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Evaluation of Antioxidant Properties of Novel Probiotic Food Products and Their Role in Rural Community Development

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Research Article

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ABSTRACT

In recent years, there has been a growing awareness among Indian consumers about the importance of nutrition, health, and quality of food they consume. In modern-day lifestyle, the market has witnessed an increase in the sale of health products amongst the health-conscious consumers globally creating new health food categories. The knowledge about their use and technology to prepare them in a convenient form for domestic use have to be imparted in the Indian rural areas as an affordable product mainly within the lower income group people. Regular use of probiotics could improve the quality of life and reduces the dependence on drugs and medical expenses. Adoption of a Probiotic culture in a country like India to prevent various diseases rather than seeking a cure for them through the production of probiotic foods at the household level is quite inevitable. The present study assesses the feasibility of blueberry, mulberry and noni as a raw substrate for the production of the probiotic blueberry jam, probiotic mulberry juice and probiotic noni juice by lactic acid bacteria (Lactobacillus plantarum DB-2, Lactobacillus fermentum J-1, Pediococcus acidilactici M-3, Lactobacillus plantarum SK-3, and Pediococcus pentosaceus SM-2). We found that the isolated LABs are optimal probiotics for fermentation with blueberry jam, mulberry juice and noni juice. In this investigation, the results could be an indicator of the development of health-promoting fruit jam and juices. These lacto-fermented jam and juices are a low-cost healthy food product, provide better nutrition and good health to the population

INTRODUCTION

Blueberry (*Cyanococcus*) belongs to the genus Vaccinium of the family Ericaceae and sub-family Vacciniaceae. They are dark purple in colour after maturing and are covered in a protective coating of powdery epicuticular wax known as the "bloom." Blueberries have high antioxidant activity and phenolic content. These findings led to further investigation of the health benefits of blueberry on human health. Studies on blueberry supplementation in diets have been associated with metabolic syndrome, improving brain health and memory, reducing the risk of cancer, improving visual acuity, enhancing gut health and muscle repair and weight management.

Mulberry (*Morus nigra L.*) belongs to the genus Morus of the family Moraceae. It is also known as black mulberry and is widely distributed in Asia, Africa, Europe, North America, and South America. There are at least 24 species of Mulberry and is extensively used for sericulture in east, central and south Asia. In India and China, farmers cultivate mulberries for silkworm, but European farmers cultivate them for fruit. Mulberries are grown considerably at higher altitudes in the Himalaya-Hindu Kush region but are widespread in northern India where the tree and fruits are known by Persian-derived names toot (mulberry) and shahtoot (superior mulberry), respectively. Black mulberry have high natural antioxidants and phenolic compounds in the form of bioflavonoids and non-anthocyanin, respectively, having bioactive functions and are responsible for their medicinal properties.

Noni (*Morinda citrofolia L.)* belongs to the family Rubiaceae. Noni juice is derived from the fruit of the Morinda citrofolia tree indigenous to Southeast Asia and Australasia but is cultivated in India, Polynesia, central and northern South America and the Caribbean [1].

Different parts of noni (fruit, root, leaves, and bark) contain different biologically active compounds, having many health benefits such as to stimulate the immune system, scavenge free radicals, inhibit LDL (low-density lipoprotein) oxidation, regulate blood cholesterol level, and provide anti-inflammatory benefits. Traditionally noni juice was prepared by natural fermentation of noni fruit in sealed containers for 1-2 months at ambient temperature, but oxygen, temperature, and microorganisms during fermentation can cause undesirable chemical reactions which reduces the health benefits of noni juice.

In Asia, any bacterial strain of a known species that are traditionally used can be added to the food for the preparation of probiotic food product. Nowadays, probiotic food products are promoted in the form of dairy products such as fermented milk and yoghurt. Lactose intolerance and cholesterol content are two significant disadvantages related to fermented dairy food products.

People with special needs of vegetarians and people with allergic reactions to milk proteins have found a good substitute in the form of fruit and vegetable food products containing probiotics. Fruit products have a high amount of antioxidants, dietary fiber, vitamins, minerals, and other useful nutritional substances and thus improve the health of the host. Recently, various raw materials have been explored to ascertain the suitability of the substances to produce novel non-dairy probiotic products. Beverages based on fruits and vegetables have been reported as a novel suitable carrier medium for probiotic.

