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# Study on preparation, nutrient analysis and shelf life of biovinegar and its formulations

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**ABSTRACT**

Biovinegar enables the utilization of pineapple peels, (which are usually discarded) sugar and starter culture (Acetobacter xylinum). The study was carried out to elicit the acceptability and shelf life of Biovinegar. Antimicrobial property and the nutritive value of the Biovinegar were assessed and compared with vinegar which is commonly used. Formulation and comparison of sensory quality of recipes made with Biovinegar and vinegar was done. Comparison of nutritive value of recipes made with Biovinegar and vinegar was studied. Results show that the sensory attributes for Biovinegar like appearance, color, texture, flavour and taste was rated as excellent by the taste panel members. Biovinegar was more effective in inhibiting the growth of Enterobacter when compared with vinegar. Both Biovinegar and vinegar was effective in inhibiting the growth of *E.coli, staphylococcus, salmonella typhi* and *klebsiella*. The nutrient analysis of Biovinegar revealed that Biovinegar contains vitamin-$B_1$, folate, vitamin-C, sodium, flavonoids, essential amino acids, dietary fibre, and proteolytic enzyme bromelain. Biovinegar contain more calorie and sodium when compared to vinegar. Concentration of acetic acid, in Biovinegar ranges from 4percent to 8percent. Incorporation of Biovinegar into recipes, adds to the nutritive value of recipes. The Biovinegar has distinct features such as highly acceptable and palatable, contains Bromelain and more nutrients than vinegar, microbiologically safe for consumption and it is low cost and easy to prepare.

**Key words:** Vinegar, acetobacter xylinum, pineapple peel, Biovinegar, fermentation.

## INTRODUCTION

Pineapple is a compound fruit consisting of many berry like structures fused together and surrounding a stem[1]. Fresh pineapple juice contains the proteolytic enzyme bromelain which aids digestion and has several therapeutic properties including malignant cell growth, thrombus formation, inflammation, control of diarrhoea, dermatological and skin debridement[2, 3]. Bromelain in pineapple can be used in food preparations as it contains essential nutrients. Evidence indicates bromelain is well absorbed orally with its therapeutic effects and if successfully incorporated in foods, it could become more acceptable as a nutraceutical product.

Bromelain is not only present in pineapple fruit, but also in the outer peel and centre core which are usually discarded as waste. It accounts for half of the total fruit weight. The pineapple waste contains high fibre content, soluble carbohydrates, but it is low in protein[4]. Pineapple waste is a by-product of the pineapple processing industry and it consists of residual pulp, peel and skin. These wastes can cause environmental pollution problems if not utilized. Pineapple peel is rich in cellulose, hemicellulose and other carbohydrates[5]. Biovinegar can be used in the place of ordinary vinegar obtained by acetic acid fermentation of alcohol-containing solutions. Biovinegar are good for salad, ginger and onion dressings since it has sweet-sour taste. Regular vinegar mainly processes from ethanol while Biovinegar is obtained by fermenting pineapple peel with the starter culture (Acetobacter xylinum).

The pineapple peel vinegar (Biovinegar) enables the utilization of pineapple peels, sugar and starter culture. The conversion of pineapple waste

into Biovinegar therefore presents an attractive option for decreasing wastage and producing a valuable product. Untreated pineapple waste has impact on the environment due to its potential for methane generation; therefore this product is eco friendly since it makes use of pineapple waste that contaminates the environment. Apart from acetic acid, Biovinegar also contains carbohydrates, vitamin-$B_1$, folic acid, vitamin-C, sodium, potassium, acetic acid, flavonoids, essential amino acids, dietary fibre and bromelain.

## MATERIAL AND METHODS

The study was carried out with the objective of producing Biovinegar using pineapple peel, sugar and starter culture (Acetobacter xylinum). Antimicrobial property and shelf life of Biovinegar was assessed and acceptability of Biovinegar was evaluated. Formulation and comparison of recipes made with Biovinegar and with vinegar was studied and acceptability of formulated recipes was done. The nutritive value of the Biovinegar and formulated recipes was also assessed.

