPLORATION ON DEVIATION IN CERTAIN PHYSICO-CHEMICAL PROPERTIES OF *DAUCUS CAROTA* JUICE SUPPLEMENTED WITH PROBIOTIC *LACTOBACILLUS ACIDOPHILUS*

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ABSTRACT

In recent days, efforts are being made to efficiently employ the probiotic bacteria in non-dairy products either as supplemental additive or for fermentation which might have several advantages over the probiotic dairy products such avoiding milk protein allergies, lactose intolerance or hyper cholesterolemia. *Daucus carota* or carrots are one of the most important vegetables appreciated as nutritious diet world over especially in India for flavor, richness in minerals, vitamins and dietary fibers. Thus the present study aimed at evaluation of some physicochemical properties of carrot juices when supplement with lactic acid bacteria after certain period of time on nutritional and medicinal perspectives. The harvested cells of *Lactobacillus acidophilus* (MTCC-447) cultured in MRS broth were used to inoculate in fresh carrot juice followed by incubation at ambient temperature for 30 days. The physicochemical evaluation of carrot juice samples under study was done for colour, smell, texture, pH, total soluble solids (TSS), and estimation of total flavonoidal content in comparison to the carrot juice added with 1% benzoic acid, and control with no additives. The quality of carrot juice sample with *L. acidophilus* were reported to be more closer to the properties of fresh juice in terms of pH, TSS and TFC followed by samples the 1% benzoic acid whereas control samples without any additive was quite deteriorated in due course of 30 days. It could be elucidated that fruit juices like carrot juice may also serve as an excellent medium for probiotic delivery that could be improved practically upon further extensive exploration.

Keywords: Probiotics, *L. acidophilus*, Carrot Juice, TFC.

INTRODUCTION

Certain living organisms from genera LAB or Lactic Acid Bacteria including Leuconostoc, Enterococcus, *Lactobacillus, Lactococcus, Bifidobacterium, Pediococcus* and *Streptococcus*, which when used as food additives or supplemental food imparts health benefits to the consumers by maintaining the microbial equilibrium within the gastrointestinal tract. Such beneficial microbial species are regarded as probiotic microorganisms [1,2].

Probiotic strains of Lactobacillus, Bifidobacterium and Saccharomyces are considered GRAS (Generally Recognized as Safe), where Genus Lactobacillus have been extensively investigated that includes

genetically and physiologically diverse variety of bacteria which are characterized Gram- positive, non-sporing, forming, cocci or rod shaped, Catalase-negative and fastidious organisms that have been used as starter culture for fermentation of a wide range of food production including milk and milk products [3-5].

Probiotic microorganism is generally employed in dairy products like flavored milks, yogurts, icecreams, cheese etc., in general in order to have the therapeutic benefits of probiotic functional food [6]. But in recent days, efforts are being made to efficiently employ the probiotic bacteria in non-dairy products either as supplemental additive or for fermentation which might have several advantages over the probiotic dairy products such avoiding milk protein allergies, lactose intolerance or hyper cholesterolemia.

As the consumers are now becoming conscious regarding the diet for nutrition, health support and safety and hence getting aware of consuming food added with functional components, such as probiotic cultures or prebiotic components. Recently, fruits and vegetables have been suggested as option for incorporating probiotic microorganism since they are highly rich in mineral nutrients, vitamins and several polyphenolic compounds and behaves an excellent medium for probiotics [7].

