DR-1690
[1-7]

RESEARCH ARTICLE Asian Journal of Dairy and Food Research

Health-promoting Effects of Developed Probiotic Orange Beverage: An *in vitro* Study

Deeptimayee Mahapatra, Mamoni Das

10.18805/ajdfr.DR-1690

ABSTRACT

Background: Probiotic food has evolved as the new trend among the health fanatics because of their proven benefits in preventing many diseases. With change in time the way of consuming probiotics has also changed. Unlike past dairy is not the only option for commercial probiotic production, recently fruit juices have become the popular choice for it. So the current study aimed to assess the feasibility of orange juice (*Citrus reticulate*) as a potential probiotic carrier for the production of probiotic orange juice with lactic acid bacteria.

Methods: Three test samples (TS) were developed with different combination of lactic acid probiotic bacteria *viz*. test sample 1 (TS1) (*L. bulgaricus* and *L. casei*), TS2 (*L. bulgaricus, L. casei* and *L. gasseri*) and TS3 (*L. bulgaricus, L. casei, L. gasseri* and *L. fermentum*). The orange juice was pasteurized for 2 min at 90°C and was inoculated at a rate of 10% inoculum. All the test samples were fermented for 4 hrs at 37°C and the physicochemical and nutritional characteristics were evaluated along with their *in vitro* hypocholesterolemic and *in vitro* hypoglycemic efficacies.

Result: The probiotic orange test samples did not show inferior properties than the control in terms of physicochemical and nutritional properties. The bacterial count was decreased with time but remained above standard limit (10^7cfu/100ml) until 28th day of refrigerated storage. All the test samples showed promising antioxidant activity, *in vitro* hypocholesterolemic activity and *in vitro* hypoglycemic activities. Hence orange juice could be used as a suitable probiotic carrier for production of novel probiotic beverages.

Key words: Hypocholesterolemic efficacy, Hypoglycemic efficacy, *In vitro*, Nutritional characteristics, Orange beverage, Probiotics.

INTRODUCTION

Now-a-days healthy food means “functional foods”, which provides specific health promoting effects other than their traditional nutritional benefits. In addition to the well established functional ingredients like vitamins, minerals, and micronutrients probiotics have emerged as a new class of active functional ingredients (Jankovic *et al.,* 2010). In Latin the term probiotic means “for life” which means these are essential for life and can be bacteria (Lactobacillus, bifidobacterium *etc*.) or yeast (Saccharomyces). Literature suggested that the use of probiotics in the form of fermented food was started from Egyptian and Tibetan times as a method to carry milk for long treks (Guo *et al.,* 2016). Since that time dairy has always been exploited for probiotic food production. But recently the introduction of concepts like high cholesterol percentage in milk products, increase of cardiac disease incidence rates, diets like vegan and conditions like lactose intolerance, has accentuated the need for non-dairy functional food options (Granato *et al*., 2010). Hence fruit and vegetable juices come into existence as an alternative for probiotificated dairy drinks. Besides juices are a good source of both macro (sugars and fiber) and micro (vitamins and minerals) nutrients, which are valuable components to human health. Again the current craze of new generations towards healthier diets in less time and labor has made juices an important natural food alternative.

Different fruit juices are a more agile and effortless way of consuming a required dietary portion of fruits for a day and addition of probiotic to fruit juices would increase the nutritional properties of the juices and would provide additional health benefits (Zuntar *et al*., 2020). Several researches are present indicating fruit juices like mango, watermelon, grape, pomegranate, peach, noni, mulberry and pineapple *etc*. as potential probiotic carriers (Okereke *et al.,* 2016, Mousavi *et al.,* 2011, Thakur and Sharma *et al.,* 2017, Pakbin *et al.,* 2014, Chaudhary *et al.,* 2019, Ghafari and Ansari, 2018). But fruits from citrus family are experimented less for probiotification due to their acidic nature. So an attempt was made to develop probiotificated orange juice in the present study because of their vast health effects. Orange juice is one of the most consumed fruit juice around the world (Galaverna and Dall’Asta, 2014). It is a good source of vitamin-C, folate and polyphenols (Ohrvik *et al*., 2008, Galaverna, *et al*., 2014). Studies have reported long term consumption of orange juice can reduce total

Department of Food Science and Nutrition, Assam Agricultural University, Jorhat -785 013, Assam, India.

