Technology Development for Black Rice Vinegar Production

Dissertation 1 Report

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CERTIFICATE

This is to certify that **Pravin kumbhar** (Registration No.11711919) has personally completed M.Tech. Predissertation entitled, "**Technology Development for Black Rice Vinegar Production"** under my guidance and supervision. To the best of my knowledge, the present work is the result of his original investigation and study. No part of pre-dissertation has ever been submitted for any other purpose at any University.

The project report is appropriate for the submission and the partial fulfillment of the conditions for the evaluation leading to the award of Master of Food Technology.

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CONTENTS

1. INTRODUCTION

Black rice is a kind of rice species *Oryza sativa L.* cultivated mainly in Asian countries. The outer region is black because presence of pigment anthocyanin. It has an antioxidant property. It is named by different names as prized, purple, forbidden, imperial, kings and heaven rice. There are nearly about 200 types of varieties of black rice all over world. The most of black rice (62% of global production) is cultivated by china. India and Thailand the Southeast Asia countries have long history of cultivation of black rice. In black rice nutrient content such as protein, minerals and dietary fibers are present in more amount as compare to white and brown rice . There is none other type of rice which contains that much high amount of nutritional quality near black rice. This rice is gluten free, Cholesterol free, sugar, salt and fat content is low. The nutrient present in higher amount are fiber, anthocyanin, antioxidants, vitamin E, iron, thiamine, magnesium, vitamin B, niacin and phosphorous.

The anthocyanins of black rice are extracted from the aleurone layer. The anthocyanin component in black rice is about 26.3%. The cyanidin-3-O-glucoside and peonidin-3-O-glucoside are the main effective constituents accounting for about 90%. Anthocyanin is supportive to guard arteries and stop the DNA injury and it has the ability to inhibit the formation of reactive cell damaging radicals. The high concentration of fiber lowers the risk of cancer. In many countries around world Black rice is typically used as a dressing, decoration and condiment, for various types of desserts. Black rice has rich flavor, sweet in taste and slightly sticky texture than white rice its flavor is richer and sweeter than white rice and texture is slightly sticky. Comparing black rice among white rice, black rice contain minerals such as P, Zn, Fe, Mn, and has a higher variability in mineral content that depend upon varieties and soil type of planting area.

Dark rice contains different phenolic acids that are ferulic corrosive, p-coumaric corrosive, vanillic corrosive, p-hydroxybenzoic, gallic corrosive and protocatechuic corrosive and ferulic corrosive has been recognized as transcendent phenolic cancer prevention agent (Butsat and Siriamornpun 2010). Natural dissolvable, for example, (CH3)2CO, ethanol, methanol, are frequently used to concentrate phenolic mixes on the grounds that they are efficient and helpful (Tananuwong and Tewaruth 2010). The main mounting confirmation is the advantages of cancer

anticipation agent utilization might be exaggerated. Utilization of cell reinforcement does not straightforwardly build the level of cancer anticipation agent in blood plasma.

Preservation of the fruits and vegetable in form of pickle is traditional method, so vinegar is used as preservative agent. It is also identified as liquid healthy for human utilization, there are different type of vinegars like as sherry wine vinegar, red wine vinegar, grapes wine vinegar etc. vinegar is formed by diverse method, raw materials that is wine (white, red, sherry wine), cider, fruits musts, malted barley, pure alcohol are used for acetic acid fermentation as a substrate.

At present the application of vinegar is to preserve food, whether naturally formed through fermentation or deliberately added, it is contribute the sensory properties to the foods and also retard the microorganism growth. There are different foods which contain vinegar like sauces, ketchup, mayonnaise, pickle etc. (De Ory et al., 2002).

Vinegar plays vital function in salad dressing, ketchup, hot sauce and other type of sauces. According to today's scenario and demands of industrialized fermentation system are able to produce bulk quantity vinegar, to get better industrialized production of vinegar many techniques have been developed , by using acetic acid bacteria some of industries trying to speed up the conversion of ethanol to acetic acid (Tesfaye *et al.*,2002).