MATERIALS AND METHODS

Fermented foods belong to a category of foods called functional foods that are known to have a beneficial effect on health. Probiotics are the bacteria (termed lactic acid bacteria) used to ferment traditional foods, and they are the most reported and researched. Thus, fermented foods and probiotics closely related and co-exist despite the increased commercial interest in probiotics due to the health attributes associated with them. However, the efficacy of probiotics is enhanced when taken in the form of fermented food rather than as probiotics alone. LAB fermentation is a traditional method used for over 1000 years throughout the world to preserve indigenous foods. The benefits of fermented food usage include prevention of lactose intolerance, immune system boost, reduced malnutrition, and diarrheal diseases caused by bacteria and rotavirus. They also play a great role in infant weaning as they prevent infections that are foodborne. It is believed that fermented foods and probiotics can immensely contribute to food security and by enhancing the livelihoods of the rural and urban communities in developing countries like India.

Lack of knowledge on fermented foods and how they are made deprives the rural communities of the opportunity to fermented foods correctly in order to reap the health benefits offered by these foods. Although this is one of the oldest technologies used for food preservation for a number of centuries, it has not been efficiently transferred to newer generations. Instead, most communities have adapted exotic Western diets as they have lost their traditional roots and seldom use these foods. The newer generation may also be unaware of their nutritional benefits. However, in certain rural commodities of India, fermentation is still embraced as a means to prepare complementary foods for young children and infants. Research reports showed that in the regions where rural communities mostly subsist on specific traditional foods, the pattern of diseases is different to those who consume modern exotic diets. Given the benefits that can be derived from traditional fermented foods, there is a need to promote their preparation, perception and usage among both rural communities [2].

The importance of probiotics is known across civilizations and strata of societies over centuries in the form of practice of consuming preserved foods obtained through the process of fermentation. The traditional fermented foods are mainly obtained from dairy products viz. yoghurt, Dahi, kefir, cheese (after long storage), fermented vegetable or from vegetable juices and from non-fermented fruit and berry juices. Indigenous fermented foods have been prepared and consumed for thousands of years, and are strongly linked to culture and tradition. Amongst Asian countries, the most prominent ones which consume probiotics are Japan, Korea, Indonesia, India, Nepal, and Sri Lanka. In India, idli, dosa, dhokla, khaman are certain popular traditional fermented foods which are consumed throughout India, particularly in southern and western parts of India. In certain parts of India, fermented rice was mixed with buttermilk and salt for direct consumption. The jalebis and kanji are mainly consumed by northern India. These fermented products are consumed unknowingly as, probiotic food or drink, by local people from ages due to enormous health benefits associated with them and have now been realized.

At present, there is limited information available with the consumer regarding the benefits of probiotic foods. The population statistics clearly reveal that of the 121 crore Indians, 83.3 crores live in rural areas while 37.7 crore stays in urban areas, said the Census of India's 2011 Provisional Population Totals of Rural-Urban Distribution in the country, released by Union Home Secretary RK Singh. This data shows that nearly 70% of the country's population lives in rural areas. Till date, only 30% of the population has easy access to probiotic products. The most common problems rural India faces are poor sanitary conditions and limited health facilities in terms of unavailability of modern medicine. Due to improper hygienic conditions, there is a concomitant outbreak of intestinal diseases. In this direction, there is a need to develop a simple and accessible method to improve the quality of these economically challenged people.

Diverse Indian traditional fermented foods and beverages, produced using different types of fermentation, have been used since antiquity because of their numerous nutritional values. Lactic Acid Bacteria (LAB) isolated from these products has emerged as a welcome source of antimicrobials and therapeutics, and are accepted as probiotics. Probiotics are defined as live microbial food supplements which beneficially affect the host by improving the intestinal microbial balance. Currently, popular probiotics are derived from fermented milk products. However, with the growing number of consumers with lactose intolerance that is affected by dietary cholesterol from milk products, there is a growing global interest in probiotics from other food sources.

Probiotic bacteria are the source that enhances the medicinal and flavor properties of fermented foods. They aid digestion and nutrient assimilation. These bacteria are also well-known for their beneficial effects on the immune system and health. Many of them produce antimicrobial bioactive molecules that make them effective bio-preservatives and produce nutraceuticals to create functional foods with increased bioavailability of nutrients. For example, lactic acid bacteria have incontestable benefits.

