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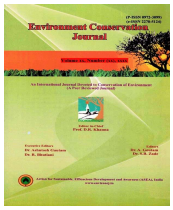

Lactic acid bacteria as an adjunct starter culture in the development of metabiotic functional black pearl grapes beverage

Ritika Modi ✉
Department of Microbiology, Punjab Agricultural University, Ludhiana, Punjab, India.
Ishwerpreet Kaur
Department of Microbiology, Punjab Agricultural University, Ludhiana, Punjab, India.
ParamPal Sahota
Department of Microbiology, Punjab Agricultural University, Ludhiana, Punjab, India.

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ABSTRACT

Black pearl Grapes are highly nutritious and one of the richest sources of polyphenols, but due to being delicate with very high loss at harvest and during distribution, is not consumed adequately. This study intended to develop functional lactic acid starter culture based fermented grapes beverage, in order to improve the quality and stability of this low pH fruit and to develop a fermented non-dairy beverage. Results showed that grapes blend was an excellent matrix for LAB growth with more than 9.38 $\log_{10}$ CFUml^{-1} of viability at the end of fermentation. LAB fermentation affectedly enhanced the total polyphenols and flavonoids content. Likewise, antioxidants capacities based on DPPH and FRAP activity were considerably increased correlating with each other, impacting the color and sensory properties of the grapes beverage. This way, the lactic acid fermentation can be considered as an appropriate tool for developing black pearl grapes based novel bio-intervention with enhanced antioxidants, polyphenols and flavonoids with anti-proliferative activity and antagonistic efficacy against recurring food borne pathogen in this post-antibiotic era.

Introduction

The appetite of nutraceutical enriched, functional fermented plant based food and beverages are on the upsurge apart from traditional milk based products. Demand for non-dairy functional fermented beverages is growing in parallel with the on-going trend of veganism and increasing health problems associated like lactose-intolerance, allergy to milk proteins or high cholesterol content. Fermented foods with plant origin have been evaluated as vectors for administration of functional Lactic Acid Bacterial (LAB) cultures following the proficiency of the production of vegetable and fruit based fermented products (Soccol *et al.,* 2010; Yoon *et al.,* 2006). Fruits and vegetables are excellent matrices rich in carbohydrates, polyphenols, vitamins, minerals, dietary fibres and antioxidants, providing a suitable growth substrate for LAB in parallel with a strong health appeal. Lactic acid fermentation, using pure functional LAB, as one of the appropriate approach for the utilisation of functional potential of plant based matrices, improving the bioactivity, bioavailability of phytochemicals and augments the plant matrices with functional bacterial secondary metabolites that are able to exert putative benefits on human health (Filannino *et al.,* 2018). Intestinal microbiome, rightly called "our second genome" comprises of trillions of mini life forms and their genetic material and is tightly regulated by the mysterious cross-talk between gut microbiome and intestinal epithelial cells. Dysbiosis of the gut microbiota therefore leads to various intestinal and extra-intestinal ailments such as colitis, inflammatory bowel disease, colon cancer and metabolic syndrome. Both clinically and experimentally, it has been observed that regaining

Corresponding author E-mail: *ritimodiii@gmail.com*
Doi: https://doi.org/10.36953/ECJ.10102225

the gut balance via oral supplementation of the fermented fruits and vegetables beverages is becoming increasingly popular for various gastrointestinal diseases, reducing risk of certain cancers and cardiovascular diseases in this post antibiotic era.

Black pearl grapes being rich in phenols particularly quercitin (12-15 mg/kg), kaempferol (2 mg/kg), myricetin (4.5 mg/kg), catechins (19 mg/kg) and coumaric acid (1-3 mg/kg) (Gil, 2000) and impressive antioxidative properties, correlated with the polyphenols and anthocyanins (ACNs) reconciles its usage as suitable substrate for lactic acid fermentation. Moreover, the ability of the phytochemicals (flavonoids specially resveratrol) in inducing human protective enzymes and the protective effects against cardiovascular diseases, cancers, and other age-related diseases (Yao *et al.,* 2004) substantiates the functionality of grape juice. As Grapes are not available in winter and are very delicate with very high loss at harvest and during distribution. Bio preservation through lactic acid fermentation of grapes harvested in summer can be a great prospect for consumers to acquire the benefits of nutraceutically enriched grapes throughout the year.

