

THE EFFECT OF NATURAL AND ARTIFICIAL PRESERVATIVES AND STORAGE TEMPERATURE ON THE pH AND MICROBIAL LOAD OF FRESHLY PRODUCED APPLE (*Malus domestica*) JUICE

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ABSTRACT

Apple (*Malus domestica*) juice was treated with 0.5 g/ml garlic, 0.5 g/ml ginger, 0.25 g/ml mix of ginger and garlic and 0.05% (w/v) of sodium benzoate respectively where the ginger, garlic and sodium benzoate serves as natural and artificial preservatives. Their effects on the apple fruit juice were evaluated during 8 days of storage at 4°C and at room temperature (28±2°C). The microbial counts ranged from 4.0×10⁴ to 7.0×10³ cfu/ml and from 1.0×10⁴ to 2.0×10³ cfu/ml for bacteria and fungi, respectively in the samples treated with ginger, garlic and sodium benzoate and from 1.2×10⁴ to 2.2×10⁴ cfu/ml in the apple juice without treatment. Juice treated with sodium benzoate had the least microbial contamination while the untreated juice had the highest. There was marginal decrease in pH values across treatments in stored apple juice with juice stored at 4°C showing the least change in pH compared to that stored at room temperature. The results obtained show that the preservatives exhibited antimicrobial effect on the microbial load and that pH has a significant impact on the shelf-life of the fruit juice as the microbial load was least when stored at low temperature the pH as acidity tends to be inclined towards low pH.

Key words: apple juice, pH, microbial load, sodium benzoate, temperature

INTRODUCTION

The apple tree (*Malus domestica*) is a deciduous tree in the rose family best known for its sweet, pomaceous fruit, the apple (GRIN, 2012) and stands 1.8 to 4.6 m (6 to 15 ft) tall in cultivation and up to 39 ft (12 m) in the wild (GRIN, 2012). It is cultivated worldwide as a fruit tree, and is the most widely grown species in the genus *Malus*. About 80 million tons of apples were grown worldwide in 2013, and China produced almost half of this total (FAO, 2015). The United States is the second-leading producer, with more than 6% of world production. Apples are often eaten raw but can also be found in many prepared foods (especially desserts) and drinks. The fruit matures in late summer or autumn, and varieties exist with a wide range of sizes. Commercial growers aim to produce an apple that is 7.0 to 8.3 cm (2.75 to 3.25 in) in diameter, due to market preference. Some consumers, especially those in Japan, prefer a larger apple, while apples below 5.7 cm (2.25 in) are generally used for making juice and have little fresh market value. The skin of ripe apples is generally red, yellow, green, pink, or russeted although many

bi- or tri-colored varieties may be found (Jules *et al.*, 1996). The flesh is generally pale yellowish-white, though pink or yellow flesh is also known (Jules *et al.*, 1996). Apple juice is one of the most common fruit juices in the world, with world production led by China, Poland, the United States, and Germany (USDA, 2005). The nutritional benefits of apple juice are often underestimated. It contains 83.1% moisture, 9.2% sugar, 1.87% protein, 2.0 mg sodium, 8.7 mg potassium, 2.7 mg calcium, 0.22 mg iron, 0.11 mg copper, 5.1 mg phosphorous and 4.5 mg vitamin C per 100 g of whole fruit (Zahid *et al.*, 2008). When closely evaluating the composition of apple juice and other apple products, it becomes apparent that the lack of fat, cholesterol and sodium are just a few of the many reasons these products are an important part of a healthful dietary routine. Fruit sugars such as those found in apple products serve as an immediate source of energy and because apple products taste so good, they are readily consumed by infants and children. Apple juice also serves as an excellent means of providing essential fluids to the body and is unlikely to cause allergic reactions.

Moreover, apple products have a nutrient/calorie ratio superior to many of the alternative snacks being consumed by children and the US Department of Agriculture's (USDA, 2008) new food pyramid My Pyramid states that 100% juice can be substituted for other fruits. A recent national survey of pediatric health professionals has confirmed that apple juice is most often recommended as the first juice for infants (USDA, 2008). It is easily accepted and pleasing to infants. Also, since babies love the sweet taste of apple juice, it may help motivate them to use a cup and accept new tastes and foods.

