

Possibility of Wine Production from Underutilized Wild Plant *Carissa spinarum* and Phytochemical Analysis of Its Leaf, Fruit Extract

Parul¹, Manbir¹, Puneet², Harpreet Kaur³

¹Research Intern, Rapture Biotech Pvt Ltd, Mohali, Punjab, India

²Senior Scientist, Rapture Biotech Pvt Ltd, Mohali, Punjab, India

³Centre Director, Rapture Biotech Pvt Ltd, Mohali, Punjab, India

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ABSTRACT

Carissa spinarum(garne) is wild variant of plant *Carissa carandas* an underutilized fruit found in various regions of north India, with plenty of therapeutic and functional properties. The present research aimed to see the possibility of wine production from the fruits of *Carissa spinarum* and examine various parameter such as temperature, pH, sugar addition, and aging time affecting wine production. This research was conducted from 2024-2025. Result showed that the supplementation of sugar 8%, pH 3.5, temperature 25°, aging in 22 days. Wine obtained high ethanol content (8.01±0.02 % v/v). wine from this valuable garne would be beneficial for health by moderate consumption.

Keywords: wine, *Carissa spinarum*, phytochemical test, alcohol estimation, spectrophotometer, wine production

I. INTRODUCTION

Carissa spinarum is a small spiny shrub belongs to Apocynaceae family. Commonly known as Garne in various region of Himachal Pradesh, Uttarakhand, and Jammu. It is also known as bush plum or conkerberry. It is also known as karonda in various southern parts of India, referring to the related karanda (*Carissa carandas*). *Carissa* contains 5 subspecies, 410 genera, and 5556 species.(Ansari & Patil, 2018). It is an underutilised plant been showing source of various drugs such as atropine, codeine, digoxin, morphine, quinine and vincristine(Fatima et al., n.d.) various properties been discussed antioxidant, anti-

inflammatory, anti-diabetic (Bachheti et al., 2023) possibility of wine production is also been discussed as Phytonutritional and Sensorial Assessment of the plant shows that beverage formed using *carissa spinarum* consist of 40% fruit and 16 brix is most suitable for further beverage production(Mamoona et al., 2023)

Taxonomical hierarchy

Kingdom: Plantae

Subkingdom: Viridaplantae

Phylum: Tracheophyta

Class: Magnoliopsida

Subclass: Asteridae

Order: Gentianales

Family: Apocynaceae

Genus: *Carissa*

Species: *Spinarum*.

Morphology

Plant consists of forked branches and thorny shrub height ranging 2-3m. thorns are 3.2cm long at the base greenish to brown and toward the tip deep brown in colour. Leaves ovate and 3.5cm long 1.5cm broad, leathery, leaves exude a white latex which have highly adhesive property. Flowers are Short-stalked, sweetly scented, bisexual, complete, and white. . Fruit: An ovoid berry, 5–12 mm in length, 6 mm in diameter, green when unripe, and Shining black when completely ripe, sour and sweet in taste(Ansari & Patil, 2018)



Figure 1 *Carissa spinarum* in its natural habitat in Cholthra village of Mandi district, Himachal Pradesh, India A) shrub of *Carissa spinarum* B) Part of branch showing ripe fruits and leaves C) Part of branch showing unripe fruit

II. MATERIAL AND METHODS

Collection of sample: fresh sample of leafs and fruit of *Carissa spinarum* were collected from Cholthara village of district Mandi Himachal Pradesh. The sample is thoroughly washed to remove dust and impurities. Now plant and fruit extract is prepared with aqueous base for further analysis and juice is obtained from fresh fruit via crushing it in mortar and pastel for the production of wine.

Preparation of leaf and fruit Extract: sample is crushed in mortar and pestle, distilled water is added as required. Filter that out and few ml distilled water. Then heated in water bath for 20 minutes at 70°C. Sample is kept in refrigerator overnight and aqueous extract is ready for further testing

PHYTOCHEMICAL ANALYSIS OF LEAFS OF *Carissa spinarum*

TEST FOR SAPONIN: A sample extract is diluted with distilled water and shaken vigorously for almost 15 minutes. Appearance of foam confirms saponin components in extract

TEST FOR TANIN: Add few drops of ferric chloride (FeCl_3) to the leaf extract. A blackish precipitate shows the presence of tannins. Blackish blue: gallic tannins. Greenish blackish: catechol tannins