Corresponding Author: Deeptimayee Mahapatra, Department of Food Science and Nutrition, Assam Agricultural University, Jorhat -785 013, Assam, India. Email: d.mahapatra.hm18@aau.ac.in

How to cite this article: Mahapatra, D., Das, M., (2021). Health-promoting Effects of Developed Probiotic Orange Beverage: An *in vitro* Study. Asian Journal of Dairy and Food Research. DOI: 10.18805/ajdfr.DR-1690.

Submitted:19-04-2021 **Accepted:** 08-11-2021 **Online:** 28-12-2021

cholesterol, LDL, LDL/HDL ratio and apo B, with significantly increase the folate and Vit-C content of blood (Aptekmann and Cesar, 2013). Evidences also suggested that orange juice consumption can also increase the concentration of HDL cholesterol (Ali *et al*., 2015, Kurowska *et al*., 2000) which makes it a healthier choice.

MATERIALS AND METHODS

The experiment was conducted at the laboratories of Assam Agricultural University, Assam during the session of 2019-2020. Mature and healthy oranges were selected randomly from a lot of oranges for the study.

Determination of physical parameters of oranges (*Citrus reticulate*)

Weight of the fruits

Weights were recorded for 7 oranges consecutively and the mean was expressed in gram per fruit.

Peel percentage

Peel percentage was determined by taking the peel weight of 7 oranges consecutively.

$$\text{Peel percentage (\%)} = \frac{\text{Peel wt}}{\text{Fruit wt}} \times 100$$

Juice content

Juice content was determined by extracting juice of 500 g fruit each for 3 times and expressed as percent juice content (Gupta *et al.,* 2009).

$$\text{Juice content (\%)} = \frac{\text{Juice content}}{\text{Fruit wt}} \times 100$$

Preparation of starter culture

The strains were revived in 50 ml MRS broth separately and incubated for 45 hrs at 37°C as per the method used by Shukla *et al.,* (2017) with little modification. All the strains were sub-cultured and incubated for 24 hrs before used for fermentation of orange juice.

Fermentation of orange beverage

Freshly pressed orange juice was autoclaved at 90°c for 2 min according to the method outlined by Deshpande *et al.,* (2019). A 10% (v/v) inoculum was used to ferment all the three test samples (Deshpande *et al.,* 2019). Three different combinations of strains were used to ferment orange juice test samples and were named as test sample 1(TS1), test sample 2 (TS2) and test sample 3 (TS3). TS1 contained L. *bulgaricus* and L. *casei* (1:1), TS2 contained *L. bulgaricus*, L. *casei* and L. *gasseri* (1:1:1) and TS3 contained L. *bulgaricus*, L. *casei*, L. *gasseri* and L. *fermentum* (1:1:1:1) respectively. After inoculation the test samples were incubated for 4 hrs at 37°C and were then stored at 4°C till further evaluations.

Enumeration of viable bacteria

The viable cell count of each orange test sample was evaluated through standard pour plate method using the formula-

$$\text{CFU/ml} = \frac{\text{Average no of colonies found}}{\text{Dilution factor}} \times \text{Aliquot of sample taken}$$

Physico-chemical analysis of probiotic orange beverage

Physico-chemical properties like pH, TTA, colour and TSS were determined using standard methods outlined by AOAC (2005).

Nutritional analysis

Test samples were subjected to nutritional evaluations using different suitable methods. The total carbohydrate was evaluated through anthrone method (Hofreiter, 1962). Crude protein, crude fat and crude fiber contents were evaluated by the methods outlined by Ekanem and Ekanem in 2019. The potassium content of the sample was measured through the method described by Nerdy in 2018 and Vitamin-C content was measured through standard AOAC method.

Antioxidant activity

Antioxidant activity/DPPH free radical scavenging activity was determined as per the procedure described by Delgado *et al*., (2010). 12 ml of juice was blended with 3 ml methanol and water solution (4:1) and mixed well.100 ml of methanol was taken and 0.0024 g DPPH (2, 2-diphenyl-1-picrylhydrazyl) was added to prepare DPPH solution. 300µL of ethanol blended sample was added to 2.7 mL of DPPH solution. After addition of DPPH solution the test tubes were kept in dark at room temperature for 1 hr. After 1 hr the absorbance of sample, DPPH solution and blank [methanol/water (4:1)] were measured at 517 nm. DPPH radical scavenging activity was calculated from the graph of DPPH radical scavenging activity percentage *vs*. extract concentration.