Despite, an ideal source, the survivability of the LAB in fruit-based matrix is complex and is influenced by the fermentation bio-process conditions, such as fermentation temperature, pH, medium composition and bacterial species. As pH has a strong influence on the LAB growth, keeping this point, the products were developed either via fortification (without fermentation) or by fermentation using a single lactic acid bacteria strain. LAB must survive and retain their functional properties during the entire bio-processing and storage and the formulated product must contain at least 10^6cfu/ml of the living functional lactic acid bacteria at the time of consumption. Therefore, reckoning the value of simple biotechnological fermentation technique and wish to prolong the shelf- life of grapes juice, pure culture application of functional LAB could be valuable in augmenting its availability and functionality.

In this context, the role of microorganisms in fermented grapes beverage health promoting properties was studied with particular attention to the metabolic contribution by LAB in the development of functional grapes beverage and to comprehend the potential beneficial effect and bioactive properties of metabiotic functional grapes beverage, its antioxidant capacity, the spectrum of functional bacteria, in relation to the chemical composition. The specific aim of this study was to investigate substrate metabolism, antagonistic (against disease causing pathogens) and bacterial composition of grapes beverage fermented via selected consortium of ten lactic acid bacteria (which have been evaluated for compatibility and individual growth in the beverage) as a basis for explicating the mechanisms underlying its functional properties. This attempt was made to develop lactic acid starter cultures based fermented grapes beverage manifesting high antioxidant activity, nutraceutical properties of the Black Pearl Grapes and bioactive compounds (secondary metabolites) of lactic acid bacterial fermentation with the beneficial functional properties of the bacteria.

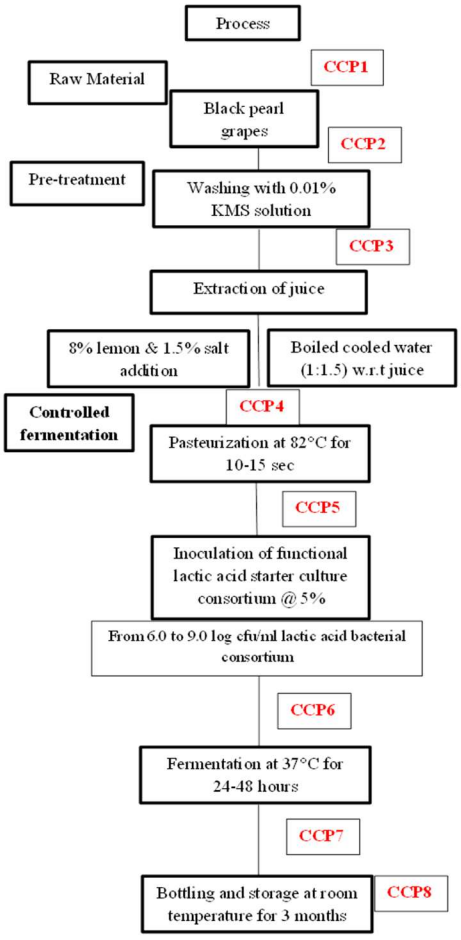
Material and Methods

Plant Material

Black pearl grapes [*Vitis vinifers* var. NS6] were acquired from Department of Fruit and Vegetable Science, PAU, Ludhiana, India along with lemons (var. *PAU Baramasi*). The nearly ripened grapes and other plant materials were harvested manually and washed properly with sodium hypochlorite solution to remove any surface microbial load. Afterwards, the substrates were refrigerated before beverage formulation.

Starter culture activation

Ten strains belonging to different species of lactic acid bacteria - LAB1 (*Pediococcus lolii* MH752471), LAB2 (*Lactobacillus plantarum*,), LAB3 (*P. acidilactici strain* 5560), LAB4 and LAB5 (*Enterococcus sp.*), LAB6 (*P. acidilactici* MK028218), LAB7 (*P. acidilactici* strain 8613), LAB8 (*P. acidilactici*), LAB9 (*P. acidilactici*) and LAB10 (*P. pentosaceous* strain L16)] were singly used for the fermentation. The bacterial stock cultures were stored frozen (-20°C) in MRS (Mann Rogassa Sharpe) with glycerol (20%) and by means of double passage on MRS were reactivated when required. For preparation and activation of functional starter culture for fermentation, grapes juice was extracted to which equal volume of sterilised water, tested for potability using BWTK (*Sahota et al*., 2010), was added. The diluted juice