Black rice consumption has declined through time due to its exotic nature and production of alternative foods will improve its consumption .white rice vinegar is already available in market. Black rice has not been explored for its utility for vinegar making which may render health benefits to the customers. Thus current project will attempt to develop black rice vinegar by the application of submerged state fermentor. The vinegar production will be optimized by modifying the carbon and nitrogen content for higher production and enhanced medicinal properties. Further, verity of products through seasoning will be developed in form of seasoned vinegar and shelf life analysis for these products will be undertaken.

2. PROBLEM BACKGROUND

Black rice is not explored for the production of vinegar, as the chemical composition shows that it containing huge quantity of starch and carbon sources which plays important role in alcoholic fermentation. Black rice contains different type of nutrient (iron, magnesium, etc.). Few years back black rice consumption was low however, increased health conscious has resulted in healthy consumption and shift of diet to unconventional sources of food.

3. REVIEW OF LITERATURE

Vinegar was globally used as a flavouring and food preservative agent. Vinegar is defined as "a liquid fit for human consumption, produced from a suitable raw material of agriculture origin containing starch , sugar by the process of double fermentation, alcoholic and acetous and contain a specific amount of acetic acid"(Joint FAO/WHO food standards programme, 1982).Vinegar is typically used as a preservative because it slow down microbial development and contributes sensory properties to a number of foods. There are different products which contain vinegar like ketchup, mayonnaise, sauce etc. and the present drop in wine consumption has favored a raise in vinegar production (De Ory *et al.,* 2002).

3.1 Black rice

Black rice is species of Oryza sativa L. however; it is gluten free rice as their own natural composition, filled with elevated level of nutrients. The outer part layer (pericarp) of kernel is black because of a pigment, which is recognized as anthocyanin. It is also work as an Antioxidant. This rice has different names which are forbidden rice, purple rice, Imperial rice, heaven rice, king's rice and prized rice. It has elevated nutritive quality and healing effect so due to this quality this rice is believed to improve the durability of life therefore in addition it is known as elongated life rice. The nutrient contents like protein, minerals (Ca, P, Fe, and Zn) and dietary fiber are in high amount in black rice as match up to white and brown rice .

3.1.1 Chemical composition of black rice

Black rice has high amount of vitamins like vitamin A, vitamin B and minerals such as iron, which are useful for overall health and the anticipation of cardiovascular Disease (Chen et al., 2003). It is an gluten free grain. In the black rice chemical components are not homogeneously distributed in grain.

Table 3.1 Proximate composition of black rice

Name of the components	Amount (%)	References
Moisture	11.07 ±0.2	(Rachel *et al*., 2013)
Ash	0.90 ±0.2	(Rachel *et al*., 2013)
Protein	8.16 ±0.3	(Rachel *et al*., 2013)
Fat	0.07 ±0.2	(Rachel *et al*., 2013)
Carbohydrate	78.26 ±0.6	(Rachel *et al*., 2013)
Total Dietary Fiber	8.47 ±0.2	(Rachel *et al*., 2013)
Anthocyanin	97.50 ± 0.3	(Zang *et al*., 2010)

3.1.2 Black rice in human nutrition

Many studies suggest that black rice bran has powerfully antioxidant activities due to phenolic and Anthocyanin-rich flavonoids. In latest decades, diseases related with the circumstances of hyperlipidemia and hyperglycemia, as well as heart disease and diabetes, has been growing drastically. Anthocyanin wealthy plant food may have healing use in the cure of cardiovascular diseases by intensifying cellular cholesterol deletion via apolipoproteins. Flavonoids and phenols are the key compounds in rice (mostly colored rice) bran have been recommended to have anti-cancerous properties for numerous types of cancer cells. (Pratiwi et al., 2017)

3.2 Vinegar

It is the food product which is prepared by using *acetobacter* genus for conversion of alcohol (wine) into acetic acid. Therefore, the formation of vinegar can be occurred by alcoholic material such as various fruit wines. Vinegar color and aroma are generally depending upon the material used. Vinegar is world's traditionally oldest cooking constituent and food preservative element. As stated by (Vinegar institute 2005), from nearly about 10,000 years studies carried on use of vinegar, and from almost 5000 years the flavored vinegar have been manufactured, sold .There

are extensive vinegars verities are existing since the 6th century BC , Babylonians are making vinegar and sell flavored vinegar with fruit and the honey malt to cuisine of moment. Several records are showed that it is used for medicinal purposes (Conner 1976).