Calculation:

$$\text{DPPH radical scavenging activity} = \frac{\text{A (DPPH)} - \text{A (Sample)}}{\text{A (DPPH)}} \times 100$$

Where,

A_{DPPH} = Absorbance of DPPH

A_{Sample} = Absorbance of sample

In vitro hypocholesterolemic efficacy

In vitro hypocholesterolemic efficacies of all the samples were performed according to the procedure cited by Liong and Shah (2005) with some modifications. MRS broth was prepared and 0.3% oxgall (bile salt) was added and autoclaved at 121°C for 15 minutes. Cooled MRS media was supplemented with water soluble cholesterol procured from Hi media. 1 ml of orange juice was added to 9 ml of cholesterol mixed broth and incubated for 24 hr at 35°C. After incubation the mixture was centrifuged for 10 min at 10000 rpm. 1 ml of supernatant was transferred to a sterilized test tube. 1 mL of KOH (33%w/v) and 2 mL of absolute

ethanol was added to the supernatant, the mixture was vortexed for 1 min to ensure mixing. Then the mixture was incubated for 15 min at 35°C. The mixture was cooled down a little and 2 mL of distilled water and 3 mL of hexane was added to it. The mixture was vortexed for a min and set aside for 1-2 min. Two separate layers were appeared among which hexane formed the upper layer. 1 ml of hexane layer was taken and the contents were evaporated. The residue was dissolved in 2 mL of o-phthalaldehyde solution. Concentrated sulphuric acid (0.5 mL) was added to the mixture and then incubated at room temperature for 10 min. Absorbance of the mixture was measured at 550nm.

Calculation

$$\text{Cholesterol reduction (\%)} = 1 - \frac{\text{A (culture supernatant)}}{\text{A (control)}} \times 100$$

***In vitro* hypoglycemic efficacy**

10 ml of sample was added with 10 ml of MRS broth containing 20% glucose in it. Mixture of sample and MRS broth was incubated at 37°C and aliquots were collected at 0 and 24 hrs and the remaining glucose in the medium were measured by a Blood glucose meter (Wang *et al.,* 2020).

RESULTS AND DISCUSSION

Physical and physicochemical characteristics

The orange variety *Citrus reticulate* is used for the production of probiotic orange beverage. The average weight for the orange is 120.41±7.04 g, the average peel percentage is 28.79±1.23% and the average juice content is 47.14±2.54% respectively (Table 1). The TSS and pH of orange juice decreased during the fermentation process in the fermented and non-fermented (Control) orange beverage samples (Table 2). But no significant change was observed among the test samples. The decrease of pH and elevation of total titrable acidity during lactic acid fermentation was due to accumulation of organic acid especially lactic acid in the beverage sample. The results obtained are in agreement with findings by Do *et al.,* 2019, Chaudhary *et al.,* 2019, and Silva *et al.,* 2016. The fermented and non-fermented orange beverage samples showed little difference in their colour. The fermented test samples showed positive values for a* and b* and higher value of b* indicated that the test samples had desirable yellow-orange colour. The L* value of control was significantly lower than the test sample which indicated that the lightness was increased in the test sample and this could be due to the application of thermal processing/ pasteurization, which is supported by the findings of Lee *et al.* (2003).

Table 1: Physical parameters of oranges (*Citrus reticulate*).

