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Comparative study of probiotic green and red cabbage juice – a nutraceutical approach

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ABSTRACT

The present study was conducted to compare the nutritional value of green cabbage juice with red cabbage juice. After fermentation, qualitative analysis of phytochemicals of fermented red cabbage juice and green cabbage juice, antioxidant potential, percentage of sugar, antagonistic activity, and shelf-life study were performed. After fermentation, qualitative analysis of phytochemicals of both the juices showed presence of various essential component and antimicrobial activity. Based on morphological and, biochemical characterization and on referring Bergey's manual of determinative bacteriology, isolates from fermented cabbage juice were identified as *Lactobacillus spp.* Fermented red cabbage juice had greater ability to survive under gastrointestinal conditions than fermented green cabbage juice.

Keywords: *Brassica oleracea*, probiotic, phytochemicals, antioxidant, and *Lactobacillus spp.*

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INTRODUCTION

Brassica oleracea (cabbage) is one of the most important vegetables consumed all over the world. Cabbage grows best in a moist and cool climate and can withstand significant frost. Cabbage is cooked as a green vegetable, eaten raw and frequently preserved as in sauerkraut or pickle. *Brassica* vegetables are significantly regarded for their nutritional value; they are a rich source of Vitamin. C and soluble fibre. They contain multiple nutrients and phytochemicals (Chauhan et al., 2016). Phytochemicals are the compounds derived from plants hypothesized to be responsible for much of the protection against various diseases. They are present in diets high in fruits, vegetables, cereals, and plant-based beverages. Different types of phytochemicals are present in cruciferous vegetables. Glucosinolates, flavonoids, indoles, ascorbic acid, carotenes, and tocopherol being the most abundant groups of phytochemicals found in cabbage. Probiotic meaning "for life" and it is generally used for the bacteria associated with beneficial effects for humans. Probiotics are defined as live microbial feed such as *Lactobacillus plantarum*, *L. casei*, *L. acidophilus*, and *Streptococcus lactis*, which are supplemented by food that beneficially affects the host by improving its intestinal balance (Swain et al., 2014). Consuming fermented foods as a part of a healthy, balanced diet appears to be associated with a lower risk of heart disease and it helps in maintaining the blood pressure and improving the cholesterol balance. Processing by microorganisms affects the content and bioavailability of many vegetable

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components such as macronutrients, as well as compounds present in food in small amounts, such as polyphenols and vitamins (Eliza et al., 2023). This study will be helpful to improve nutrition for good health.

MATERIALS AND METHODS

Fresh red cabbage and green cabbage collected from the local market (Market yard, Pune) and preserved at 4°C for further study.

Preparation of cabbage juice

Fresh red cabbage and green cabbage were washed and cut into small pieces. After chopping, it was ground using a grinder. Distilled water was added to make the juice homogeneous. A pinch of salt was added to the juice (500ml) for preservation. Both the juices were filtered through a cloth and the pulp was discarded. Then both red cabbage and green cabbage juices were filled in sterile airtight bottles for fermentation.

Fermentation of cabbage juice

Red cabbage juice and green cabbage juice bottles were kept in the dark at room temperature for 8 days. After eight days physiochemical analysis was done.

Qualitative tests for phytochemicals in cabbage juice

Alkaloids (Wagner's test)-To a few ml of filtrate, a few drops of Wagner's reagent were added by the side of the test tube (Chauhan et al., 2016).

Glycosides-To 5ml of aqueous extract of the samples, 5 ml of Benedict's solution, and a few drops of dilute HCl were added and heated for minutes (Chauhan et al., 2016).

Saponins- In a test tube containing about 5 ml of an aqueous extract of the juice, a drop of sodium bicarbonate solution was added. The mixture was shaken vigorously and left for 3 minutes (Chauhan et al., 2016).

Flavonoids- The stock solution (1 mL) was taken in a test tube and to it were added few drops of dilute NaOH solution (Hossain et al., 2013).

Triterpenoids- The dry crude plant extract (5 mg) was dissolved in chloroform (2 ml) and then acetic anhydride (1 ml) was added to it. Concentrated sulphuric acid (1 ml) was added to the solution (Hossain et al., 2013).

Estimation of the antioxidant potential of cabbage juice

To 1,000 µL of DPPH(2,2 -Diphenyl -1-picryl hydrazyl) solution 800 µl of Tris-HCl buffer (pH 7.4) was added in a test tube and then 200 µl of testing sample solution was added and mixed quickly. The solution was kept at room temperature for 30 min. The absorbance of the solution was recorded at 517 nm. A mixture of 1,200 µl of ethanol and 800 µl of Tris-HCl buffer (pH 7.4) was used as blank. The inhibition ratio (%) was obtained from the following equation: Inhibition ratio (%) = (A1 − A2) × 100⁄A1,

Where A1 is the absorbance of ethanol instead of the testing sample and A2 is the absorbance of the testing sample solution (Fan Xiao1 et al., 2020).

