Year: 2014; Volume: 1; Issue: 1
Article ID: MI14 12; Pages: 1-10

BioMed Research

The Open Access Publisher

www.bmrjournals.com

BMR Microbiology

Research Article

Isolation, Characterization and Identification of Potential Probiotics from Fermented Tender Coconut Water

Shiny Esther EA[1], Maria Margaret Joseph[1] and Priya Iyer[2]

[1]Department of Home Science,[2]Department of Biotechnology, Women`s Christian College, Chennai-600006.

Correspondence should be addressed to Priya Iyer

Received 25 July 2014; Accepted 30 July 2014; Published 5 September 2014.

Abstract:

Tender Coconut Water is a refreshing beverage that comes from tender coconuts. It is a powerhouse of nutrition containing a complex blend of vitamins, minerals, amino acids, carbohydrates, antioxidants, enzymes, health enhancing growth hormones, and other phytonutrients. History and folklore credit coconut water with remarkable healing powers, which medical science is now confirming. Published medical research and clinical observation have shown that coconut water, Makes an excellent oral rehydration sports beverage; Aids in exercise performance; Reduces swelling in hands and feet; Aids in kidney function and dissolves kidney stones; Protects against cancer; Helps balance blood sugar; Provides a source of ionic trace minerals; Improves digestion; Contains nutrients that feed friendly gut bacteria (Fife, 2007). Within India the demand for tender coconut water has been increasing over the years. Of the total production of coconut in India, nearly 20 per cent is harvested in tender form, which was only 5 per cent in 2000. At present about 3200 million tender coconuts are consumed annually in India. In a metro city like Mumbai, the annual tender coconut turnover handled by some 40-50 wholesalers is estimated to be around Rs. 750 million. The nutrient content of fresh and fermented tender coconut water was identified. Identification of microorganism was done on fresh tender coconut water and fermented tender coconut water. Standardization of the fermented tender coconut water was done. The formulated drink was tested for its antimicrobial property with E.coli, Salmonella typhi and Enterococcus faecalis and it was seen that it had good effect. The pH of the fermented tender coconut water was found to be 3.98 and the alcohol percentage 2.91 %. Sensory evaluation was done for the fermented tender coconut water in combination with fruit juices in the ratio 1:1 and it was rated as good on a score of 5 by the panel members. Therefore fermented tender coconut water is a nutritious drink that has good acceptability and palatability. It is an easy to prepare fermented drink which has potential probiotics in it which is good for the gut.

Keywords: Coconut Water, potential probiotics, phytonutrients, antimicrobial property.

ISSN 2349-4603

Introduction:

Tender Coconut Water is a refreshing beverage that comes from tender coconuts. It is a powerhouse of nutrition containing a complex blend of vitamins, minerals, amino acids, carbohydrates, antioxidants, enzymes, health enhancing growth hormones, and other phytonutrients. Because its electrolyte (ionic mineral) content is similar to human plasma, it has gained international acclaim as natural sports drink for oral rehydration. As such, it has proven superior to commercial sports drinks. Unlike other beverages, it is completely compatible with the human body, in so much that it can be infused directly into the bloodstream. In fact, doctors have used coconut water successfully as an intravenous fluid for over 60 years.

Fermentation is everywhere, always. It is an everyday miracle, the path of least resistance. Microscopic bacteria and fungi are in every breath we take and every bite we eat. They are ubiquitous agents of transformation, feasting upon decaying matter, constantly shifting dynamic life forces from one miraculous and horrible creation to the next. Fermented foods are those foods which have been subjected to micro-organisms or enzymes so that desirable biochemical changes cause significant modification to the food. However, to the microbiologist, the term “fermentation” describes a form of energy-yielding microbial metabolism in an organic substrate. Fermentation gives us many of our most basic staples, such as bread and cheese, and our most pleasurable treats, including chocolate, coffee, wine and beer. The process of fermentation makes food more digestible and nutritious (Katz 2003).

The process of fermentation uses natural ingredients to encourage growth of these beneficial microorganisms, or probiotics as they are commonly known. They help to restore the correct balance of bacteria in the gut. Many conditions such as lactose and gluten intolerance, asthma, irritable bowel syndrome, constipation and allergies are believed to be linked to an imbalance of bacteria in the gut. Fermenting food is like partially digesting it before we eat it. This is why some people can tolerate yoghurt but not milk - the lactose in milk is broken down as the milk ferments and turns into yoghurt. Fermented foods contain increased levels of folic acid, which is very important for pregnant women in preventing birth defects, along with various other vitamins including the B vitamins. They are also rich in enzymes, which are needed for digestion and absorption of food .