In fact, recent research suggests that it is the antioxidants that are plentiful in apple juice which are responsible for improving cognitive function. Apples are rich source of antioxidants; and researchers reported that one apple packs more cancer-fighting antioxidant capability than a 1,500 mg dose of vitamin C (Zahid *et al.*, 2008). Efforts have been made by technologists all over the world to providing methods of processing tropical fruits and vegetable through pre-processing storage techniques, preparative processes, extraction, pasteurization, packaging and preservations using chemical and natural preservatives (Ihekoronye and Ngoddy, 1985). Literature abounds on fruit crop processing, fruit juice extraction, efficacy of different artificial preservatives in improving the appearance and storage quality, retarding microbial growth in foods and drinks (Nwachukwu *et al.*, 2007; Ogiehor *et al.*, 2008; Onyeagba *et al.*, 2004). Studies showed that the practice of mixing different exotic fruits and/or certain food preservatives positively impact on the flavor and taste of fruit and fruit products (Nwachukwu *et al.*, 2007; Ogiehor *et al.*, 2008). Little is however reported in literature on the use of natural food products as preservatives in subsistent food and fruit processing especially in fruit juice extraction. This study was therefore carried out to compare the performance of a synthetic (Sodium benzoate) and selected natural (garlic and ginger) preservatives on the shelf life quality of locally produced apple fruit juice and to determine the effect of these preservatives and temperature on the pH and microbial load of locally produced apple fruit juice.

MATERIALS AND METHODS

Collection and Processing of Fruit

The Garlic bulbs, Ginger rhizomes and mature, ripe healthy Apple fruits were repurchased from different sales point in Uyo metropolis, Akwa Ibom State, Nigeria and transported to the Food Processing and Analytical Laboratory of the Department of Food Science and Technology, University of Uyo, where the research work was carried out. The Apple fruit were washed with distilled water to remove adhering soils, dirt and extraneous materials. The apples were sorted, trimmed, peeled and cut to small pieces before the extraction of juice.

Preparation of Fruit Additive/Preservative

The ginger rhizomes and garlic bulbs were washed with potable water repeatedly. Their outer covering was peeled off with a sterile knife and then sliced into cutlets and dried using a hot air oven at 65°C for 48 h. An electric blender was used to pulverize the dried ginger and garlic bulb into powder.

Production of Apple Juice

The pieces of apple were introduced into a juice extractor and the juice extracted. The juice was then filtered using clean muslin cloth into sterile conical flasks.

Treatment of Apple Juice with Natural and Chemical Preservatives

Natural preservative: Exactly 0.5g of ginger powder was added into 100ml of apple juice and 0.5g of garlic powder was added into another 100 ml of the apple juice. Similarly, a combination of 0.25g of ginger powder and 0.25g of garlic powder were measured and added to 100ml of apple juice. Chemical preservative: 0.05% (w/v) sodium benzoate (Sigma chemical company) was aseptically added to another 100ml of apple juice with another 100 ml of apple juice container serving as a control (without preservative).

Storage of Sample

The apple juice samples with the preservatives were stored at room temperature (28±2°C) and refrigeration temperature (4°C) for 8 days. The room temperature was measured using the Thermo Pro TP55 Digital Hygrometer Indoor Thermometer and the temperature was maintained at 28±2°C. The effect of treatment on pH of apple juice during storage and the effect of treatment on microbial population of apple juice during storage were monitored throughout the duration of storage.

Microbiological Analysis of Fruit Juice

Determination of microbial counts of the juice

The number of microorganisms present in the fruit juice were determined using the spread plate count method. Nutrient Agar was used for the enumeration of total aerobic bacteria while potato dextrose agar was used for fungal count. The agars were prepared according to the manufacturer's instruction. The fruit juice sample were serially diluted up to the 5th dilution and the agar was allowed to cool to 15°C. Each experiment was carried out in duplicates. Exactly 0.1ml of each of the dilution was aseptically introduced into sterile petri dishes using spread plate technique. The plates were inverted and incubated at 37°C for 24-48hrs for bacterial count and at 25°C for 3-5 days for fungal count. Colonies that developed on the plates were counted and expressed as colony forming units per milliliter (CFU mL⁻¹) of the sample. The process was repeated at two days intervals for 8 days (Cheesbrough, 2000).