The present study demonstrates the fermentation of blueberry, mulberry and noni fruit with probiotic lactic acid bacteria to select an appropriate starter culture for developing lacto- fermented blueberry jam. Antioxidant assay, and *in vitro* cholesterol reduction were carried out during fermentation. The focus of this research is to specifically highlight the suitability of Indian fermented jams and juices as a viable source of novel probiotics and their role in rural community development.

Preparation of blueberry substrate

Fresh blueberry fruit was procured from fruit market, New Delhi, India. After arrival at the laboratory, blueberry fruits were appropriately washed. The blueberry jam (without supplementary water or nutrient) was prepared by putting them in a saucepan over heat. The jam was sterilized by autoclaving at 121°C for 15 min.

Preparation of mulberry substrate

Fresh mulberry fruit were procured from the Kurukshetra University, Haryana. After arrival at the laboratory, mulberry fruit were appropriately washed. The mulberry juice (without supplementary water or nutrient) was prepared by the juicer. The juices were sterilized by autoclaving at 121°C for 15 min.

Preparation of noni Substrate

Fresh noni fruit were procured from the fruit market, Delhi. After arrival at the laboratory, noni fruit were appropriately washed. Noni fruit was peeled. The seeds from the noni fruit were separated from manual splitting. The noni juice (without supplementary water or nutrient) was prepared by the juicer. The juice were sterilized by autoclaving at 121°C for 15 min.

Probiotic lactic acid bacteria

Probiotic Lactic Acid Bacteria *L. plantarum* DB-2, *L. fermentum* J-1, P. acid lactici M-3, *L. plantarum* SK-3 and P. pentosaceus SM-2 were isolated from Dosa batter, Jalebi batter, Maida dough, Sauerkraut, and Soymilk, respectively. Probiotic attributes such as acid tolerance, bile tolerance, antibiotic susceptibility, hemolytic activity, gelatinase activity, autoaggregation, co-aggregation studies, hydrophobicity, bacteriocin production, lactic acid and hydrogen peroxide production, exopolysaccharide production, were studied on all the five isolates. Genotypic characterization was done of all the five isolates. Analysis of the 16S rRNA sequences revealed that lactic acid bacteria isolated from Dosa batter, Jalebi batter, Maida dough, Sauerkraut, and Soymilk showed 99%, 99%, 97%, 100% and 99% homology with *Lactobacillus plantarum*, Lactobacillus fermentum, Pediococcus acidilactici, Lactobacillus plantarum and Pediococcus pentosaceus respectively. The 16S rRNA gene sequence was submitted to Genbank and assigned accession number MK246169, MK353735, MK461878, MK246167 and MK461882 for isolate DB-2, J-1, M-3, SK-3 and SM-2, respectively.

Fermentation of probiotic blueberry jam

All the five isolates were sub-cultured in MRS broth at 35°C till the colony count reaches up to 108 CFU/ml. 100 g of the blueberry jam was inoculated separately in triplicates. The probiotic enriched blueberry jam prepared in the present study was divided into three sets, i.e., Set A was inoculated with L. plantarum DB-2 (108 CFU/ml), Set B was inoculated with P. pentosaceus SM-2 (108 CFU/ml), and Set C was inoculated with consortia of probiotic strains *viz. L. plantarum* SK-3, L. fermentum J-1, P. acidilactici M-3 and P. pentosaceus SM-2. Set D was control (without probiotic bacteria). The fermentation process was executed at 35°C for 72 h. The fermentation was terminated by keeping these four sets at 4°C.

Fermentation of probiotic noni juice and mulberry juice

Both isolates were sub-cultured in MRS broth at 35°C till the colony count reaches up to 108 CFU/ml. 100 mL noni juice and mulberry juice were inoculated separately in triplicates. The probiotic enriched noni juice and mulberry juice was divided into three sets, i.e., Set A was inoculated with *L. plantarum* SK-3 (108 CFU/ml), Set B was inoculated with P. acidilactici M-3 (108 CFU/ml), and Set C was inoculated with consortia of probiotic strains viz. *L. plantarum* SK-3, L. fermentum J-1, P. acidilactici M-3 and P. pentosaceus SM-2. Set D was control. The fermentation process was executed at 35°C for 72 h. The fermentation was terminated by keeping these four sets at 4°C.