3.2.1 Formation of vinegar and its types

Acetic acid bacteria are known to spoil the alcoholic beverages according to their ability because they are produce excess amount acetic acid from the alcohol (wine) and from other compound of wine (Joyeux *et al.,* 1984).

Acetic acid bacteria is used for the development of Vinegar, it is gram negative bacteria and rod shape cell that have required aerobic metabolism with the help of oxygen (Gonzalez *et al.*, 2004). There are various type of vinegars are famous according to their characteristics (Crisco company 2005).

- Balsamic vinegar
- Cane vinegar
- Champagne vinegar
- Cider vinegar
- Coconut vinegar
- Distilled vinegar
- Malt vinegar
- Rice wine vinegar
- Sherry vinegar
- Wine vinegar

3.2.2 Medicinal Uses of Vinegar

3.2.2.1Antimicrobial effect

Vinegar is well known for antimicrobial properties; it also useful in ear infection, cleaning, treating of nail fungi, head lice and warts (Rutala et al., 2000). Now a day it is used as a natural preservative method for inhibit the development of the microorganism in food material. Many type of organic acid are present natural form in verity of fruits and other in fermented food, including acetic lactic, ascorbic, malic, citric, propanic acid etc, and in no excess levels. Not any

of above acids are hazardous to individual health (Escudero, 1999). Many of research stated that the vinegar is used for prevention of pathogenic microorganisms on fresh fruits and vegetable.

3.2.2.2 Cardiovascular Effects

Vinegar ingestion influences other hazard components for cardiovascular malady in people is not known. Hu and colleagues announced an essentially bring down hazard for lethal ischemic coronary illness among members in the Attendants Wellbeing Study who expended oil-and-vinegar serving of mixed greens dressings as often as possible (5-6 times or more for each week) contrasted and the individuals who seldom devoured them Successive utilization of mayonnaise or other velvety plate of mixed greens dressings was not altogether connected with hazard for ischemic coronary illness in this populace. The review creators fight that since oil and vinegar dressings are a noteworthy dietary wellspring of dietary alpha-linolenic corrosive, an antiarrhythmic operator, alpha-linolenic corrosive may possibly be the advantageous element of this sustenance. However, velvety, mayonnaise-based serving of mixed greens dressings are additionally rich in alpha-linolenic corrosive and did not demonstrate a similar hazard advantage as the oil and vinegar dressings.

3.2.2.3 Anti obesity Effect

In a review revealed by Johnston (2006), human subjects expending 2 tablespoons of red raspberry vinegar every day with unreservedly access to sustenance and water for 4 wk shed pounds though the control bunch devouring a comparable measure of cranberry squeeze day by day for 4 week had a slight weight pick up.

3.2.2.4 Anti-infective properties

The utilization of vinegar for battle contaminations and other intense conditions goes back to Hippocrates (460-377 BC; the father of current prescription), who prescribed a vinegar arrangement for cleaning ulcerations and for the curing of wounds. Oxymel, a well known antiquated prescription made out of nectar and vinegar, was endorsed for tireless hacks by Hippocrates and his peers, and by doctors up to current day. The definition of oxymel was itemized in the English Pharmacopeia (1898) and the German Pharmacopeia (1872), and, as

indicated by the French Codex (1898), the solution was set up by blending virgin nectar, 4 sections, with white wine vinegar, 1 section, focusing and elucidating with paper mash.

3.2.2.5 Antioxidant effect

In the recent study hydrogen peroxide, superoxide and hydroxyl radical are the reactive oxygen genus that have been reported to affect lipid, protein, and DNA resulting in leading cancer, aging and degenerative disorder (Buonocore et al., 2010). Some of bioactive compounds within food may lead to reduce incidences of degenerative illness by providing the antioxidant effect. Examination of conventional balsamic vinegar indicated an antioxidant activity, which was mostly due to melanoidins, futher investigation report that melanioidins prevent absorption and prooxidant, cytotoxic effect to human in simulated gastric digestion of the meat(Xu et al ., 2004)

3.2.2.6 Anti diabetic effect

Insulin affectability has been enhanced through vinegar treatment in 19% of citizens with sort 2 diabetes and 34% of citizens with prediabetes (Johnston and others 2004). Late reviews in both creatures and people have demonstrated that vinegar may be used for diabetic treatment (Salbe and others 2009).

