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MICROBIAL AND PRESERVATIVE SAFETY OF FRESH AND PROCESSED FRUIT SALADS, FRUIT SOFT DRINKS AND JAM IN KENYA

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ABSTRACT

Fresh fruits provide vitamins A and C which are anti-oxidants and other phytochemicals that form a healthy diet and lower the risk of cancer and chronic heart diseases. Kenya's horticulture production provides 3.82 million tonnes of fruits and vegetables to the local market and 0.13 million tonnes to export market. Thirty percent is lost annually due to lack of transport and processing. The FAO/WHO Expert consultation has recommended a daily intake of at least 400g of fruit and vegetables which means at least 200g fruit per capita per day. In Eastern Africa, fruit consumption is less than 50g of fruit per adult per day. Consumer confidence is eroded by poor hygienic conditions in growing, handling or transporting the produce. Processing fruit by drying loses up to 88% of water and yielding a product safe for storage and results in a more intense flavour. Canning involves sterilization. Common preservatives used in fruit processing are benzoic acid and sulphates. The objective of this research was to assess safety of fresh and processed fruit products marketed in Kenya in terms of content of microorganisms, and residual chemical preservatives compared to FAO/WHO Codex standards and Kenya standards. The products were tested according to AOAC *Official Methods of Analysis* for total plate count, *Staphylococcus aureus*, yeast and mould, coliforms as well as benzoic acid and sulphurous acid. Results showed that chilled fresh juice had nil coliforms while processed fruit and fruit drinks from supermarkets had nil total plate count and, therefore, met safety requirements. Unrefrigerated fresh fruit salad in street kiosks had coliforms in the range 0.50 x10^2 to 2.10 x10^3 cfu per g. All processed fruit products apart from strawberry jam had levels of microorganisms and preservatives below the maximum residue limits. The strawberry jam had sulphurous acid at 133 ppm which is above 100 ppm, the maximum residue limit. The producers and traders of fresh fruit and the processers should implement quality management practices and safety standards in farming, fresh fruit, processing and storage. This is to ensure safety, enhance consumption of fruits and fruit products for health of consumers and eliminate wastage.

Key words: Fresh fruit, vitamins, spoilage, preservation

INTRODUCTION

Fresh fruits and vegetables refer to fresh produce that is likely to be sold to consumers in an unprocessed or minimally processed form [1]. Over 90% of smallholder farmers in all but arid regions of Kenya produce horticultural products. Fewer than 2% do so directly for export [2]. Local market accounts for 3.82 million tonnes and export market takes 0.13 million tonnes. This translates into 96% for local markets and 4% for exports. Thirty percent is lost annually due to lack of transport and processing [3, 4].

Processing fruit by drying loses up to 88% of water to a level of 18-25%, which is safe for storage and results in a more intense flavour [5]. Canning involves washing the fruit, peeling, blanching and then cutting into pieces or comminuting as pulp or juice before placing in the can for sterilization. Chemical preservatives such as benzoic acid are also added [6].

Ripening of fruit is characterized by changes in solubility of pectic substances resulting in softening of fruit texture. Hydrolysis of polymeric constituents such as starch causes conversion of it and organic acids to glucose, or breakdown of proteins, particularly enzymes and evolution of aromatic volatiles [7]. Fresh fruits provide anti-oxidants, other phytochemicals and vitamins that may lower risk of cancer and chronic heart diseases [8]. FAO/WHO Expert Consultation recommended a daily intake of at least 400g of fruit and vegetables [9]. This is regarded to provide 200g of fruit on average. Both vitamins A and vitamin C enhance iron utilization, and therefore enhances iron status of children [10]. In Eastern Africa, fruit consumption is less than 50g of fruit per adult per day [11].

Consumer confidence is eroded by poor hygienic conditions in growing, handling or transporting the produce [12]. In many countries, fresh vegetables or fruit salads for example apples were contaminated by *E. coli* O157:H7 through cow manure, well water, irrigation water, flood water or overload of organic material either at washing, growing, storage or transport**.** Yeasts and moulds invade and grow on grains, nuts, beans, and fruits in the field before harvest, in store, and in processed foods [13]. In spoilage, fruit becomes too soft due to action of pectolytic enzymes deposited on them by microorganisms and gives off an unpleasant smell due to rotting. Yellow fruit turns grayish due to mould attack, or the juice becomes too sour due to fermentation [14].