Determination of pH

The pH of each group of containers with the different treatment was determined with a pH meter (Jenway pH meter, Model 3310) for the storage period (8 days), at two days interval from the first day. Ten milliliters of the juice was dispensed into a beaker and the pH was determined with a previously standardized pH meter. The pH meter was calibrated using phosphate buffer of pH 4.0 and 7.0 (AOAC, 2005).

RESULTS AND DISCUSSION

Microbial Population Counts

The effect of different treatments and temperature on the microbial load of the apple juice throughout the period of storage is shown in Figures 1-4. The addition of the preservatives helps to reduce the bacterial load in all juice sample treatments stored at 4°C and room temperature (28±2°C) with juice and sodium benzoate showing the highest reduction from 4.0 × 10⁴ to 8.0 × 10³cfu ml⁻¹ and 4.0 × 10⁴ to 7.0 × 10³cfu ml⁻¹ for juice stored at 4°C and room temperature respectively. There was no increase in the bacterial load for control samples stored at 4°C but increase in the sample stored at room temperature 4.2 × 10⁴ to 4.0 × 10⁴cfu ml⁻¹ and 4.1 × 10⁴ to 6.0 × 10⁴cfu ml⁻¹. The same trend was seen in the fungal load with juice treated with sodium benzoate having the highest reduction 1.0 × 10⁴ to 2.0 × 10³cfu ml⁻¹ and 1.2 × 10⁴ to 3.0× 10³cfu ml⁻¹ for samples stored at 4°C and room temperature respectively as compared to other treatments with the control sample having an increase in the fungal load 1.2 × 10⁴ to 2.2 × 10⁴cfu ml⁻¹ for the period of storage.

Effect of Preservatives on pH during Storage

Figures 5 and 6 shows the effect of the different treatments on the pH of apple juice. pH of juice sample stored at 4°C with ginger ranges between 4.97 to 5.16, juice with garlic ranges between 4.50 to 4.89. The juice with combination of ginger/garlic ranges between 4.74 to 4.91, juice with sodium benzoate ranges between 4.57 to 4.88 while juice alone (control) ranges between 4.43 to 4.81. For juice samples stored at room temperature (28±2°C), the pH ranges between 4.67 to 4.97, 4.97 to 5.16, 4.61 to 4.89, 4.67 to 4.91 and 4.53 to 4.88 for control sample, juice and ginger, juice and garlic, juice and ginger/garlic, juice and sodium benzoate respectively.

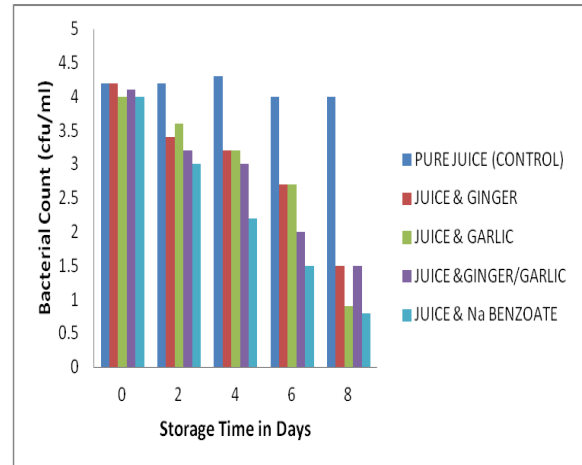


Figure 1: Effect of preservatives on the bacterial count (10⁴) at 4°C during storage

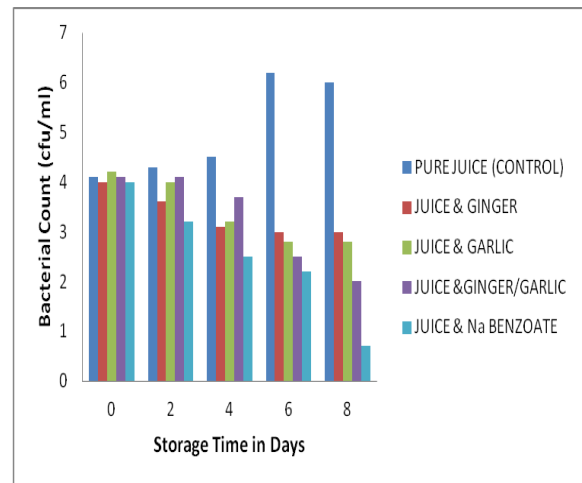


Figure 2: Effect of preservatives on the bacterial count (10⁴) at (28±2°C) during storage