Estimation of the total phenolic content of cabbage juice

0.5 mL of extract solution and 0.1 mL of Folin-Ciocalteau reagent were mixed thoroughly. After 15 min, 2.5 mL of saturated Na_2CO_3 (75 g/L) was added, and then the mixture was allowed to stand for 30 min at room temperature (RT). The absorbance was measured at 760 nm using a colorimeter. The concentration of the total phenolics was calculated as mg of gallic acid equivalent by using an equation obtained from the gallic acid calibration curve (Rafiquzzaman, 2015). The total phenolic contents in all samples were calculated using the formula: C = c (V/m) where, C = total phenolic content mg GAE/g dry extract, c = concentration of gallic acid obtained from calibration curve in mg/mL, V = volume of extract in ml, m = mass of extract in grams.

Estimation of protein by Bradford method

5 ml of Bradford reagent was added to 1 ml of cabbage juice. Absorbance was taken at 595 nm using a colorimeter. The concentration of protein was calculated by using an equation obtained from the standard graph of BSA as protein.

Detection of sugar using a Bricks meter: A drop of cabbage juice was taken on a Bricks meter and the percentage of sugar was determined.

Antimicrobial activity of cabbage juice: Pure suspensions of *Pseudomonas aeruginosa, Bacillus subtilis, Escherichia coli and Staphylococcus aureus* were prepared. Bacterial suspensions were spread on sterile Muller Hinton agar plates. Using a sterile cork borer, wells of 8 mm were made in each plate. 100 µL of juice was added to respective wells. Streptomycin was added in one of the wells as positive control and negative control well was kept blank. The plates were placed in the refrigerator for 30 min to let the sample diffuse into the agar properly. The plates were incubated at 37°C for 24h (Abdullah et al., 2021).

Shelf-life study of cabbage juice: Fermented cabbage juice samples were taken. Dilutions of both samples at room temperature as well as low temperature cabbage juice were done aseptically.10^{-2} and 10^{-4} dilutions were used for spreading on MRS agar plate and incubated anaerobically. The viability of probiotic cultures in fermented juice was determined and expressed as colony forming unit (CFU) (Mohan et al., 2013).

Isolation and characterization of bacterial isolates from juices

Isolation of bacterial isolates: Samples were inoculated on MRS agar by streak plate method and incubated in a desiccator (anaerobic conditions) for 48 hrs. A single colony was streaked on different agar media and incubated in a desiccator for 48 hrs. The pure cultures were selected and preserved at 4°C for further study.

Characterization of bacterial isolates:

Morphological characterization of bacterial isolates: The bacteria were characterized by morphological characteristics such as colony characters, Gram nature, and motility.

Biochemical characterization of bacterial isolates: By referring to Bergey's manual of determinative bacteriology, biochemical tests were performed. Sugar fermentation (Glucose, Fructose, Sucrose, Lactose, and Maltose) nitrate reduction, methyl red, Voges-Proskauer, catalase, oxidase, citrate utilization, urease, gelatin hydrolysis and Starch hydrolysis tests were performed (Holt et al., 1994).

Survival of bacterial isolates under Gastrointestinal Conditions: Isolates were screened for their ability to withstand gastrointestinal conditions i.e., low gastric pH. Isolates were cultivated in MRS broth to get the desired cell number (10^9CFU/mL) after 18 h of incubation at 37^{o}C. The simulated gastric juice (SGJ) containing pepsin (3 g/L) was prepared in sterile saline (0.85%, w/v NaCl) and adjusted to pH 2, 3 by using 1 M HCl.1 ml of overnight cell suspension was added in 9 ml of simulated gastric juice under aseptic conditions and incubated at 37^{o}C for 2 hours. Absorbance was noted at 620 nm (Yasmin et al., 2020).

RESULTS AND DISCUSSON

Fermentation of cabbage juice: After 8 days, turbidity and sedimentation were observed in both the juices. After fermentation, both juices showed a lighter colour as compared to the original colour.

Physiochemical Analysis of cabbage juice: From the (table1), it is observed that the acidity of red and green cabbage juice is increased after fermentation.

