

A STUDY ON DEVELOPMENT OF WATER KEFIR AND EVALUATION OF ITS ANTIOXIDANT PROPERTIES

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ABSTRACT

Tibicos or water kefir is a traditional drink. It is slightly sweet, acidic, fruity, sparkling fermented beverage produced with water kefir grains, and the latter consisting of polysaccharide and micro organisms, made of water and symbiotic cultures of bacteria and yeast (SCOBY) held in polysaccharide biofilm matrix created by the bacteria. The present study was aimed to study the antioxidant properties of fermented coconut water kefir. water kefir was fermented in tender coconut water for 48 hours, colony forming unit, antioxidant content by DPPH assay, alcohol content using density meter were estimated. Sensory evaluation of the developed product was conducted by 20 panelists. Results showed 6×10^7 CFU of micro organisms were present in the sample, water kefir have high antioxidant activity, IC_{50} 's of the sample showed 10000 for 625 μ g/ ml. Alcohol content was 0.0633 / 100 ml. It has many health benefits , it is dairy free can be used in lactose intolerance, boosts gut health, promotes immune functions, helps fight cancer, it has antihyperglycemic and antihyperlipidemic properties. Thus, it was concluded that fermented coconut water kefir had high antioxidant content.

INTRODUCTION

Tibicos is a slightly sweet, acidic, fruity, sparkling fermented beverage produced with water kefir grains, and the latter consisting of polysaccharide and micro organisms. Water kefir grains are polysaccharide grains. Water kefir or Tibicos is a traditional water sucrose based fermented drink made with water and symbiotic cultures of bacteria and yeast (SCOBY) held in polysaccharide biofilm matrix created by the bacteria.

Common name of Tibicos: Tibicos or Tibi is commonly known as water kefir.

Other names of water kefir : Water kefir grains, sugar kefir grains, Japanese water crystals, and California bees and in older literature as beebes, African bees, Australian bees, ginger bees, Vinegar bees, Japanese beer seeds, Beer plant, Ale nuts, Eternity grains, and balm of Gelead (David Laureys et al., 2014).

Origin of water kefir: There are two prevalent theories as to the origin of water kefir, but the exact source remains unknown. The first source is that soldiers returning from the Crimean War brought grains to Western Europe, while the second proposes the spontaneous formation of grain on the pads of the Opuntia Cactus in Mexico. Regardless water kefir grains were passed from household to household and this is still the most common means by which the grains and beverages are acquired as water kefir production has yet to be commercialized on a significant scale. Tibicos culture is also made from a bacteria cultured from known stocks with similar properties (Alan Marsh et al., 2013).

WATER KEFIR GRAINS MICROFLORA:

The micro organisms involved in water kefir fermentation comprise yeast, lactic acid bacteria, Bifidobacteria and acetic acid bacteria. The microbiota of kefir grains varies depending on its origin and culture media used for fermentation. (Sladana Davidovic et al., 2014 ; David Laureyset al., 2014).

Exopolysaccharides (EPS) production by water kefir microorganisms: Bacteria can produce polysaccharides by the action of enzyme glucosyl transferase and excreted extracellularly. The microbial polysaccharides are called exopolysaccharides (EPS) (Waldherr et al., 2010).

FOOD FERMENTATION:

Fermentation is one of the oldest food processing technologies which has been used by human for thousands of years for the production of foods and beverages with desirable properties (Prajapati and Nair, 2003; Smid and Hugenholtz, 2010).

Fermentation method: The basic preparation method includes adding tibicos to a sugary liquid and allowing it to ferment for 24 to 48 hours. Ingredients that inhibit the process of fermentation are checked. Precautions should be taken to keep the cultures healthy.

Precautions: Precautions should be taken to keep the cultures healthy. Use of reactive metals such as aluminum, copper, zinc, should be minimized. Since the acidity of the solution can draw these metals out damaging the cultures. Instead plastic, lead free ceramic, or glass containers should be used. Culturing grains in a glass jar and using clean plastic or wooden utensils when handling the grains is recommended”.

Products of fermentation: During fermentation changes in the composition of ingredients occur. The microbes present in tibicos act in symbiosis to maintain a stable culture. Tibicos can do this in many different sugary liquids, feeding off the sugar to produce lactic acid, alcohol (ethanol), and carbon dioxide which carbonates the drink.. Non alcoholic beverage means a beverage which contains a maximum of 2.8% by

volume ethyl alcohol (The alcohol act, 2001).

TENDER COCONUT WATER:

Coconut (*Cocos nucifera* L.) has been described as the most important and extensively grown palm tree all over the world. It belongs to the family Arecaceae. Traditionally Tender Coconut Water was used as an emergency glucose supply in absence of sterile glucose. It was used as intravenous fluid to counteract the effect of drug overdose, poisoning and adverse drug reaction, extensively used as the refreshment drink after physical exercise. Till date it has been known that TCW is a good source of various biomolecules like Sugar, Vitamins, Electrolytes, Plant hormones like Zeatin, Kinetin etc (Vishak et al., 2014).