**The study was carried out in two parts**

**Part I**

**The preparation of Biovinegar**

The peels were cut into thin strips and placed in sterilized bottles. Aluminum or iron pots should not be used. About 200g sugar was added depending on the weight of the peels. The bottles were then inoculated with the starter culture and sealed. The inoculated pineapple peels were fermented at room temperature for about ten days. The pineapple peels were removed when the color change is noticed in the product i.e. from light yellow to brown color. The inoculums were left in the product till the end of fermentation period. The fermentation period varies with the climatic conditions. The Biovinegar was ready after ten days.

**Determination of the acceptability of Biovinegar**

Twelve post graduate students from the Department of Home Science, Women's Christian College, Chennai were chosen as panel members to score the sensory attributes of Biovinegar. A single portion of Biovinegar was served in a glass bowl. The judges were instructed to fill the five point hedonic score card for the Biovinegar. The options on the score card for the evaluation were excellent, very good, good, fair and poor for each sensory attributes such as appearance, color, texture, flavour and taste. A glass tumbler filled with drinking water was provided to rinse the mouth. Individual scores were given for appearance, color, texture, flavour and taste.

**Shelf life of Biovinegar**

The Biovinegar was prepared under sterile conditions and the shelf life was assessed. The shelf life of the Biovinegar was assessed qualitatively by standard plate count and most portable number. The medium used was Nutrient agar, Brilliant green lactose bile broth and Potato dextrose agar.

**Assessment and comparison of antimicrobial activity of Biovinegar and commercial vinegar**

Nutrient agar and nutrient broth was used to get the pure culture of the organisms to study the antimicrobial property of Biovinegar and vinegar. The antimicrobial property of the Biovinegar and vinegar was assessed using the Agar Well Diffusion method. The Muller Hinton agar was prepared and left to cool at 45º. The agar was then poured into sterile Petri plates. The medium was pre-seeded with the organism using a sterile cotton swab dipped in the inoculum. The well puncture was dipped in ethanol and sterilized by flaming. Two wells were made and spaced out at equal intervals. With the help of a micro pipette, 20 micro liter of the Biovinegar and vinegar was pipette into well. The plates were left in an incubator at 37 º for 24 hours and observations were made. The zone of clearance of Biovinegar and vinegar was compared.

**Nutrient analysis of Biovinegar and comparison with nutritive value of commercial vinegar**

Quantitative analysis of nutrients present in Biovinegar was assessed. The nutrients include carbohydrates, vitamin-$B_1$, vitamin-$B_6$, vitamin-$B_3$, folic acid, vitamin-C, vitamin-K, iron, sodium, potassium, acetic acid, flavonoids, essential amino acids, dietary fibre, and bromelain was carried. The bromelain content of the pineapple peel used in the preparation of Biovinegar was also assessed. The nutritive value of vinegar is obtained from[6] and compared with Biovinegar.

**PART II**

**Formulation and comparison of sensory quality of recipes made with Biovinegar and vinegar**:

Nine recipes were formulated and standardized using Biovinegar and vinegar. Sensory evaluation for these recipes was done by the panel of judges. The score obtained for the sensory qualities were assessed and compared such as appearance, color, texture, flavour, taste and overall acceptability. The recipes formulated and standardized include

1. Chilled rice salad - Cereal based product
2. Strawberries with vinegar – Fruit based product
3. Carrot and fruit salad – Vegetable and fruit based product
4. Potato salad with vinegar – Root vegetable based product
5. Sliced cucumber pickles – Vegetable based product
6. Vegetable salad with orange juice and vinegar salad dressing – Vegetable and fruit based product
7. Vinegar chicken – Poultry based product
8. Mutton vandaloo - Meat based product
9. Prawn pickle – Sea food based product

Recipes with milk were not done as bromelain curdles milk.

