CHEMICAL PROPERTIES OF VINEGAR PRODUCED FROM SWEET ORANGE PEELS (*CITRUS SINENSIS*)

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ABSTRACT

This study reports the chemical properties of vinegar produced from sweet orange (*Citrus sinensis*). The peels were digested with α- and β- amylases, amyloglucosidase and fermented with *Saccharomyces cerevisae and Acetobacter aceti* for 2, 4, 6, 8, 10, 12 and 14 days. Acetic acid, total acidity, pH, total solids, total sugar, ethanol content, ester value, oxidation value and iodine value of the vinegar were determined. After 8 days of fermentation, vinegar produced gave acetic acid (5.00%) and total acidity (15.00%) values which were not significantly different ($p>0.05$) from those of cider vinegar (R) (5.10% and 15.33% respectively). Vinegar produced at 14 days fermentation gave pH (3.46) and total solid content (8.70%) values of no significant difference ($p>0.05$) from R (3.56 and 8.87% respectively). At the end of 14 days of fermentation, total sugar content reduced gradually from 2.22 to 0% for all the samples which falls within the range for R (0 – 2.50%). Sweet orange peels fermented for 14 days produced vinegar that contained 0.60 and 0.78% respectively for ethanol and 20.04 and 20.00 ml/100ml respectively for oxidation value with no significant differences ($p>0.05$) from R (0.63 and 20.00 ml/100ml respectively). At 14 days fermentation, the vinegar produced gave ester (4.13 ml/100ml) and iodine (8.05 g/100g) values which were not significantly different ($p>0.05$) from R (4.00 ml/100ml and 8.00 g/100g respectively). This study revealed that vinegar could be produced from sweet orange peels as fermentation for 14 days gave the highest yield of 75% v/v. Thus, sweet orange peel vinegar could be of high importance as import substitute for cider vinegar and value addition in the citrus industry which provides an alternative economically viable use of citrus fruit peels that are usually regarded as wastes and of no value.

Key Words: Fermentation, Digestion, Acetic Acid, pH

INTRODUCTION

Vinegar is a food grade preservative that has been used to preserve pickled vegetables, sausages and food emulsions like salad cream and mayonnaise. Vinegar is made from sugary or starchy materials by an alcoholic fermentation process followed by acetous fermentation (Okafor, 1987). Merry weather *et al*. (2005) also described vinegar as a fermented condiment that is essentially a solution of greater than (up to 95 %) or equal to four (4) percent of acetic acid. Vinegar has long been used worldwide as a basic seasoning in the preparation and cooking of certain foods such as sauces, pickles, vegetables and fish products, which it preserves due to the properties of acetic acid. Vinegar is also used as an additive in a wide variety of foods such as spiced fruits, ketchups, other tomato products, relishes, fish products, barbecued poultry, marinated and pickled meats, breads, sauces, cheese dressings,

and soft drinks (Macrae *et al*., 1993). Shomatsu (2008) also explained that vinegar can effectively eliminate fatigue by eliminating excess pyruvic acid produced in the Citric Acid Cycle process.