Hazard	Preventive measures
[1]Microbiological contamination Pathogenic spoilage	Use only high quality chemical free appropriate plant matrices Sort out any damaged or rotten fruits Tested using BFTK
[2]Biological cross contamination	Boiled potable water (tested using BWTK) with 0.01%KMS kill vegetative pathogens
	Clean & sanitised equipment prevent cross contamination
[3]Biological: pathogenic, fungal, mold contamination cross contamination	Clean & sanitised utensils and equipment Keep containers covered with clean & sanitised porous cloth (cheese cloth) Prevent overhead contamination
[4]Biological: mold or wild culture cross contamination	Adequate heating procedure Cooling parameters also need to be monitored
[5]Biological: Spore-formers may be heat shocked and germinate	Dry heat sterilization at 120-180°C, The addition of active fermentation culture will outcompete spore-formers to prevent growth
[6]Biological: mold or wild culture cross contamination	Use of active @5-10 % starter culture will outcompete wild culture growth
[7]Biological: Pathogens, mold or wild culture growth Chemical: Lactic acid can leach metal	Ferment in a safe, non-metallic food grade fermenter Keeping environment should be also be free from contamination
[8]Biological safety and shelf life stability	Store in sterile glass bottles Prevent overhead contamination during filling of the bottles

Figure 1: HACCP plan for bio-process of lactic acid fermented Grapes beverage
***CCP-Critical control point, HACCP-Hazard Analysis Critical Control Point**

was pasteurised at 82°C for 15-20 min and further cooled to room temperature of 28± 2°C. After cooling, the grapes juice was inoculated with the consortium of functional lactic acid starter culture and incubated at 37°C for 24 h for activation.

Beverage formulation and fermentation

Prior to the beverage formulation, refrigerated grapes were thawed at 4° C for 8 h and juice was extracted. The optimized bioprocess parameters using Response Surface Methodology (data not shown here), comprised of grapes blend [grapes juice (100ml); lemon juice (8% v/v)]; dilution ratio (1:1.5 with boiled cooled water), and condiment (salt) concentration (1.2%) for osmotic stable decoction in the fermented grapes beverage and then pasteurization at 82ºC for 10-15 sec and (5% v/v) consortia of ten potential lactic acid bacterial strains as functional starter culture (3.8×10^7 log CFU/ml) for controlled fermentation at 37ºC for 28h. Thus, changes in active culture counts, phytochemical composition and antioxidant activity were evaluated fortnightly throughout the fermentation process every 4-h interval and control was kept without lactic acid fermentation. The final product was tested for retention of functional LA Bacteria, titrable acidity, ºbrix, shelf stability, nutraceutical enrichment and sensory evaluation on nine point hedonic scale.

Measurement of fermentation characteristics

Microbial growth

Total plate count method was used to determine the bacterial growth during the fermentation process.

Concisely, every four hours samples were drawn from the formulated beverage and LAB count with antioxidant capacity, phenols and flavonoids concentration was determined.

pH, total titrable acidity (TTA) and acidification kinetics

pH was estimated using a digital pH meter and TTA defined as quantity of lactic acid (g) per 100 mL was determined by taking (10 ml) homogenized sample with 50 mL of distilled water, titrated with 0.1 M NaOH using (0.1%) phenolphthalein as an indicator.

The acidification kinetics was modelled according to the Gompertz equation:

$$y = k + A \exp \{- \exp[(Vmax e/A) (\gamma - t) + 1]\}$$

where, y is the acidification value at time t expressed as dpH/dt (units of pH/h); k is the initial level of the dependent variable to be modelled (pH units); A is the difference in pH (units) between inoculation and the stationary phase (dpH); Vmax is the maximum acidification rate expressed as dpH/h; t is the time and k the length of the lag phase expressed in hours.

Phytochemical concentration assay

Total Polyphenols concentration (TPC)

The Folin-Ciocalteu method explained by Aydın and Mammadov, (2017) was used for the determination of total polyphenols. A 2 mL freshly prepared (1:1 v/v) Folin-Ciocalteu reagent was added to 100 µL FGB samples after which 2 ml $NaCo_3$ was subsequently added, vortexed for 1 min and incubated at room temperature for 30 min and absorbance was recorded at 760 nm using UV spectrophotometer. Total polyphenols were expressed as milligram of Gallic acid equivalent per 100ml of FGB.