**Comparison of nutritive value of recipes made with Biovinegar and vinegar**

The nutritive value of the formulated recipes using Biovinegar and vinegar was calculated by using the nutritive value[7] and both were compared.

## RESULTS AND DISCUSSION

**Determination of the acceptability of Biovinegar**

Sensory attributes are the most significant quality parameters for determining consumer acceptance. In the present study, a five point hedonic scale was used to evaluate the acceptability of Biovinegar. The five point hedonic scale was scored as 1 – poor, 2 – fair, 3 – good, 4 – very good and 5 – excellent. The taste panel in this study consisted of twelve post graduate students from the Department of Home Science, Women's Christian College, Chennai.

Table 1 show that, the appearance of the Biovinegar was rated as 4.5±0.5 on a total score of five using the five point hedonic scale. The appearance was rated excellent by the taste panel members. The color of the Biovinegar was given a rating of 4.4±0.5 on a total score of five using five point hedonic scale. The color of Biovinegar was rated as excellent by the taste panel members. The color of Biovinegar was indicated a pale yellow color by the taste panel members which is due to the presence of yellow color pigment called carotenoids that comes from the pineapple fruit[8]. Texture of the Biovinegar was rated as 4.6±0.5 on a total score of five. The texture of Biovinegar was rated as excellent by the taste panel members With regard to flavour; Biovinegar was rated as 4.8±0.4 on a total score of five. The flavour of Biovinegar was rated as excellent by the taste panel members. With regard to taste, Biovinegar was rated 5±0 on a total score of five. Thus the taste of Biovinegar was scored excellent by the taste panel members. The taste of Biovinegar was judged as sweet–sour taste by the taste panel members. This can be due to the presence of acetic acid which gives the sour taste to the product. The Overall acceptability of the Biovinegar was rated as 4.7±0.3 by the taste panel members. The acceptability of food can be highly subjective. Thus the overall acceptability of Biovinegar was excellent. On the overall basis the Biovinegar was acceptable for its appearance, color, texture, flavour and taste.

**Shelf life of Biovinegar**

The main organisms known to be associated with food poisoning are – Organisms of the genus *Salmonella typhi, Staphylococcus aureus, Escherichia coli, Vibrio parahaemolyticus* and

**Table 1: Acceptability and palatability of biovinegar**

| Sensory attributes | Mean | Standard deviation |
|---|---|---|
| Appearance | 4.5 | 0.5 |
| Color | 4.4 | 0.5 |
| Texture | 4.6 | 0.5 |
| Flavor | 4.8 | 0.4 |
| Taste | 5 | 0 |
| Overall acceptability | 4.6 | 0.3 |

*Clostridium* species. In the present study, Biovinegar was examined for the presence of microorganisms. Qualitative tests were carried out to detect the presence of microorganisms. The techniques used were standard plate count and most probable number. Confirmatory tests were not carried out as the qualitative tests read negative.

**Standard plate count**

Pour plate technique was used for the estimation of standard plate count. The plates were observed after an overnight incubation at 37°. The tests were carried out on the 1st day, 30th day and 60th day. The plates of 1st day, 30th day and 60th day showed no bacterial growth. It can be seen from table 2 that there was no growth of microorganisms in the nutrient agar plate and potato dextrose agar plate for all the three days, this shows that Biovinegar is free from microbial contamination.

**Most portable number**

Brilliant green lactose bile broth was used to estimate the presence of coli form in the Biovinegar. Gas production after an overnight incubation at 37° in the tubes indicates the presence of E.coli which would then be confirmed using ImVic test. The results are shows that there was no gas production in the test tube. This confirms the absence of E.coli in Biovinegar. Therefore the Biovinegar is free from microorganisms and has a shelf life quality of about two months.

