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FULL PAPER

Study on the pre-formulation of *Sonneratia caseolaris* as ready-to-drink fruit beverage

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Abstract

The uses of mangrove fruit in food and beverages are rarely published in scientific papers. One of the mangrove fruits used is *Sonneratia caseolaris* (berembang) or mangrove apple. Each part of the mangrove apple is unique and believed to have many uses, such as the sepal showing high antioxidants property. Some villagers eat the fruit as a vegetable although it is not very well known. Ripe fruit could destroy intestinal parasites in combating diarrhoea and semi-cooked fruit could cure cough. Few mangrove communities in Malaysia can process *S. caseolaris* fruit into food products such as jams and syrups. This research aimed to formulate mangrove apple (*S. caseolaris*) fruit puree and process it into a refreshing drink which complied with consumer acceptance and a longer shelf-life. Processing procedures for handling delicate *S. caseolaris* fruits and fruit puree formulations were developed for commercialization to ensure sustainability of raw material supply. Besides quality control and safety studies, the selection of ripe fruit with the appropriate size along with processing of the starting material is important to increase the quality of the product to be produced. Paste is the most suitable form to handle because the fruit of *S. caseolaris* has a soft texture apart from the sour taste with its own unique aroma. Ready-to-drink (RTD) fruit beverage product from *S. caseolaris* was developed through the support of scientific value through chemical, bioactivity, formulation, sensory, product safety and quality studies. Formula selection of RTD fruit beverage product is also an important factor in meeting local demand, ensuring it comply with customer requirements and are marketable. This study is expected to produce better quality herbal products that meet standards and penetrate the global market.

1. Introduction

Berembang tree or *Sonneratia caseolaris* from the family of Lythraceae is one of the native mangrove plants usually found in muddy bank areas of mangrove forests in Malaysia, South China, the northern continent of Australia and the Pacific islands. *S. caseolaris* has distinctive features as it is now a "magical" tourism product of Malaysia, where it attracts many fireflies at night to feed on the fruit nectar of the trees. *S. caseolaris* fruit or mangrove apple has a triangular-shaped calyx on the outer sepals. The young fruit is very sour and is used as a flavouring in cooking (Bunyapraphatsara *et al.,* 2003; Hastuti *et al.,* 2013). *S. caseolaris* flowers throughout the year with two peak flowering and fruiting seasons. The fruits collected from the first fruiting season generally had a higher germination success than the second one (Premjith *et al.*, 2017).

The *Sonnneratia* genus is a rich source of tannins with antimicrobial and contains high antioxidant activities. Every part of this plant is said to be useful. It is a Malay remedy for sprains, swellings and worms. Old fruit walls were used to treat worms. Meanwhile, the half -ripe fruits treat coughs. The fruits were used to make poultices and leaves were crushed, mixed with salt, and also applied as poultices onto cuts and bruises. The pounded leaves were used as a treatment for haematuria and smallpox (Ken Fem, 2014). The leaves had antimicrobial properties that could treat infectious diseases caused by microorganisms such as *Escherichia coli, Staphylococcus aureus*, and *Bacillus cereus* and contained anti-diabetic properties that inhibited α-

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glucosidase. An antioxidant *Sonneratia* study in FRIM showed mangrove apple fruit samples contained a high percentage of activity to trap DPPH radicals. According to a research paper by Manalu (Hamsah, 2013), the fruit *Sonneratia* sp. contained 84.76% water, 77.5% carbohydrates, 9.21% protein, 8.4% ash, and 4.82% fat, and vitamin C at 56.74 mg/ 100 g. Presently, this species is at serious risk of extinction as no systematic attempt has been made to conserve them.