Total Flavonoid concentration (TFC)

The total flavonoids were estimated using colorimetric method adopted by Kwaw *et al*. (2017). 300 µL of sodium nitrite (50g/l) with 4 mL dw was added to one ml FGB, vortexed for 1 min and let stand for 5 min. Then one ml of $AlCl_3$ (100 g/l) was further added, vortexed and let stand again for another 5 min. Afterward, 2 mL of 1M sodium hydroxide and 2.4mL of dw were added and the resultant mixture was incubated at room temperature for 2 minutes with intermittent shaking. The absorbance was read at 510 nm using UV spectrophotometer after 10 min. The TFC was expressed as milligram of Quercetin equivalents per 100ml FGB.

Antioxidant activity assay

DPPH radical scavenging activity

Radical (%) scavenging activity of the grapes blend against stable DPPH free radicals was determined by method described by Zhang and Xu (2015). The free radicals in the DPPH solution absorbs light at 517 nm and as a result of scavenging free radicals in the presence of samples the decreasing absorbance was measured as antioxidant activity. A 0.6mM of DPPH stock solution was prepared from DPPH crystals (0.0238g) dissolved in 95% ethanol. After 30 min of incubation, the absorbance of the negative (A_0) control was measured with 0.5ml ethanol and 2.5ml of the DPPH solution. However, the effect of the blend color was excluded by measuring the absorbance of a mixture of 0.5ml different sample concentrations with 2.5ml of ethanol and measured as (A_2). 2.5ml of DPPH solution was mixed with 0.5 ml of blend at different concentration (50, 100, 150, 200 and 250µg/ml) and the absorbance was measured as A1. The percentage inhibition was calculated using the expression in Eq. (1).

$$\textbf{Scavenging activity, DPPH (\%)} = (1 - [A_1 - A_2])/A_0 \times 100\%$$

Ferric reducing antioxidant power assay

The method explained by Suarez *et al.* (2010) was adopted for estimation of ferric reducing antioxidant power. The working FRAP reagent was prepared freshly by mixing 5.0 ml of TPTZ (10 mM in 40 mM Hydrochloric acid), 5.0 ml of Ferric chloride (20 mM), and 50 ml Sodium acetate buffer (300 mM, pH 3.6). The FRAP reagent (3.6 ml) was first maintained at 37°C and then mixed with 0.4 ml of each sample and mixed vigorously, incubated for 4 at 37°C min and then absorbance was measured at 593 nm. The FRAP anti-oxidant activity was expressed as mM Ferrous sulphate equivalents using standard curve prepared by using known concentration of Ferrous sulphate solution (Ferrous sulphate 1 mM= 1.51 mg/10 ml).

Color assessment

The study used the Konica Minolta colorimeter (CR-410, Konica Minolta Inc. Japan) to quantify the color characteristics (L*, a* and b*) and the index of hue angle (H°) was calculated as $\tan^{-1}(b/a^*)$ with chroma (C) as $(a^{*2} + b^{*2})^{1/2}$ and total color difference (ΔE) calculated as $[(L_o-L)^2 + (a_o-$

$a)^2 + (b_o-b)^2$] ½ (Fazaeli, Hojjatpanah, and Emam-Djomeh., 2013).

Antagonistic Activity of formulated metabiotic grapes beverage

The antagonistic activity of the metabiotic grapes beverage was assessed using strains *Staphylococcus aureus* MTCC3906, *Listeria monocytogenes* MTCC657, *Klebsiella pneumoniae* MTCC109, *Escherichia coli* MTCC443 and *Aeromonas hydrophila* MTCC173 was examined using agar well diffusion. The test pathogens containing 2×10^7 cfu/mL were seeded on molten Muller Hilton agar plates and wells were bore on seeded plates after solidification. The beverage samples along with erythromycin as a positive control were introduced into the wells and first incubated for 60 min at 4 °C, allowing the test material to diffuse in the agar, and then incubated for 18h at 37°C and clear zones were measured.

Human sensory evaluation

The sensory evaluation was carried out according to the method of Kwaw and Sackey (2013). Briefly, a panel of both male and female members (25-45 age group) comprising of staff, students of the Department of Microbiology were offered with coded samples. Each panel evaluated samples (10 ml) for color, flavor, taste, aroma, bouqet, astringency and overall acceptability using a 9-point hedonic according to the ISO8586-1 (1993) sensory analysis guidelines and assessments were recorded on the designed sensory analysis form.