**Table 2: Results of standard plate count for biovinegar**

| Media | 1st day (CFU/ ml) (colony forming unit) | 30th day (CFU/ml) (colony forming unit) | 60th day (CFU/ml) (colony forming unit) |
|---|---|---|---|
| Nutrient agar | No growth | No growth | No growth |
| Potato Dextrose Agar | No growth | No growth | No growth |

**Table 3: Zone of inhibition by biovinegar and vinegar on test organism**

| Test organism | Zone of inhibition (mm)(millimeter) | | | |
|---|---|---|---|---|
| | Biovinegar (1ml) | Resistance | Vinegar (1ml) | Resistance |
| Enterobacter | 15 | Intermediate | 13 | Intermediate |
| *E.coli* | 16 | Susceptible | 17 | Susceptible |
| *Staphylococcus* | 15 | Intermediate | 20 | Susceptible |
| *Salmonella typhi* | 8 | Resistant | 22 | Susceptible |
| *Klebsiella* | 8 | Resistant | 22 | Susceptible |

**Assessment and comparison of antimicrobial activity of Biovinegar and commercial vinegar**:

The antimicrobial property of the Biovinegar and vinegar was detected using the Agar Well Diffusion method using the Muller Hinton agar. The results are obtained after incubating the plates at 37º for 24 hours. Interpretation of inhibition zones of test cultures was adopted from Johnson and Cas[9], (1995). Diameter zone of inhibition of 10 or less indicates test product being resistant to test organism, diameter zone of inhibition of 11 to 15 indicates test product being intermediate resistance to test organism, diameter zone of inhibition of 16 or more indicates test product being susceptible resistance to test organism. The zone of inhibition by Biovinegar and vinegar on test organism is presented in the table 3. From the table 3, we can infer that the zone of inhibition produced by Biovinegar (15mm) and vinegar (13mm) against Enterobacter exhibited a intermediate resistance (11-15mm) against the test organism, however Biovinegar (15mm) exhibited higher zone of

inhibition compared to vinegar. The zone of inhibition produced by Biovinegar (16mm) and vinegar (17mm) against E.coli exhibited a susceptible resistance (>16mm) against the test organism, however vinegar (17mm) exhibited higher zone of inhibition compared to Biovinegar. The zone of inhibition produced by Biovinegar (15mm) exhibited an intermediate resistance against staphylococcus, while vinegar (20mm) exhibited a susceptible resistance against the test organism. The zone of inhibition produced by Biovinegar (8mm) exhibited a resistance against salmonella typhi, while vinegar (22mm) exhibited a susceptible resistance against the test organism. The zone of inhibition produced by Biovinegar (8mm) exhibited a resistance against Klebsiella, while vinegar (22mm) exhibited a susceptible resistance against the test organism. Biovinegar can effectively inhibit the growth of Enterobacter, Escherichia coli, Salmonella typhi, Klebsiella and Staphylococcus aureus. The lactic and acetic acids have antimicrobial properties[10]. Both vinegar and Biovinegar possess antimicrobial property indicating release of antibiotics components into the surrounding medium and organisms were susceptible to both vinegar and Biovinegar, thus forming a zone of growth inhibition around Biovinegar.

**Nutrient analysis of Biovinegar and comparison with nutritive value of commercial vinegar**

The nutritive value of the Biovinegar was analyzed. Table 4 shows the nutrients present in the Biovinegar. It can be observed from the table 4 that the Biovinegar provides about 97 kilocalories per 100ml. About 100 ml of Biovinegar contains 24.12g of carbohydrates. Biovinegar also contains vitamin B1 (0.0098 mg), folic acid (1.1mcg), vitamin C (4.214mg), sodium (25.5mg), potassium (5.66mg), flavonoids (0,44mg), essential amino acids (35.7mg), dietary fibre (0.98g) and the proteolytic enzyme Bromelain (1.9mg). About 100 ml of Biovinegar contains 6.5g of acetic acid. Vinegar must contain at least 4percent acetic acid[11]. A comparison of nutrient composition of Biovinegar which was analyzed in the present study with commercial vinegar indicates that Biovinegar has 97 kilocalories/100ml compared to 12 kilocalories/ 100ml in commercial vinegar. Potassium is less in Biovinegar when compared with vinegar. Sodium is higher in Biovinegar (25.5mg) compared vinegar (1mg). The bromelain content of the pineapple peel analyzed in the laboratory is found to be 5.78mg in 100g of pineapple peel. After fermentation the bromelain content decreased and it was 1.9mg in 100ml of Biovinegar. Pineapple peel which is not edible was fermented to produce Biovinegar. Biovinegar was found to contain more nutrients than vinegar.