Vinegar is a product formed by a two- part fermentation process. First is the ethanolic fermentation by yeast (*Saccharomyces cerevisae*) which produces ethanol. In the second stage, ethanol is oxidized by *Acetobacter aceti* into acetic acid and a number of flavour compounds. Vinegar may be produced from any raw material containing sufficient sugar or alcohol. Examples are starchy vegetables (potatoes, etc), malted cereals (barley, rye), sugars (molasses, honey, etc), alcoholic beverages or dilute ethanol and fruit juices (apple, citrus, etc) (Sutherland *et al.*, 1986). Citrus fruits are the fleshy and juicy fruits produced on trees of the genus *citrus*, which include oranges, lemons, grapefruit, limes, tangerines, mandarins and many other hybrid varieties (Merryweather *et al*., 2005). As reported by Hanibaal (2004), sweet orange peels contain carbohydrates such as pectic substance, 'hemicellulose' and 'cellulose'. Upon hydrolysis, the pectic substances yield arabinose, galactose and galacturonic acid. Sweet orange peel contains 25g of carbohydrate (Hanibaal, 2004). In Dangriga Town, Belize, Central America, and the citrus industry's former practice involved dumping of 100,000, 000 kg of citrus peel wastes annually into a North Stann Creek River. This act led to the death of numerous fishes and became a real problem when it showed up in the water supply to the town. The industry has since abandoned this practice, in favour of decomposing the citrus peels at new compost site near the river, which also produces leachate that contaminates the watersheds (William, 2007). In Nigeria, most consumers of citrus fruits usually discard the peels indiscriminately as waste materials after consumption, thereby constituting avoidable pollution. Therefore, it is worthwhile evaluating the potential of sweet orange peel in vinegar production instead of discarding the peels as waste materials. This study however made use of α– and β-amylase, amyloglucosidase, *Saccharomyces cerevisae* and *Acetobacter aceti* to produce vinegar from the citrus peels.

OBJECTIVE

To determine the chemical properties of vinegar produced sweet orange peels.

MATERIALS AND METHODS

Materials

The materials used were ripe sweet orange fruits (*Citrus sinensis*). The sweet orange fruits were obtained from Kuto and Lanfenwa markets in Abeokuta, Ogun State, Nigeria. Yeast (*Saccharomyces cerevisae*) and *Acetobacter aceti* were obtained from the Institute of Agricultural Research and Training (IAR&T) in Ibadan, Nigeria, while α– and β- amylases and amyloglucosidase were obtained through Sigma Laboratory, Ikeja, Lagos, Nigeria.

Methods: Hydrolysis of Sweet Orange Peels

Sweet orange fruits were peeled and the peels digested or hydrolyzed according to the modified method of Ishiwu and Iwouno (2006). Matured and ripe sweet orange fruits were washed with potable water and peeled. Two kilogrammes of sweet orange peels was wet

milled using the attrition mill. The slurry was divided into seven different containers of 0.7 litre capacity each. To each of the container, 1.5 g each of alpha amylase, 0.03 g each of β-amylase and 0.15 g each of amyloglucosidase were added and left for 2, 4, 6, 8, 10, 12 and 14 days, respectively at ambient temperature 28 ± 2 ^{o}C. The samples were then sterilized at 121 ^{o}C for 15 mins and cooled to 28 ± 2 ^{o}C.

Fermentation of Sweet Orange Peels

In the first fermentation, the yeasts (*Saccharomyces cerevisae*), 23 g were added to each container with vigorous stirring for 10 mins at ambient temperature (28 ± 2 ^{o}C) for 2, 4, 6, 8, 10, 12 and 14 days. After which the samples were sterilized also at 121 ^{o}C for 15 mins and cooled to 28 ± 2 ^{o}C. In the second (acetous) fermentation a loopful of the pure culture of *Acetobacter aceti* were added to the sterilized sample in each of the containers and stirred for 10 mins under aerobic condition at room temperature (28 ± 2 ^{o}C) and left for 2, 4, 6, 8, 10, 12 and 14 days. The vinegar obtained was then pasteurized at 100 ^{o}C for 10mins, filtered, bottled, sterilised at 121 ^{o}C for 15 mins and cooled to room temperature (Ishiwu and Iwouno, 2006).

CHEMICAL ANALYSES

Acetic Acid

The sample (10ml) was pipetted into a 100 ml volumetric flask and diluted to 100 ml with distilled water. It was mixed well by inversion. 10 ml of the diluted sample was then pipetted into a conical flask. 3 drops of phenolphthalein were added and titrated against 0.1 M sodium hydroxide solution (James, 1996).

The acetic acid content is given by:

$$\% \, Acetic \, acid \left(\frac{m}{v}\right) = Tx0.6$$

T = mean titre (in ml) of 0.1 M sodium hydroxide solution required to neutralise the acidity in 10ml of the diluted sample.