Statistical Analysis

All the microbial, physic-chemical analyses were performed in triplicate and expressed as mean ± standard deviation (SD) and analysed using SPSS (version 16.0). The significance of difference was tested by one-way ANOVA and Tukeys post hoc test was performed for post comparisons. Results with $p\leq0.05$ were considered to be statistically significant.

Results and Discussion

The microbial growth kinetics, pH, TTA and acidification kinetics

During the fermentation process, the growth pattern of the Lactic Acid Bacteria gives important information regarding the properties of on-going fermentation. The fermentation kinetics for Lactic Acid Bacterial consortium is presented in Figure (2a). The differences between the pre-culture and the fermentation medium might have induced nutritional stress and there resulted into decreased microbial growth rate during initial stages of fermentation process (first 2h). This stage was clearly defined for the Lactic Acid Bacterial during which minor metabolic activity is observed and the bacterial culture attempts to acclimatise with the new fermentation conditions. An exponential growth of the lactic acid bacteria in the grapes beverage after 2h up to 28 h was experiential, reaching the count of 9.44 log10 CFU/ml, which relates to the normal growth curve of microbes and were higher than the minimum (10^6) recommended value of viable LAB in fermented product. The elevated viable cell counts advocates the supply of sufficient nutrients and favourable conditions for the exponential growth of functional Lactic Acid Bacterial consortium.

The Figure (2b) shows the results for pH and TTA dynamics during the fermentation of grapes blend using lactic acid bacterial at 37ºC for 28 h. The pH values decreased from 4.32 (initial) to 2.64 (28h). The initial TTA value was (0.448) and increased to (0.63) after 28 h of fermentation (Figure 2b). The highest value of ΔpH (1.683) and V_{max} (0.089) occurred on the 28^{th} hour and 16^{th} hour, respectively (Table 1), representing maximum growth rate of fermentation at the 16^{th} hour during which the LAB grows exponentially. Similar increases were found during lactic acid fermentation of rice-based beverage (Gosh *et al.,* 2015). The variation in pH and Titrable acidity, with the microbial growth during the fermentation bio-process, suggests the usage of sugars present in the blend by Lactic Acid Bacteria to produce lactic acid.

Antioxidant capacity and active phytochemical concentration during lactic acid fermentation of Grapes blend

Total antioxidant activity is a unique parameter widely used in phytomedicine for the determination of scavenging activities of bioactive formulations. DPPH-SA (1- Diphenyl -2- picryl hydrazyl-radical scavenging activities) and FRAP (Ferric Reducing Antioxidant Power) assay are among the most widely accepted methods for determining the antioxidant capacity of the food and beverages. The changes in antioxidant capacity during 28 h

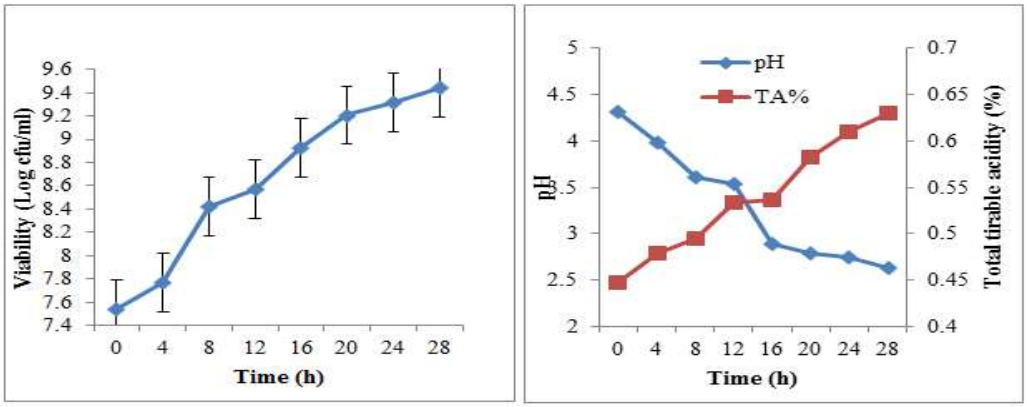


Figure 2: **Lactic acid bacterial viability (A), pH and total titrable acidity (B) during lactic acid fermentation of the Grapes beverage.**

Table 1: Acidification kinetics of lactic acid bacterial consortium during 28 h of Grapes beverage fermentation.