Efforts were made to process the mangrove fruit into a variety of food products and generate income for locals. Mangrove fruit tastes vary depending on the type of plant. Therefore, not all mangrove fruits can be processed into the same food type. Thus, the taste of the fruit must be adapted to the type of food to be made. *S. caseolaris* can be eaten raw or consumed in juice form in Sulawesi and other countries like Sri Lanka and Malaysia. It will appear acidic when it is cooked (Priyono *et al.,* 2010). Extracted fruit juice or fruit pulp as the base material can be made into a wide range of drinks. Many are consumed as pure juice without adding other ingredients, but some are added with syrup. There are two basic types of drinks made from the fruit, the RTD after opening and the small amount used when required. The former types should not require any preservatives if they are processed and packaged correctly. However, the latter type contained permitted preservatives to maintain a long shelf-life after opening. The different drink types are classified according to each of the above products preserved by a combination of natural acidity, pasteurization, and packaging in sealed containers. Some drinks such as syrups and squashes also contain high concentrations of sugar, which helps to preserve them. This research aimed to formulate mangrove apple (*S. caseolaris*) fruit puree and process it into a refreshing beverage that complied with consumer acceptance and a longer shelf-life.

2. Materials and methods

2.1 Selection and fruit processing

Sonneratia caseolaris ripe fruits were collected from a mangrove forest in Sg. Acheh Nibong Tebal, Penang. The fruit obtained were sorted according to the ripeness and size of more than 44 mm, the soft texture of the fruit and not being infected by fruit fly larvae. The fruits were washed with clean water and the fruit petals were separated. The peeled skin was crushed with water to separate the puree from the seeds. The cut surfaces must not be exposed to air for long periods, or they might turn brown and discolour the beverage. The puree was kept frozen for storage. Figure 1 shows the preparation of *S. caseolaris* frozen fruits puree.

2.2 Paste preparation

The fruit puree was then poured into a pot with water, stirred until boiling, and sugar was added. The ratio between developing fruit puree and granulated sugar is 1: 1. The cooking was continued for several hours until the paste was caramelized or became thick and reddish. Figure 2 displays the preparation of *S. caseolaris* paste from fruit puree.

2.3 Beverage Pre-formulation

In the starting stage for product development and to determine a formula or recipe for *S. caseolaris* fruit beverage, approximately 10 to 60 g of paste were weighed. Different water ratios were added to the paste

A B C

Figure 1. Preparation of *S. caseolaris* frozen fruit puree. A. *S. caseolaris* fruits washing and cutting process, B. Separating the puree from the seeds by crushing it with water, C. The puree was kept frozen for storage

A B C

Figure 2. Preparation of *S. caseolaris* paste from fruit puree. A. Ingredients (Puree, sugar, and water), B. All ingredients were added and stirred until boiling, C. Cooked for several hours until it is caramelized or thick

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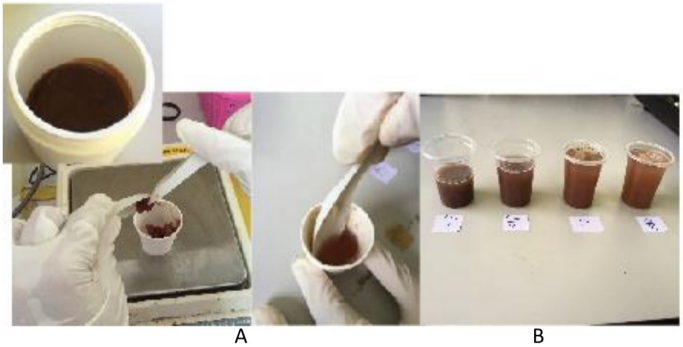

Figure 3. Pre formulation processes. A. Fruit paste is weighed and mixed with water, B. Samples ready for organoleptic assessment.

and mixed until dissolved (Figure 3 and Figure 7). The organoleptic test was done for every sample to obtain the right formulation for the fruit beverage. Each sample was evaluated according to taste, aroma, and physical appearance. Figure 4 shows the formulation processes for RTD fruit beverage. The beverage was then prepared as prototyped, and at this point, potassium sorbate was added as a preservative. The beverage is filtered through a muslin cloth or a stainless steel filter to make it clear. Although the natural juice was cloudy in nature some consumers prefer a clear product.