TEST FOR PHENOLIC FLAVONOIDS (LEAD ACETATE TEST): Take 1ml plant extract. Add 10% lead acetate, Brown precipitate indicates presence of phenolic flavonoids

TEST FOR FLAVONOIDS/ ALKALINE REAGENT TEST: Take 1ml plant extract. Add 2N 500 μl NaOH. Yellow coloured sample will be observed. Now add few drops of concentrated HCl. Colourless sample represent positive result . Yellow shows negative result

TEST FOR ALKALOID/ WAGNER TEST: the wagner reagent is prepared by dissolving 2gm of iodine and 6 gm of potassium iodide in 100ml of distilled water. Take 1ml plant extract add few drops of Wagner 's reagent. Reddish brown precipitate represent positive result

TEST FOR TERPENOID : Extract (5 ml) was mixed with chloroform (2 ml), and concentrated sulphuric acid (3 ml) was carefully added to form a layer. A reddish brown coloration of the inter face was formed to show positive results for the presence of terpenoids

DRAGENDROFFS (KRAUT'S TEST): It is a test used to detect alkaloids and other nitrogen containing compounds. Dragendroff consist of bismuth subnitrate, potassium iodide and tartaric acid. Take 1ml filtrate

add 1-2ml dragendroffs reagent . reddish brown precipitate represent positive result for alkaloid.

H₂SO₄ TEST for FLAVONOIDS: Mix a small amount of wine sample with chloroform. Carefully add few drops of concentrated H₂SO₄ along the side of test tube allowing it to form a separate layer. Observe the bright yellow colour interface indicates the presence of flavonoid add few drops of HCl yellow colour disappears confirms the presence.

PHYTOCHEMICAL ANALYSIS OF FRUIT OF CARISSA SPINARUM

Benedict test: 0.5ml filtrate is added into test tube now 0.5ml of benedict reagent is added and heated for two minutes. Green, yellow ,red colour show positive result

Fehling test: 1ml of fehling solution is added into 1ml of filtrate. Heat the solution for few minutes. Red precipitate indicates positive result

Protein test{ninhydrin test}: take 2ml of filtrate add 2drops of ninhydrin solution. Purple colour precipitate indicates positive result.

Molish test; take 2ml of filtrate add two drops of alcoholic α naphthol. Now add 1ml concentration of H₂SO₄. Violet coloured ring indicates positive result

PREPARATION OF WINE

This experiment is performed in laboratory of rapture biotech Mohali. Firstly the fruit is washed and cleaned then crushed in mortar and pastel to obtain fruit juice. It is filtered out and the seeds and peel of fruit is removed. Adjustment of brix was done by adding jaggery (TSS 20° Brix and pH 4.0). Now the pasteurization of juice is done at 70°C for 30minutes. Now the juice is kept overnight.(PATIDAR et al., 2023) traditional activated yeast is added into the sample, but different strains of *saccharomyces cerevisiae* can be used for max. alcohol extraction and stability. Commercial wine strains selected as a result of long breeding work have a number of advantages over natural isolates, such as high fermentation efficiency, resistance to high concentrations of alcohol, sugar, sulfite, low temperatures, etc.(Eldarov & Mardanov, 2020). Now it is kept for fermentation

process for few days and alcohol content is being recorded

Phytochemical analysis of wine produced: the wine prepared is analysed for the presence of alkaloid, saponin, tannin, steroids, flavonoid, protiens etc(Ram Krishna Rao, 2018)

TEST FOR SAPONIN: A sample extract is diluted with distilled water and shaken vigorously for almost 15 minutes. Appearance of foam confirms saponin components in extract

TEST FOR TANNIN: Add few drops of ferric chloride (FeCl₃) to the wine extract. A blackish precipitate shows the presence of tannins. Blackish blue: gallic tannins. Greenish blackish: catechol tannins.