ANTIOXIDANT PROPERTIES:

The increased interest in natural antioxidants has given rise to the screening of microbial sources for compounds to replace the synthetic compounds currently in use as food antioxidants. Antioxidant activity is increased by fermentation process of foods and addition of micro organisms. Water kefir is a result of fermentation which contains lactic acid, acetic acid, and yeast which produces important molecules such as polypeptide, polysaccharide, organic acid and other compounds. Thus water kefir can provide benefit micro organisms and bioactive molecules and help in health improving (Muneer Alsaydi et al., 2013).

HEALTH BENEFITS:

Water kefir is a health promoting drink. Its consumption has been associated with numerous health benefits including lower levels of inflammation, anticarcinogenic effects, low serum cholesterol levels, improved digestion and gut health, reduction in hypertension and regulation of reactive oxygen species (Conor Slattery et al., 2019).

OBJECTIVES

- To ferment water kefir in coconut water.
- To maintain and check favourable conditions for fermentation.
- To assess CFU (colony forming unit).
- To assess antioxidant properties.
- To estimate alcohol content.
- To assess the sensory attributes of the developed product.
- To create awareness on health benefits of water kefir.

MATERIALS AND METHODS

PROCUREMENT OF THE SAMPLE:

The sample kefir is procured from local market of North India i.e Srinagar.

FERMENTATION OF WATER KEFIR:

- In a glass bottle 5 gms of dehydrated water kefir grains were taken.
- 1000 ml of tender coconut water was added to the grains.
- It was mixed thoroughly.
- Bottle was closed using a lid.
- Glass bottle was kept in dark place in the corner of the room.
- It was fermented for 48 hours.
- After the process of fermentation it was kept in refrigerator for further use. (DavidLaurius et al.,2014) (urban platter –water kefir probiotic health elixir tin)

TO MAINTAIN AND CHECK FAVOURABLE CONDITIONS FOR FERMENTATION:

- Glass bottle was used.
- Room temperature was maintained as it was kept in the dark place at the corner of the room.
- After the process of fermentation the bottle was kept in refrigerator for further use.

TO ASSESS CFU (colony forming unit):

A colony-forming unit (CFU) is a unit used in microbiology to estimate the number of viable bacteria or fungal cells in a sample. Viability is defined as the ability to multiply via binary fission under the controlled conditions. Counting with colony-forming units requires culturing the microbes and counts only viable cells. Sample was cultured.

The CFU/ml was calculated using the formula:

$$\text{Cfu / ml} = (\text{no. of colonies} \times \text{dilution factor}) / \text{volume of culture plate}$$

TO ASSESS ANTIOXIDANT PROPERTIES:

Antioxidant properties were assessed by DPPH (diphenyl 2 picryl hydrazyle) method.

DPPH Procedure:

Preparation of test substance:

In 1ml of DMSO, 10mg of test substances were dissolved separately to obtain the concentration of 10000 μ g/ml. Further stock solution was serially diluted to obtain 10000 –625 g/ml to get the lower concentration.

Preparation of standard:

In 1ml of DMSO, 10mg of Ascorbic acid was dissolved to obtain solutions of 1000 μ g/ml which was serially diluted to get lower conc. Containing 62.5, 125, 250, 500 and 1000g/ml.

Preparation of 2, 2-Diphenyl 1-picryl hydroxyl solution (DPPH, 100 M):

4 mg of DPPH was accurately weighed and dissolved in 100 ml of methanol to obtain 100 M DPPH solution.

Antioxidant activity for DPPH Assay:

In the test and test blank 0.01ml of different conc. of test substances and standards were taken separately, in control and control blank 0.01ml of DMSO was taken in place of test sample. To the test and control 0.2 ml of DPPH was added whereas to the test blank and control blank 0.2ml of methanol was added in place of DPPH.

All the tubes were incubated at 37 $^{\circ}$ C for 30 minutes. After incubation 0.1ml of reaction mixture was pipette out to microtitre plate. Absorbance was measured at 490 nm using microtitre reader. Same procedure was repeated for standard by replacing test sample with standard. Test and control were performed in triplicate and test blank and control blank were conducted in singlet.

(Jaishree vaijayanathappa et al., 2008)

TO ESTIMATE ALCOHOL CONTENT:

Alcohol content is estimated by using density meter. Digital density meter is analytical tool that is used to determine the amount of alcohol in a sample.