Total Acidity

Each sample of 10ml was diluted to 80 ml with distilled in a conical flask. Two drops of phenolphthalein were also added. The sample was then titrated with 0.1 M sodium hydroxide (James, 1996). The total acidity is given by:

$$TA = 10xT$$

T = mean titre (in ml)

pH

The pH of each sample was determined using a Jenway pH meter which was standardized by a buffer solution of pH 7.0 and pH 4.0. The sample was then poured into a 25 ml beaker and the reading was taken by inserting the electrode into the sample (James, 1996).

Total Solids

Each sample of 25ml was evaporated in a crucible and dried to a constant weight in an oven at 100 ^{o}C (James, 1996).

$$\% \, Total \, Solids = 100 - \% \, Moisture$$

$$\%\ Moisture = \frac{(W2 - W3)x100}{W2 - W1}$$

W1 = Initial weight of empty crucible, W2 = weight of crucible + sample and W3 = weight of crucible + residue (solids).

Total Sugar

The non-reducing sugars in the vinegar were first hydrolysed to reducing sugars by pipetting 100 ml of the vinegar into a conical flask, adding 10 ml dilute HCl and boiling for 5 min. After cooling, the vinegar was neutralized to phenolphthalein with 10 % NaOH and made up to volume in a 250 ml volumetric flask. The burette was filled with vinegar. 10 ml of the mixed Fehling's solutions was pipetted into a conical flask and 4 drops of 1 % methylene blue were added. The solution was brought to boil. Whilst boiling, vinegar was added from the burette until there was a sharp colour change. Vinegar (12 ml) was added to the boiling mixed Fehling's solutions. 4 drops of methylene blue indicator were also added. The mixture was reboiled and vinegar from the burette was titrated against mixed Fehling's solutions in increment of 0.25 ml until the end-point was reached (James, 1996).

$$\%\ Total\ Sugars\ (as\ glucose) = \frac{4.95x250x0.25}{TxW}$$

T = titre value of vinegar and W = weight of vinegar used.

Ethanol

The sample of 100ml was poured into 100 ml volumetric flask. It was then transferred into a distillation flask and about 95 ml of the distillate was distilled into a 100 ml volumetric flask. The flask was filled to the mark with distilled water and mix by inversion. The specific gravity of the sample was determined and the corresponding alcohol value was read from the alcohol table (James, 1996).

Ester Value

Ester Value was the number of millilitres of 0.01 M potassium hydroxide required to saponify the esters contained in 100 ml of the vinegar sample.

The sample of 25ml was poured into a flask. The sample was made alkaline with 0.01 M potassium hydroxide. Two drops of phenolphthalein were also added. The pink colour was removed by the drop wise addition of 0.02 M hydrochloric acid. Potassium hydroxide (10 ml of 0.01 M) was also added and then heated under reflux for 2hrs on a boiling water bath to saponify the esters. It was then cooled and titrated with 0.02 M hydrochloric acid (A ml) after adding additional phenolphthalein. A blank titration was carried out at the same time (blank titration = B ml). Ester Value = 8 (B-A ml) (Ronald and Ronald, 1991).

Iodine Value

The distillate of 5ml from the vinegar (or 10 ml from the spirit vinegar) was poured into a 250 ml glass-stoppered bottle and make just neutral to litmus with 10 M potassium hydroxide.10 ml of 10 M potassium hydroxide and 10 ml of 0.1 M iodine were also added. It

is allowed to stand in the dark for 15 mins and then added to 10 ml dilute sulphuric acid. The liberated iodine was then titrated with 0.2 M thiosulphate (an ml) using starch near the end-point. Blank was carried out at the same time (b ml).
Iodine Value =20 (b-a) (Ronald and Ronald, 1991).