Daucuscarota or carrots are one of the most important vegetables appreciated as nutritious diet world over especially in India for flavor, richness in minerals, vitamins and dietary fibers. Carrots are rich sources of provitamin A, and carotenes (a-, b- , g- and z-carotenes, lycopene and b-zeacarotene), terpinoids (terpenes, sesquiterpenes) and polyphenols like neochlorogenic acid (3'-caf-feoylquinic acid), chlorogenic acid (5'-caffeoylquinic acid), 3'-p-coumaroylquinic acid, 3'-feruloquinic acid, 3'4'-dicaffeoylquinic acid, 5'-feruloylquinic acid, 5'-p-coumaroylquinic acid, 4'-feruloylquinic acid, 3'5'-dicaffeoylquinic acid, 3'4'-diferuloylquinic acid and 3'5'-diferuloyoquinic acid [8,9]. Because of the richness of provitamin-A, carrot juices are used in production of alpha-tocopherolbeta- carotene drinks (ATBC-drinks) due to its excellent physical and chemical stability [10]. Thus the present study aimed at evaluation of some physicochemical properties of carrot juices when supplement with lactic acid bacteria after certain period of time on nutritional and medicinal perspectives.

MATERIALS & METHODS

Sample Collection:

For present experiment, the MTCC culture of *Lactobacillus acidophilus* (MTCC-447) was used. The carrots were purchased from local market of Bhopal for preparation of fresh carrot juice.

Mass Culture of Lactobacillus spp.:

The lyophilized MTCC culture of *L.acidophilus* was first revived on MRS broth (HiMedia, India Pvt Ltd) by inoculating them in tube containing 5 ml of sterile MRS broth followed by incubation of 24-48

hours at 37^{o}C. Upon gaining the turbidity in the medium small volume of revived culture was used to scale up the propagation of *L. acidophilus* strain in 100 ml volume in condition of nutrition and incubation till the turbidity reaches for absorbance value of 1 at 600 nm in colorimeter. The *L. acidophilus* bacterial cells were them harvested by centrifugation at 5000 rpm for 15 minutes at ambient temperature, the cell pellets were retained by discarding supernatant followed by re-suspending the cells in 1 ml of sterile normal saline solution. This suspension of cells was used to inoculate the juice samples.

***Addition of Lactobacillus in Carrot Juice*:**

The carrot roots were washed cleaned and peeled followed by preparing juice in domestic food processor/juicer. The juice was filtered through clean muslin cloth twice and the cleared juice was pasteurized by steaming in steam bath for 20 minutes. The juice was now divided into 3 sets in sterile 125 ml conical flasks each containing approx. 50 ml of juice. One of the flasks was inoculated with 1 ml of harvested *L. acidophilus* culture suspension, second flask was added with benzoic acid at a rate of 1%while other flask was left uninoculated to be used as control. The sets were left at room temperature maintaining aseptic conditions for 30 days and used for assessment by then.

Physicochemical and Microbial Evaluation of Juice:

The physicochemical analysis and microbial evaluation of carrot juice samples under study was done after 30 days for colour, smell, texture, pH, total soluble solids (TSS), and estimation of total flavonoidal content.

Estimation of TFC

The total flavonoid content of carrot juices were done were determined spectrophotometrically by aluminum chloride method [11]. For this, small amount of juice were suitably diluted with distilled water upto 4 ml to which 0.3 mL of 5% $NaNO_2$ solution; 0.3 mL of 10% $AlCl_3$ solution was added after 5 min of incubation, and the mixture was allowed to stand for 5 min. Then, 2 ml of 1M NaOH solution were added and final volume of 10 ml with double-distilled water was made. The absorbance of the mixture was measure at 510 nm after 15 minutes of incubation. The total flavonoid content was calculated by comparing the absorbance of the samples with standard curve of quercetine.

RESULTS AND DISCUSSION

From the experiment it was observed that the addition of microbial strain in have influence on the quality of carrot juice after 30 days of incubation at room temperature. The physical assessment of juice was done by visual and sensory observation, where pH was measured by digital pH meter (Electronic India) while TSS was measured by refractometer (Irma, Japan) as per the method suggested by AOAC, (1995) the results of which are depicted in table 1.

Table 1: Results of Physical evaluation of carrot juices after 30 days of incubation at room temperature.