Procedure:

- Fermented water kefir sample was taken in a small glass beaker.
- Tube of the density meter was dipped into the sample.
- Measuring cell was filled with the sample.
- After few seconds final value was displayed on the screen.
- The value was substituted in the specific

density formula. Specific density of alcohol =
Obtained value / alcohol density.

TO ASSESS SENSORY ATTRIBUTES OF THE DEVELOPED PRODUCT:

Sensory evaluation is a combination of different senses of perception coming into play in choosing and eating a food. Appearance, flavor and mouth feel decide the acceptance of food.

Selection of panelists: 20 Panelists were selected for a single trial of evaluation of sensory attributes. The panelists were residents of Venkatapuram, Alwal, Secunderabad. Sensory evaluation was done using Hedonic rating scale.

Sample was placed in front of panelists with a score card to rate the recipe. A glass of water was provided to drink in between the assessment to get the exact taste.

Development of score card: The developed product was tasted and rated on a scale of 5 based on the following sensory attributes like appearance, taste, colour, consistency, overall acceptability. Score card (Annexure i) consists of name of the judge, date and time.

TO CREATE AWARENESS ON HEALTH BENEFITS OF WATER KEFIR:

- Nutrition education was given using visual aid.

RESULTS AND DISCUSSION

The results of the conducted studies are depicted as follows.

FERMENTATION:

Water kefir grains were fermented in tender coconut water for 48 hrs at 25- 30° C. As temperature plays an important role in fermentations as it creates a favourable environment for growth of fermenting micro organisms. Fermentation temperature of a food product influences the rate of fermentation and the species of organisms involved.

Glass bottle was used for the process of fermentation instead of aluminium, copper containers. Glass bottles keep the cultures healthy. Whereas, aluminium, copper bottles damage the cultures because these metals will be drawn out by the acidity of the solution (David Laurius et al., 2014) (urban platter – water kefir probiotic health elixir tin). Tuncay Cevik et al., 2019 fermented water kefir with dried sugary fruits and sugar, to study the effect of different sugars on water kefir grains.

Fig 3: Water Kefir



Fig 4: Fermented Coconut Water Kefir



COLONY FORMING UNIT:

In order to obtain the potential health benefits, the population of probiotics in a product, the viability of probiotic microorganisms and their ability to activate at the desired site in the alimentary canal are very important. The initial inoculum size of probiotics in the selected food item is critical. The effective daily dose of probiotics is considered to be 10^9 - 10^{11} CFU (Sanders, 1999). Hence, consumption of 100 ml or a g of a product bearing the therapeutic minimum (10^6 - 10^8 CFU/ml or g of the product), would satisfy the daily requirement. The viable cell counts for fermented coconut water kefir were found to be 6×10^8 CFU/1000 ml. Showing that it could be used successfully as a vehicle for probiotics (Aishwarya Gangavar et al., 2018). Sakel Munna Md et al., 2014 followed the method as mentioned earlier for CFU.

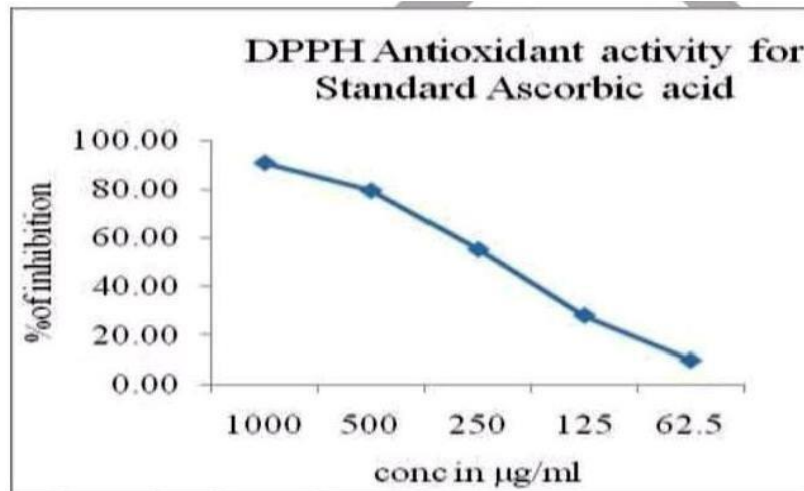
ANTIOXIDANT CONTENT:

Antioxidant activity of coconut water kefir drink is measured using DPPH (2, 2-Diphenyl 1- Picrylhydroxyl) assay. DPPH is one of the free radicles widely used for testing preliminary radical scavenging activity of a compound. In the present study, fermented coconut water kefir showed potencial free radical scavenging activity. This effect is showed by the antioxidants of micro organisms. In this study water kefir inhibit ascorbate autoxidation. Below given table 2 gives information about antioxidant activity of test substances for DPPH assay and figure 5 gives graphical representation of DPPH inhibitory activity of standard Ascorbic acid.