The fermented blueberry jam extract was analyzed for anti-oxidative properties viz. total antioxidant property, reducing power, and anti-scavenging property. Samples were taken at 0, 7, 14, 21, and 28 days for analysis.

The fermented noni juice and mulberry juice were centrifuged at 8000 × g for 5 min at room temperature. The supernatant of each juice was extracted and analyzed for antioxidative properties viz. total antioxidant property, reducing power, and anti-scavenging property. Samples were taken at 0, 7, 14, 21, and 30 days for analysis [3].

The ABTS antioxidant activity of lacto-fermented blueberry jam was determined using the method of Miller and Rice-Evans. The antioxidant activity was measured by adding 1 ml of each peroxidase (Sigma-Aldrich), Hydrogen peroxide, 100 M ABTS [2,2-azinobis (3-ethylbenz-thiazoline-6-sulphonic acid)] (Sigma-Aldrich) and distilled water. After proper mixing, the reaction mixture was incubated in the dark at 25°C for 1 h. 1 ml of each blueberry jam extract, mulberry juice supernatant and noni juice supernatant were added separately. Finally, the absorbance was measured at 734 nm.

The reducing power of lacto-fermented blueberry jam, probiotic mulberry juice and probiotic noni juice was determined following the method of Duh and Yen. It was determined by adding 0.5 ml of 0.5 M phosphate buffer, 2.5 ml of potassium hexacyanoferrate solution and 1 ml of blueberry jam extract, mulberry juice supernatant and noni juice supernatant, separately. The reaction mixture was heated at 50°C for 20 min. After the mixture was cooled to room temperature, the reaction was terminated by adding 0.5 ml of 10% trichloroacetic acid. After centrifugation at 3000 x g for 10 min, 1 ml of supernatant was mixed with 1 ml of distilled water and 0.1 ml of 0.1% ferric chloride. The reaction mixture was incubated for 10 min at room temperature. The absorbance was measured at 700 nm using a spectrophotometer.

Free radical scavenging activity

Free Radical Scavenging Activity (FRSA) was performed according to the method of Shimada et al., (1992). It was measured by adding 1 ml of blueberry jam extract, mulberry juice supernatant and noni juice supernatant, separately with 5 ml of 0.1 mM DPPH-methanolic solution. The mixture was kept in the dark for 1 h, and absorbance was measured at 517 nm by using a spectrophotometer.

In vitro cholesterol-lowering property was performed by following the method. The 0.3% oxgall (HiMedia) was added to MRS broth and autoclaved at 121°C for 15 minutes. Water-soluble cholesterol (after filter sterilization) was added to autoclaved MRS broth (HiMedia). 10% of the lacto-fermented blueberry jam extract, mulberry juice supernatant and noni juice supernatant, separately was added to it and incubated at 35°C for 24 h. Centrifugation was performed at 5000 × g for 10 min at 4°C. 1 ml of supernatant was taken into a sterilized test tube and added with 1 ml and 2 ml of KOH (33% w/v) and absolute ethanol, respectively. The mixture was incubated at 35°C for 15 min after vortexing for 1 min. The reaction mixture was cooled to room temperature. Then, it was mixed with 2 ml of distilled water and 3 ml of hexane. Vortexing was done for 1 min. 1 ml of hexane layer was separated and evaporated under nitrogen gas. The residue was dissolved in 2 ml of o-phthalaldehyde solution. 0.5 ml of conc.

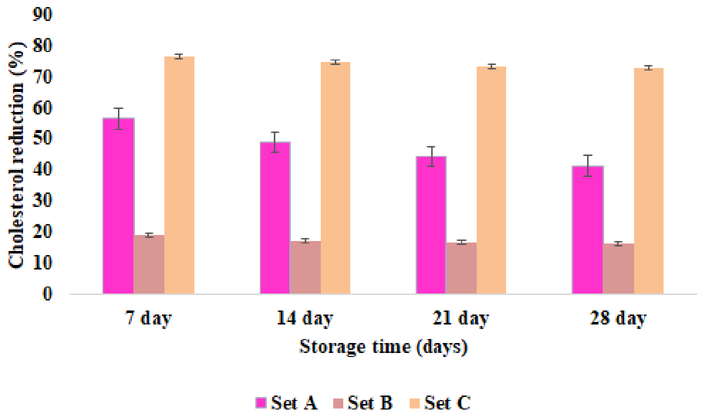
STATISTICAL ANALYSIS

All the experimental results were recorded as mean ± SD (Standard Deviation). For every observation, 3 determinations were used. The data were statistically analyzed. The significant differences between means were calculated by a one-way analysis of variance (ANOVA) using Duncan's multiple range test at $P < 0.05$.

