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Studies on bio-clarifying & antimicrobial efficacy of moringa seed powder on shelf stability of mixed carrot-beetroot juice

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Abstract
The most important aspect of this study is clarification and increasing the shelf stability of mixed carrot-beetroot juice with moringa seed powder (MSP).To clarify and to increase shelf life of juice, moringa seed powder added with different proportions (0.5 g,1 g,1.5 g) in 100 ml mixed carrot-beetroot juice. After addition of MSP in juice, it kept to clarification for 1 hour. The juice was subjected to turbidity test, anti nutritional factors and microbial analysis. The juice clarified with 0.5g of MSP is more accepted. Based on the results, MSP could be highly recommended as natural clarifying agent and has antimicrobial efficiency. The juice with 0.5g is showing better results compared to 1 g and 1.5 g.

Keywords: Moringa seed powder, antimicrobial efficiency & bio-clarification

Introduction
Moringa tree (*Moringa oleifera* Lam.) is the most important species belongs to the *Moringaceae* family, popularly known as "Tree of life". Some parts of the plant, like leaves, roots and seeds, have several nutritional and medicinal properties used with industrial and medicinal purposes. Moringa seeds can consume fresh or cooked and their nutritional composition is similar to leaves in various minerals and amino acids. The root is rich in nutrients like phosphorus, magnesium, calcium and vitamin C.

M. oleifera seeds act as a natural absorbents and antimicrobial agent, with the ability to reduce 87% of Escherichia coli colonies in contaminate water. M. oleifera seeds are also acting as antimicrobial agent against variety range of bacteria and fungi. The seed contain number of benzyl isothiocyanate and benzyl glucosinolate which act as antibiotic.

Moringa seeds contain 1% active polyelectrolyte's that neutralize the negative charged colloid in the juice. This protein can therefore be a nontoxic natural polypeptide for sedimentation of mineral particles and organics in the purification of juice. It is believed that the seed is an organic natural polymer. The active ingredients are dimeric proteins. The protein powder is stable and totally soluble in juice. Moringa oleifera seed powder has shown a signifcant reduction of turbidity and coliform count when it was used at smaller concentrations without altering the pH of the water.

Beetroot (*Beta vulgaris* L.) belongs to the Chenopodiaceae family. It has bright crimson colour. Beetroot is commonly known as beet, chard, spinach beet, sea beet, garden beet, white beet. It has very medicinal properties which give some positive effect on the human body. Beetroot can be eaten raw, boiled, steamed and roasted. Beetroot is a rich source of minerals (magnesium, manganese, sodium, potassium, iron, copper), which boosts the energy as it has one of the highest nitrates and sugar contents plant (Yadav *et al*., 2016) [9]. Beetroot makes an excellent dietary supplement as it is not only rich in minerals, vitamins and nutrients but it also has unique Phytochemical compounds (carotenoids, phenolic acids, ascorbic acid) which have many medicinal uses.

Carrots (*Daucus carota)* are high in dietary fiber & trace mineral molybdenum, which is uncommon in vegetables. Molybdenum is necessary for iron absorption & helps in fat & carbohydrate metabolism. Magnesium & manganese are abundant in this fruit. Magnesium is required for bone formation, protein synthesis, B vitamin activation, nerve & muscle relaxation, blood coagulation, & energy generation. Carrot consumption is gradually rising as a result of its recognized as a valuable source of natural antioxidants with anticancer properties.

Material and Methods

The raw materials used are beetroot, carrot and sugar are generally procured from the local areas and where as moringa seeds were procured from nearby orchids.

Preparation of moringa seed powder

The preparation of moringa seed powder prepared in the laboratory. Moringa seeds of 50 g were soaked in water for 6 hours and these soaked seeds are dried in tray dryer using 90 min, 50 ºC, 1.5 m/s as the ranges of contact time, heater and fan speed respectively and then it is milled into fine powder.