TEST FOR PHENOLIC FLAVONOIDS (LEAD ACETATE TEST): Take 1ml wine extract. Add 10% lead acetate, Brown precipitate indicates presence of phenolic flavonoids

TEST FOR FLAVONOIDS/ ALKALINE REAGENT TEST: Take 1ml wine extract. Add 2N 500 μ l NaOH. Yellow coloured sample will be observed. Now add few drops of concentrated HCl. Colourless sample represent positive result . Yellow shows negative result

TEST FOR ALKALOID/ WAGNER TEST: the wagner reagent is prepared by dissolving 2gm of iodine and 6 gm of potassium iodide in 100ml of distilled water. Take 1ml wine sample add few drops of Wagner 's reagent. Reddish brown precipitate represent positive result

TEST FOR TREPENOID : Salkowski test was used to detect terpenoids. Extract (5 ml) was mixed with chloroform (2 ml), and concentrated sulphuric acid (3 ml) was carefully added to form a layer. A reddish brown coloration of the inter face was formed to show positive results for the presence of terpenoids

MAYER'S TEST: it is test primarily used to detect the presence of alkaloids in a sample. It involves using mayers reagent which is solution of potassium mercuric iodide(K₂HgI₄). Take few ml sample add 1-2ml mayers reagent , cream white/yellow ppt represent positive result

DRAGENDROFFS (KRAUT'S TEST): It is a test used to detect alkaloids and other nitrogen containing compounds. Dragendroff consist of bismuth subnitrate, potassium iodide and tartaric acid. Take 1ml filtrate add 1-2ml dragendroffs reagent . reddish brown precipitate represent positive result for alkaloid

H₂SO₄ TEST for FLAVONOIDS: Mix a small amount of wine sample with chloroform. Carefully add few drops of concentrated H₂SO₄ along the side of test tube allowing it to form a separate layer. Observe the bright yellow colour interface indicates the presence of flavonoid add few drops of HCl yellow colour disappears confirms the presence.

AMMONIA TEST FOR FLAVONOIDS: Mix a small amount of the wine sample with distilled water. Add few millilitre of dilute ammonia solution to the mixture. Carefully add small amount of concentrated H₂SO₄. If yellow colour appear and then disappear upon standing this indicates the presence of flavonoids.

XANTHOPROTEIC TEST: It is used to detect presence of protein, especially those containing aromatic amino acids. Take few ml wine sample mix it with concentrated nitric acid. The mixture is heated and cooled. A strong base, sodium hydroxide is added to the solution, a yellow to orange colour change indicates a positive result i.e presence of aromatic amino acid.

FEHLING TEST: Used to detect the presence of reducing sugars. Fehling solution is prepared by combining equal volume of Fehling's solution A (copper sulphate solution) and Fehling's solution B(potassium sodium tartrate in alkaline solution). A

BENEDICT TEST: we take benedict reagent which is sodium citrate, sodium carbonate and copper(II)sulphate pentahydrate. A sample is mixed with benedict reagent and heated. The colour change indicates the presence and concentration of reducing sugars.

NINHYDRIN TEST: This test is used to detect presence of free amino acid. Dissolve 0.2g of ninhydrin in 10ml of ethanol or acetone. Add few drops of ninhydrin solution to the wine sample. Heat

the mixture in warm water bath for few minutes. A purple colour indicates presence of amino acid

Estimation of alcohol using spectrophotometer

Estimation of alcohol content using spectrophotometer: A spectrophotometer is an instrument used to measure the absorbance or transmittance of light by a sample, typically at specific wavelengths. It works by passing a beam of light through a sample and measuring how much light is absorbed or transmitted. This information is then used to quantify the concentration of a substance in a solution or to analyze the color and other properties of a material.

Estimation of alcohol concentration using spectrophotometer is done by colorimetric reaction between alcohol and potassium dichromate(IV) measuring the absorbance of the resulting solution and comparing it to a calibration curve. Ethanol reacts with chromium ion and forms a coloured product. The decrease in absorbance is observed at 600nm wavelength (Sriariyanun et al., 2019) various readings are observed at different span of time.

Preparation of chromatic acid: for Preparing chromatic acid (26ml) we will take 1g of potassium dichromate in conical flask will add 20ml distilled water into it and carefully add 5ml H₂SO₄. Prepare test tubes heat at 60-70 for 5-15 minutes. We will prepare ethanol of various concentration 5%, 8%, 10% etc for comparing the ethanol concentration of unknown sample. Now we will add few ml ethanol in various test tube will add dichromatic acid into it and distilled water. Will heat it for 20 minutes at 60-70°C.