Probiotics are live microorganisms (in most cases, bacteria) that are similar to beneficial microorganisms found in the human gut. They are also called "friendly bacteria" or "good bacteria." Probiotics are available to consumers mainly in the form of dietary supplements and foods. The digestive system is the home of more than 500 different types of bacteria. They help keep the intestines healthy and assist in digesting food. They are also believed to help the immune system. “Probiotics can improve intestinal function and maintain the integrity of the lining of the intestines,” (Stefano Guandalini, MD, professor of paediatrics and gastroenterology at the University of Chicago Medical Centre). These friendly organisms may also help fight bacteria that cause diarrhoea.

Review of Literature:

The production is 12,147.6 million nuts. The productivity is 6,285 nuts per hectare. In Karnataka, area under coconut cultivation is 3.79 hectares. Production is 1,549 million nuts and productivity is 4,078 nuts per hectare. Out of this, tall trees constitute 90% and 10% are dwarf and hybrid varieties which is only tender coconut cultivation. From the 90% tall trees, growers pluck tender coconut of four to six months. Coconuts are grown in the four southern states (Kerala, Tamil Nadu, Andhra Pradesh and Karnataka) which constitute 92% of the acreage. But under the new plantation scheme, efforts are on by the Board to increase the cultivation of tender coconut by 50% where tall

ISSN 2349-4603

trees would constitute 60% of the acreage, 20% dwarf and 20% hybrid. The consumption pattern indicates raw nut is 50% and tender nut is 8-11%. Going by the present demand for tender coconut the rate of consumption would definitely increase to 25%.

Tender *Cocos nucifera* L. (Palmacea) water, was tested for its ability to scavenge free radicals, inhibit lipid peroxidation and protect hemoglobin from nitrite-induced oxidation. Fresh sample of CW scavenged 1, 1-diphenyl-2-picrylhydrazyl (DPPH) 2, 2′-azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid) (ABTS) and superoxide radicals but promoted the production of hydroxyl radicals and increased lipid peroxidation. The activity was most significant for fresh samples of CW and diminished significantly upon heat, acid or alkali treatment or dialysis. Maturity of coconut drastically decreased the scavenging ability of CW against DPPH, ABTS and superoxide radicals. CW protected hemoglobin from nitrite-induced oxidation to methemoglobin when added before the autocatalytic stage of the oxidation. Acid, alkali or heat treated or dialyzed CW showed a decreased ability in protecting hemoglobin from oxidation. The scavenging ability and protection of hemoglobin from oxidation may be partly attributed to the ascorbic acid, which is an important constituent of CW. As CW is a rich source of vitamins, amino acids and enzymes, *etc.*, more than one active principle may be involved (Mantena et al. 2003).

Probiotic bacteria are sold mainly in fermented foods, and dairy products play a predominant role as carriers of probiotics. These foods are well suited to promoting the positive health image of probiotics for several reasons: 1) fermented foods, and dairy products in particular, already have a positive health image; 2) consumers are familiar with the fact that fermented foods contain living microorganisms (bacteria); and 3) probiotics used as starter organisms combine the positive images of fermentation and probiotic cultures. When probiotics are added to fermented foods, several factors must be considered that may influence the ability of the probiotics to survive in the product and become active when entering the consumer's gastrointestinal tract. These factors include 1) the physiologic state of the probiotic organisms added (whether the cells are from the logarithmic or the stationary growth phase), 2) the physical conditions of product storage (eg, temperature), 3) the chemical composition of the product to which the probiotics are added (eg, acidity, available carbohydrate content, nitrogen sources, mineral content, water activity, and oxygen content), and 4) possible interactions of the probiotics with the starter cultures (eg, bacteriocin production, antagonism, and synergism). The interactions of probiotics with either the food matrix or the starter culture may be even more intensive when probiotics are used as a component of the starter culture. Some of these aspects are discussed in this article, with an emphasis on dairy products such as milk, yogurt, and cheese (Heller, 2001).

Probiotics produce antimicrobial agents or metabolic compounds that suppress the growth of other microorganisms [Spinler et al. 2008; O'Shea et al. 2011), or compete for receptors and binding sites with other intestinal microbes on the intestinal mucosa (Collado et al. 2007). Probiotic *Lactobacillus* strains enhance the integrity of the intestinal barrier, which may result in maintenance of immune tolerance, decreased translocation of bacteria across the intestinal mucosa, and disease phenotypes such as gastrointestinal infections, IBS and IBD (Lee and Bak, 2011).

