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Full Length Research Paper

Effect of pure microbial isolates recovered from carbonated and non-carbonated fruit juices on freshly produced pineapple juice

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Spoilage microorganisms were isolated from local fruit juice samples and identified. *Bacteria*, *Moulds* and *Yeasts* spp. were amongst the identified organisms and their effect on freshly produced clarified pineapple juice was studied. Though carbonation was able to prolong the shelf life of the juice, the results from the experiment showed that the isolates were able to cause spoilage and as such confirmed them as spoilage microorganisms by their high acid tolerance characteristics. Total Titratable Acidity (TTA), Turbidity and Total Dissolve H_2S tests were determined amongst other biochemical parameters. *Bacillus* non-reactive showed high turbidity, high flocculation with TTA value of 0.61 at pH 2.87, *Bacillus licheniformis* on the other hand showed the least TTA of 0.31 at pH 2.88. These and other isolates showed high acid tolerance properties confirming them as spoilage organisms. They were also able to produce hydrogen sulphide (H_2S).

Keywords: Pineapple fruit Juice, Carbonation, Isolates, Spoilage microorganisms, Pectinase.

INTRODUCTION

Fruit Juices are extracts from fruits and they are of important values to man and the environment. Fresh fruits are living dynamic system even after detachment from the parent plants. Nutritionally, fruits and vegetables contribute considerable amounts of vitamins A, C, Thiamine (B_1), Niacin (B_3), Pyridoxine (B_6) and Minerals (Salunkhe *et al.*, 1984). They also supply trace elements, starch, sugars and are important sources of dietary and crude fibres. Organoleptically, they have attractive flavour and aroma, crisp, texture, colours and appeal to human sense of smell, taste, touch and sight. They respire and transpire after harvest; loss of substrate and moisture are not replaced hence deterioration occurs. Fruits on the other hand can be defined as the matured fleshy seed-bearing part of a plant used for food. Fruits can become contaminated with microorganisms capable of causing human diseases while still on the plant in fields or orchards, or during harvesting, transport, processing, distribution and marketing, or in the home. Bacteria such as *Clostridium botulinum, Bacillus cereus* and *Listeria monocytogenes*, all capable of being transferred into juice during processing thereby causing illness, are normal inhabitants of many soils, whereas *Salmonella, Shigella, Escherichia coli* and *Campylobacter* reside in the intestinal tracts of animals, including humans, and are

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more likely to contaminate raw fruits through contact with faeces, sewage, untreated irrigation water or surface water (Cliver, 1997; Speer, 1997). The objectives of the study were to isolate microbial isolates from carbonated, and non-carbonated fruit juices, demonstrate the abilities of the isolates to cause spoilage of clarified friuit juices, produce pectinase enzymes for clarification of fruit juices, and also to confirm carbonation as a method for preserving fruit juice drinks.

MATERIALS AND METHOD

Pectinase Production

The pectinase enzyme production medium was assembled as follows; $ZnSo_4.7H_20$ (0.124g), $FeSo_4.7H_20$ (0.126g), $CuSO_4.5H_20$ (0.016g), distilled water 70ml, Corn pomace (50g), Soy bean powder (5g), Pectin powder (2g), pH adjustment to 4.5, Autoclave, tween 80, Muslin bag, Petri-dish and *Aspergillus niger.* The mixture was placed in a muslin cloth and sterilized by autoclave at 121^0C for 80 minutes. The sterile medium was allowed to cool to room temperature (25±3) ^{0}C under sterile condition. Sterile distilled water of 80 ml containing 4 drops of tween 80 was aseptically used to transfer the 5 days old *Aspergillus niger* (strain AA1) spores from Malt Extract Agar (MEA) culture into the production medium. The inoculum was mixed with the medium in a sterile aluminium tray, covered with aluminium foil and allowed to sporulate for 5 days at room temperature (25±3) ^{0}C under aseptic condition. After the sporulation period, the heavily sporulated culture was dissolved in 10L of sterile distilled water and allowed to stand for 30 minutes. The mixture was filtered with clean sterile muslin cloth. The filtrate (crude enzyme) was preserved with 15g of sodium benzoate and stored in a refrigerator prior to use.

Procedures for fruit juice production

Extraction of juice
All the fruits and materials are washed with sodium metabisulphite (1g in 1L of distilled water). Pineapple fruits were peeled. The juice of the edible part of each fruit was extracted (Binatone Juice Extractor).

Pasteurization of juice and enzyme digestion

The pulp was aseptically mixed with the juice and pasteurized at 60^0C for 1 hr. The pulps were allowed to cool to 45^0C, mixed with 1.5% sodium benzoate (preservative) before 10% of the spinned crude pectinase (pH 4.5) was added and mixed thoroughly. The treated pulps in the sterile muslin cloth were placed inside hydrolyser (FIIRO Fabricated Model) set at 45^0C, pH 5.5 for 24 to 48 hrs. The hydrolysed juices were aseptically collected into the beaker. These was pasteurized at 80^0C for 10 minutes, allowed to cool then chilled.