Absorbance is taken typically at around 600nm because at this wavelength colour change from orange to blueish green is most prominent, indicating reaction between the dichromate ions and ethanol, higher absorbance is absorbed as the ethanol content increase.

Estimation of alcohol per volume using refractometer: refractometer is used to calculate brix or specific gravity of a solution. Specific gravity also known as

relative density is a dimension quantity that compares the density of a substance to the density of a reference substance. Brix is a measure of the total soluble solids TSS, primarily sugars, in a liquid solution often used in the food and beverages industries to assess sweetness or maturity. One degree brix (°Bx) represents 1 gram of sucrose in 100 gram of solution. Alcohol per volume can be calculated if we know the specific gravity of a solution via using formulae (Tupe et al., 2023)

Original specific gravity- current specific gravity/0.0074 = alcohol percentage

(Original specific gravity – current specific gravity)* 131.25= alcohol by volume

Health Benefit of *Carissa spinarum* wine

Fruit of *Carissa spinarum* consist of Reducing and non reducing sugar, glucose, galactose, glycine, alanine, Phenylalanine, vitamin C, Pectin, Carissol, Lupeol, cerine, malonic acid, oxalic acid, tartaric acid, malic acid, glycolic acid and citric acid. Which leads to various health benefits including.

Antioxidant properties:- *Carissa spinarum* is rich in antioxidants, including flavonoids, phenolic compounds, and Vitamin C, all of which are present in the fruit and likely retained in the wine.

Potential anti-inflammatory effects Extracts from various parts of *Carissa spinarum*, including the roots and leaves, demonstrate anti-inflammatory properties:- This suggests that the fruit, and subsequently the wine, might also contribute to reduced inflammation. However, further research is needed to confirm this in the context of wine consumption.

Potential antidiabetic activity Studies, primarily in animal models, suggest that *Carissa spinarum* may help regulate blood sugar levels, reducing blood glucose and lipid profiles. However, human trials are needed to confirm these effects with *Carissa spinarum* wine consumption (Beck, 2016) although *Carissa spinarum* wine contains ethanol, and excessive alcohol consumption is harmful So moderation is required.

III.RESULTS AND DISCUSSIONS

Calculation of ethanol content using specific gravity

Refractometric analysis was conducted across a fermentation span of 22 days. Initial Total Soluble Solids (TSS) were adjusted to 20°Brix using jaggery. Specific gravity (SG) readings and Brix values were recorded periodically to monitor sugar conversion and alcohol development. Alcohol percentage and alcohol by volume (ABV) were calculated using standard formulas. By Day 12, a significant alcohol yield of 8.01% ABV was achieved, which remained stable through Day 22, indicating the completion of fermentation. The plateau in alcohol content and unchanged specific gravity readings beyond Day 12 confirm that maximum fermentation efficiency had been reached.

Time(in day)	Specific gravity	brix	Alcohol percentage	Alcohol by volume
Day 1 18 th dec	1.083	20	..	0
Day 5 23 rd dec	1.030	7.6	7.1621	6.96%
Day 12 31 th dec	1.022	5.6	8.2432	8.01%
Day 17	1.022	5.6	8.243	8.01%
Day 22	1.022	5.6	8.243	8.01%

Original specific gravity- current specific gravity/0.0074 = alcohol percentage

(Original specific gravity – current specific gravity)*

131.25= alcohol by volume

Calculation of ethanol content in wine using spectrophotometer (Caputi et al., 1968)

To prepare the calibration curve, standard ethanol solutions of 5%, 8%, and 10% were prepared. Each test tube contained 50 µL ethanol solution, 3950 µL distilled water, and 1 mL chromic acid. Absorbance was measured using a spectrophotometer at 595 nm against a blank. Wine samples collected on different fermentation days were processed similarly. The

increasing absorbance values over the fermentation period clearly indicate the progressive increase in ethanol content. By comparing sample absorbance values with the standard curve, the ethanol content in the wine samples was estimated to range between approximately 5–9% over time, aligning closely with commercially available wine. As shown in the table below and graph is drawn from it.