Moringa seeds
↓
Deshelling
↓
Soaking in water (6hrs)
↓
Drying (Tray dryer-90 min at 50 ℃)
↓
Grinding
↓
Sieving (250 µ) & packing

Flowchart for preparation of moringa seed powder

Preparation of mixed beetroot-carrot juice

Beetroot and carrot were taken according to the standardized procedure as listed in table and are manually cleaned and this are grinded to acquire juice.

Formulation of mixed beetroot-carrot juice

S.No.	Ingredients	Quantity
1.	Beetroot	250 g
2.	Carrot	250 g
3.	Sugar	100 g
4.	Water	400 ml

Carrot and beetroots
↓
Washing
↓
Peeling
↓
Cutting
↓
Juice extraction
↓
Addition of sugar
↓
Mixing

Flowchart for preparation of mixed beetroot-carrot juice

Clarification of juice

Mixed carrot-beetroot juice
↓
Addition of moringa seed powder/Agar agar/Gelatin
↓
Kept for 1 hour (clarification)
↓
Strain the juice with muslin cloth
↓
Collect the clarified juice

Flowchart for clarification of mixed beetroot-carrot juice

Clarification of mixed carrot-beetroot juice

To standardize the clarification of mixed carrot-beetroot juice with moringa seed powder with different proportions and comparing with chemical clarifying agents with same proportions to know the efficiency of clarification with moringa seed powder.

Various clarifying agents used in different proportions

Sample	Moringa seed powder	Agar-agar	Gelatin
Control sample	-	-	-
Sample 1	0.5	0.5	0.5
Sample 2	1	1	1
Sample 3	1.5	1.5	1.5

Physico-chemical analysis

Measurement of TSS

Total Soluble solids content was measured using Digital hand-held pocket Refractometer (ATAGO) in % Brix. The range of the refractometer is 0 to 85%.

Determination of Turbidity

Turbidity Meters technically known as nephelometers-emit light and measure the amount scattered by particles in the sample. The units depend on the wavelength of the light and the angle of the detector(s) 13; the most common units are Nephelometric Turbidity Units or Formazin Nephelometric Units.

Determination of pH

pH measures the concentration of hydrogen ions in water. An ion is an atom or molecule that has gained or lost electrons, and thus has a negative or positive charge. The pH scale measures the concentration of those charges, assigning them a value from 0 to 14. Wash the electrode with distilled water and wipe with the tissue paper. Dip the electrode in the standard solution (Buffers solutions (pH 4, & pH 9). Again wash the electrode with water and clean. Dip the electrode in the test solution. Record the pH.

Estimation of phytic acid

The phytate is extracted with trichloroacetic acid and precipitated as ferric salt. The iron content of the precipitate is determined colorimetrically and the phytate phosphorous content calculated from this value assuming a constant 4 Fe: 6 p molecular ratios in the precipitate.

$$\text{Phytate P (mg/100 g)} = \frac{\text{µg Fe} \times 15}{\text{Weight of the sample}}$$

Estimation of oxalate

In this method oxalic acid is determined by titration with NaOH solution. The purpose of an acid- base titration is to determine the concentration of the acidic solution by titrating it with a basic solution of known concentration until neutralization occurs.

$$\text{Calcium oxalate content} = \frac{\text{Titration vol. (ml)} \times \text{Normality of } KMnO_4 \times \text{Molecular wt. of calcium oxalate} \times \text{DF}}{\text{Weight of sample}}$$