Table 1: Analysis of pH

Day	pH of fermented red cabbage juice	pH of fermented green cabbage juice
0	6	5
8	2.18	4.53

Qualitative tests for phytochemicals in fermented red and green cabbage juice (Table 2)

Alkaloids- Formation of reddish-brown precipitate was observed in both fermented cabbage juices, which confirmed the test as positive

Glycosides-Formation of red colour was observed in fermented red cabbage juice, which confirmed test as positive. Test was negative for green cabbage juice.

Saponins- Formation of honeycomb-like froth was formed in both fermented cabbage juices, which confirmed the test as positive.

Table 2: Phytochemical analysis of fermented red and green cabbage juice

Phytochemicals	Fermented red cabbage juice	Fermented green cabbage juice
Alkaloids	+	+
Glycosides	+	-
Saponins	+	+
Flavonoids	-	+
Triterpenoids	+	-

Flavonoids- In fermented green cabbage juice**,** after adding few drops of dilute NaOH solution; an intense yellow coloured appeared and became colourless on addition of a few drops of dilute acid that confirmed the test as positive. Test was negative for red cabbage juice.

Triterpenoids- Formation of reddish-violet colour was observed in fermented red cabbage juice, which confirmed the test as positive. Test was negative for green cabbage juice. Qualitative analysis of fermented red cabbage juice and green cabbage juice showed that alkaloids and saponins were present in both the juices. Glycosides and triterpenoids were found to be present in fermented green cabbage juice while flavonoids were found to be present in fermented red cabbage juice. Lucho-Constantino et al., (2017) suggested that phytochemicals are naturally occurring biologically active compounds in plants that are released as a means of protection in response to environmental stress or pathogens but are also said to provide several health benefits when consumed.

Estimation of antioxidant potential of fermented cabbage juices

Antioxidant activity performed using DPPH assay and inhibition ratio was calculated using formula. The reducing ability of antioxidant towards DPPH can be evaluated by monitoring the decrease of its absorbance at 530 nm.

Table 3: Antioxidant activity of fermented red and green cabbage juice

Fermented cabbage juice	Absorbance at 530 nm	Inhibition ratio (%)
Fermented red cabbage juice	0.81	45.26%
Fermented green cabbage juice	0.70	52.69%

The inhibition ratio of fermented green cabbage juice was more than fermented red cabbage juice (Table 3).The edible parts of *Brassica* plants are a rich source of phytochemical compounds, which possess strong antioxidant potential. These plants contain a variety of phytochemical compounds including phenolics, polyphenols, phenolic acids, flavonoids, carotenoids, alkaloids, phytosterols chlorophyll, glucosinolates, terpenoids, and glycosides. Among various species of the genus *Brassica* studied for their phytochemical composition and antioxidant activity, *Brassica oleracea* leaves, florets and seeds have better phytochemical and antioxidant profiles. The phytochemical profile and antioxidant potential of *Brassica* plants make them the preferred candidates for nutritional and pharmaceutical applications (Nawaz et al., 2018).

Estimation of total phenolic content of cabbage juice

Total phenolic content was calculated using Standard graph equation of gallic acid i.e. Y=0.0038X + 0.017 From the Table 4. It is observed that the total phenolic content of fermented red cabbage juice was more than fermented green cabbage juice.

Table 4: Total phenolic content of fermented red and green cabbage juice

Fermented cabbage juice	Absorbance at 754 nm	Total phenolic content(mg GAE)
Fermented red cabbage juice	1.65	0.43
Fermented green cabbage juice	1.60	0.41

Estimation of protein by Bradford method: The concentration of protein was calculated by using an equation obtained from BSA standard graph of protein i.e. Y=0.5176X + 0.0041.

Table 5: Estimation of protein content in fermented red and green cabbage juice by Bradford method

Fermented cabbage juice	Absorbance at 595 nm	Concentration of protein(mg/ml)
Fermented red cabbage juice	1.20	2.31
Fermented green cabbage juice	1.18	2.27

Table 5 shows that the concentration of protein in fermented red cabbage juice was more than in fermented green cabbage juice.

Estimation of Sugar

Using Bricks meter, it was found that sugar content of fermented green cabbage juice was more than fermented red cabbage juice (Table 6)

Table 6: Determination of sugar content of fermented red and green cabbage juice

	Sugar content (%)
Fermented red cabbage juice	3%
Fermented green cabbage juice	4%

Qualitative detection of Vitamin C in cabbage juice: Iodine titration method shows that if vitamin C is present, the brown colour of the iodine solution will become colourless, vitamin C serves as a reducing agent and reduces iodine to iodide ions (colourless in solution). If there is no vitamin C (or very little), the blue-black colour appears immediately. The immediate formation of blue-black colour was observed in both fermented red cabbage juice and fermented green cabbage juice, which indicated that the concentration of vitamin C reduced after fermentation.