Good Management Practice includes "Good Agricultural Practices" in field and storage operations and "Good Manufacturing Practices". Composting, appropriate aging, and other treatments may reduce pathogens in manure. Domestic animals should be excluded from fresh produce fields, vineyards, and orchards during the growing season. Mud and debris should be removed from processing equipment daily. Fruits should be washed or rinsed with clean chlorinated water immediately after harvest to remove field heat and contaminants, and packed before shipping to reduce microbial spoilage [15].

The most heat resistant microorganisms in medium acid foods such as fruit juice are spores of *Bacillus coagulance*, a food coagulating spoilage bacteria and those most heat

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resistant in low acid foods are spores of *Clostridium sporogeness, or of Cl. botulinum* a poisoning bacterium [16]. In insufficient thermal and chemical treatments *Staphylococcus* vegetative cells a food poisoning bacterium may survive [17]. Chemical hazards include pesticides, veterinary drug residues, food additives and industrial contaminants that cause neurological diseases and cancer [18]. Worldwide, the persistent *Dichlorodiphenyl trichloroethane* (DDT) has been banned [19].

The objective of this research was, therefore, to assess safety of fresh and processed fruit products marketed in Kenya in terms of content of microbial total plate count, yeast and mould, coliforms and *Staphylococcus aureus*, and the preservatives; benzoic acid and sulphurous acid compared to FAO/WHO Codex standards and Kenya standards.

MATERIALS AND METHODS

Fruit salad bought from the street, chilled unpasteurized fruit juice and selected brands of processed fruit drinks and canned fruit bought from Uchumi super market were tested for microorganisms and preservatives such as benzoic acid and sulphurous acid contents. The samples for analysis of each specified product type were ten packages analyzed in triplicate.

Total plate count

The products were tested according to Association of Official Analytical Chemists (AOAC) *Official Methods of Analysis* [20]. Sampling and plating equipment were sterilized with dry heat in hot air oven, at 160^{o}C for 2h. Rubber corks were autoclaved at 121^{o}C for 15 min. Plate medium for growth was prepared using tryptone, yeast extract, glucose, sodium chloride, Agar bacterial grade and distilled water. The pH of medium was readjusted using sodium hydroxide (IN) and hydrochloric acid (1N).The medium was placed in bottles autoclaved to sterilize and stored in refrigeration.

Dilution bottles were filled with phosphate buffer so that after sterilization, each contained 99ml blanks. To the initial dilution, 1ml or 1g of material was added using pipette so that dilution was 1**:**100. Subsequent dilution of 1:1000 was prepared by taking 1ml of the previous to a subsequent bottle.

The growth medium in the container was melted over boiling water. 15ml of liquefied medium at 44^{o}C was introduced into the plates. As each plate is poured, it was thoroughly mixed with the test portions in the petri dish. The mixture was allowed to solidify. The plates were then inverted and placed in the incubator. Plates were incubated at 37.0±0.5^{o}C, for 48h. After incubation, all colonies were counted and recorded.

Staphylococcus aureus

Staphylococcus aureus was tested by using AOAC (2002), Official method 987.09 [21]. Unthawed 50g food was aseptically weighed into jar. To the jar was added water measuring 450 ml and phosphate buffer to dilute and homogenized. This formed 1 to

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10, or 10^{-1} dilution that was used to prepare other serial decimal dilutions 10^{-2} to 10^{-6}. Inoculation of food from serial dilutions of the sample was done in triplicate into tubes of trypticase soy broth that had 10% NaCl and 1% Sodium pyruvate.