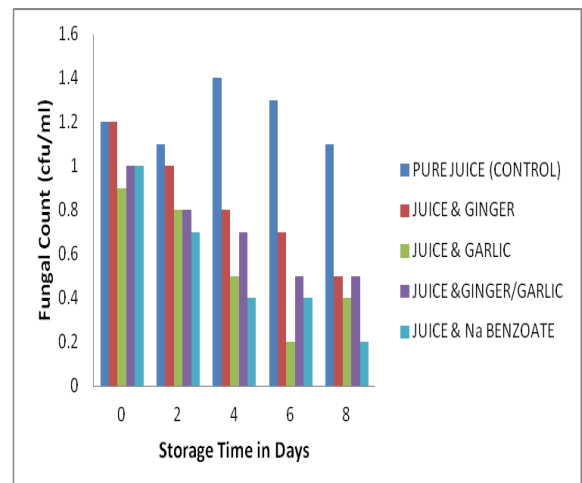


Figure 3: Effect of preservatives on the fungal count (10⁴) at 4°C during storage

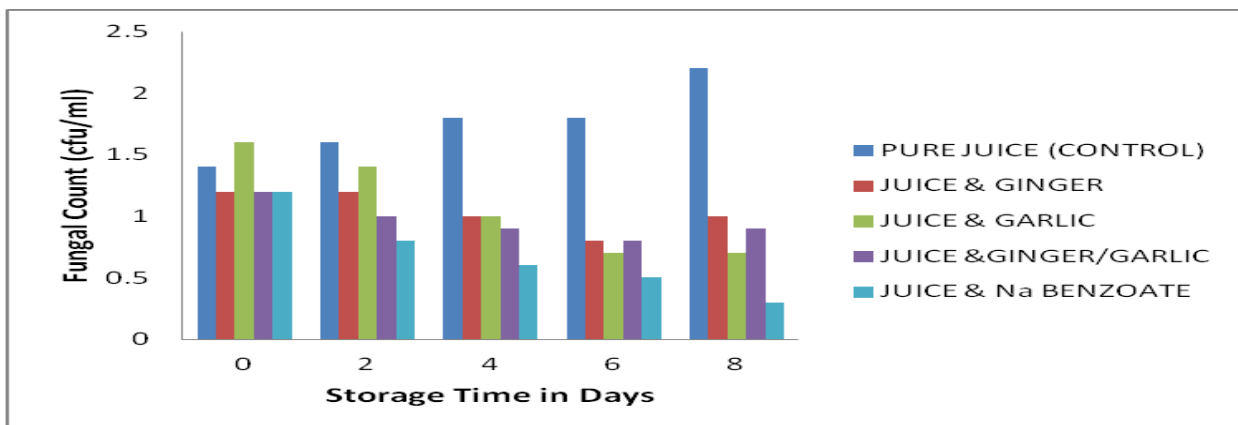


Figure 4: Effect of preservatives on the fungal count (10^4) at ($28\pm 2^\circ\text{C}$) during storage