Oxidation Value

Oxidation Value was the number of millilitres of 0.02 M potassium permanganate used by 100 ml of the sample in 30 mins. 5 ml of the sample was added to a 250 ml glass-stoppered bottle. 10 ml of dilute tetraoxosulphate (VI) acid and 15ml of 0.002 M potassium permanganate were also added. The solution was allowed to stand at about 18 ^{0}C for 30 mins. 5 ml of 10 % potassium iodide solution was then added. The liberated iodine was then titrated with 0.02 M thiosulphate (A ml) using starch near the end point. A blank titration was also carried out at the same time (blank titration = B ml). Oxidation Value = 40(B-A) (Ronald and Ronald, 1991).

Percentage Yield: The percentage yield of the vinegar was calculated using:

$$\%\ Yield = \frac{(Volume\ of\ Vinegar\ Produced)x100}{Volume\ of\ Citrus\ Peels + Volume\ of\ Water}$$

RESULTS AND DISCUSSION

The acetic acid values ranged from 5.60 to 13.00% as shown in figure 1. There were significant differences ($p < 0.05$) in the acetic acid values as the days of fermentation increased from 2 to 14 days. The acetic acid value of vinegar obtained on the 8th day of fermentation was not significantly different ($p > 0.05$) from that of the reference sample (cider vinegar) (R) (5.10%). The acetic acid values at 2, 4, 6, 8 and 10 days of fermentation were in agreement with the range of 3.90 to 9.00% reported for vinegar from cider (apple) by Ronald and Ronald (1991). Acetic acid, which usually occurs as a by-product of yeast and bacteria metabolism, may impart a vinegar-like aroma or flavour, which is detectable only in wines spoiled by microorganisms (Aline *et al.*, 2010).

Total Acidity

The total acidity ranged from 15.00 to 29.00%as shown in figure 1. There were significant differences ($p < 0.05$) in the values of the total acidity obtained as the days of fermentation increased from 2 to 14 days. Vinegar from sweet orange peels produced total acidity values of 15.30% and 15.00% at 14 days and 8 days of fermentation, respectively, that compared favourably with R (15.33%). The high values recorded in the vinegar with high total acidity values could be partly due to high amount of other organic acids. Macrae, *et al*. (1993) reported that sweet orange peels contain large quantities of citric acid and tartaric acid.

pH

pH ranged from 2.15 to 3.66 as shown in figure 1. There were significantly differences ($p < 0.05$) in the pH obtained as the days of fermentation increased from 2 to 14 days. The pH at 2, 8 and 14 days of fermentation were not significantly different from R (3.56). pH is strongly

dependent on organic acids such as acetic acid, malic acid or lactic acid levels (Hufnagel and Hofmann, 2008).

Total Solids

Total solids ranged from 7.87 to 8.70% as shown in figure 2. There were significant differences ($p < 0.05$) in the values of the total solids as the days of fermentation increased from 2 to 14 days. Total solids obtained were in agreement with the report of Macrae *et al.* (1993).

Total Sugar

Total sugar content ranged from 0.00 to 2.20 % as shown in figure 2. At the end of 14 days of fermentation, the total sugar content reduced gradually from 2.20 to 0.00%, which still fell within the range recommended for cider vinegar (0.00 to 2.50%). Total sugar contents were within the range specified by Ishiwu and Iwouno (2006). The low value of the sugar content in all the vinegar produced is an indication of the effectiveness of acetous fermentation stage by *Acetobacter aceti*. Polysaccharides, monosaccharides and disaccharides also influence vinegar, enhancing sourness and viscosity (Nurgel and Pickering, 2005).

Ethanol Content

As the days of fermentation increased from 2 to 14 days, the ethanol content decreased gradually from 1.82 to 0.60% as shown in figure 2. Ethanol content of vinegar obtained at 14 days of fermentation (0.60%) was not significantly different ($p > 0.05$) from the cider vinegar (R) (0.63%). The results obtained for ethanol were still within the range specified by Macrae *et al.* (1993). The level of alcohol tolerance by yeast varies from 5% to about 21% depending on yeast strain (Joyeux, 1999). Alcohol (glycerol) contributes to the aromatic profile of vinegar (Solieri and Giudici, 2008). Alcohols (e.g. ethanol) produce aromatic constituents, such as esters, during the ageing process of wine and ethanol itself has an influence on wine sensory properties (Simone *et al*., 2009). The interaction between alcohols and carboxylic acids produces esters (Graham and John, 1984).