Parameters	Values
Fruit weight (g)	120.41±7.04
Peel percentage (%)	28.79±1.23
Juice content (%)	47.14±2.54

Nutritional characteristics of probiotic orange beverages

The experiment was conducted to examine the effect of fermentation by lactic acid bacteria on the proximate composition of orange juice and also for evaluating the effect of different combination of probiotic strains on the proximate composition of the same. Moisture content of the fermented test samples (TS1, TS2 and TS3) was recorded to be significantly decreased than non fermented orange juice but no such significant difference was noticed among the test samples. The decrease could be explained by the fact that use of thermal processing reduces the moisture content of juice, which was previously documented in several other thermally processed probiotic beverages like pine-apple juice, sweet lime juice and blueberry and carrot blended juice (Ghafari and Ansari, 2018, Khatoon *et al.,*2015, Mauro *et al.,* 2016). The carbohydrate content of non fermented juice (control) was 9.20% which was significantly higher than the fermented test samples. The reduced values of carbohydrate showed the utilization of available sugar by the probiotic strains for their growth and multiplication. Similar trend was recorded by several other researchers (Mauro *et al.,* 2016, Wang *et al.,* 2009). No significant difference was seen among the test samples, and this could

Table 2: Physico-chemical properties of probiotic orange beverage.

Parameters	Control	Test samples		
		TS1	TS2	TS3
TSS(°Brix)	13^{a}±0.00	12^{b}±0.00	12^{b}±0.00	11.83^{b}±0.28
TTA (% citric acid)	0.40^{c} ±0.01	0.46^{b}±0.01	0.45^{b}±0.01	0.50^{a}±0.01
pH	4.14^{a} ±0.00	3.63^{b} ±0.05	3.66^{b} ±0.05	3.63^{b}±0.05
Colour L*	98.22^{b}±0.01	98.33^{a}±0.01	98.31^{a}±0.01	98.31^{a}±0.02
a*	0.19±0.01	0.17±0.01	0.18±0.01	0.18±0.01
*b	2.33±0.01	2.34±0.01	2.34±0.01	2.33±0.01
*Hue	85.31^{b}±0.01	85.64^{a}±0.01	85.60^{a}±0.01	85.58^{a}±0.01
Chroma	2.33±0.01	2.34±0.02	2.34±0.01	2.33±0.01

Values are mean of triplicate determinations ± SD.

Means are separated by Duncan's multiple range test P=0.05.

L* ranging from 0 (black) to 100 (white), a* ranging from red (+a*) to green (-a*), b* ranging from yellow (+b*) to blue (-b*).

be due to shorter time of fermentation. Fermentation showed no significant effect on the crude protein content of fermented and non fermented orange beverage test samples. The results were supported by the findings of Mauro *et al.,* in 2016. Similarly no significant difference was observed in the crude fat content between the non fermented and fermented test samples, which was previously reported in a probiotic pine-apple juice (Ghafari and Ansari in 2018). Such a non significant difference was also recorded for the crude fiber and potassium content of the non fermented and fermented test samples (Table 3).

Ascorbic acid/ Vitamin-C the major vitamin available in oranges was evaluated to compare the effect of fermentation on the Vit-C content of the test samples. A non significant relationship was recorded among the test samples but a significant decrease was recorded in the fermented sample compared to the non fermented sample (Table 3). Many studies revealed that application of thermal processing and pasteurization resulted in decreased Vitamin C content of the orange juice (Igwemmar *et al.,* 2013, Martýnez *et al.,* 2006,) which could be a possible explanation for the findings of the present study.

Health promoting effects of probiotic orange beverage

Antioxidant properties

The percentage free radical scavenging activity of the test samples was lower than the control (Fig 1) and this could be explained with the fact that application of thermal processing and pasteurization decreases the free radical scavenging activity as it affects the vitamin C content (Igwemmar *et al.,* 2013, Meena *et al.,* 2017, Martýnez *et al.* 2006). Among the test samples TS3 showed highest DPPH free radical scavenging activity which could be due to the presence of several specific strains. Literature documented that lactic acid bacteria namely *L. casei*, *L. fermentum* and *L. gasseri* posses some antioxidant activity (Lin and Yen, 1999, Lee *et al.,* 2005, Wang *et al.,* 2009, Chooruk, *et al.,* 2017, Rastogi *et al.,* 2020).

Hypocholesterolemic activity

The in vitro hypocholesterolemic activity of all the developed orange test samples ranged from 25%-28% and among all TS3 had the highest hypocholesterolemic activity (Fig 2). The hypocholesterolemic properties for the probiotic lactic acid bacteria used for the study were previously described by several other researchers with different mechanisms like, incorporation of cholesterol to cell surface and conversion of cholesterol to coprostanol *etc*. (Lye *et al.,* 2010).