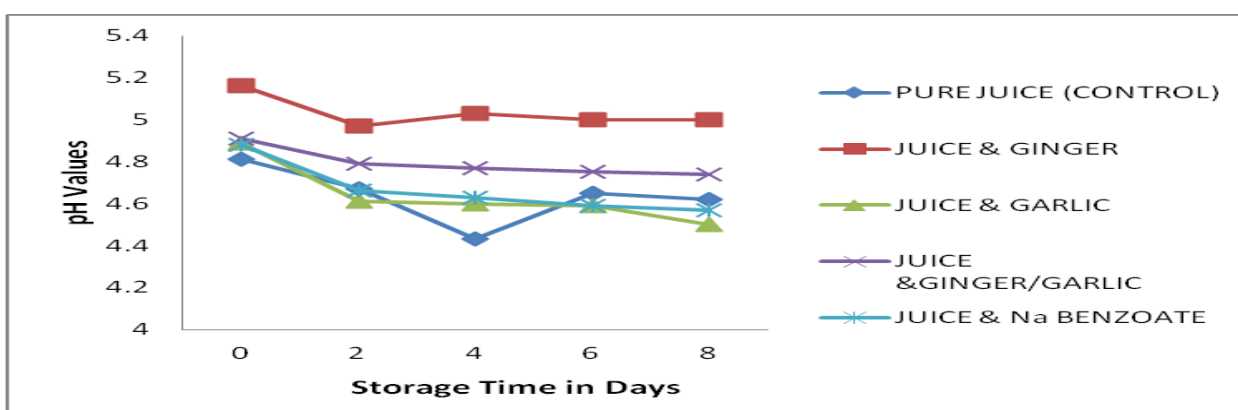


Figure 5: Effect of preservatives on pH at 4°C during storage

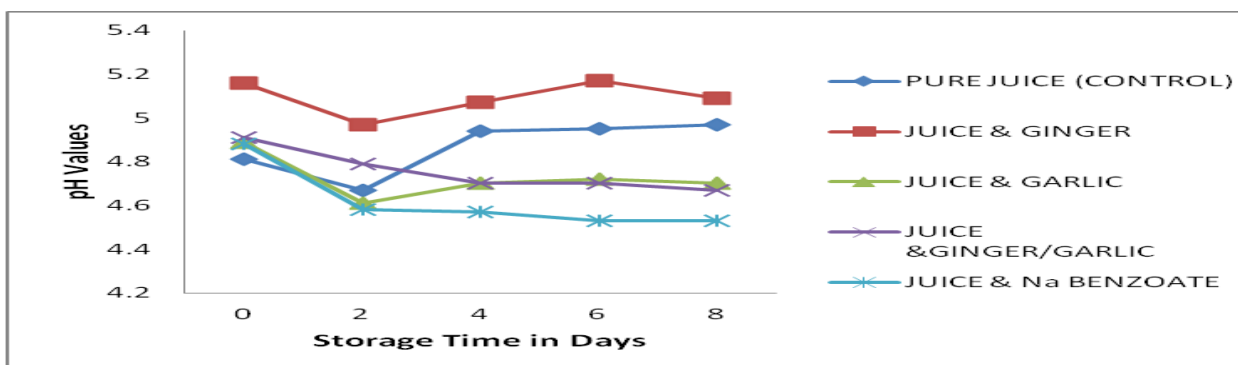


Figure 6: Effect of Preservatives on pH at ($28\pm 2^\circ\text{C}$) During Storage

Treatment Formulation Table

Sample	Treatment
Pure juice	No treatment (control)
Juice+ ginger	0.5g ginger
Juice+ garlic	0.5g garlic
Juice+ ginger/garlic	0.25g ginger + 0.25g garlic
Juice + sodium benzoate	0.05% (w/v) sodium benzoate

All the treatments were carried out in duplicates

The microbial load data obtained from the apple fruit juice suggest that both the natural preservatives (ginger, garlic and their mixture) and

the synthetic sodium benzoate preservative were effective in prolonging the shelf-life of the apple fruit juice with the sodium benzoate showing a better bacteriostatic and fungistatic performance at both temperatures of storage. The reason for this observation may be attributed to the insolubility of natural preservatives used for the study. The use of some chemical preservatives such as sorbate and benzoate to improve the shelf life of drinks has been reported (Dougheri *et al.*, 2007; Nwachukwu and Ezeigbo, 2013). According to Krebs *et al.* (2006), Sodium benzoate is a bacteriostatic and