Inoculated tubes were incubated at 35°C for 48h. Using 3 mm loop; a loopful of each growth was transferred to Baird–Parker medium plates. If growth was visible, it was streaked to obtain isolated colonies. Growths of *S. aureus* are typically circular, smooth, convex, moist and black. To confirm *S. aureus* a colony from each plate was picked and transferred to tubes containing 0.2 ml BHI (Brain Heart Infusion) broth and agar slants with trypticase soy; and the culture suspension was incubated at 35°C for 18 to 24h. To BHI culture was added 0.5ml reconstituted coagulase plasma with SDTA, mixed and incubated at 35–37°C and examined periodically at 6 hours interval for clot formation. Clot is positive reaction. The most probable count number was then reported.

Yeast and mould

The test for yeast and mould used the method of the FDA/CFSAN [22]. Food sample was diluted with 0.1% peptone water to dilution of 10^{-1}. It was homogenized and pipetted to prelabelled 15x100 mm petriplates. Dichloran 18% (DG18), agar (184) was added to the plates, which then were whirled to mix contents and incubated in the dark at 25°C for 5 days, and growth counted.

Coliforms

Coliforms content in fruit products was analysed by AOAC, 2002, Official methods of analysis, 990.11 Determination of total coliforms and *Escherichia coli* [23]. The sampling and plating equipment were sterilized with dry heat in hot air oven at 160°C for 2h. Media and materials such as rubber cork, were sterilized by autoclaving at 121°C for 15 minutes.

The diluent Physiological Salt Solution, 500 ml, was made by dissolving NaCl, 0.85 g in every 100 ml distilled water (Oxoid, UK). Dilution bottles were prepared and each filled with 9ml of the diluent. Two plates were prepared per sample dilution for test, so that total colonies on a plate would be between 30 and 300.

Sample serial decimal dilutions

Test sample 25g was dissolved in 225ml physiological salt solution to obtain a 1:10 or 10^{-1} homogenate. The second dilution was made by using sterile pipette to take 1 ml of sample dilution 10^{-1} and adding to a bottle having 9 ml dilution blank, so that dilution was 1:100 or 10^{-2}. Subsequent dilution was prepared by taking 1 ml of the previous sample dilution to a subsequent bottle of the diluent blank of 9 ml.

Inoculation and incubation

One ml of each sample dilution was transferred using a pipette into culture growth plate followed by the Violet Red Bile Lactose (VRBL) agar (Oxoid UK). The sample dilutions cultured in specific plates were the 10^{-3} to 10^{-5} in duplicate. From first sample dilution to pouring of last dilution on plate took less than 15 minutes. The

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plates were incubated at 35ºC for 48h. The characteristic colonies were reddish maroon. The colonies were counted at 48 hours of incubation. The number of colonies in one ml of the sample was calculated taking account of the dilution factors. The data was presented as means and ranges of microorganisms count.

Tests for chemical preservatives in processed fruit products

Benzoic acid

Benzoic acid was tested according to AOAC, official method [24]. A sample of 10 ml fruit juice was placed in 50 ml centrifuge tube and centrifuged for 5 min at 1500 x g. C_{18} cartridge was preconditioned by using 10 ml syringe to pass 2 ml methanol through it followed with 5ml water. 1ml test sample was pipeted into the syringe and through the cartridge. The cartridge was washed with 3ml of 2% acetonitrile hexane by pushing with plunger to elute drip and the eluate discarded. Excess hexane in cartridge was eliminated by blowing air through cartridge. 3ml methanol was added to syringe and used to eluate cartridge and eluate collected in 5ml graduated centrifuge tube. Eluate volume was adjusted by adding methanol to 3ml. The eluate was passed through 0.45 µm filter into vial.

A spiked juice sample was divided in two portions. One portion was filtered through 0.45 µm as a control. The other was passed through C_{18} cartridge and 0.45 µm filter. Recovery of benzoic acid was calculated based on the difference between amount determined in control and amount obtained after C_{18} cartridge clean up. The eluted test sample was injected in duplicate of 10 µl to the LC, after injecting the standard. Calculation of:

Benzoic acid, is 10 µml = (A/A’) xCx3x (1/R). Where A, A’ = peak area of test sample and standard, respectively; C = concentration of the standard, µg/ml; 3 = dilution factor; and R = recovery rate.