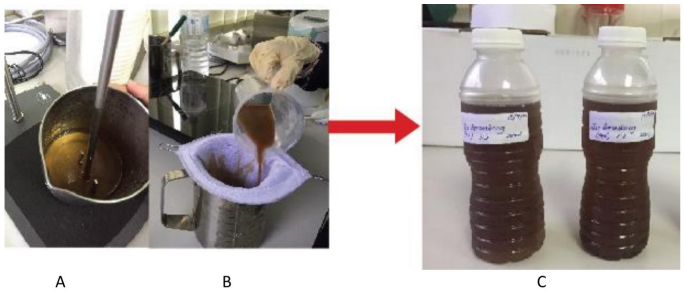

Figure 4. Formulation processes. A. Mixing the paste with water and Potassium sorbate, B. Filtering the juice with a muslin cloth, C. RTD fruit beverage prototype product.

2.4 Quality control and safety study

The fruit beverage samples were then sent for quality control and safety study. In the quality assessment, the microbial load and heavy metal test were tested. In the safety test, the beverage went through a toxicity test. In addition, the beverage was also tested for antioxidant and nutritional facts.

3. Results and discussion

The total weight of the fleshy and globular *S. caseolaris* fruit was about 92% consisting of the internal heavy seeds, as mentioned by Azlen and Mohd Fadzelly (2018). Figure 5 shows the fruit puree, sugar, and water were processed into a paste. They formed a thick and reddish or caramelized paste. Results for the organoleptic test with the amount of paste dissolved in water was shown in Figure 7. The organoleptic test involved the assessment of the flavour, odour, appearance, and

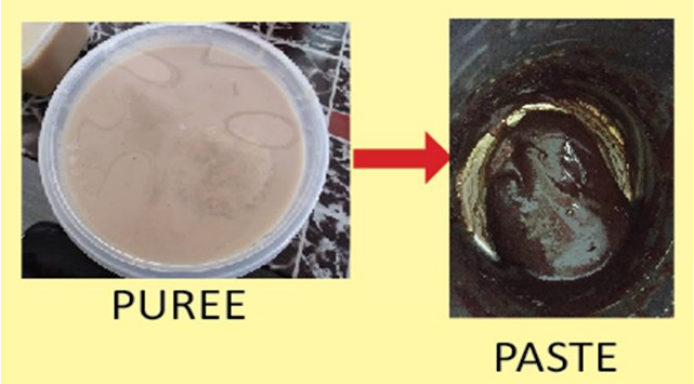

Figure 5. The processed fruit puree into a paste

mouthfeel of food or drink products (NSAFD, 2019) to ensure the RTD fruit beverage products complied with customer requirements. When the juice or puree was collected, it was necessary to prepare the product batch according to the chosen recipe. This recipe was carefully chosen and very much a matter of choice and judgment to suit local tastes. RTD fruit beverage was formulated from the *S. caseolaris* fruit paste, a sweet mixture processed into a refreshing beverage and consumed on a 'one shot' basis. Essentially, they consisted of a 30% mixture of fruit puree and sugar syrup to produce a final sugar level of about 12-14%. The actual sugar levels varied from fruit to fruit and with the stage of fruit ripeness. They also varied within the same fruit grown in different parts of the world. In order to achieve the recommended levels for preservation, the addition of sugar to the fruit puree should also consider the amount of sugar already present in the fruit. All fruits contained sugar, usually around 8-10% (NPCS, 2012). It was important to achieve the minimum sugar level to prevent bacteria growth. However, once that level was achieved, adding more sugar was done if the consumers required a sweeter product. The manufacturer usually will decide the amount of sugar added. In all cases, sugar should be added to the fruit juice in a form of sugar syrup. Filtering the RTD fruit beverage through a muslin cloth before mixing removed the precipitate in order to give a clearer and higher quality product. Figure 6 shows the RTD *S. caseolaris* fruit beverage prototype. Sodium and potassium sorbate were widely used to inhibit the growth of moulds and yeasts. The RTD fruit beverage prototype