fungistatic preservative underneath acidic environment and is mostly used in acidic foods like salad dressings (vinegar), carbonated munchies (carbonic acid) and condiments. The inability of the apple juice to stay for a long period without preservatives could synergize the nutrient status of the fruit juices creating the appropriate food base for the colonization by microorganisms (Andargie *et al.*, 2008; Fasolin and Cunha, 2012). Ginger (*Zingiber officinale*) and garlic (*Allium sativum*) are traditionally used as spices in food preparation but also have both antioxidant and antimicrobial properties (Kolapo *et al.*, 2007). Ginger was reported to have bactericidal effect against *Escherichia coli* and *Streptococcus* (Smith-Palmer *et al.*, 1998). Benzoates are preferable in the preservation of fruit juices due to the solubility of their salts. Benzoates are used at low temperatures to extend the shelf-life of minimally processed juices (Uma *et al.*, 2011). The irwide usage is due to their broad-spectrum activity against some microorganisms, as well as their non-alteration of food flavor. The reduction in the microbial loads over storage period in juice treated with both natural preservatives and the synthetic preservative may be attributed to the antimicrobial and phytochemical properties of the preservatives and apple juice respectively as antimicrobials can effectively extend the shelf-life of fruit products (Tagoe *et al.*, 2010; Ukwo *et al.*, 2010). The results of this investigation also revealed that untreated apple juice (control) had a higher level microbial load during the period of storage. This could probably be due to the microbial activities favored by the absence of refrigeration in the juice stored at room temperature and preservatives. This finding therefore suggests that the addition of these preservatives to apple juice might prolong the shelf-life of the juice.

The fruit preservation performance of these phytogens showed no difference in the reduction of the microbial loads in fruit juices treated with ginger, garlic and mixture of both respectively. The pH results (figures 5 and 6) showed a gradual acidic inclination for the period of storage in samples stored at room temperature and 4°C except for juice treated with ginger. This is in consonance with the reports of Dennis *et al.* (2013) and Fleet, (2003) that the addition of ginger and garlic moves the pH towards acidity and also reduces the microbial load profile in the fruit juice. Environmental factors, activities of microorganisms together with the incomplete dissolution of the natural preservatives may also alter the pH of the juice during storage (Ukwo *et al.*, 2010). The excellent keeping quality of fruits and soft drinks is influenced by low pH (Bates *et al.*, 2001). The near anaerobic condition of the study may have favored the early appearance of bacteria species and the late emergence of fungi

isolates as equally observed in the microbiological results from the work of Dennis *et al.* (2013) on a homemade soursop (*Annona muricata* L.) fruit juice.

CONCLUSION

Home-made non-commercial juices that are processed by using mechanical or electrical juice extractor, squeezing, macerating or aqueous/solvent extractions, with or without the application of heat have short shelf life and low organoleptic property as found in the present study. The findings of this study generally indicate that combination of chemical and natural preservation together with refrigeration is suitable for preservation of apple juice for a long time. These methods considerably decreased the microbial count. Since these methods are simple, inexpensive and convenient, they can be adopted for industrial use in the processing and preservation of apple juice. The utilization of the preserved juice should be encouraged as health/therapeutic drink. Above all, preservation of apple juice is important because of the seasonality of its production which makes it abundantly available during its season and scarce during off season. Chemical preservatives can be replaced with natural preservatives such as ginger and garlic.

REFERENCES

- Andargie G., Kassu A., Moges F., Tiruneh M. and Huruy K. (2008) Prevalence of bacteria and intestinal parasites among food-handlers in Gondar Town, Northwest Ethiopia. *Journal of Health and Population Nutrition*, **26**, 451–455
- AOAC. (2005). Official Methods of Analysis of AOAC International. 18th Edn., AOAC International, Gaithersburg, MD., USA., ISBN-13: 978-0935584752
- Bates R.P., Morris J.R. and Crandall P.G. (2001). Principles and practice of small- and medium-scale fruit juice processing. *FAO Agricultural Service Bulletin*, **146**, 1–221
- Cheesbrough M. (2000). *District Laboratory Practice in Tropical Countries*, Part 2. Cambridge University Press, pp. 52-70
- Dennis E.V., Omrefosa O.O. and Juliet N.A. (2013). The effect of garlic and ginger phytochemicals on the shelf life and microbial contents of homemade soursop (*Annonamuricata* L) fruit juice. *International Journal of the Nigerian Society for Experimental Biology*, **25** (2), 31–38
- Dougheri J.H., Alabi G. and Elmahmood A.M. (2007). Effect of some chemical preservatives on the shelf-life of Zobo drink. *African Journal of Microbiology Research*, **2**, 37-41
- FAO (2015). FAO production data, FAO. Retrieved 2 July 2015
- Fasolin L.H. and Cunha R.L. (2012). Soursop juice stabilized with soy fractions: a rheological approach. *Ciênc. Tecnol. Campinas*, **32**, 558–567
- Fleet GH (2003) Yeast interactions and wine flavour. *Int. Journal of Food Microbiology*, **86**, 11–22