Ester Value

Ester values increased gradually from 2.00 to 4.13 ml/100ml as shown in figure 3. An ester value at 14 days of fermentation was not significantly different ($p > 0.05$) from R (4.00ml/100ml). Each ester value obtained was the extent to which the alcohol in each peel could interact with the carboxylic acid present. Esters also contribute to vinegar flavour. However, esterification process should be checked to avoid loss of acetic acid, which is the major component of vinegar. The ester values were within the range (0.00-20.00 ml / 100 ml) specified by Macrae *et al.* (1993). Each ester value obtained was the extent to which the alcohol in each peel could interact with the carboxylic acid present. Esters also contribute to vinegar flavour. However, esterification process should be checked to avoid loss of acetic acid, which is the major component of vinegar. The ester values were within the range (0.00-20.00 ml / 100 ml) specified by Macrae *et al.* (1993).

Iodine Value

As the days of fermentation increased from 2 to 14 days, the iodine values increased gradually from 7.50 to 8.05g/100g as shown in figure 3. Iodine values of vinegar from sweet orange peels from 6 to 14 days of fermentation were not significantly different ($p > 0.05$) from each other and the cider vinegar (R) (8.00g/100g). All the iodine values were still within the limit (5.00 – 30.00 g / 100 g) specified by Ronald and Ronald (1991). The iodine value measures the degree of unsaturation of the fatty acids in a fat. The iodine value in vinegar is mostly influenced by acetylmethylcarbinol and diacetyl (Macrae *et al.*, 1993).

Oxidation Value

Oxidation value increased gradually from 12.00 to 20.04ml/100ml as shown in figure 3. Oxidation value at 14 days of fermentation was not significantly different ($p > 0.05$) from R (20.00 ml/100ml). The oxidation values were also in agreement with the range specified for cider vinegar by Ronald and Ronald (1991). Hollis (2006) reported that oxidation processes, triggered by oxygen, occurred in vinegar as a result of chemical changes in the polyphenolic compounds.

Yield (%)

Percentage yield ranged from 50 to 75 % as shown in figure 4. Percentage yield of vinegar from sweet orange peels at 14 days of fermentation was significantly higher ($p < 0.05$) than all other samples. From the result obtained at 14 days of fermentation, 285.7g of sweet orange peels milled with 714.3 ml of water produced 750ml of vinegar.

CONCLUSION

The results obtained indicate that commercially viable vinegar could be produced from sweet orange peels if they can be properly digested with α-and β-amylase and amyloglucosidase, and fermented with *Saccharomyses cerevisae* and *Acetobacter aceti.* Considering all the quality indices, sweet orange peels at 14 days of fermentation gave the best vinegar. It should be noted that any material of plant origin that contains appreciable amounts of carbohydrate could yield vinegar provided it is properly digested and fermented with appropriate enzymes and microorganisms. It could be deduced that sweet orange peels – water combination of ratio 1: 2.5 respectively gave the highest yield of 75% v/v. Thus, sweet orange peels vinegar could be of high importance as importsubstitute for cider vinegar and value addition in the citrus industry which provides an alternative economically viable use of citrus fruit peels that are normally regarded as wastes of no value.

REFERENCES

1. Aline C.T. Biasoto; Rodrigo R. Catharino; Gustavo B. Sanvido; Marcos N. Eberlin and Maria Aparecida A.P. da Silva, 2010. Flavour Characterization of Red Wines by Descriptive Analysis and ESI Mass Spectrometry. *Food Quality and Preference*. 21 (7): 755-762.

