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RESEARCH ARTICLE.....!!!

MICROBIAL STATUS OF MANGROVE FRUIT (*SONNERATIA APETALA)* JELLY

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KEYWORDS:

Sonneratia apetala, Jelly, Total Aerobic Bacterial Count,Ttotal Anaerobic Bacterial Count.

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ABSTRACT

Sonneratia apetala is a mangrove tree found abundantly in the western part of Indian Sundarbans. The fruit of the species appears in the monsoon period and is extensively consumed by the island dwellers of Indian Sundarbans. With the vision to develop small scale mangrove based industry in Indian Sundarbans, jelly was prepared from the pulp of the fruits and analyzed for microbial quality. The total aerobic, anaerobic bacteria, coliform, fecal coliform, *Escherichia coli*, *Streptococcus* sp, *Vibrio* sp. and *Salmonella* sp. of prepared jelly were analyzed after three months. Absence of *E.coli*, *Streptococcus* sp., *Vibrio* sp. and *Salmonella* sp. ensures the standard quality of the product.

INTRODUCTION:

S. apetala is a woody mangrove tree found in Indian Sundarbans and is locally known as Keora (Fig. 1). The species is found in various countries like Sri Lanka and Bangladesh. It is also abundantly found in India, especially in the western part of the Indian Sundarbans, at the apex of Bay of Bengal, where salinity is relatively low (average water salinity is ~11 psu) compared to the central sector (average value is ~18 psu) [1-5].

Figure 1: Fruits of *Sonneratia apetala*

The fruit of this species is seasonal. It only appears in monsoon season, during the month of August to October. The fruit is sour in taste with greenish yellow pulp. The fruits are mainly preferred by deer population of Indian Sundarbans, but a large percentage of island dwellers consume it after processing and cooking. Jelly was prepared as per the standard method from the pulp of these fruits and subject to microbial analysis as a part of quality control of the finished product.

Fruit jam, jellies have become an important part of the modern diet in almost all countries of the world. Because of their good taste and nutrient level, they form a significant component in the list of balanced diet. Microbial studies of such items are very important as several incidents of outbreaks of illnesses due to consumption of unpasteurized food have been reported [6,7]. Jelly prepared from *S. apetala* fruit pulp contains high amount of vitamin C, major and trace elements compared to jelly from other citrus fruits[8]. The sufficient nutrients in the jelly could support microbial growth[9]. Several factors encourage, prevent or limit the growth of microorganisms. The storage temperature of the product is not always best for maintenance of the desirable quality of the fruits. Water used for jelly preparation can be a major source of microbial contaminants such as total coliform, fecal coliform, streptococci etc. [7]. Environmental factors may also make the product unsafe and may have a role in the spread of *Stretococcus, Vibrio, Salmonella, Escherichia coli* and other diseases as well fruit spoilage[9].

In view of the threat posed by bacterial pathogens in jellies, demand for jellies in the list of modern diet and urgent need for alternative livelihood for the island dwellers of Indian Sundarbans, the present work was undertaken to assess the microbial quality of jelly prepared from the fruit pulp of *S. apetala* so that marketing linkage can be established on the foundation of quality.

MATERIALS AND METHODS

Study Site

The Indian Sundarbans (21˚13′N to 22˚40′N and 88˚03′E to 89˚07′E) at the apex of Bay of Bengal is a mangrove dominated delta in the lower Gangetic region. This mangrove forest has been declared as the World Heritage Site by IUCN in 1987, Biosphere Reserve under Man and Biosphere programme by UNESCO 1989 and is a proposed RAMSAR site[10].

The western sector of the Indian Sundarbans, adjacent to Hooghly estuary sustains dense growth of *S. apetala*, from where the ripe fruits were collected during September, 2013.

Microbiological Analysis

The collected fruits were processed for preparation of jelly by mechanically squeezing fresh fruits. Water, sugar, permitted colour and preservatives were added to the pulp extract[11].

Total aerobic bacteria count

Mullar Hilton agar medium were used for the enumeration of total aerobic bacteria present in the jelly sample. 10 gm jelly was suspended in a 500 ml conical flask containing 100 ml of sterile water. The flask was shaken thoroughly by mechanical shaker for 5 min. In each case the suspension was allowed to stand for 15 min to settle down the heavy particles and then the stock solution was prepared. Sample from each stock solution was then serially diluted. During dilution, 1.0 ml suspension was taken and added to 9.0 ml of sterilized distilled water in test tube and thus 10^{-1} diluted jelly sample was prepared. Similarly, up to 10^{-5} dilution was prepared. Then 0.1 ml of each diluted sample was spread in the respective labeled sterilized Mullar Hilton agar petriplate under aseptic condition. Three replicas were made for each dilution of every sample.

Enumeration of anaerobic bacteria

Jelly sample was diluted up to 10^{-5} dilution using 0.025 (w/v) % cystein supplemented with peptone buffer. Total anaerobic bacteria were enumerated by direct plate count method on selective media (Himedia, M-1396) under anaerobic condition (Gas pack method). The gas pak is highlighted in Fig. 2.