Test tube	ethanol	Distilled water	Chromatic acid	Absorbance at 595nm
blank	0	4000ul	1ml	0
Test tube 1 (5%)	50ul	3950ul	1ml	0.223
Test tube 2(8%)	50ul	3950ul	1ml	0.359
Test tube 3(10%)	50ul	3950ul	1ml	0.468
Wine sample day 1	50ul	3950ul	1ml	0.240
Wine sample day 5	50ul	3950ul	1ml	0.380
Wine sample day 12	50ul	3950ul	1ml	0.391
Wine sample day 22	50ul	3950ul	1ml	0.397
Bottled wine from market with(8-9%)	50ul	3950ul	1ml	0.415

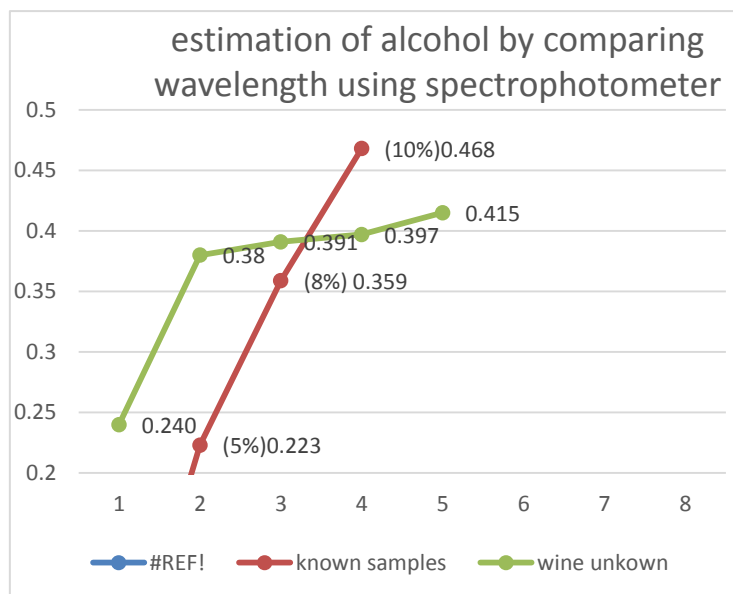


Figure 2 Test tubes containing dichromate acid and ethanol showing colour change due to the oxidation reaction between alcohol and dichromate ions

pH measured of wine produced

pH is a crucial factor in winemaking, impacting various aspects of wine quality, including color, microbial stability, sensory characteristics. Wine produced have 4 PH which is considered ideal for wine making (Baca-Bocanegra et al., 2022)

colour: pH of red wine affects the stability of anthocyanin which are responsible for the red colour. Higher pH levels can lead to decrease in anthocyanin concentration and shift in color

microbial growth: pH is a crucial factor in determining the growth of spoilage microorganisms in wine. Lower pH level inhibit the growth of microbes including many bacterias, while higher pH can create more hospitable environment for microbes.

Sourness: directly impacts the sourness of wine. Lower pH wines will taste more tart or acidic, while higher pH wines will be perceived as less acidic.

Bitterness and Astringency: pH can indirectly affect bitterness and astringency, which are also important sensory attributes of wine. These attributes are often linked to the phenolic compounds in wine, which are themselves affected by pH.

Protein Stability: pH influences the solubility of proteins in wine, which can lead to the formation of haze or sediment.

Tartrate Stability: pH affects the solubility of tartrate salts, which can precipitate out of solution and cause tartrate crystals to form in the wine.

AST PERFORMED ON WINE SAMPLE

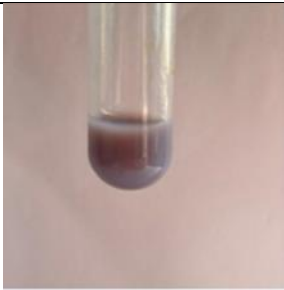
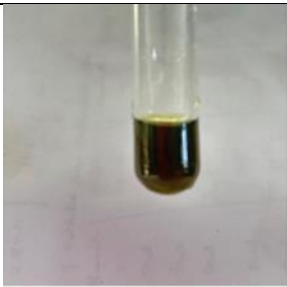
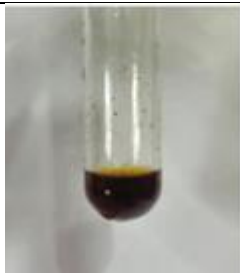
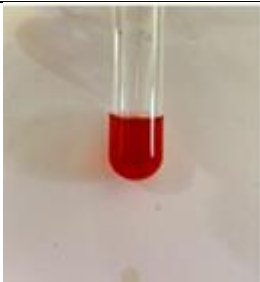
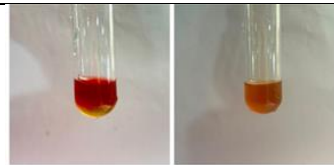
Antibiotic susceptibility test is a crucial laboratory procedure used to determine which antibiotic are effective against a specific bacterial isolate. Kirby-Bauer disc diffusion method in which antibiotic impregnated paper disks are placed on an agar plate inoculated with bacteria. Zones of inhibition are observed showing the effectiveness of antibiotic. When it comes to wine, antibiotic susceptibility testing is not a standard practice for the wine itself in the same way it is used in medicine to determine which antibiotic will effectively fight a bacterial infection. wine consist of phenolic compound, ethanol