Yeast, a microscopic, one-celled organism belonging to the group of organisms called fungi. There are many kinds of yeasts, some of them of great importance to humans. Yeast is necessary to make leavened bread, beer, cheese, wine, and whiskey. It is rich in B vitamins; a form of yeast called brewer's yeast is used as a diet supplement. Yeast is also used in genetic engineering to produce large quantities of certain hormones and enzymes, which are used for such medical purposes as healing wounds and reducing inflammation. Yeast enzymes chemically break down the sugars into products that the cell can use. Other yeast enzymes can make simple sugars out of disaccharides (double sugars), which are found in certain

ISSN 2349-4603

organisms. Yeast contains trehalose (Myrback, 1949) and atleast three polysaccharides (Whistler & Smart, 1953).

Materials and Methods:

The method of fermentation for the fermented tender coconut water was standardized. In the standardization of the fermented tender coconut water the first phase consisted of the preparation of the fermented tender coconut water. All the equipment needed was cleaned well and the bottles were sterilized using the autoclave. The water was taken from the tender coconut and kept in a sterilized bottle. 1tsp of sugar was measured and added to the tender coconut water. The bottle was closed with a muslin cloth and left for 24hours for fermentation. In phase two, the tender coconut water fermented with sugar was streaked in nutrient agar medium and gram's staining was done for the same. It was seen that there were no potential probiotic found in the tender coconut water fermented with sugar. Therefore the fermentation procedure for fermenting tender coconut water was altered and 0.5g of yeast was added to the tender coconut water instead of sugar. All the equipment's needed were cleaned well and the bottles were steam sterilized using the autoclave. The water was taken from the tender coconut and kept in a sterilized bottle. 0.5g of dry yeast was measured and added to the tender coconut water. The bottle was closed with a muslin cloth and left for 24hours for fermentation.

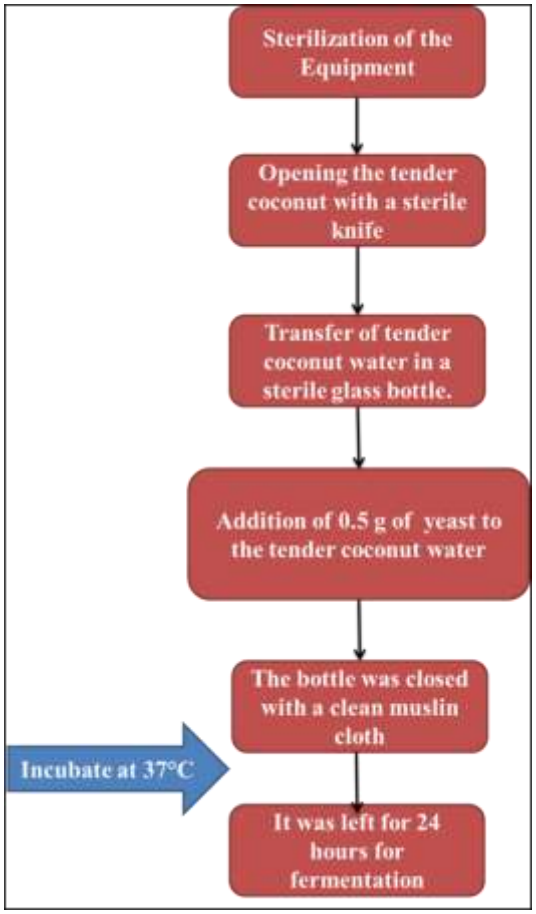

Fig 1: Steps Followed in Preparation of Fermented Tender Coconut Water

After the fermentation procedure, the acceptability of the fermented tender coconut water was determined. Twenty post graduate students were chosen as panel members to score the sensory attributes as appearance, colour, texture, flavour and taste of the fermented tender coconut water. A single portion of the fermented tender coconut water was served. The judges were instructed to fill the five point hedonic score card for the fermented tender coconut water. The options on the score card for the evaluation were excellent, very good, good, fair and poor. Individual scores were given for appearance, colour, texture, flavour and taste.