3.2.3 Vinegar quality characteristics

Vinegar aroma

Vinegar quality is depend upon its double fermentation process, it include rate of fermentation, acetification fastness. A frequency in fermentation enhances the sensory properties of the finishing vinegar. The vinegar odor and essence is depending on method of processing, it may be traditional or submerged, aging time, basic row material used for vinegar creation. Vinegar is not only containing acetic acid but also containing additional organic acid.

3.3 Acetator

In most of Mediterranean countries wine vinegar is produced and widely used as a preservative, condiment, and acidifying agent. Conventional production method needs maturation within wood for some years to gain a high acetic degree and the resulting produce is also costly. To conquer this complexity new technologies are being developed to producing vinegars with an analogous quality and less expensive. This technique of manufacturing of vinegar involve the application of constant aeration system and submerged bacterial culture which enhancing yield and superiority of the finishing product. (Tesfaye., et al 2002).

3.3.1 Production Methods

Vinegar is made with two phase fermentation process, at initial stage the sugar is converted into ethanol by application of yeasts culture, generally *Saccharomyces species* and in next stage the oxidation of ethanol is done with bacteria, generally *Acetobacter species*. As a technical point of view, there are two well defined methods for vinegar making: traditional (slow) process (Orleans Process) and submerged (quick) process (Generator Process) methods.

3.3.1.1 Orleans Process

The process called orleance is the sluggish process of acetifying wine and from 1670 in France this method has been used. Orleans process is the only method to produce wholesome wine vinegar. It was reported that it is the top method to make excellent quality table vinegar (Hickey and Vaughn 1954). In this method the wood barrels are utilized and filled with alcohol fermenting liquid to approximately ¾ full.

At initial, a few inches over the fluid surface and at the ends of the barrel a holes are made. The holes are stayed open and roofed with screen. After that nearly about 20-30% of clean and fresh vinegar filled in barrel (Muspratt 1871). The reason behind addition of fresh and clean vinegar is to acidify the fluid to point of finest expansion of vinegar bacteria. At 70 ºF to 85 ºF the fermentation is run for near about 1 to 3 months. Following this time about 1/4 to 1/3rd of the liquid vinegar drained out for purpose of bottling and an equal quantity of alcoholic fluid filled. The alcohol source should continuously be added to the vinegar may initiate to oxidize (Cruess 1958).

3.3.1.2 Submerged Fermentation

At present, majority familiar production technique is submerged culture from which enhance the general fermentation settings like aeration, stirring, heating, etc. As we know the generator culture systems are time-consuming and costly, submerged culture fermenters have become extensively used at industrial levels. In this method, the mash is mixed and aerated repeatedly. The fermenters are generally fixed with a heat exchanger for the upholding of the optimal temperature throughout the fermentation operation (De Ory *et al* 1999).

The word submerged vinegar fermentation display the connection to the processes utilized in the making of antibiotic, yeast etc. Just as in those procedures the term expresses that the organism, in case the vinegar bacteria, do their work in a liquid, in this case in an alcoholic mash. Submerged vinegar fermentation does with any filler substance like shavings, Birchwood, etc. as it is used in packed generators for holding the vinegar bacteria. The vinegar bacterium is forever submerged in fermenting liquid where they reproduce and oxidize the alcoholic mash into vinegar. For oxidation and safeguarding of their activity, the bacteria require oxygen. So one of the necessary prerequisite of submerged vinegar fermentation is a consistent and intensive insertion of air into the fermenting liquid (Frings).

To make 11 to 12 % vinegar the temperature needed for industrial making was nearly about 86°F (30°C) (Allgeier *et al* 1960; Adams 1985). If the temp rise above 86°F will causes harm to bacteria. In adding up to this, the bacterial state may also affect the concentration of acetic acid formed (Fregapane *et al* 2001).