Figure 6. RTD *S. caseolaris* fruit beverage prototype product

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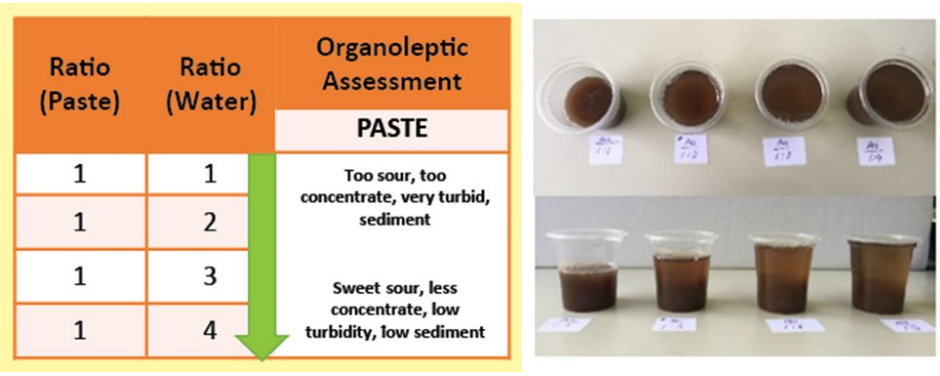

Figure 7. Organoleptic test with series of paste dissolved in water

was tested for its nutritional content (Figure 8). It showed the nutritional composition such as energy (50 kcal/100 mL), total fat (0.1 g/100 mL), protein (0.2 g/100 mL), carbohydrate (12.4 g/100 mL), total sugar (8.2 g/100 mL), dietary fibre (0.8 g/100 mL) and antioxidants enriched with Vitamin C (0.55 mg/ 100 mL). The laboratory test showed that the microbial load and heavy metal test of the RTD fruit beverage were within the safety limit (Tables 1 and 2). Cytotoxicity studies were done using Vero and WRL-68: kidney and hepatic–like cell lines MTT Test (Table 3 and Figure 9). The result showed that the RTD fruit beverage was safe because its therapeutic dose was higher than the cytotoxic value. As for the antioxidant activity (Table 4), the RTD fruit beverage recorded DPPH free radical and superoxide scavenging activity above 90% at 2.5% of the beverage concentration which was good for health and well-being. Table 5 shows the ORAC value of the RTD fruit beverage was 950 µmol TE/100 g which was higher

Maklumat Kandungan Khasiat/ Nutrition Information

Hidangan satu pek : 330 ml *Serving Per Pack : 330 ml*	Saiz Hidangan : 250 ml *Serving Size : 250 ml*	
	Setiap Hidangan *Per Serving*	Setiap 100 ml *Per 100 ml*
Tenaga/ *Energy*	125 kcal (523 kJ)	50 kcal (210 kJ)
Protein	0.5 g	0.2 g
Jumlah Lemak/ *Total Fat*	<0.25 g	< 0.1 g
Karbohidrat/ *Carbohydrate*	31 g	12.4 g
Ash	0.75 g	0.3 g
Moisture	217.75 g	87.1 g
Jumlah Gula/ *Total Sugar*		
• Fructose	10.5 g	4.2 g
• Glucose	10 g	4.0 g
Serat Permakanan/ *Dietary Fiber*	2 g	0.8 g
Vitamin C	1.38 mg	0.55 mg

Figure 8. Nutritional content of RTD *S. caseolaris* fruit beverage prototype

Table 1. Heavy metal test result for RTD fruit beverage

Elements	Results (Mg/Kg)	Criteria DRGD 2016; Rev Jan 2019.
Plumbum (Pb)	<0.01	NMT 10.0 mg/kg
Cadmium (Cd)	<0.01	NMT 0.3 mg/kg
Mercury (Hg)	0.08	NMT 0.5 mg/kg
Arsenic (Ar)	0.09	NMT 5.0 mg/kg