**Formulation and comparison of sensory quality of recipes made with Biovinegar and vinegar**

Nine recipes were formulated using both Biovinegar and vinegar. Five point hedonic scales was used for sensory evaluation and the score of the formulated recipes obtained from the panel of judges for sensory qualities such as appearance, color, texture, flavour, taste and overall acceptability was compared between recipes. From table 5 we can infer that there is no significant difference in mean scores for sensory qualities rating for chilled rice salad with Biovinegar and vinegar with regard to appearance, color, texture, flavour, taste and overall acceptability. There is a significant difference between the taste of strawberry made with Biovinegar and vinegar. The difference was significant at 1percent level. The overall acceptability

**Table 4: Comparison of nutritive value of commercial vinegar with biovinegar**

| Nutrients | Vinegar (100 ml) | Biovinegar (100 ml) |
|---|---|---|
| Energy(Kcal) | 12 | 97 |
| Carbohydrates(g) | 5 | 24.12 |
| Vitamin $B_1$(mg) | 0.0 | 0.0098 |
| Folic acid(mcg) | 0.0 | 1.1 |
| Vitamin C(mg) | 0.0 | 4.214 |
| Sodium(mg) | 1 | 25.5 |
| Potassium(mg) | 15 | 5.66 |
| Magnesium (mg) | 22 | 0.0 |
| Copper (mg) | 0.04 | 0.0 |
| Selenium (mg) | 0.50 | 0.0 |
| Acetic acid(g) | 0.0 | 6.5 |
| Flavonoids(mg) | 0.0 | 0.44 |
| Dietary fibre(g) | 0.0 | 0.98 |
| Essential amino acids (mg) | 0.0 | 35.7 |
| Bromelain(mg) | 0.0 | 1.9 |

of strawberry made with Biovinegar was higher (4.8±0.2) when compared to strawberry made with vinegar (4.5±0.2). The difference was significant at 1percent level. There is a significant difference between the mean score of color of carrot and fruit salad made with Biovinegar and vinegar. The difference was significant at 5percent level; Biovinegar had a higher mean score of (4.5±0.5) for color. The color of Biovinegar adds on to the color of the recipe were as the normal vinegar is colorless therefore there is a significant difference between the color of carrot and fruit salad made with Biovinegar and vinegar. There is a significant difference between the mean score of flavour and taste for carrot and fruit salad made with Biovinegar and vinegar. The difference was significant at 1percent level. The pineapple flavour in Biovinegar adds on to the flavour of the recipe and therefore there is a difference in the flavour of recipe made out of Biovinegar and vinegar. The taste of carrot and fruit salad made with vinegar is sour compared to the taste of carrot and fruit salad made with Biovinegar which gives a sweet-sour taste to the recipe. The mean overall acceptability of carrot and fruit salad made with Biovinegar was higher (4.4±0.3) when compared to carrot and fruit salad made with vinegar (3.9±0.2). The difference was significant at 1percent level. There is a significant difference between the mean score of taste for potato salad with dressing made with Biovinegar and vinegar. The difference is significant at 5percent level. The overall acceptability of potato salad with