Fermentation time (h)	Acidification kinetics	
	ΔpH (pH units)	Vmax (ΔpH/h)
0	0^{g}	0^{f}
4	0.336 ± 0.01^{f}	0.084 ± 0.003^{b}
8	0.709 ± 0.010^{e}	0.088 ± 0.001^{a}
12	0.783 ± 0.03^{d}	0.065 ± 0.002^{d}
16	1.43 ± 0.015^{c}	0.089 ± 0.001^{a}
20	1.53 ± 0.01^{b}	0.076 ± 0.001^{c}
24	1.576 ± 0.03^{b}	0.0656 ± 0.001^{d}
28	1.683 ± 0.015^{a}	0.060 ± 001^{e}

Table 2: Antioxidant activity and active phytochemical composition of lactic acid fermented Grapes beverage during 28h of fermentation

Antioxidant activity			Phytochemical ingredients	
Fermentation time (hours)	**DPPH-SA (%)**	**FRAP (μM of ferrous sulphate equi.)**	**TPC (mg/100ml)**	**TFC (mg/100ml)**
0	58.51 ± 0.59^{d}	64.92 ± 0.04^{e}	35.16 ± 0.71^{e}	33.08 ± 0.11^{d}
4	60.85 ± 0.41^{cd}	73.26 ± 0.03^{d}	45.11 ± 0.21^{d}	39.65 ± 0.51^{c}
12	63.77 ± 0.79^{c}	81.9 ± 0.01^{c}	48.86 ± 0.54^{c}	42.27 ± 0.35^{b}
20	67.82 ± 0.67^{b}	98.2 ± 0.07^{b}	52.36 ± 0.35^{b}	44.41 ± 0.39^{a}
28	69.70 ± 0.77^{a}	100.3 ± 0.01^{a}	52.29 ± 0.32^{a}	46.96 ± 0.06^{a}

DPPH-SA- 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity, FRAP-ferric reducing antioxidant power, TPC-total phenolic content, TFC-total flavonoid content

FRAP quantified as- μM Ferrous sulphate equivalent antioxidant capacity

Data expressed as mean ± standard deviation.

Means that do not share the same letter are significantly different at $P \leq 0.05$

fermentation of the grapes blend are shown in Table 2. The free radical scavenging activity increased during the 28 h fermentation and then remained stable during storage the maximum value of 69.70 %SA was found after 28 hours. The drift in FRAP activity was characterized by an increment during 28 hour of fermentation. It reached its highest value (100.3 μM of ferrous sulphate

equivalents) at 28th hour of fermentation. Torres *et al.* (2015) has correlated the increased profile of phenols and flavonoids in fermented fruits with antioxidant capacity. The total phenolics (TPC), total flavonoids (TFC) changes during the fermentation process have also been determined (Table 2). The total polyphenols in the grapes based beverage was 52.29 mg quercetin equivalent per100ml of beverage former to fermentation (28 hours). The variation in TPC content was in conjunction with fermentation process as initially it increased rapidly while more stable lately. It increased during the fermentation period and then remains stable in synchrony during the fermentation phase. The study by Ng *et al.* (2011) exhibited an increase in the total phenolic concentration of the plant substrates after fermentation hence, an experiential increment in the antioxidant activity. It has been also proposed by Chu and Chen (2006) that LABs transformation and depolymerisation of plant compounds could account for the increased content of phenolic compounds during the fermentation process.

The TFC content of grapes beverage varied in similar manner as TPC. It enhanced as the fermentation progresses (28 hours) and remained stable. The maximum value of TFC was 46.96 mg Gallic acid equivalents per 100ml, whereas the TFC level at 0 h was 33.08 mg/100ml. The lactic acid bacterial enzymes and acids could have facilitated the release of flavonoids from their complex compounds and make them more available in the fermenting medium (Katina *et al.,* 2007). Therefore, lactic acid bacterial metabolism can increase the flavonoids during fermentation of grapes beverage and resulted in a high-quality nutritionally enriched fermented beverage.