RESULTS AND DISCUSSION

In this study, the antioxidative activity of cell-free extract of lactic acid bacteria was determined using various antioxidant assays. The antioxidant activity of blueberry jam, noni juice and mulberry juice, respectively using different starter cultures of *L. plantarum* DB-2, L. fermentum J-1, P. acidilactici M-3, *L. plantarum* SK-3, and P. pentosaceus SM-2. Fermented blueberry jam, mulberry juice and noni juice exhibited high antioxidative activity in terms of total antioxidant activity, reducing power and DPPH radical scavenging activity irrespective of the starter culture used in them. Antioxidant activity of blueberry jam (fermented with all the five strains), mulberry juice (fermented with the four strains), and noni juice (fermented with the four strains) reduced with the storage time. Blueberry jam showed a reduction of 2.77%, 4.43% and 3.82% in Set A, Set B, and Set C, respectively whereas Set D (Control) showed a decrease of approximately 5.69% over 28 days of storage time at 4 °C. The antioxidant activity of mulberry juice exhibited a reduction of 6.43% in Set A, 6.13% in Set B, 4.80% in Set C and 5.91% in Set D (Control) over 28 days of refrigerated storage. Similarly, the noni juice also showed the reduction in the antioxidant activity over 28 days of storage time at 4 °C. The reduction of 4.66%, 2.81%, 4.31%, and 3.88% was shown by Set A, Set B, Set C, and Set D (Control) (Figure 1) [4].

Figure 1. Cholesterol reducing property of probiotic mulberry juice



- Set A: Juice fermented with Lactobacillus plantarum SK-3
- Set B: Juice fermented with Pediococcus acidilactici M-3
- Set C: Juice fermented with consortia of Lactobacillus plantarum SK-3, Lactobacillus fermentum J-1, Pediococcus acidilactici M-3, and Pediococcus pentosaceus SM-2

The reducing power of the blueberry jam, mulberry juice, and noni juice showed a reduction during storage at 4°C for 28 days. Radical scavenging activity of blueberry jam, mulberry juice, and noni juice was varied with that of control because of the starters used. Set C showed good DPPH scavenging activity in comparison with Set A and Set B. Four weeks of storage at 4°C reduced the DPPH scavenging activity of blueberry jam, mulberry juice, and noni juice. Free radicals play an essential role in numerous chronic pathologies as they are involved in the process of lipid peroxidation. A compound with radical scavenging property serves as a potential antioxidant (Table 1).

Table 1. Antioxidative activity of probiotic mulberry juice.