S. N.	Juice Sample	Additions	Physical Parameters after 30 days Compared to Fresh Juice				
			Colour	Smell	Texture	pH	TSS
1	DC -1	*L. acidophilus*	Reddish Pink	Organic	Viscid	5.2	8.1%
2	DC -2	Benzoic acid	Brownish Pink	Organic	Non-Adhering	6.4	8.3%
3	DC -3	Control	Reddish Brown	Unpleasant	Slimy	4.8	9.4%
4	DF	FJ Control	Reddish Pink	Organic	Dense Smooth	5.8	7.4%

The evaluation as mentioned in table 1 including pH and TSS of carrot juices supplemented with probiotic *L. acidophilus* indicates that its properties are more or less similar to the properties of fresh juice even after the 30 days of incubation at room temperature compared to juice with 1% benzoic acid and juice samples with no preservatives. pH of juice is related to the acidic taste of juice according to Rosnah*et al*., (2012)[12] where in present work, the pH of juice with probiotic bacteria was reported to be 5.2 after 30 days which is comparable to fresh juice with pH 5.8 contrary to juice with chemical preservative and control samples which showed a larger deviation in pH change. Similar was the case of total soluble solids.

Estimation of TFC

Total flavonoids content was calculated as quercetin equivalent (mg/ml) using calibration curve graph (figure 1) based on the equation:

$$\mathbf{Y=0.040X + 0.009,\ R^2=0.999},$$

Where: X = absorbance and Y =quercetin equivalent (QE).

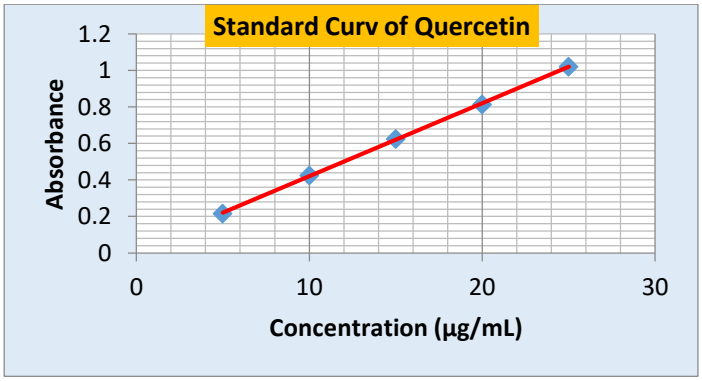

Figure 1: Standard Curve of Quercetin for Estimation of Total Flavonoid Content at 510 nm by Aluminum Chloride Precipitation

Table 2:Results of total flavonoid estimation in carrot juices after 30 days of incubation at room temperature.

S.N.	Juice Sample	Additions	TFC after 30 days In mg/ml
1	DC -1	*L. acidophilus*	1.36 mg/ml
2	DC -2	Benzoic acid	1.14 mg/ml
3	DC -3	Control	0.71 mg/ml
4	DF	FJ Control	1.54 mg/ml

The results of total flavonoid estimation in carrot juices with different addition compared to fresh juice is depicted in table 2. It was observed that the TFC values carrot juice samples when supplemented with *L. acidophilus* culture remains stable more than the samples with benzoic acid like preservatives where control samples showed least concentration of TFC when compared to TFC in fresh juice. TFC estimation suggest that when carrot juices allowed to stand on shelf results into deterioration of beneficial flavonoid components due to several reasons, but addition of probiotic may improve the stability of flavonoids in juices.

The changes in physico-chemical parameters carrot juice samples in terms of investigation are somewhat coinciding the outcomes of Leahu, *et al*., (2013)[13] who estimated total phenolic compounds. Lu, *et al*., (2018)[14] recently while working with fruit beverage & probiotics stated that selection of appropriate probiotics can be an alternative way to develop new functional beverages from star fruit juice with specific aroma notes.

CONCLUSION

From the outcome of present investigation it can be concluded that addition of probiotic microbial species like lactobacillus could possibly improve and maintain the nutritional and medicinal quality fruit juices like carrot juice. The fruit juices may also serve as an excellent medium for probiotic delivery that could be improved practically upon further extensive exploration.

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