2. Graham, C. H and John, S. H., 1984. Chemistry in Context. Thomas Nelson and Sons Ltd, Walter-on-Thames, Surrey KT125PL. 3rd Edition. pp 495.

3. Hanibaal Diab, 2004. Nutritional Facts on Citrus Peels. http://www.nutritionanalyser.com/food composition /? Fid=09156. 1-15.

4. Hollis Cline (2006). Sperry and Hebb: Oil and Vinegar. *Analitica Chemica Acta.*557 (1-2). 360-366.

5. Hufnagel, C. and Hofmann, T., 2008. Quantitative Reconstruction of the Nonvolatile Sensometabolome of a Red Wine. *Journal of Agricultural and Food Chemistry* 56 (19): 9190–9199.

6. Ishiwu, C.N. and Iwouno, J.O., 2006. Physico-Chemical and Sensory Properties of Vinegar Produced from Pineapple Peels. *Nigerian Food Journal*. 24(1). 127-130.

7. James, C.S., 1996. Analytical Chemistry of Foods. Blackie Academic and Professional, an imprint of Chapman & Hall, Wester Cleddens Road, Bishopbriggs, Glasgow G64 2NZ, UK. 2nd Edition. pp 73-74, 163-167.

8. Joyeux. A., 1999. Alcoholic Fermentation by Yeast. http://www.Yobrew.co.uk/ Alcoholicfermentation.-by-yeast.htm. Last modified in January 1999. 1-5.

9. Macrae, R.; Robinson, R. K. and Sadler, M. J., 1993. Encyclopeadia of Food Science and Technology and Nutrition. 2nd Edition. Academic Press. pp 4773-4778.

10. McPhee, John. 1966. Orange (Fruits) Focuses on Florida Grove. Http/www.orangensw. Com/history.html. 1.

11. Merryweather, L.M., Hill, S.H.A., Robinson, A.L., Spenser, H.A., Rudge, K.A., Taylor, G.R.J, Wheatcroft, A.J.D., Weeks, J.J., Sahibdeen, S.S., Flynn, P.J., Blowers, M., Murphy, L.I., Murray, J.T., Waites, B.A., Goodchild, M.E., Morgan, C., Horgarth, S.L., Buckley, D.S. and Rogers, F.C., 2005. Dictionary of Food Science and Technology. Blackwell Publishng, UK. 1st Edition. pp 84.

12. Nurgel, C and Pickering, G., 2005. Contribution of Glycerol, Ethanol and Sugar to the Perception of Viscosity and Density Elicited by Model White Wines, *Journal of Texture Studies* 36 (3): 303–323.

13. Okafor, N., 1987. Vinegar in "Industrial Microbiology". 1st Edition. University of Ife Press Ltd., Ile-Ife, Nigeria. pp 222-229.

14. Ronald, S.K and Ronald S., 1991. Pearson's Composition and Analysis of Foods. Longman Scientific and Technical, Longman House, Burnt Hill, Harlow, England. 9th Edition. pp 462-465.

15. Shomatsu O., 2008. Vinegar for a Longer and Happier Life. www.naturodoc.com/library/lifestyle/umeboshi_vinegar.htm. Last modified on 30/12/2008. 1-12.

16. Simone Rochfort, Vilnis Ezernieks, Susan E.P. Bastian and Mark O. Downey., 2009. Sensory Attributes of Wine Influenced by Variety and Berry Shading Discriminated by NMR Metabolomics *Food Chemistry* 121 (4): 1296-1304.

17. Solieri, I. and Giudici, P., 2008. Yeast Associated with Traditional Balsamic Vinegar: Ecological and Technological Features. *International Journal of Food Microbiology*. 125 (1): 36-45.

18. Sutherland, J.P., Varnam, A.H. and Evans, M.G., 1986. A Colour at Last of Food Quality Conttrol. Wolf Publishing Limited. 1st Edition. pp 132.