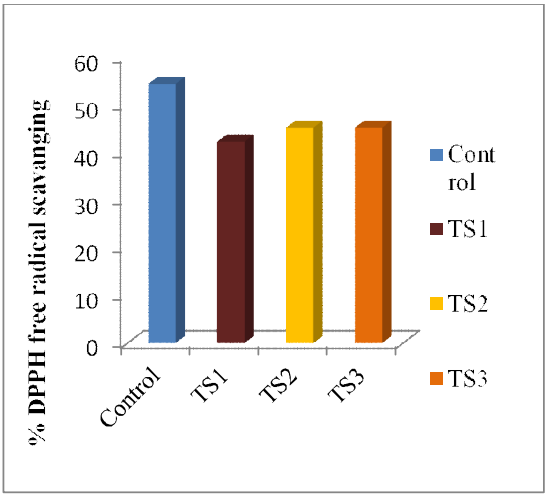

Fig 1: Antioxidant activity of test samples.

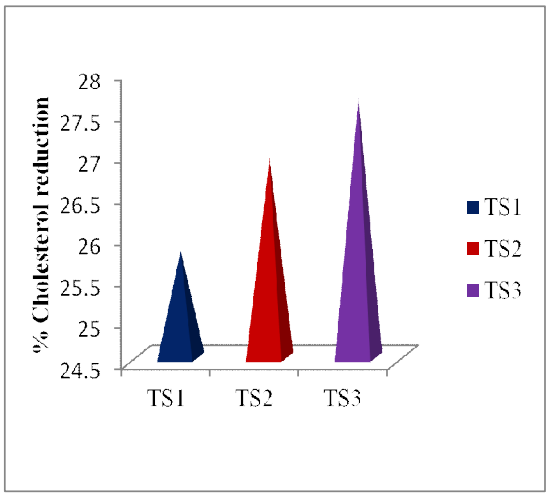

Fig 2: *In vitro* Hypocholesterolemic activity of test samples

Table 3: Nutritional characteristics of multi-strain probiotic orange beverages.

Parameters	Control	Test samples		
		TS1	TS2	TS3
Moisture (g/100 g)	94.17^{a}±0.01	92.70^{b}±0.00	92.70^{b}±1.74	92.70^{b}±0.00
Carbohydrate(g/100 g)	9.20^{a}±0.00	8.45^{b}±0.00	8.43^{b}±0.02	8.45^{b}±0.05
Protein (g/100 g)	0.40±0.00	0.35±0.00	0.35±0.00	0.35±0.00
Fat (g/100 g)	0.21±0.00	0.21±0.00	0.21±0.00	0.21±0.00
Crude fibre (g/100 g)	0.21±0.00	0.21±0.00	0.21±0.00	0.21±0.00
Pottasium (mg/100 g)	180^{a}±0.00	180^{b}±0.00	180^{b}±0.00	180.33^{b}±0.57
Vit-C (mg/100 ml)	40.50^{a}±0.50	38.28^{b}±0.70	38.26^{b}±0.34	38.16^{b}±0.57

Values are mean of triplicate determinations ± SD.

Means are separated by Duncan's multiple range test P=0.05.

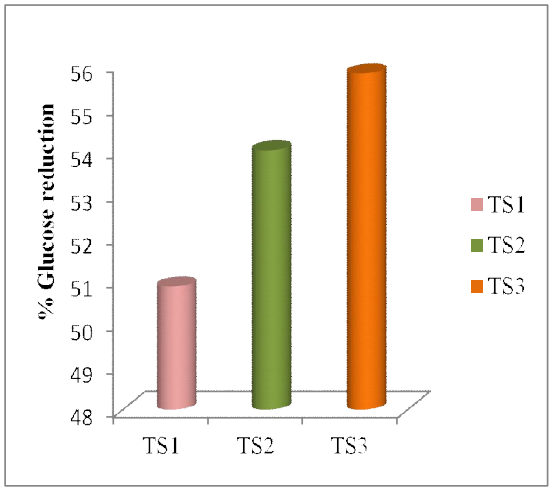

Fig 3: *In:vitro* hypoglycemic activity of test samples.