Antimicrobial activity of cabbage juice:

Table 7: Antimicrobial activity of fermented red and green cabbage juice

Microorganisms	Zone of inhibition	
	Fermented red cabbage juice	Fermented green cabbage juice
Pseudomonas aeruginosa	2 mm	3 mm
Bacillus subtilis	3 mm	3 mm
Escherichia coli	2 mm	3 mm
Staphylococcus aureus	2 mm	2 mm

The agar well diffusion method was used to screen the antibacterial and antifungal activities of different solvent extracts. (Daoud et al., 2015). From table 7. It was observed that fermented green cabbage juice has more antimicrobial activity against pathogens than fermented red cabbage juice.

Shelf- life study of cabbage juice

Key: RT RC-Room Temperature Red Cabbage, RT GC- Room Temperature Green Cabbage, LT RC- Low Temperature Red Cabbage, LT GC- Low Temperature Green Cabbage. The number of colonies was reduced after 20 days. Contamination was observed in the fermented cabbage juice after 30 days. Higher contamination was observed at room temperature than at low temperatures (Table 8).

Table 8: Shelf- life study of cabbage juice

Dilutions of cabbage juice	CFU/mL	
	Day 20	**Day 30**
10^{-2} RT RC	21×10^{3}	5×10^{3}
10^{-2} RT GC	101×10^{3}	2×10^{3}
10^{-4} RT RC	21×10^{5}	11×10^{5}
10^{-4} RT GC	95×10^{5}	15×10^{5}
10^{-2}LT RC	8×10^{3}	3×10^{3}
10^{-2}LT GC	6×10^{3}	3×10^{3}
10^{-4}LT RC	6×10^{5}	3×10^{3}
10^{-4}LT GC	3×10^{5}	2×10^{5}

Isolation and characterization of bacterial isolates from juices

Two isolates RC1 and GC1 were morphologically and biochemically characterized by using biochemical tests (Sugar fermentation (Glucose, fructose, sucrose, lactose and maltose), nitrate reduction, methyl red, Voges-Proskauer, catalase, oxidase, citrate utilization, urease, gelatine hydrolysis, and Starch hydrolysis tests.Both promising isolates RC1 and GC1 were positive for the Sugar utilization test and starch hydrolysis test and negative for nitrate reduction, methyl red, Voges-Proskauer, catalase, oxidase, citrate utilization, urease, and gelatin hydrolysis. Based on morphological and biochemical characterization and referring to Bergey's manual of determinative bacteriology, these isolates were identified as *Lactobacillus* spp (Table 9 and 10).

Table 9: Morphological characterization of bacterial isolates from fermented red and green cabbage juices

Colony characters	Isolates	
	Fermented red cabbage juice (RC1)	**Fermented green cabbage juice (GC1)**
Size	1 mm	1 mm
Shape	Circular	Circular
Colour	Cream white	Cream white
Margin	Entire	Entire
Elevation	Convex	Convex
Opacity	Opaque	Opaque
Consistency	Sticky	Sticky
Gram character	Gram positive	Gram positive
Motility	Non-motile	Non-motile

Table 10: Characterization of bacterial isolates from fermented red and green cabbage juices

Colony characters	Isolates	
	Fermented red cabbage juice (RC1)	Fermented green cabbage juice (GC1)
Sugar utilization test:	+	+
Glucose		
Fructose	+	+
Sucrose	+	+
Lactose	+	+
Maltose	+	+
Nitrate reduction	-	-
Methyl red	-	-
Voges-Proskauer	-	-
Catalase	-	-
Oxidase	-	-
Citrate utilization	-	-
Urease	-	-
Gelatin hydrolysis	-	-
Starch hydrolysis	+	+

Survival of bacterial isolates under Gastrointestinal Conditions

Table 11 shows that microorganisms in fermented red cabbage juice have greater ability to survive under gastrointestinal conditions than the microorganisms in fermented green cabbage juice. In fermented red cabbage juice, it is observed that survival rate is more at pH 3 than pH 2. While, survival rate was less and unaffected in fermented green cabbage juice.