19. William Y., 2007. Industrial Pollution Imperils Belize's Water Supply. http://www.reporter.bz/index.php?option=content&task=view&id=2196&Itemid=2. Last modified on Friday, 24th August, 2007. 1-3.

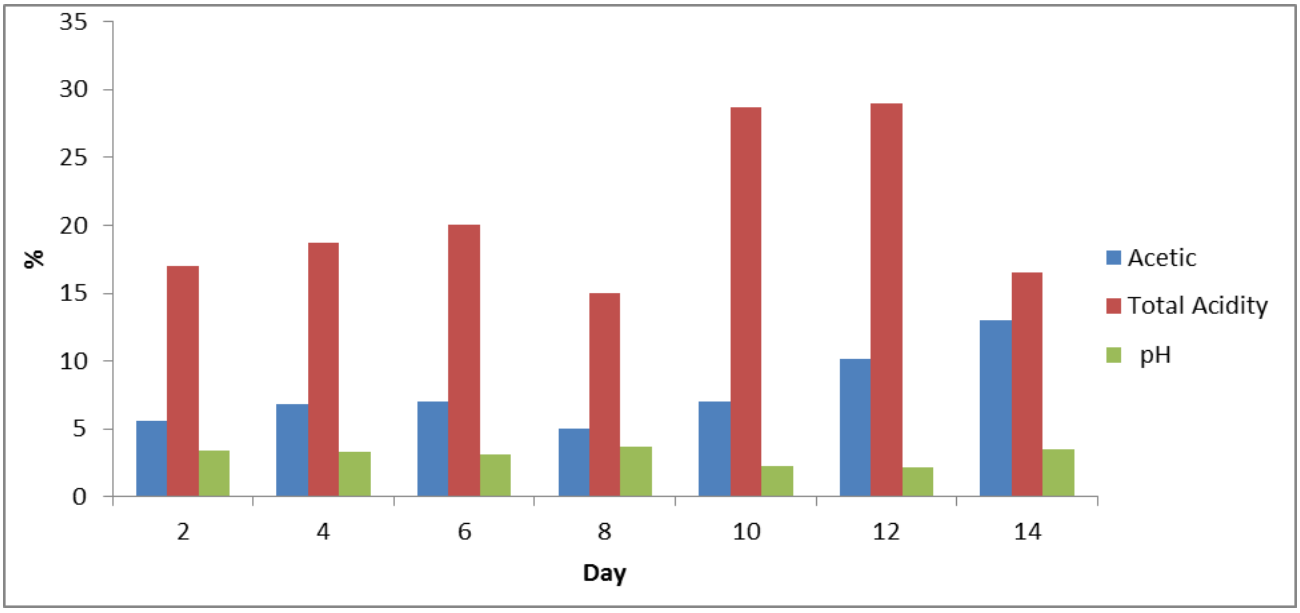

Fig. i: Acetic Acid (%), Total Acidity (%) and pH of Vinegar from Sweet Orange Peels