Hypoglycemic activity

The hypoglycemic efficacy of developed probiotic orange test samples TS1, TS2 and TS3 were 50.85%, 54% and 55.78% respectively (Fig 3). All the test samples showed promising results that could be described with the fact that glucose is the primary substrate for growth of probiotic bacteria although the glucose utilization capacity is different from strain to strain (Merkel and Perry, 1977).

CONCLUSION

The research demonstrated that all the three probiotic orange beverage test samples maintained viability till 4th week of storage which proves that orange juice can be a potential substrate for probiotic lactic acid bacteria without any nutrient supplementation. The promising antioxidant activity of all the test samples by virtue of both orange juice components (Vit-C and phenols) and probiotic mixtures requires further exploration in terms of their potential use for managing several oxidative stress associated diseases. The results of *in vitro* hypo-cholesterolemic and hypo-glycemic properties that are recorded in the present study needed to be investigated more with proper animal models. Overall, orange juice with probiotics can be used as healthy non-dairy probiotic drink in the future.

ACKNOWLEDGEMENT

The authors acknowledge Assam Agricultural University, Jorhat, Assam, India, for facilitating the research work.

Author contributions

Ms Deeptimayee Mahapatra conducted the experiments, analyzed the data and drafted this paper. Dr. Mamoni Das designed the experiments, revised the manuscript and approved the final version for publication.

Conflicts of interest

The authors declare no conflict of interest.

REFERENCES

AOAC. (2005). Official Methods of Analysis of the Association of Official Analytical Chemists International. USA: Maryland.

Ali, Z., Naeem, N., Farooq, Z. and Masood, S. (2015). Effect on lipid profile parameters by the addition of orange Juice in Diet of Hypercholesterolemic Patients. Journal of Nutrition and Food Sciences. 5(6). 5-10. https://doi.org/10.41721559600.1000428.

Aptekmann, N.P. and Cesar, T.B. (2013). Long-term orange juice consumption is associated with low LDL-cholesterol and apolipoprotein B in normal and moderately hypercholesterolemic subjects. Lipids in Health and Disease. 12(1): 1-10. https://doi.org/10.1186/1476-511X-12-119.

Chaudhary, A., Sharma, V. and Saharan, B.S. (2019).Probiotic potential of Noni and Mulberry juice fermented with lactic acid bacteria. Asian Journal of Dairy and Food Research. 38(2): 114-120. https://doi.org/10.18805/ajdfr.dr-1435.

Chooruk, A., Piwat, S. and Teanpaisan, R. (2017). Antioxidant activity of various oral Lactobacillus strains. Journal of Applied Microbiology. 123(1). 271-279. https:// doi.org/10.1111/jam.13482.

Delgado, T., Malheiro, R., Pereira, J.A. and Ramalhosa, E. (2010). Hazelnut (*Corylus avellana* L.) kernels as a source of antioxidants and their potential in relation to other nuts. Industrial Crops and Products. 32(3): 621-626.https://doi.org/10.1016/j.indcrop. 2010.07.019.

Deshpande, H.W., Hruyia, L., Katke, S.D. and Tamboli, N.M. (2019). Process Standardization and Quality Evaluation of De-bittered Probiotic Sweet Orange Juice. International Journal of Current Microbiology and Applied Sciences. 8(05): 836-850. https://doi.org/10.20546ijcmas.2019.805.099.

Do, T.V.T. and Fan, L. (2019). Probiotic viability, qualitative characteristics and sensory acceptability of vegetable Jjice mixture fermented with *Lactobacillus* strains. Food and Nutrition Sciences. 10(04): 412-427. https://doi.org/10.4236/fns.2019.104031.

Ekanem, J. and Ekanem, O. (2019). Proximate analysis and sensory evaluation of freshly produced apple fruit juice stored at different temperatures and treated with natural and artificial preservatives. Global Journal of Pure and Applied Sciences. 25(1): 31. https://doi.org/10.4314/gjpas.v25i1.5.

Elez-Martínez, P., Soliva-Fortuny, R.C. and Martín-Belloso, O. (2006). Comparative study on shelf life of orange juice processed by high intensity pulsed electric fields or heat treatment. European Food Research and Technology. 222(3-4): 321-329. https://doi.org/10.1007/s00217-005-0073-3.