Sulphurous acid

Sulphurous acid was tested with use of AOAC official Method 962.16 [25]. The test portion was placed in distillation flask and boiled for at least 1 h in slow current of N_2. Sulphur dioxide was liberated and passed through reflux condenser and the refluxed vapour passed through U-tubes and condensate collected in the second U-tube. Hydrogen peroxide was added to react with sulphur dioxide to form sulphuric acid. This was titrated with 0.1 m NaOH from a burette. The end was when methyl red turned yellow.

RESULTS

Microbiological safety of fresh unprocessed fruit juices and street fruit salads

Chilled passion fruit juice had TPC 10^4 cfu per ml of product and was free from coliforms and staphylococcus. The street fruit salad had TPC 10^4 and coliforms 10^3. The results are presented in Table 1.

Microbiological safety of processed fruit products
Processed fruit drinks and canned fruit slices had nil TPC and nil yeasts and moulds while jam had 125 TPC and <10 yeasts and moulds per g. These conformed to safety requirements. Results of analysis are presented in Table 2.

Chemical preservatives safety of processed fruit products
The tests revealed that residues of sulphurous acid were nil in fruit slices and fruit juice, while it was 3.8 ppm in fruit flavour drink and 19 ppm in plum jam. These were within the standard Maximum Residue Limit (MRL) of 100 ppm. Sulphurous acid in strawberry jam was 337ppm which was higher than MRL. Benzoic acid residue in fruit slices and fruit juice was nil while its MRL is not applicable, and in fruit flavor it was 54 to 295ppm compared to MRL of 1000 ppm. Benzoic acid in jam was nil within MRL of 500 to 1000ppm. The results are presented in Table 3.

DISCUSSION

Total microorganisms counts in fresh street fruit salad were higher than in processed fruit products. Microorganisms in fresh chilled juice were lower than in fruit salads, an indication that using chill temperatures is good. This shows that good hygiene practice including chilling of fruit products should be implemented in street kiosks. It also shows that thermal and chemical preservation reduced the microorganisms. However, the fruit juice and street fruit salad and processed fruit products had tolerable levels of TPC coliforms and *staphylococcus and yeast and moulds.* The amounts of microorganisms in the products were low compared to assumed maximum load of 10^{12} colony forming units of *Clostridium botulinum* spores in fresh foods [26]. However, in further storage fresh produce could spoil and develop poison.

Among products tested, strawberry jam failed on safety because of its content of preservative sulphurous acid of higher level than standard requirement. Apparently the manufacturer had added excess amount of the preservative to the product. Food preservation is best achieved when the processor takes account of the type and concentration of antimicrobial agent, storage time and temperature, food pH and buffering capacity, integrity of food and presence of other agents for shelf life [27]. Results of analysis of pesticide residues in fruits showed that the fruits were chemically safe for eating.

CONCLUSION AND RECOMMENDATION

Laboratory analysis of fresh fruit salads, juice and packaged fruit products revealed that fruit and fruit products were generally safe. The chilled fresh salad and processed fruit had no pathogenic microorganism detected in them. Street salad unchilled had some coliforms in the range 0.50×10^2 to 2.10×10^3. Strawberry jam was found to have preservative sulphurous acid (SO_2) above maximum residue limits.

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Producers should implement Good Agricultural Practice and Good Manufacturing Practice, as well as quality and safety standards in farm production stages, material, processing, storage and labeling to improve consumer confidence in fresh unprocessed fruit salads and juice and processed fruit products.

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Table 1: Results of test for microorganisms in fresh unpasteurized juices and fruit salads

Fruit Product	Microorganisms count (cfu per ml of juice or g of salad)			
		Total Plate Count	**Staphylococcus aureus**	**Coliforms**
Mixed fruit salad (Mango, pineapple, melon and banana)	**Range**	4.45×10^2 to 9.47×10^4	0.00 to 2.51×10^3	0.50×10^2 to 2.10×10^3
	Mode	9.24×10^4	0.88×10^3	0.98×10^3
	Mean	9.23×10^4	2.20×10^3	1.88×10^3
Passion fruit fresh Juice (chilled)	**Range**	5.38×10^3 to 8.5×10^4	No growth was noted in all dilutions 10^{-1} to 10^{-4}	No growth was noted in al dilutions
	Mode	6.52×10^4	nil	Nil
	Mean	6.73×10^4	nil	Nil