Microbiological analysis

Microbial analysis was carried out in FDSQ lab in College of

Food Science and Technology, Rudrur by using standard procedure of AOAC, 1995 [10]. The stored mixed beetroot-carrot juice were subjected to microbial analysis for standard plate count (SPC) using nutrient agar. The equipment used for microbial analysis are laminar air flow, autoclave, incubator and colony counter. The Nutrient agar were mixed in Double distilled water and sterilized in autoclave at 121 °C for 15mins and cooled. The sterilized media was poured in the sterilized petri plates before solidification and kept for 15 minutes in laminar air flow for solidification. Then incubate the plates at 30 °C for 24 hours for growth of bacteria. Dilutions were made in the 0.85% saline water. Microbial analysis was carried out with 10-1 to 10-9 dilutions. For 10-1 dilution, take 1ml of sample into a sterilized glass test tube containing 9 ml of saline water. For 10-2 dilutions, sample of 1ml from 10-1 dilution into another sterilized glass test tube containing 9ml of saline water. For 10 -3 dilution, 1ml from 10-2 dilution sample was aseptically taken into a sterilized glass test tube containing 9ml of saline water. Similarly same dilutions were followed upto 10-9 dilution. Prepared dilutions were poured in a set of sterile petri plate.

By using spread plate method, 0.1 ml of samples of each were taken from 10-7, 10-8, 10-9 dilutions and used for spread plating on the nutrient agar and cooled. The plates then were incubated in an incubator at 30 °C for 24 hrs and number of bacterial colonies formed was recorded.

Results and discussion

The results obtained by adopted systematic approach for 'Studies on Bio-Clarifying & Antimicrobial efficiency of Moringa seed powder on shelf stability of mixed Beetroot-Carrot Juice' were presented. The clarified juice was subjected to determination of turbidity, pH, brix, antinutrional factors & shelf life studies.

Antinutritional factors

Antinutritional factors	Raw seeds	Soaked seeds
Phytic acid	196 mg /100 g	125 mg/100 g
Oxalate	158 mg/100 g	97 mg/100 g

The anti-nutritional factors phytic acid & oxalate contents were decreased in the soaked moringa seeds compared to the raw seeds. Level of anti-nutritional factors was decreased on soaking of moringa seeds for 6 hours. Soaking reduces the anti-nutrient phytochemicals due to leaching of water-soluble vitamins and minerals seeds and grains. The phytic acid content of raw moringa seeds is 196 mg /100 g, is decreased to 125 mg/100 g. The oxalate content is reported as 97 mg/100 g in soaked moringa seeds.

Clarification of mixed carrot-beetroot juice by various clarifying agents

The clarification of mixed carrot-beetroot juice was carried out with moringa seed powder, agar-agar and gelatin. And compared the moringa seed powder and other chemical clarifying agents with same proportions to know the clarifying and antimicrobial efficiency.

When compared to other chemical clarifying agents, the MSP provides the juice with better characteristics based on the sensory evaluation (9-point hedonic scale).

Clarification of mixed carrot-mixed beetroot juice with MSP in different proportions

Parameters	Control sample	MSP (0.5 g)	MSP (1 g)	MSP (1.5 g)
Brix	13.9	12.7	12	11.6
pH	6.08	6.30	6.20	6.0
Turbidity	580 NTU	467 NTU	429 NTU	382 NTU

The parameters like brix, turbidity was decreased after addition of moringa seed powder in the juice where as the pH was increased.

Microbial analysis

The microbial study of standard plate count (SPC) conducted for 1 month as specified below

Storage period	Control sample (cfu/ml)	MSP (0.5 g) (cfu/ml)
Day-1	-	-
Day-10	30×10^{-7}	22×10^{-7}
Day-20	124×10^{-7}	56×10^{-7}
Day-30	TMC	138×10^{-7}

Conclusion

The results of present study revealed that the clarification of juice with moringa seed powder (0.5 g) can reduce the turbidity of juice from 580 NTU to 467 NTU. The dehulling of moringa seeds and soaking in water reduces the phytate and oxalate content.

The moringa seed powder acts as an antimicrobial agent which delays the growth of pathogens & other spoilage organisms, there by increases the shelf life of mixed carrot-beetroot juice for 10 days. The use of moringa seed powder has an added advantage over the chemical treatment of juice because it is biological and edible. The beetroot juice blended with the carrot enhanced their nutritional quality. The moringa seed powder acts as a natural clarifying & antimicrobial agent and is economic when compared to the chemical clarifying agents.

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