Galaverna, G. and Dall'Asta, C. (2014). Production Processes of Orange Juice and Effects on Antioxidant Components. In: Processing and Impact on Antioxidants in Beverages, [Preedy, V.R., Ed.] Elsevier, Amsterdam, 203-214. http://dx.--doi.org/10.1016/B978-0-12-404738-9.00021-0.

Ghafari, S. and Ansari, S. (2018). Microbial viability, physico-chemical properties and sensory evaluation of pineapple juice enriched with *Lactobacillus casei*, *Lactobacillus rhamnosus* and inulin during refrigerated storage. Journal of Food Measurement and Characterization. 12(4): 2927-2935. https://doi.org/10.1007/s11694-018-9908-z.

Granato, D., Branco, G.F., Nazzaro, F., Cruz, A.G. and Faria, J.A. (2010). Functional Foods and Nondairy Probiotic Food Development: Trends, Concepts, Products. Comprehensive Reviews in Food Science and Food Safety. 9: 292-302. https://doi.org/10.1111/j.1541-4337.2010.00110.x.

Guo, L., Li, T., Tang, Y., Yang, L. and Huo, G. (2016). Probiotic properties of Enterococcus strains isolated from traditional naturally fermented cream in China. Microbial Biotechnology, 9(6): 737-745. https://doi.org/10.1111/1751-7915.12306.

Gupta, N., Trivedi, S., Gaudani, H., Gupta, M., Patil, P. and Gupta, G. (2009). Orange: Research analysis for wine study. International Journal of Biotechnology Applications. 1(2): 10-15.

Hofreiter, B.T. and Hedge, J.E. (1962). In: Carbohydrate Chemistry 17 (Eds Whistler R.L. and Be Miller, J.N.) Academic Press New York.

Igwemmar, N.C., Kolawole, S.A. and Imran, I.A., (2013). Effects of heating on vitamin C content of some selected vegetables. Int. J. Sc. Tech. Res. 2: 209-212.

Jankovic, I., Sybesma, W., Phothirath, P., Ananta, E. and Mercenier, A. (2010). Application of probiotics in food products-challenges and new approaches. Curr. Opin.Biotechnol. 21: 175-181.

Khatoon, N. and Gupta, R.K. (2015). Probiotics beverages of sweet lime and sugarcane juices and its physiochemical, microbiological and shelf-life studies. Journal of Pharmacognosy and Phytochemistry. 4(3): 25-34.

Kurowska, E.M., Spence, J.D., Jordan, J., Wetmore, S., Freeman, D.J., Piche, L.A. and Serratore, P. (2000). HDL-cholesterol-raising effect of orange juice in subjects with hyper--cholesterolemia. American Journal of Clinical Nutrition. 72(5): 1095-1100. https://doi.org/10.1093/ajcn/72.5.1095.

Lee, H.S. and Coates, G.A. (2003). Effect of thermal pasteurization on valencia orange juice color and pigments. LWT - Food Science and Technology. 36(1): 153-156. https://doi.org/10.1016/S0023-6438(02)00087-7.

Lee, J., Hwang, K.T., Chung, M.Y., Cho, D.H. and Park, C.S. (2005). Resistance of *Lactobacillus casei* KCTC 3260 to Reactive Oxygen Species (ROS): Role for a metal ion chelating effect. J. Food Sci. 70: 388-391. doi: 10.1111/j.1365-2621.2005.tb11524.x.

Liong M.T., Shah N.P. (2005). Acid and bile tolerance and cholesterol removal ability of lactobacilli strains.J. Dairy Sci. 88: 55-66.

Lin M.Y., Yen, C.L. (1999). Antioxidative ability of lactic acid bacteria. J. Agric Food Chem. 47(4): 1460-6. doi: 10.1021/jf981149l. PMID: 10563999.

Lye, H.S., Rusul, G. and Liong, M.T. (2010). Removal of cholesterol by lactobacilli *via* incorporation and conversion to coprostanol. Journal of Dairy Science. 93(4), 1383-1392. https://doi.org/10.3168/jds.2009-2574.

Mauro, C., Guergoletto, K., and Garcia, S. (2016). Development of Blueberry and Carrot Juice Blend Fermented by Lactobacillus reuteri LR92. *Beverages*. 2(4): 37. https://doi.org10.3390/beverages2040037.