Effect of lactic acid fermentation on color characteristics of the Grapes beverage

The effect of fermentation on color characteristics (L*, a* and b*) of FGB were determined along with total color difference (ΔE). The observations (Table 3) revealed a decrease in L*and b* and an increment in a*of the fermented sample compared to the control. Pereira *et al.* (2011) observed a lightness (L*) reduction in cashew apple juice due to the higher turbidity caused by bacterial growth and hence, the decrease in brightness. Grapes beverage color is attributed to the tannins, alkaloids, flavonoids and phenols and increase in a* could be due to the increase in phytochemical concentration among the FGB compared to control (Table 2).

Antimicrobial activity of lactic acid fermented grapes beverage against selected food grade spoilage microorganisms

In the present study the efficacy of FGB fermented using LAB consortium at the rate 5%w/v (10^8 CFU/ml), was evaluated against microorganisms responsible for causing food infection and food intoxication (Table-4). The microorganisms selected were *Staphylococcus aureus* MTCC3906, *Listeria monocytogenes* MTCC657, *Klebsiella pneumoniae* MTCC109, *Escherichia coli* MTCC443 and *Aeromonas hydrophila* MTCC173. The inhibition zones were compared with Erythromycin (positive control) after 24 hours of incubation at 37ºC.

The antimicrobial effect against *Escherichia coli* was observed with 15.0±2.6mm and 10±2.3mm zone of inhibition with fermented and unfermented samples respectively, followed by *Listeria monocytogenes* (13mm), *Staphylococcus aureus* (11mm). Strains of *Klebsiella pneumonia* and *Aeromonas hydrophila* displayed inhibition zones (9.0mm and 5.0mm respectively) only with fermented sample. The results are in concordance with earlier studies reported by *Mantzourani et al.,* 2019 where Cranberry juice fermented with potentially isolated probiotic *Lactobacillus paracasei* K5 exhibited greater antimicrobial efficacy against *Enterobacter faecalis* and *Staphylococcus aureus* as compared to unfermented juice with zone of inhibition ranging from 10.75-15.45mm. This antagonistic response is believed to be derived from organic acids, bacteriocins, antimicrobial peptides and hydrogen peroxide action on the cell membrane of bacteria, playing role in maintain the membrane potential while inhibiting the active transport (Parvez *et al.,* 2006; Vasconcel*os et al.,* 2018; Muhammed *et al.,* 2018).

Sensory assessment of lactic acid fermented Grapes beverage

Sensory assessment (Figure 3) showed that NFGB and the FGB were above six (like) on the 9 points hedonic for all the sensory attributes. The FGB was

Table 3: Colorimetric properties of lactic-acid fermented Grapes beverage

Sample	L*	a*	b*	H°	C*	ΔE
NFGB	16.63±0.37	3.5±0.47	6.76±0.35	62.90±0.85	7.61±0.44	-
FGB	6.6±0.11 *	7.66±0.32 *	7.56±0.05 *	44.30±0.73 *	10.73±0.20 *	10.89±0.35

Data expressed as mean ± standard deviation
NFGB-non-fermented grapes beverage, FGB-lactic acid fermented grapes beverage
L*- Lightness, a*- Redness, b*- Yellowness, C*- Chroma, H°- Hue angle, ΔE- color difference
Means with different superscripted letter (a,b) on the same column shows significant difference at P≤0.05

Table 4: Inhibition zones for lactic acid fermented grapes beverage (1000mg/ml+5%w/v consortium) against selected food grade spoilage microorganisms

Isolate	Accession number	Zone of inhibition (mm)		
		Erythromycin (15mcg)	NFGB (10ul)	FGB (10ul)
Staphylococcus aureus	MTCC3906	15.0±2.6	7.0±0.8	12.0±1.2
Listeria monocytogenes	MTCC657	14.0±2.1	11±1.4	11±2.0
Klebsiella pneumoniae	MTCC109	18.0±3.0	ND	9.0±1.0
Escherichia coli	MTCC443	10.0±1.4	9.5±2.3	14±2.5
Aeromonas hydrophila	MTCC1739	8.0±0.3	ND	6.0±0.8

***Values reperesented as mean±SD**
*** Antibiotic disk: HIMEDIA Laboratories pvt. Ltd (HX023-1PK)**
***Incubation for 24h at 37°C**
***ND-not detected**