which have shown to possess antimicrobial properties (Boban et al., 2021). These phenols are plant secondary metabolites possessing various health benefits including antioxidant, anti allergic, anti inflammatory, anti cancer. Among polyphenol classes e.coli showed sensitivity to phenolic acids. although biological effects are rarely studied but they still show some resistance.

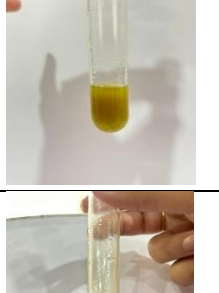
In our experiment we have used agar- well diffusion method, we could not demonstrate antimicrobial effectiveness for wine produced. Inhibition zones were not detected around the wells. The small amount of wine maybe the cause of no result. And that's why AST is not used for testing antimicrobial resistance testing. But MIC is considered to be better option. (Santoro et al., 2020)

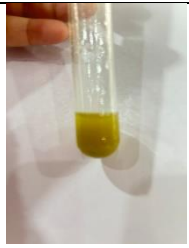
**WINE PHYTOCHEMICAL TESTS**

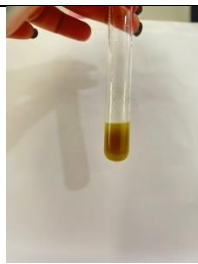
Tanin or braymer test	Positive	
Saponin test	Negative	

Lead acetate test	Positive		H ₂ SO ₄ test	Negative	
Alkaline reagent test	Negative		Ammonia test	Negative	
Trepanoid test	Positive		Benedict test	Negative	
Wagner test	Negative		Fehling test	Positive	
Mayers test	Negative		Ninhydrin test	Positive	
Dragendroffs test	Negative		Xanthoproteic test	Positive	

LEAF PHYTOCHEMICAL TESTS

SAPONIN	Negative	
TANIN	Positive	
FLAVNOID	Positive	
PHENOLIC FLAVANOID	Negative	
ALKALOID MAYERS REAGENT TEST	Negative	
TREPNOID	Negative	

DRAGENDROFF TEST	Negative	
AMMONIA	Negative	
H ₂ SO ₄ TEST	Positive	
BENEDICT TEST	Positive	
XANTHOPROTIEC TEST	Negative	
POTASSIUM DICHROMATE TEST	Positive	
FEHLING TEST	Positive	

NINHYDRIN TEST	Negative	
MOLISH TEST	Positive	

IV. CONCLUSION

The present study successfully demonstrated the potential of *Carissa spinarum* fruit for wine production using traditional fermentation methods and yeast strains of *Saccharomyces cerevisiae*. The fermentation process, monitored through refractometry and spectrophotometric analysis, revealed a progressive increase in ethanol concentration, stabilizing at approximately 8–8.5% alcohol by volume (ABV) by the 12th day of fermentation. The ethanol levels were found to be comparable to those in commercial wines.

Additionally, preliminary phytochemical screening of both the fermented wine and aqueous leaf extract of *Carissa spinarum* indicated the presence of various bioactive compounds, such as alkaloids, flavonoids, tannins, saponins, and phenolic compounds, suggesting potential antioxidant, antimicrobial, and therapeutic properties. The retention of certain phytochemicals even after fermentation highlights the medicinal value of the final wine product.

Overall, the study supports the feasibility of utilizing *Carissa spinarum*, a locally available wild fruit, in the development of value-added functional beverages. Further research is encouraged to explore its full nutraceutical potential, sensory acceptability, and long-term shelf stability.

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