Meena, V.S., Sharma, P.C. and Jalgaonkar, K. (2017). Influence of process factors on antioxidant and chemical properties of blended aonla (*Emblica officinalis Gaertn.*) juice and its quality retention during storage. International Journal of Chemical Studies. 5(6): 2082-2089.

Merkel, G.J., Perry, J.J. (1977). Effect of growth substrate on thermal death of thermophilic bacteria. Applied and Environmental Microbiology. 34: 626.

Mousavi, Z. E., Mousavi, S.M., Razavi, S.H., Emam-Djomeh, Z. and Kiani, H. (2011). Fermentation of pomegranate juice by probiotic lactic acid bacteria. World Journal of Microbiology and Biotechnology. 27(1): 123-128. https://doi.org/10.1007/s11274-010-0436-1.

Nerdy. (2018). Determination of Sodium, Potassium, Magnesium, and Calcium Minerals Level in Fresh and Boiled Broccoli and Cauliflower by Atomic Absorption Spectrometry. IOP Conference Series: Materials Science and Engineering. 288(1): 0-8. https://doi.org/10.1088/1757-899X/288/1/012113.

Okereke, H.C., Ajunwa, K.V. and Owanta, J.O. (2016). Physico-chemical properties of fresh and probioticated fruit juices with *Lactobacillus casei.* Asian Journal of Natural and Applied Sciences. 5(2): 9-15.

Ohrvik, V. and Witthoft, C. (2008) Orange juice is a good folate source in respect to folate content and stability during storage and simulated digestion. European Journal of Nutrition. 47: 92-98. http://dx.doi.org/10.1007/s00394-008-0701-3.

Pakbin, B., Razavi, S.H., Mahmoudi, R., and Gajarbeygi, P. (2014). Producing probiotic peach juice. Biotechnology and Health Sciences. 1(3). https://doi.org/10.17795/bhs-24683.

Rastogi, S., Mittal, V. and Singh, A. (2020). *In vitro* assessment of antioxidant and antimicrobial potential of *Lactobacillus gasseri* strains isolated from human milk and infant faeces. Journal of Pure and Applied Microbiology. 14(2). 1305-1315. https://doi.org/10.22207/JPAM.14.2.26.

Silva, S.B. and Ferrari, J. (2016). Development of probiotic grape juice and *Lactobacillus paracasei* viability under cold storage. 3-9. https://www.researchgate.net/publication/312042441.

Shukla, P. and Kushwaha, A. (2017). Development of probiotic beverage from whey and orange juice. Journal of Nutrition and Food Sciences. 7(5): 5-8. https://doi.org/10.4172/2155-9600.1000629.

Thakur, M. and Sharma, R.K. (2017). Development of probiotic pomegranate beverage and Its physico-chemical and microbial characterization. International Journal of Pure and Applied Bioscience. 5(1): 35-41.

Wang, C.Y., Ng, C.C., Su, H., Tzeng, W.S. and Shyu, Y.T. (2009). Probiotic potential of noni juice fermented with lactic acid bacteria and bifidobacteria. International Journal of Food Sciences and Nutrition. 60(6): 98-106. https://doi.org/10.1080/09637480902755095.

Wang, A.N., Yi, X.W., Yu, H.F., Dong, B. and Qiao, S.Y. (2009). Free radical scavenging activity of *Lactobacillus fermentum in vitro* and its antioxidative effect on growing-finishing gs. Journal of Applied Microbiology. 107(4): 1140-1148. https://doi.org/10.1111/j.1365-2672.2009.04294.x.

Wang, L., Shang, Q., Guo, W., Wu, X., Wu, L. and Wu, L. (2020). Evaluation of the hypoglycemic effect of probiotics *via* directly consuming glucose in intestines of STZ-induced diabetic mice and glucose water- induced diabetic mice. Journal of Functional Foods. 64*:* 103 614. https://doi.org/10.1016/j.jff.2019.103614.

Žuntar, I., Petric, Z., Kovacevíc, D.B. and Putnik, P. (2020). Safety of probiotics: Functional fruit beverages and nutraceuticals. Foods. 9(7): 1-19. https://doi.org/10.3390/foods9070947.