Chapter 4: PROPOSED RESEARCH OBJECTIVE

1. To standardize the process for base wine production from black rice.

2. To optimize the process for production of black rice vinegar using submerged state fermentation.

3. To Develope a farm based acetator.

4. To develop a process for production of seasoned black rice vinegar.

Chapter 5: PROPOSED RESEARCH METHODOLOGY

5.1 Detailed plan work

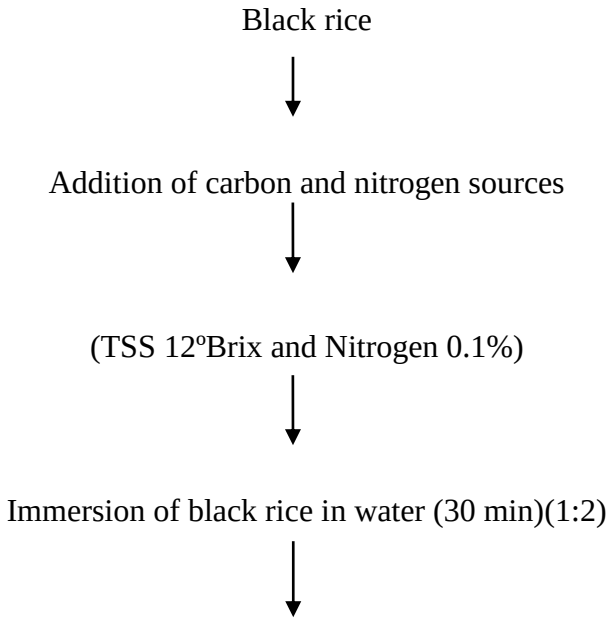
5.1.1 Objective 1: Standardize the process for base wine production from black rice

Base wine preparation: The method of vinegar production from black rice will be standardized by alcoholic fermentation (wine). Alcoholic fermentation will be done by the *saccharonomyces cerevisia* (Brewer's yeast). The wine process will be standardized by ameliorating with low cost carbon (starch and sugars) and nitrogen source (mustered cake and soya cake) comparing with defined carbon and nitrogen source, to ensure the ferment ability of black rice.

- Carbon source: The TSS will be maintained at 12º Brix (to get minimum 5% alcohol)
- Nitrogen source: The nitrogen concentration will be maintained at 0.1% (mustered cake and soya)

Black rice alcohol fermentation (Nishidai et al., 2000)

The process of production of black rice vinegar starts from addition of black rice into water followed by heating, cooling and incubation it with yeast for production of ethanol.



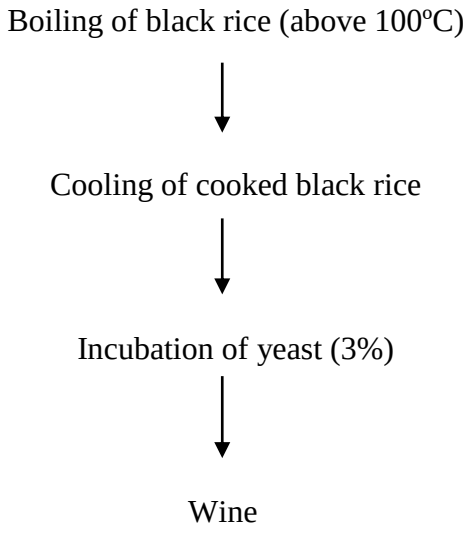


Fig. 5.1 Flow chart of base wine production

The following parameters will be evaluated after production of wine:

- Titratable acidity (San Chiang, 2005)
- Antioxidant activity (Sasmita, 2016)
- pH (San Chiang, 2005)
- Alcohol content (Ranganna, 2012)
- Total phenolic (Sasmita, 2016)
- Sensory evaluation (Tesfaye et al., 2002)

5.1.2 Objective 2: To optimize the process for production of black rice vinegar using submerged state fermentation

The term "submerged acetic fermentation" is similar to the common procedures involved in the cultivation of yeasts. In this case, bacteria carry out fermentative work on a solution, which is the alcohol blend. Bacteria are forever dipped in the liquid to ferment, where they build up and oxidize the alcohol blend into vinegar. In submerged state fermentation method, acetic acid bacteria is dipped in liquid to ferment, multiplying and drawing power from the oxidation of ethanol to acetic acid. To catalyze the reaction that provides them energy, acetic acid bacteria want a sufficient and permanent delivery of oxygen in all parts of the tank. A disturbance in the

oxygen delivery, especially in the last stages of fermentation, will influence the performance (Budak et al., 2014)