Table 11: Survival of bacterial isolates under gastrointestinal conditions

pH	Absorbance at 620 nm	
	Fermented red cabbage juice	Fermented green cabbage juice
2	0.13	0.06
3	0.30	0.06

CONCLUSION

Probiotics are live microorganisms that are intended to have health benefits when consumed. Nutraceutical is a food or part of the food that provides medical and health benefits including prevention and treatment of a disease. Red cabbage juice and green cabbage juices were fermented which improved the probiotic potential of red and green cabbage juice. Red cabbage juice was found more acidic than green cabbage juice. Qualitative tests for phytochemical analysis were performed from which we concluded that red cabbage has more probiotic potential than green cabbage juice. Antioxidant potential was higher in fermented green cabbage juice while total phenolic content and protein concentration were higher in the red cabbage juice. By using Brick's meter, it was concluded that the percentage of sugar in fermented green cabbage juice was more than fermented red cabbage juice. We concluded that vitamin C content was reduced in both cabbage juices after fermentation. Antagonistic activity of both

the fermented cabbage juices showed a zone of inhibition for all four bacterial isolates. At low temperature shelf life of both the fermented cabbage juices were increased. Isolation and characterization of isolates from fermented cabbage juice concluded that both the cabbage juices have Lactobacillus spp. that gives probiotic potential to fermented cabbage juice. The probiotic potential of isolates was assessed through their survival ability under the artificial simulated conditions of the digestive tract. Both the isolates showed sufficient viability under gastric conditions. From the results of this study, it was concluded that fermented cabbage juices are helpful as healthy beverages for vegetarians or consumers who are allergic to dairy products. From the above study, it was concluded that red cabbage has more probiotic potential than green cabbage juice.

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REFERENCES

Abdullah, A. A. 2021. Antifungal Activity and Mechanism of Action of Different Parts of Myrtus communis growing in Saudi Arabia against Candida spp. Journal of Nanomaterials, P. 1-10.

Bergey, D.H. and Holt, J.G. 1994. Bergey's manual of determinative bacteriology. 9th Edition, Lippincott Williams and Wilkins, Baltimore.

Chauhan,E., Tiwari,A. and Singh,A. 2016. Phytochemical screening of red cabbage (Brassica oleracea) powder and juice - A comparative study. Journal of Medicinal Plants Studies, 4(5), 196-199.

Eliza, Knez., Kornelia, Kadac-Czapska. and Małgorzata, Grembecka. 2023. Effect of Fermentation on the Nutritional Quality of the Selected Vegetables and Legumes and Their Health Effects. Life, 13 (655), 1-24.

Fan, X. Tao, X. Baiyi, L. Ruihai, L. 2020. Guidelines for antioxidant assays for food components. Food Frontiers.1, 60–69.

Gaanappriya, M. Guhankumar, P. Kiruththica, V. Santhiya, N. and Anita, S. 2013. Probiotication of fruit juices by Lactobacillus acidophilus. International Journal of Advanced Biotechnology and Research,. 4, 935-940.

Haq, N. M. Aslam S. and Saima, M. 2018. Phytochemical composition and Antioxidant potential of Brassica, Brassica germplasm-characterization.Breeding and Utilization, 8-26.

Iqra, Y. Muhammad, S. Wahab, Ali K. Adnan, K. Muhammad Farhan, J. Rabia, Iqbal Saima T. Saima, N. Atif, L. Tariq Mehmood, Samreen A. and Saira, T. 2020. In Vitro Probiotic Potential and Safety Evaluation (Hemolytic, Cytotoxic Activity) of Bifidobacterium Strains Isolated from Raw Camel Milk. Microorganisms, 354, 1-21.

Mohammad, A. Hossain. Khulood, A. S. A. Zawan, H. A. Afaf, M. W. and Qasim, Al- Riyami. 2013. Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of locally grown Thymus vulgaris. Asian Pacific Journal of Tropical Biomedicine, 3(9), 705-710.

Rafiquzzaman, S. M. In-Soo, Kong. and Jin-Man, Kim.2015. Enhancement of Antioxidant Activity. Total Phenolic and Flavonoid

Content of Saccharina japonica by Submerged Fermentation with Aspergillus oryzae. KSBB Journal, 30, 27-32.

Swain, M. R. Anandharaj, M. Ray, R. C and Rani, R. P. 2014. Fermented fruits and vegetables of Asia: a potential source of probiotics. Biotechnology Research International, 19.

Daoud, A. Malika, D. Bakari, S. Hfaiedh, N. Mnafgui, K. and Kadri, A. 2015. Assessment of polyphenol composition, antioxidant and antimicrobial properties of various extracts of date palm pollen (DPP) from two tunisian cultivars. Arabain Journal of Chemistry, 12(8), 3075-3086.

Lucho-Constantino G. G., Zaragoza-Martínez F., Ponce-Noyola T., et al. 2017. Antioxidant responses under jasmonic acid elicitation comprise enhanced production of flavonoids and anthocyanins in Jatropha curcas leaves. Acta Physiologiae Plantarum, 39(8),1–10.