**Table 5: The level of significant difference in sensory qualities of different recipes made with biovinegar and vinegar**

| Recipes | Sensory qualities | | | | | |
|---|---|---|---|---|---|---|
| | Appearance | Color | Texture | Flavor | Taste | Acceptability |
| Chilled rice salad | NS | NS | NS | NS | NS | NS |
| Strawberries with vinegar | NS | NS | NS | NS | 1percent | 1percent |
| Carrot and fruit salad | NS | 5percent | NS | 1percent | 1percent | 1percent |
| Potato salad | NS | NS | NS | NS | 5percent | 1percent |
| Sliced cucumber pickles | NS | 1percent | NS | NS | 5percent | 1percent |
| Vegetable salad | NS | NS | NS | 5percent | NS | 5percent |
| Vinegar chicken | NS | NS | 5percent | NS | NS | NS |
| Mutton vandaloo | NS | NS | NS | NS | 5percent | NS |
| Prawn pickle | NS | NS | NS | NS | NS | NS |

NS-Not Significant, 5percent- Significant at P<0.05, 1percent- Significant at P<0.01

dressing made with Biovinegar was higher (4.0±0.5) when compared to potato salad with dressing made with vinegar (3.9±0.4). The difference was significant at 1percent level. There is a significant difference between the color of cucumber pickle made with Biovinegar and vinegar. The difference was significant at 1percent level. There is a significant difference between the taste of cucumber pickle using Biovinegar and vinegar. The difference was significant at 5percent level. The mean overall acceptability of cucumber pickle using Biovinegar was higher (4.3±0.3) when compared to cucumber pickle using vinegar (4.0±0.2). The difference was significant at 1percent level. There is a significant difference between the flavour of vegetable salad made with Biovinegar and vinegar. The difference was significant at 5percent level. The mean overall acceptability of vegetable salad made with Biovinegar was higher (3.8±0.6) when compared to vegetable salad made with vinegar (3.6±0.4). The difference was significant at 5percent level. There is a significant difference between the texture of chicken made with Biovinegar and with vinegar. The difference was significant at 5percent level. The texture of chicken made with vinegar was soft than the texture of chicken made with Biovinegar. There

is a significant difference between the taste of mutton vindaloo made with Biovinegar and with vinegar. The difference was significant at 5percent level. The taste of mutton vindaloo using Biovinegar was sweet. There is no significant difference in sensory qualities and mean overall acceptability between prawn pickle made with Biovinegar and vinegar.

**Comparison of nutritive value of recipes made with Biovinegar and vinegar**:

The nutritive value of all the formulated recipes using Biovinegar and vinegar was calculated and compared by using the values given by ICMR, 2004. All the recipes made out of Biovinegar had higher calorie, carbohydrate, sodium, vitamin C, folic acid and fibre when compared to recipes made out of vinegar. In addition to this the recipes made with Biovinegar have bromelain, flavonoids and essential amino acids. Protein, fat, calcium and iron content of recipes made with Biovinegar and vinegar was the same. The potassium content of recipes made with vinegar was higher when compared to recipes made with Biovinegar. From this we can infer that the incorporation of Biovinegar in recipes can contribute to the overall nutritive value of the recipe. While vinegar contributes only to the potassium level of the recipe made with it. Thus recipes prepared with Biovinegar are more nutritious than recipes prepared with vinegar.

Biovinegar is produced from the natural fermentation of acetic acid bacteria. Production of Biovinegar is eco-friendly and is highly acceptable and palatable. Biovinegar is microbiologically safe for consumption. Biovinegar is economic and easy to prepare. Biovinegar has antimicrobial property. Concentration of acetic acid, in Biovinegar ranges from 4percent to 8percent. Apart from acetic acid Biovinegar also contains carbohydrates, vitamin-$B_1$, folic acid, vitamin-C, sodium, potassium, acetic acid, flavonoids, essential amino acids, dietary fibre and bromelain. Incorporation of Biovinegar into recipes, adds to the nutritive value of recipes. Biovinegar can be used in pickles, salads, dressings and sweet-and-sour sauces.

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