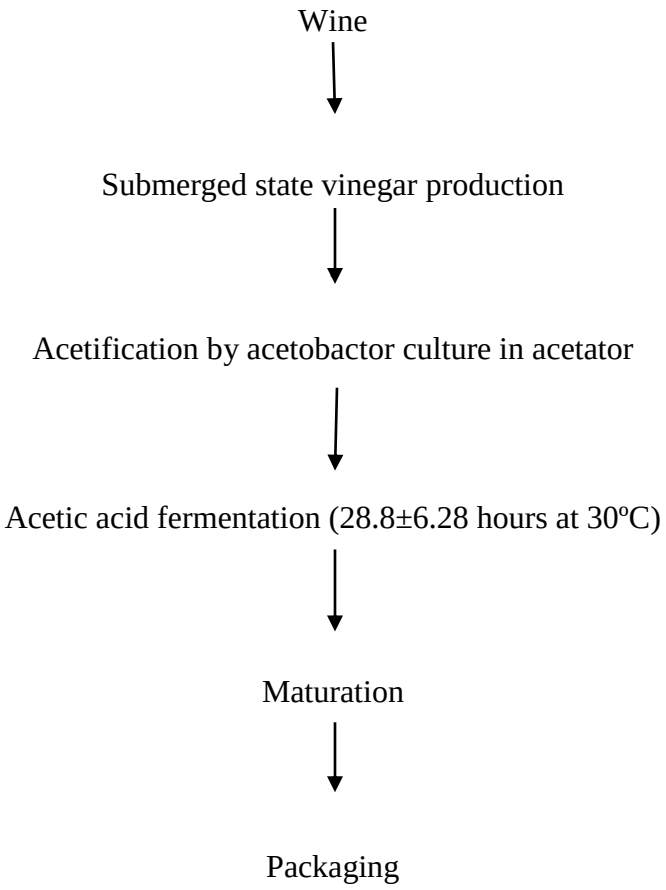


Fig. 5.2 Flow chart of black rice vinegar production

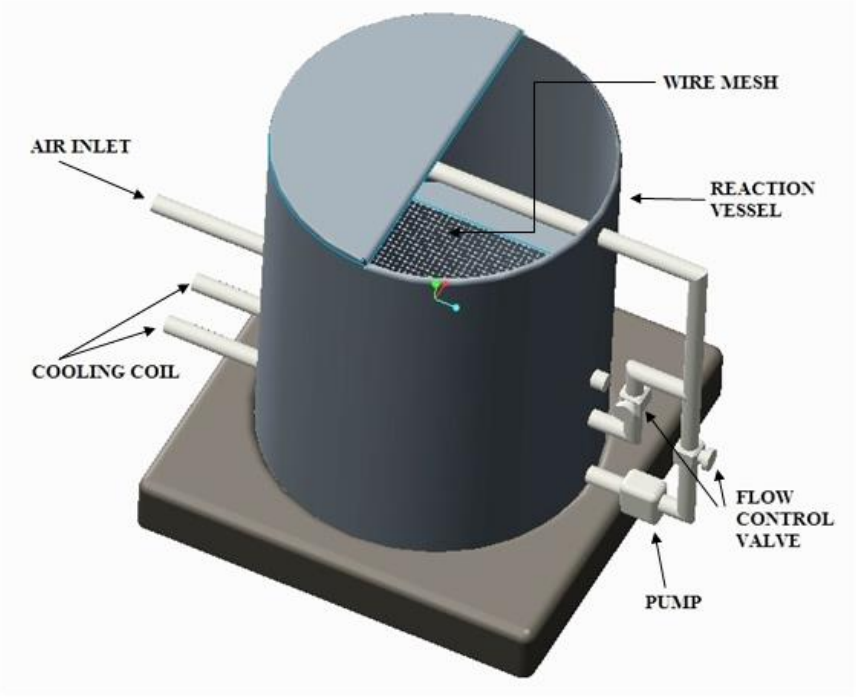


Fig 5.2 Submerged state fermentor

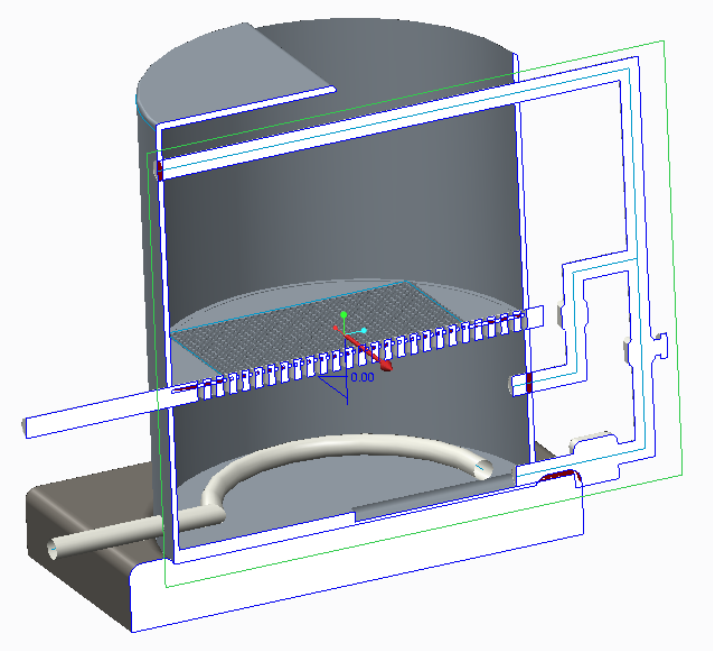


Fig.5.3 Sectional view

The following parameters will be evaluated after production of acetic acid:

- pH (San, 2005)
- Titratable acidity (San, 2005)
- Antioxidant activity (singh et al., 2009)
- Antimicrobial activity (inhibition zone) (Ghalem, 2009)
  a) Well diffusion method
  b) Disc diffusion method
- Sensory evaluation (Tesfaye et al., 2002)
- Yield of vinegar.

5.1.3 Objective 3: To Develops a farm based acetator.

Material requirement:

- Reaction vessel
- Cooling coils
- Aeration system
- Valves to control loading, unloading
- Cooling coils
- Air compressor
- pH meter
- Air Flow meter
- Centrifugal pump
- Thermocouples

Design parameters: (Wilma et al., 2015)

- The height of acetator 2 times larger than diameter
- Total volume = 8 liters
- Working volume= 6 liters
- The fermentation process set at=30±03 ºC
- Air flow rate= 3 liters/min
- The black rice wine used as raw material has pH of 3.0

- The rice wine used to produce vinegar should have 6.28 g/100 mL of an alcohol concentration, 2.8 g/100 mL of glucose content and the pH of 3.00.
- The fermentation of Acetobacter sp. is carried out in existence of fresh air.
- The process is start with about 3.4 liter of wine containing alcohol 6.28%.
- At starting add 1.6 liter of inoculums of Acetobacter sp. Contained 2.7 gram /liter of bacteria determined by measuring optical density at 600 nm.

5.1.4 Objective 4: To develop a process for production of seasoned black rice vinegar

Seasoning of the developed black rice vinegar will be done using salt, pepper, ginger; garlic, clove, and cinnamon, with accessory ingredient such as honey, olive oil, soy oil etc, and different formation will be analyzed using descriptive sensory analysis

Following parameter will be evaluated:-

- Titratable acidity (San, 2005)
- pH (San, 2005)
- Antioxidant content phenolics (Yang et al., 2014)
- Antioxidant activity (singh et al., 2009)
- Antimicrobial activity (inhibition zone) (Ghalem, 2009)
  a) Well diffusion method
  b) Disc diffusion method
- Sensory evaluation (Tesfaye et al., 2012)

Chapter 6: EXPECTED RESEARCH OUTCOME

1) Optimized process for the production of black rise base wine will be obtained.

2) Optimized process for the production of black rice vinegar will be obtained by submerged state fermentation.

3) Developed a farm based acetator.

4) The obtained final product will be having good sensory characteristics by seasoning of vinegar with different spices

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