Figure 2: Gas pack method for anaerobic bacteria

Total coliform and fecal coliform

The standard MPN procedure[12] was used for the enumeration of total coliform and fecal coliform using LTB broth and EC (*Escherichia coli*) culture respectively. Briefly, 10 ml of 10^{-1} dilution was added in test tube containing 10 ml of double strength and 1 ml of each dilution (10^{-1} and 10^{-2}) was added separately in test tube containing 10 ml of single strength LTB broth. The total sets were incubated at 35±0.5ºC for 24 hrs and examined for the presence of microbial growth accompanied by gas production. The MPN was calculated and results were expressed as "presumptive coliform MPN/100 g". Then the positive culture was inoculated into BGLB broth and the tubes were incubated at 35±0.5ºC for 24 hrs and examined for growth with gas production. The MPN of total coliform (TC) was calculated and results were expressed as "confirmed coliform MPN/100 g". To enumerate fecal coliform (FC), inocula from 24 hrs positive presumptive tubes were aseptically transferred to tubes of EC medium and incubated at 44±0.5ºC for 24 hrs and examined for the presence of growth with gas production. The results were expressed as "fecal coliform MPN/100 g".

Enumeration of enteric bacteria

The enumeration of enteric bacteria such as *E. coli*, *Streptococcus* sp., *Vibrio* sp. and *Salmonella* sp. was done by standard plate counting method using EMB agar, Azide-dextose agar, thiosulfate citrate-bile-salt agar and xylose lactose dextrose agar media respectively[13,14].

RESULT

Table 1 exhibits that total microbes in the jelly. It is observed that total aerobic and anaerobic bacteria present in jelly were 320×10^5 cfu/gm and 5.5×10^4 cfu/gm respectively (Fig. 3). Total coliforms, fecal coliforms, *E. coli*, *Streptococcus* sp., *Vibrio* sp. and *Salmonella* sp. were totally absent in the jelly.

Table 1: Different types of microbes present in fruit product jelly

Sample	Total aerobic bacterial Count (cfu/gm)	Total anaerobic bacterial Count (cfu/gm)	TC (MPN/ 100 gm)	TFC (MPN/ 100 gm)	*E.coli* / gm	*Streptococcus* sp. / ml	*Vibrio* sp./ ml	*Salmonella* sp. / ml
Jelly	320×10^5	5.5×10^4	nil	nil	nil	nil	nil	nil

TC = Total coliform; TFC = total fecal coliform; cfu = Colony forming unit; MPN = Most probable number

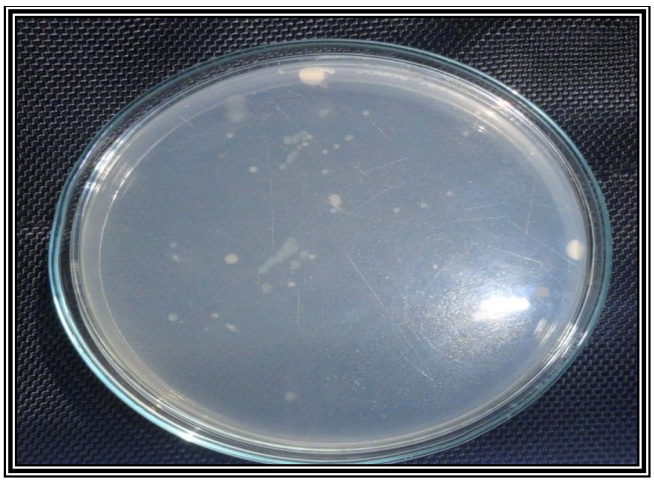

Figure 3: Anaerobic bacteria culture

DISCUSSION

The composition/ ingredients of the jelly prepared from *S. apetala* pulp are mainly water, sugar, preservative, permitted color and fruit pulp. The most commonly used preservative are benzoic acid, sorbic acid and potassium meta bisulfate[7,8]. Natural colors such as anthocyanins, betanin are used. Acid is also an essential universal constituent of juice and the most commonly used acid is Citric acid[7]. Microorganism present in the jelly may be originated from fruit pulp and water. Aerobic colony count (ACC) is defined as the total number of bacteria that are able to grow in an oxygenated or aerobic environment. It is one of the most common tests applied to indicate the microbial quality and not safety of the food. The ACC is the function of shelf life of the product. High ACC value is observed when the product is analyzed in a phase close to its shelf life. Elevated count of ACC may be due to expire of the shelf life, poor sanitation, non hygienic packing materials, post processing contamination due to poor handling and hygiene practices. The present value of ACC in the *S. apetala* jelly is alarming as per the standard recommendation (Table 2). The exact cause of this elevated value, however, could not be pin pointed. The complete absence of TCC, TFC, *E. coli*, *Streptococuus* sp., *Vibrio* sp., *Salmonella* sp. is an indication of better and safe quality fruit product.

Table 2: Microbiological Recommendations for Ready-to-eat Foods

Aerobic bacterial count (CFU/gm)			
Food category	Satisfactory	Cautionary	Unsatisfactory
Category 1*	< 10,000	< 100,000	≥ 100,000
Category 2**	< 100,000	< 1,000,000	≥ 1,000,000
Category 3***	N/A	N/A	N/A

Source: Food Quality Check Program - Microbiological Recommendations and Sampling Schedule – 2014.

*These foods are ready-to-eat and all components are fully cooked in preparation of the final product without any subsequent handling prior to sale or distribution.

**These foods contain components that are fully cooked and ready-to-eat but may have undergone some additional handling (storage, slicing or mixing) prior to the sale, distribution or consumption.

***These foods are exempt from microbial recommendations because it is expected that high aerobic colony counts would be present due to the normal microbial flora associated with these food items.

CONCLUSION

The present programme may pave the avenue of setting a small scale fruit based industry in Indian Sundarbans and can be a road map for alternative livelihood for the island dwellers. However, the quality of the product can ensure the marketing of the product. For this the essential steps are (1) regular monitoring of the microbial strains and level, (2) maintenance of proper hygienic condition during the manufacturing phase, (3) use of good quality water and ingredients, (4) collection of fruits from pollution/ free environments (to avoid the chance of bioaccumulation of conservative pollutants in the fruits).

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