Storage time (days)	**Total antioxidant activity**			
	Set A	Set B	Set C	Set D (Control)
0	77.36 ± 1.35A	75.33 ± 2.45A	78.38 ± 3.58A	76.83 ± 2.94A
7	76.73 ± 1.45A	73.66 ± 1.35A	77.74 ± 2.69A	73.24 ± 1.83A
14	74.06 ± 1.72A	72.63 ± 1.84A	76.57 ± 4.98A	72.47 ± 1.74A
21	72.96 ± 2.30A	71.00 ± 1.80A	74.83 ± 1.47A	71.34 ± 1.32A
28	70.93 ± 1.30A	69.20 ± 2.57A	73.58 ± 1.75B	70.92 ± 2.28A
	Reducing power			
	Set A	Set B	Set C	Set D (Control)
0	0.84 ± 0.09^{A}	0.85 ± 0.05^{A}	0.85 ± 0.04^{A}	0.84 ± 0.05^{A}
7	0.83 ± 0.04^{A}	0.84 ± 0.07^{A}	0.84 ± 0.02^{A}	0.83 ± 0.06^{A}
14	0.83 ± 0.05^{A}	0.83 ± 0.14^{A}	0.83 ± 0.05^{A}	0.82 ± 0.02^{B}
21	0.82 ± 0.15^{B}	0.82 ± 0.06^{B}	0.82 ± 0.06^{A}	0.81 ± 0.09^{B}
28	0.81 ± 0.11^{B}	0.82 ± 0.12^{B}	0.80 ± 0.07^{B}	0.80 ± 0.11^{B}
	Free radical scavenging activity			
	Set A	Set B	Set C	Set D (Control)
0	74.60 ± 2.20^{A}	76.70 ± 2.55^{A}	77.39 ± 2.59^{A}	71.36 ± 1.84^{A}
7	73.80 ± 2.45^{A}	75.90 ± 1.65^{A}	76.84 ±3.27^{A}	70.00 ± 1.96^{A}
14	72.06 ± 3.25^{A}	74.90 ± 3.44^{A}	75.26 ± 1.48^{A}	69.80 ± 2.23^{A}
21	71.80 ± 1.75^{B}	73.63 ± 1.70^{A}	74.43 ± 2.63^{A}	68.28 ± 1.48^{A}
28	71.10 ± 1.15^{B}	72.74 ± 2.00^{B}	73.58 ± 3.33^{A}	67.86 ± 2.25^{B}

Each value represents the mean ± SD (n = 3). Data bearing uppercase superscript letters in the same column (different sampling time) are significantly different (p <0.05). Each value represents the mean ± SD (n = 3). Data bearing uppercase superscript letters in the same column (different sampling time) are significantly different (p <0.05). Each value represents the mean ± SD (n = 3). Data bearing uppercase superscript letters in the same column (different sampling time) are significantly different (p <0.05)

The lowering of serum cholesterol level could be an important health benefit of lactic acid bacteria, demonstrated in humans as well as in animal studies. In the present study, blueberry jam fermented with probiotic lactic acid starters showed to reduce the *in vitro* cholesterol level. Similarly, mulberry juice and noni juice also exhibited the reduction in *in vitro* cholesterol level. Natural News observed the same results in cocktail fermented with L. plantarum and noted the reduction of 13.6% after weeks of consumption.81 reported the significant level of reduction in the total cholesterol and low-density lipoprotein-c level after the consumption of kimchi by the young, healthy volunteers. No literature was found for *in vitro* reduction of cholesterol by fruit jam. The cholesterol level and its fraction levels in serum were reduced after the treatment with the probiotic bacteria B. animalis VKL, B. animalis VKB and L. casei IMV B-7280 in 6-8 weeks old female BALB /c mice but the effects were strain-specific on serum lipid profiles [5].

CONCLUSION

In the present study, the survival of *L. plantarum*, L. fermentum, P. acidilactici, and P. pentosaceus probiotic strains in the blueberry jam, mulberry juice and noni juice was reported for the first time. The antioxidative and cholesterol-lowering property of different probiotic strains was examined in a blueberry jam, mulberry juice and noni juice during fermentation and under refrigerated storage. The probiotic cultures added to the jam and juices did not remarkably modify the colour and appearance of the product; however, the metabolism of Lactobacilli changes the pH which, in turn, accelerates the rate of hydrolysis of sugar into simpler ones. Fruit jam and juices represent a suitable carrier for the delivery of probiotics. Blueberries, mulberries and noni fruit are a good candidate for producing a novel and healthy non-dairy probiotic food which could effectively deliver probiotic L. plantarum, L. fermentum, P. acidilactici and P. pentosaceus strains under refrigerated conditions. Incorporation of probiotics into fruit jam and juices makes the jam and juices healthier as fruits are naturally rich in essential macro- and micro-elements. Concerning the performance, L. plantarum DB-2, L. fermentum J-1, P. acidilactici M-3, L. plantarum SK-3, and P. pentosaceus SM-2 were observed to be suitable for fermentation of blueberry jam, mulberry juice and noni juice and may have the ability for a possible industrial application in the production of lacto-fermented blueberry jam, probiotic mulberry juice and probiotic noni juice. Further work on optimizing the fermentation conditions and in vitro and in vivo functionality of the fermented blueberry jam, mulberry juice and noni juice is highly recommended. Lacto-fermented blueberry jam, probiotic mulberry juice and noni juice can increase consumer acceptability.

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