Table 2: The antioxidant activity of test substances for DPPH assay

S.No	Test substance	Parameter	IC ₅₀ (µg/ml)
1.	Fermented water kefir with coconut water	DPPH	>10000
2.	Std.Ascorbic acid	DPPH	188.97

Fig.5. Graphical representation of DPPH inhibitory activity of standard Ascorbic acid



The results indicate high ability of fermented coconut water kefir for inhibition of ascorbate autoxidation suggesting high anti oxidation activity of fermented coconut water kefir. IC₅₀ (Inhibitory concentration 50%) value denotes the concentration of sample required to scavenge 50% of the DPPH free radicals. The antioxidant activity property of fermented coconut water kefir exhibited the IC₅₀ value for DPPH is >10000 for 625 µg / ml. Muneer Alsayada et al., used DPPH method to know the antioxidant potency of water kefir. In his study Water kefir demonstrated great ability to DPPH scavenging ranged (9.88-63.17 percent). And inhibit ascorbate oxidation by (6.08-25.57 percent) increased in consequent with concentration raising. These results prime to conclude that water kefir could be promisor source of natural antioxidants with good potency in health developing (Muneer Alsayada et al., 2013)

ALCOHOL CONTENT:

It is estimated by density meter in femented coconut water kefir. Ethanol is a product of fermentation. Specific gravity of ethanol is less than water. Alcohol content of fermented water kefir is 0.063 / 100ml. Lisa Luciana Surja et al., in her study estimated ethanol content using distillation method (Lita Lusiana Surja et al., 2019).

SENSORY EVALUATION:

Any food must be give the person satisfaction and pleasure if it has to be accepted and become part of eating habits. The acceptance naturally depends primarily on those qualities that he readily perceives and experiences. These are appearance, taste, consistency and flavor which are sensory responses of the person to the foods. For the measurement of sensory responses in the form of estimates of individual dimensions of overall quality we have to rely on human panels. There were 20 panelists for the trial.

The key used to rate the product for sensory evaluation is given below:

Key:

Sensory attributes	Score
Excellent	05
Very good	04
Good	03
Fair	02
Poor	01

The result for palatability and acceptability is discussed in the following paragraph. The score obtained for fermented coconut water kefir is shown in the annexure 1.

Table 3: Results of sensory evaluation for fermented water kefir

Sensory Attribute	Mean score
Appearance	4.5
Taste	4.1
Consistency	4.9
Flavor	4.6
Overall acceptability	4.3

Appearance: It is the first appraisal of the food. The sense of sight plays an important role in the acceptability of the food. The mean score of fermented water kefir for appearance is 4.5.

Taste: Taste is the important criteria for appraisal of food. Tastes are sweet, sour, salty and bitter. According to Lowe (1955) people differ in their taste threshold. The mean score of fermented waterkefir for taste is 4.1.

Consistency: Consistency and texture are used to designate each of the imprecise used to characterize food. The consistency of food is an important factor affecting its general acceptance. Each food has its identifying consistency developing on its composition and physical state. The mean score of fermented water kefir for consistency is 4.9.

Flavor: It is one of the most important factors in the acceptability of food. The mean score of fermented water kefir for flavor is 4.6.

Overall acceptability: It is defined as experience or feature of experience by the attitude that is a liking for specific food item. If the food is palatable then it is acceptable. The mean score of fermented water kefir for overall acceptability is 4.3.

As sensory evaluation is a scientific discipline that analyses and measures human response to the composition of food and drink. It provides ideal opportunity to evaluate and give feedback on dishes, test products and experimental designs.

AWARENESS:

By distributing brochures among the people awareness is created on fermented coconut water kefir and its antioxidant properties.

SUMMARY AND CONCLUSION:

It can be concluded from the results of the present study that the Water kefir grains were procured (online) and were fermented in fresh tender coconut water for 48 hours. Temperature was maintained during the process. After the process sample was stored in the refrigerator for further use. To know the viable count of the bacteria colony forming unit was checked. 6×10^7 CFU/ml was observed. As the main quality of a probiotic is to have more than 5 billion live micro organisms. DPPH assay was performed to know the antioxidant properties of the fermented water kefir drink. The results of the assay stated that the drink has high antioxidant activity i.e $>10000/625 \mu\text{g}$. Alcohol estimation was done as ethanol is a product of fermentation. Estimation was done after 48hrs of fermentation using a density meter. Sensory evaluation was performed by 20 panelists and they evaluated the palatability and acceptability of the product. Mean score of fermented water kefir for appearance, taste, consistency, flavor, over all acceptability are 4.5, 4.1, 4.9, 4.6, and 4.3. The product has high antioxidant properties it has many health benefits. Awareness was created on health benefits of the fermented coconut water kefir using a brochure. Water kefir is a non dairy beverage so it can be used for lactose intolerance.

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