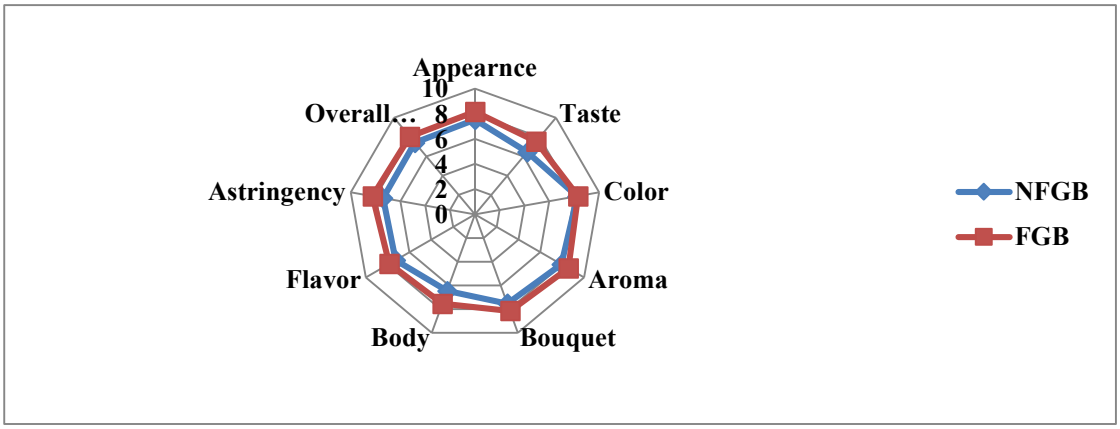


Figure 3: Radar plot for sensory assessment of non-fermented Grapes beverage and fermented Grapes beverage
NFGB-non-fermented grapes beverage, FGB-fermented grapes beverage
Asterisk indicates significant difference at P ≤ 0.05.

significantly ($p<0.05$) better in astringency, flavor, taste and overall acceptability compared to the NFGB (Figure 3). The higher color score for FGB compared to the NFGB could be accredited to its enhanced content of phytochemicals (Table 2). Though high concentrations of nutraceuticals and phytochemicals are accountable for the bitterness and acidic taste in various products (Sun-Waterhouse and Wadhwa, 2013), the high lactic acid content of FGB might have lessened its bitterness and astringency (Milkulic-Petkovsek *et al.,* 2012). As previously reported by Sun *et al.* (2016) lactic acid strains can considerably transform the aroma characters of fermented beverages with the ability to produce diverse enzymes. But FGB and NFGB have no significant difference ($p>0.05$) in terms of aroma, color and body having almost alike sensorial scores (Figure 3). The observations also showed that appearance and bouqet were significantly different ($p<0.05$) between the FGB and NFGB having a higher score for FGB. This could be due to the secondary metabolites and chemical changes and lactic acid contents of the FGB. The overall acceptability

score by the panel showed that FGB was much more preferred as compared to NFGB (Figure 3).

Conclusion

Lactic acid fermentation is commonly applied on milk, however, owing to the lactose intolerance and milk-resistant proteins related with milk consumption, is an immediate need to develop fruit and vegetable-based fermented beverages. Lactic acid fermentation can also be applied for preserving and often enhancing the organoleptic and nutritional quality of fresh vegetables and fruits and an ideal medium for the administration of probiotic lactic acid bacteria. We found grape juice as a reliable/favourable substrate supporting all nutritional requirements for potential growth of Lactic Acid Bacteria (LAB). Lactic acid bacterial fermentation enhanced the antioxidant capacity by altering the phenolic content in the grape blend. The refinement of polyphenols along with the increase in flavonoids and total phenols participates in the elaboration of grape juice offering optimised nutritional profile. Enrichment with polyphenolic compounds, flavonoids limits the risk of innumerable chronic diseases related with oxidative stress along with the metabolites released during fermentation are able to exert biological activities more pertinent to human health in reverence to their parent phenolic compounds with bioaccumulation property inside the endothelial cells retaining an intracellular antioxidant activity. Beyond the basic nutritional properties the fermented beverage can possess anti-diabetic, anti-inflammatory and anti-cancer properties with an impact on reducing obesity, firmly correlated with antioxidant activity, flavonoids and the occurrence of other phytochemicals in fermented grapes beverage which can be further tested. Hence, the resulting fermented grapes beverage was nutraceutically enriched, healthy functional food alternative providing functional lactic acid bacteria with potential probiotic properties, which might be an appropriate and effective fermentation medium providing desired characteristics even during storage time.

Conflict of interest

The authors declare that they have no conflict of interest.

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