* NMT = Not More Than

Table 2. Microbial load test result for RTD fruit beverage

Test	Results	Criteria British Pharmacopeia, 2016
Total Aerobic Microbial Count (TAMC)	<10 CFU/mL	5×10^4 CFU/mL
Total Yeasts and Moulds Count (TYMC)	<10 CFU/mL	5×10^2 CFU/mL
Bile tolerant gram-negative bacteria	<10 PN/mL	$<10^2$ CFU/mL
Escherichia coli	Absent	Absent
Salmonella spp.	Absent	Absent

*Drink complies with BP 2016 (microbiological quality of herbal medicinal products for oral use).

Table 3. *S. caseolaris* cytotoxicity study

Test Samples		IC_{50} VERO	IC_{50} WRL-68
S. caseolaris	Fruit	116.413±14.129	125.620±38.480
Standard	Paclitaxel	0.053±0.0012	0.007±0.007

*VERO and WRL-68: kidney and hepatic–like cell lines MTT Test

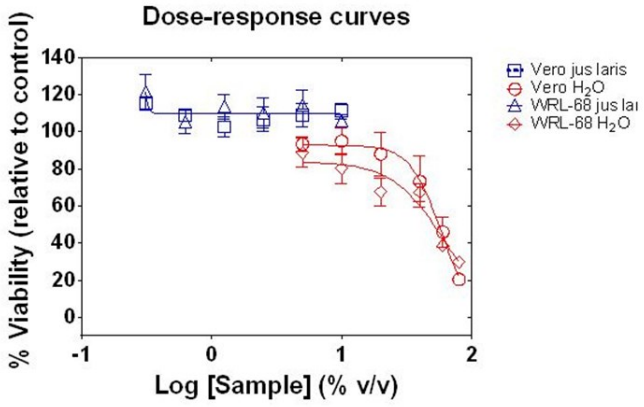

Figure 9. RTD fruit beverage prototype cytotoxicity study

Table 4. Antioxidant activity of RTD fruit beverage

Concentration	Superoxide radical scavenging activity (%)	DPPH free radical scavenging activity (%)
2.5%	93.34±2.04	95.2±1.70
Standard	Superoxide Dismutase (SOD) 75.55±0.67	Ascorbic Acid (AA) 96.55±0.00

*Antioxidant activity; low: 0-49%; moderate: 50-69%; high: 70-100%

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Table 5. RTD fruit beverage ORAC Value

Sample	Type of products	ORAC value[a] µmol TE/100 mL	MU[b]
Berembang	Juice	950	±144

*Data expressed as ORAC value µmol TE/100 mL, are mean values of triplicate wells in duplicate experiments
[a]Rounded up to nearest thousand
[b]the acceptable precision of the ORAC analysis is ≤0% measurement uncertainty (MU)

compared to the ORAC Value of raw orange juice (726 µmol TE/100 g) (Superfoodly, 2010).

4. Conclusion

RTD beverage from fruit juices were an alternative to adding fruits to our diet and keeping the body hydrated by providing fluids and all the nutrients of the fruits. The use of *S. caseolaris* fruit puree had a significant effect on the organoleptic nature of the RTD fruit beverage, including colour, aroma, texture, and likeness. Cytotoxicity study of the RTD *S. caseolaris* fruit beverage was considered safe because the percentage of cell viability was high and DPPH free radical and superoxide scavenging activity were above 90%, which was good for health and well-being. Furthermore, the RTD fruit beverage contained energy, protein, carbohydrate, sugar, dietary fibre, and Vitamin C needed as recommended by RNI Malaysia, 2017. Therefore, this study hoped to produce better quality herbal products which complied with the standards and penetrate the global market.

Conflict of interest

The authors declared no conflict of interest.

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