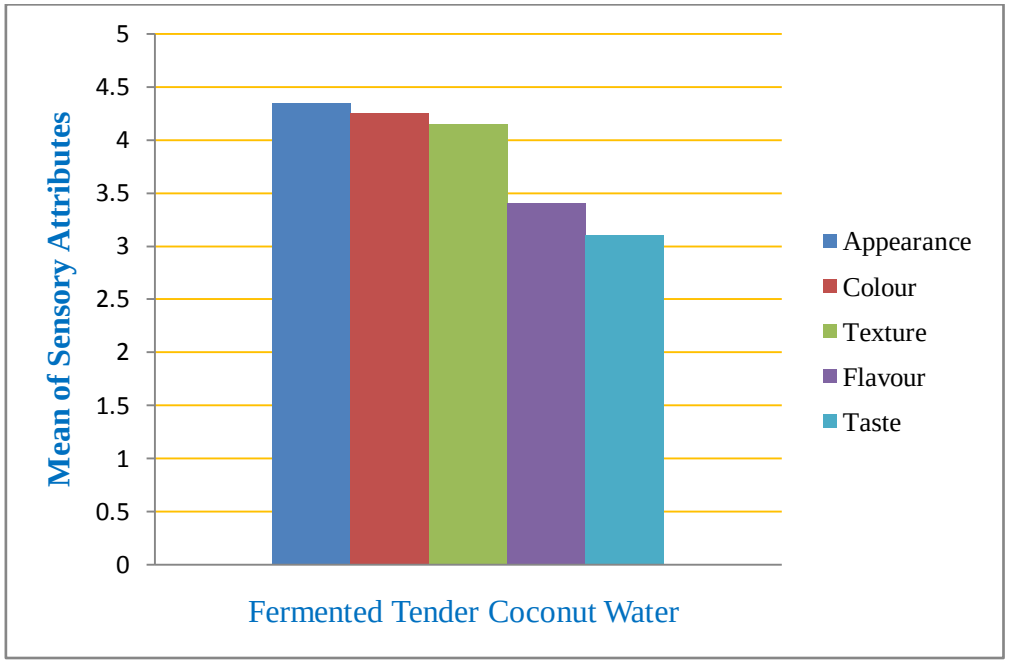

Fig 2: Acceptability and Palatability of the Fermented Tender Coconut Water

Results and Discussion:

Nutrient analysis was done for fermented tender coconut and it was seen that protein and fat content were more after fermentation. Carbohydrate content was low after fermentation due to the presence of yeast, which utilizes carbohydrate. There were traces of essential amino acids before and after fermentation. Vitamin C and vitamin K content were found to be more after fermentation. The mineral content of fresh tender coconut as such was high. It was seen that certain minerals like calcium, sodium and magnesium levels were more after fermentation. The value of calcium, sodium and magnesium of fermented tender coconut water were 32.4mg, 156.7mg, and 45.6mg respectively. Fermented tender coconut water is rich in potassium with about 156.7mg of potassium. The nutrient content of fermented tender coconut water was compared with that of fresh tender coconut values from the nutritive value of Indian foods by Gopalan et .al., 2007.The alcohol percentage of the fermented tender coconut water was found to be 2.91%.

ISSN 2349-4603

The fermented tender coconut water had antimicrobial property against *E.coli*, *Salmonella typhi* and *Enterococcus faecalis* at 100µl, 150µl, 200µl, 250µl, 300µl and 350µl respectively. The zone of clearance for *E.coli* and *Salmonella typhi* was seen in 100µl, 150µl, 200µl and 250µl, whereas *Enterococcus faecalis* required higher concentration like 300µl and 350µl for obtaining a clear zone. Therefore the fermented tender coconut has effect in maintaining good gut microflora.

Plate 1: Fermented Tender Coconut Water - Antimicrobial for *E.coli*

Plate 2: Fermented Tender Coconut Water - Antimicrobial for *Salmonella typhi*

ISSN 2349-4603

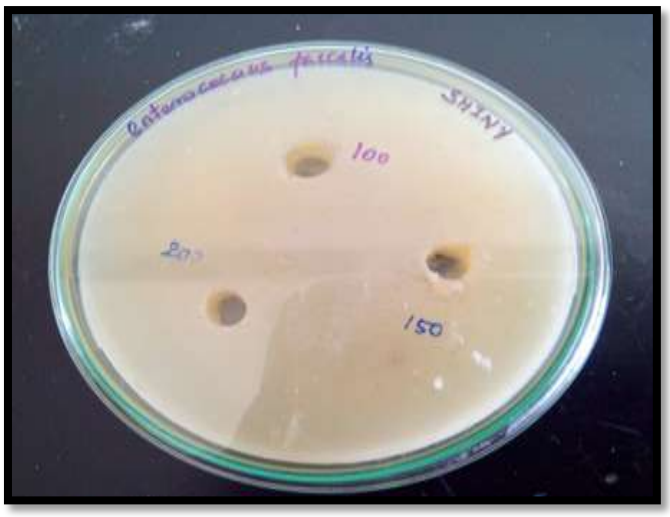

Plate 3: Fermented Tender Coconut Water - Antimicrobial for *Enterococcus faecalis*