- Germplasm Resources Information Network (GRIN). Retrieved 29 January 2012. "Pyrusmalus L."
- Germplasm Resources Information Network (GRIN). Retrieved 4 January 2012. "Maluspumilauct."
- Ihekoronye A.T. and Ngoddy P.O. (1985). *Integrated Food Science and Technology for the Tropics*. Macmillan Publishers Limited, London, UK., ISBN-13: 9780333388839, pp: 167, 171-179
- Jules J., James N., Cummins S., Brown K. and Minou H. (1996). "Chapter 1: Apples". In Jules Janick and James N. Moore. *Fruit Breeding, Volume I: Tree and Tropical Fruits* (PDF). John Wiley & Sons, Inc. p. 9. ISBN 0-471-31014-X
- Kolapo A.L., Popoola T.O.S., Sanni M.O and Afolabi R.O. (2007). Preservation of soybean daddawa condiment with dichloromethane extract of ginger. *Research Journal of Microbiology*; **6**, 13-18.
- Krebs H.A., Wiggins D., Stubbs M., Sols A. and Bedoya F. (2006). Studies on the mechanism of the antifungal action of benzoate. *Journal of Biochemistry*, **214**, 657-63
- Nwachukwu E., Onovo O.M. and Ezema C.F. (2007). Effect of lime juice on the bacterial quality of Zobo drinks locally produced in Nigeria. *Research Journal of Microbiology*, **2**, 787-790
- Nwachukwu E. and Ezeigbo C.G. (2013). Changes in the microbial population of pasteurized soursop juice treated with benzoate and lime during storage. *African Journal of Microbiology Research*, **7** (31), 3992-3995
- Ogiehor S.I., Nwafor O.E. and Owhe-Ureghe U.B. (2008). Changes in the quality of zobo beverages produced from *Hibiscus sabdariffa* (Linn.) Roselle and the effects of extract of ginger alone or in combination with refrigeration. *African Journal of Biotechnology*, **7**, 1176-1180
- Onyeagba RA, Ugbogu OC, Okeke, CU and Iroakazi O (2004) Studies on the antimicrobial effects of garlic (*Allium sativum*Linn), ginger (*Zingiber officinale* Roscoe) and lime (*Citrus aurantifolia* Linn). *African Journal of Biotechnology* **3**, 552-554.
- Tagoe D, Baidoo S, Dadzie I, Kangah V and Nyarko H (2010). A comparison of the antimicrobial (antifungal) properties of garlic, ginger and lime on *Aspergillus flavus*, *Aspergillus niger* and *Cladosporium herbarium* using organic and water-based extraction methods. *Internet Journal of Tropical Medicine* **7** (1), doi:10.5580/1099.
- Ukwo SP, Ezeama, CF and Ndaeyo NU (2010) Growth of different yeast strain during fermentation of soursop (*Annonamuricata*) juice as influenced by acetic acid bacteria. *Nature*, **5**, 23-24
- Uma T, Rama RV, Khasim BS (2011). Preservation and shelf life extension of cashew apple juice. *Internet J. Food Safety*, **13**, 275-280.
- United States Department of Agriculture, USDA (2008). New Food Pyramid: My pyramid. Pyramid/Fruits/Tips to fruits. http://www.mypyramid.gov/pyramid/fruits_tips.html.
- United States Department of Agriculture, USDA (2005). Foreign Agricultural Service. World Apple Juice Situation. 2004-2005
- Smith-Palmer A., Stewart J. and Fyte L. (1998). Antimicrobial properties of plant essential oil against five important food borne pathogen, *Letters in Food Microbiology*, **26**, 112-118
- ZahidM., Alam Z., Mohammad A., Nizakat B., Amal B. and Ihsanullah I. (2008). Effect of pasteurization and chemical preservatives on the quality and shelf stability of apple juice. *American Journal of Food Technology*, **3**, 147-153