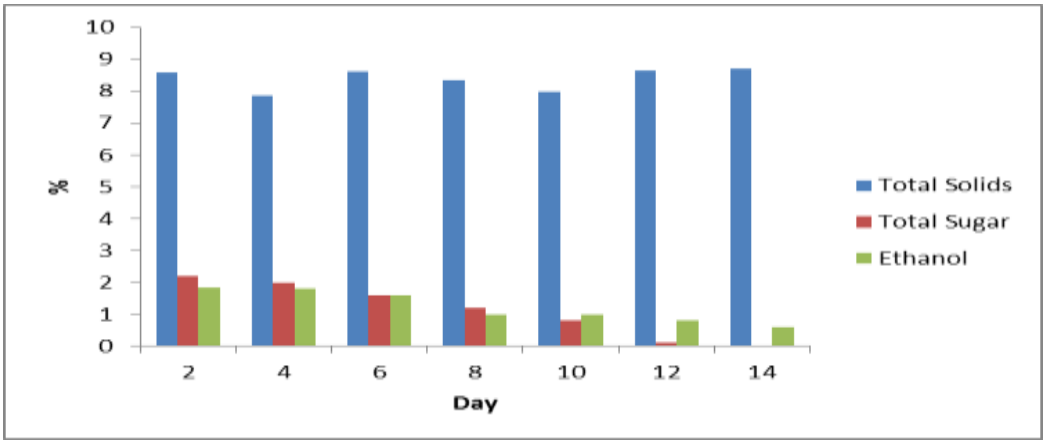

Fig. ii: Total Solids (%), Total Sugar (%) and Ethanol Content (%) of Vinegar from Sweet Orange Peels

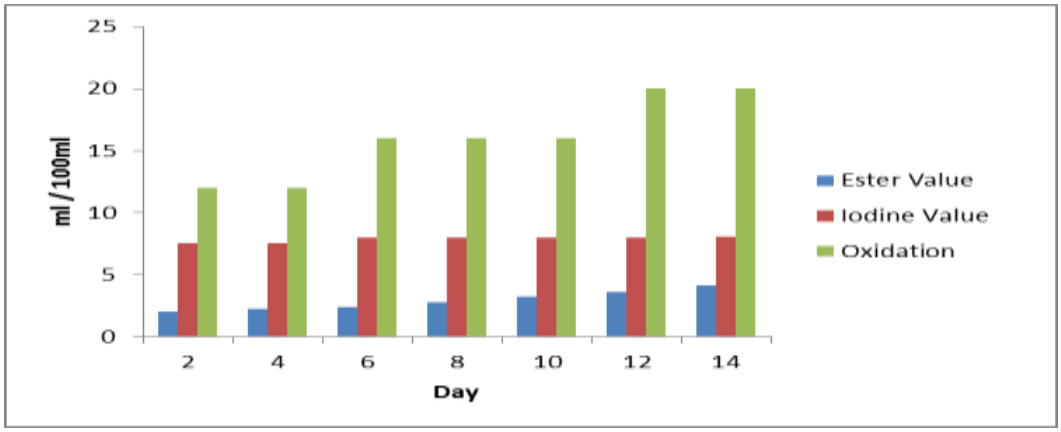

Fig. iii: Ester Value (ml/100ml), Iodine Value (g/100g) and Oxidation Value (ml/100ml) of Vinegar from Sweet Orange Peels

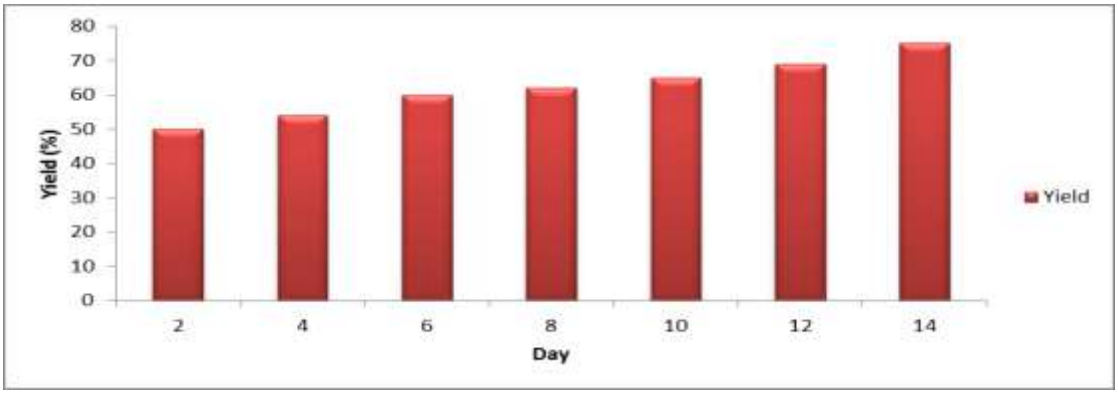

Fig. iv: Yield (%) of Vinegar from Sweet Orange Peels