Plate 4: Fermented Tender Coconut Water - Antimicrobial for *E. coli*

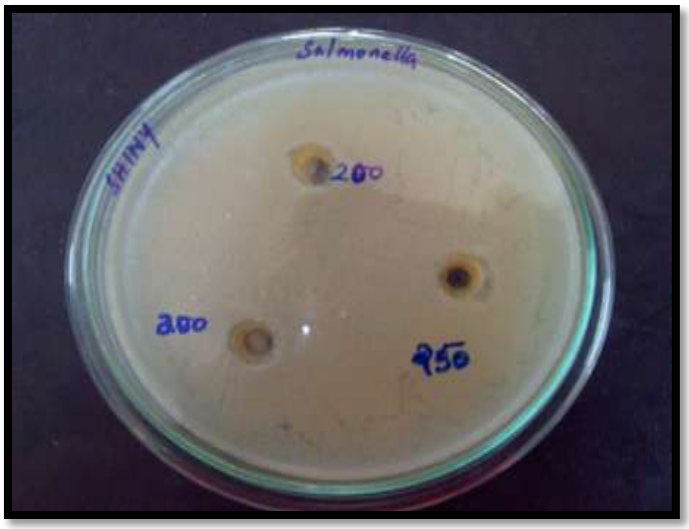

Plate 5: Fermented Tender Coconut Water - Antimicrobial for *Salmonella typhi*

ISSN 2349-4603

Plate 6: Fermented Tender Coconut Water - Antimicrobial for *Enterococcus faecalis.*

Table 1: Zone of Inhibition Exhibited by Fermented Tender Coconut Water.

ORGANISMS	CONCENTRATION OF FERMENTED TENDER COCONUT WATER					
	100µl	**150µl**	**200µl**	**250µl**	**300µl**	**350µl**
E.coli	-	11mm	14mm	16mm	*	*
Salmonella typhi	10mm	11mm	13mm	14mm	*	*
E. faecalis	-	-	-	12mm	13mm	14mm

* Since a good response was seen with 100, 150 and 200 microlitres of the fermented tender coconut water further testing was not required.

Sensory evaluation was done by twenty post graduate students for fermented tender coconut water and the four fruit juices where fermented tender coconut water had been incorporated in the ratio 1:1. The acceptability of the fermented tender coconut water was rated as good by the panel members. Among the four fruit drinks prepared with fermented tender coconut water, melon crush scored highest in the overall acceptability followed by grape delight and apple fantasy, pomegranate punch scored the least.

ISSN 2349-4603

Table 2: Overall Acceptability of the Fruit Drinks

S.No.	Fruit Drinks	Overall Acceptability (Mean±S.D)
1.	Melon Crush	4.48±0.7
2.	Grape Delight	4.0±0.8
3.	Apple Fantasy	3.94±0.8
4.	Pomegranate Punch	3.88±0.6

Fig 3: Overall Acceptability of Fruit Drinks

The overall acceptability of the fruit drinks is represented in Fig 3. Among the four fruit drinks prepared with fermented tender coconut water, melon crush scored highest in the overall acceptability followed by grape delight and apple fantasy, pomegranate punch scored the least.

Therefore fermented tender coconut water is a nutritious drink that has good acceptability and palatability. It is an easy to prepare fermented drink which has potential probiotics in it which is good for the gut.

Shelf life of both fresh tender coconut water and fermented tender coconut water need to be studied. Nutrient analysis of the same fresh tender coconut water sample needs to be analyzed. Supplementation studies could be carried out to document antioxidant and hypotensive effect of fermented tender coconut water.

References:

1. Bruce fife, Coconut Water for Health and Healing 2007, 4th edition, Piccadilly books ltd.
2. Gopalan. C, B. V. Rama Sastri, S. C. Balasubramanian., Nutritive Value of Indian Foods. National Institute of Nutrition, Indian Council of Medical Research 2007.
3. Heller. KJ, Probiotic Bacteria in Fermented Foods: Product Characteristics and Starter Organisms. American Journal of Clinical Nutrition 2001; 73: 374S-379S.

ISSN 2349-4603

4. Lee B., Bak Y., Irritable Bowel Syndrome, Gut Microbiota and Probiotics. Journal ofNeurogastroenterol Motility 2011; 17: 252–266.
5. Mantena S.K., Jagadish, Badduri S.R., Siripurapu K.B., Unnikrishnan M.K., In Vitro Evaluation of Antioxidant Properties of CocosNucifera Linn. Water, Nahurng 2003; 47: 126-131.
6. SandorEllix Katz., Wild Fermentation: The Flavor, Nutrition and Craft of Live-Culture Foods 2003; 2,3
7. Whistler, R. L. & Smart, C. L., Polysaccharide Chemistry, New York: Academic Press 1953.