Results are average of analysis of 10 packages of each brand

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Table 2: Results of analysis of microbiological safety of processed fruit products

Processed fruit product type	Specified fruit product (brand)	Total Plate Count per g or ml		Yeasts & Moulds per g or ml		Quality Standard Specifications	Remarks
		Test Results	Maximum limit	Test Results	Maximum limit		
Canned fruit slices	Canned pineapple	**Nil**	Shall be absent	**Nil**	2 max	KS 05-318 Specification for canned pineapples	Passed all
	Canned pear	**Nil**	Shall be absent	**Nil**	Shall be absent	KS 05-1174 Specification for canned pears	‘’
Fruit juice	Pineapple juice	**Nil**	50 max	**Nil**	‘’	*KS 05-556 Specification for pineapple juice preserved exclusively by physical means	‘’
	Mango juice	**Nil**	50 max	**Nil**	‘’	KS 05-404 Specification for mango juice preserved exclusively by physical means	‘’
Fruit based soft drink	Orange drink	**5**	50 max	**Nil**	‘’	KS 05-328 Specification for fruit based soft drinks	‘’
	Passion fruit drink	**Nil**	10 max	**Nil**	‘’	‘’	‘’
Fruit flavour drink	Tropical juice	**Nil**	10 max	**Nil**	2 max	KS 05-1485 Specification for fruit flavoured Drinks	‘’
	Orange cordial	**Nil**	10 max	**Not detected**	2 max	‘’	‘’
jam	Plum jam	**125 per g**	10^6 max	**Per g, 10**	100 max	KS 05-139 specification for jams jellies and Marmalade	‘’
	Strawberry jam	**5**	10^6 max	**Per g, 10**	100 max	‘’	Passed all

Values are the means of results of analysis of 10 packages of each brand, with content of each container analysed in triplicate, and acceptable repeatability of results in the range ≤ 0.05. *KS – Kenya standard

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Table 3: The amounts of preservatives in processed fruit products

Processed fruit product type	**Specified fruit product (brand)**	**Sulphurous acid ppm**		**Benzoic acid ppm**		**pH**		***Quality Standards***	**Remarks**
		Results	**Requirements**	**Results**	**Requirements**	**Result**	**Requirements**		
Canned fruit slices	Canned pineapple	Nil	N/A	Nil	N/A	4.0	Not specified	KS 05-318 Specification for canned pineapples	Passed all requirements
	Canned pears	Nil	N/A	Nil	N/A	4.0	Not specified	KS 05-1174 Specification for canned pears	‘’
Fruit juices	Pineapple juice	Nil	N/A	Nil	N/A	3.4	Not specified	KS 05-556 Spec for pineapple juice preserved exclusively by physical means	‘’
	Mango juice	Nil	N/A	Nil	N/A	4.0	Not specified	KS 05-404 Spec for mango juice preserved exclusively by physical means	‘’
Fruit based soft drink	Orange drink	Not detected	350 max	405.2	1000 ppm max	3.6	4.5 Max	KS 05-328 Specification for fruit based soft drinks	‘’
	Passion fruit base soft drink	3.2	350 max	277.1	‘’	2.4	4.5 Max	‘’	‘’
Fruit flavour drink	Tropical ready juice	3. 84	100 ppm max	54.9	‘’	4.0	Not specified	KS 05-1485 Specification for fruit flavoured drinks	‘’
	Orange cordial	19.2	‘’	295	‘’	2.7	4.5	‘’	‘’
Jam	Plum jam	12.8	‘’	Nil	‘’	3.6	4.5 max	KS 05-139 specification for jams jellies and marmalade	‘’
	Strawberry jam	133.7	‘’	Nil	500 ppm max	3.7	4.5 max	‘’	Failed by high level of sulphurous acid

Results are average of analysis of 10 packages of each brand, with content of each container analysed in triplicate, and acceptable repeatability of results in the range ≤ 0.05.

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