Carbonation and bottling

The chilled juices were aseptically filtered and gently dispensed into their various sterile bottles. Each bottled juice was carbonated with 0.5 bar CO_2 per 750 ml juice and cocked immediately. The bottled carbonated fruits juices were pasteurized at 80^0C for 15 minutes allowed to cool then chilled prior to use for the spoilage experiment

Fruit juice spoilage experimental design

Microorganisms were isolated from the followings; Clarified carbonated pasteurized pineapple fruit juice, Pasteurized mango fruit juice, Pasteurized pineapple juice Clarified pasteurized banana fruit juice, Pasteurized orange fruit juice. The microorganisms were characterised and identified using standard laboratory practices. The isolates; Bacteria, mould, and yeasts were preserved in Nutrient Agar, Potato Dextrose Agar slants respectively. These isolates were aseptically inoculated into freshly produced clarified carbonated Pineapple fruit juice. The inoculated juice bottles were re-corked and incubated for 168hours at 35^oC (Bacteria inoculation), and 28^oC (Mould and Yeast inoculation).

The juice inoculated with bacteria was different from those inoculated with mould and yeast. The microbiological and physico-chemical properties of the inoculated and incubated juices in bottles were studied thereafter incubation period.

Microbiological Analyses

The following samples were taken off the shelf after 12 - 18 months of production and standard procedure was followed for analysis; clarified carbonated pasteurized pineapple fruit juice, pasteurized mango fruit juice, pasteurized pineapple juice, clarified pasteurized banana fruit juice and pasteurized orange fruit juice from the Federal Institute of Industrial Research, Oshodi, Lagos State, Nigeria. Ten fold serial dilutions of the samples were carried out and inoculation on prepared media with appropriate dilution (1ml) of each juice samples was done in duplicates.

The culture plates were incubated at optimal growth conditions. Pure colonies were obtained and preserved on slant of Plate Count Agar or Nutrient Agar (general bacterial), Potato Dextrose Agar (fungal), DeMan Rogosa Sharpe Broth (lactic acid bacteria) and MacConkey Agar (Coliforms).

Table 1 Microbiological population of stored fruit juice samples

SAMPLE DESCRIPTION	MICROBIOLOGICAL COUNTS				
	Bacteria Count On Nutrient Agar	Mould Count On Potato Dextrose Agar.	Lactic Acid Bacteria Count On M.R.S.	Yeast Count On Malt Extract Agar.	Coliform Count On MacConkey Agar.
Clarified carbonated pineapple juice drink.	-	-	-	-	-
Pasteurized mango fruit juice.	9.6×10^{5}	4.0×10^{2}	7.2×10^{4}	1×10^{6}	-
Pasteurized pineapple juice drink.	4.0×10^{3}	3.0×10^{2}	2×10^{3}	3.6×10^{5}	-
Clarified pasteurized banana juice drink.	1.5×10^{4}	2×10^{2}	-	6.8×10^{2}	-
Pasteurized orange juice drink	1.7×10^{6}	2×10^{2}	3.0×10^{5}	3.0×10^{5}	-

Table 2 Characterization of bacteria isolated from carbonated and non-carbonated fruits juice samples

Sample Description	Microbial Tests for Bacteria			Probable Micro-organisms
	Gram	Cell Morphology	Catalase	
Clarified carbonated pasteurized pineapple Juice.	-	-	-	No microbial isolates after 12 month storage
Pasteurized mango juice.	+	Cocci	+	*Micrococus varians*
	+	Rod	+	*Bacillus megatarum*
	+	Cocci	+	*Staphylococus aureus*
	+	Rod	+	*Corynebacterium ovis*
	+	Rod	+	*Corynebacterium murium*
Pasteurized pineapple juice.	+	Rod	+	*Bacillus licheniformis*
	+	Rod	-	*Lactobacillus* spp.
	+	Rod	-	*Leuconostoc* spp.
	+	Cocci	+	*Micrococcus luteus*
Clarified pasteurized banana juice.	+	Rods	+	*Bacillus coagulans,*
	+	Rods	+	*Bacillus non-reactive*
Pasteurized Orange juice.	+	Rods	+	*Bacillus amyloliquefasciens*
	+	Rods	+	*Brevibacillus agri*

Biochemical Analyses for Identification

The following test were carried out for morphological and biochemical characterization; catalase, oxidase, gram staining, The Analytical Profile Index kit was used in identifying the isolates.

Physico-chemical Analyses

Physico-chemical parameters such as Turbidity, pH was carried out using Automated Proximate Analyser at the Step B Laboratory, Federal Institute of Industrial Research, Oshodi, Lagos.

RESULTS AND DISCUSSION

The tables below illustrated the results obtained from the analysis carried out on the juice extracts. Tables 1 and 2 showed physico-chemical properties and microbiological properties of pineapple and banana juice extracts.

Table 3 showed the sensory evaluation of banana and pineapple fruit juice extracts. Table 4 showed the population of mesophilic microorganisms on carbonated and non-carbonated fruit juices. Table 5 showed the microbial population of microorganisms isolated from the samples. Tables 6 and 7 showed characterization of bacteria and fungi respectively isolated from juice extracts. Tables 8 and 9 showed the results for bacteria

Table 3 Characterization of Fungi Isolated from Carbonated and Non-Carbonated Fruit Juice

Sample Description.	*Microbial Tests for Yeast and Moulds.*	*Probable Micro-organisms.*
	Cell Morphology	
Clarified carbonated pasteurized pineapple juice	-	*No fungi isolated*
Pasteurized mango juice.	Elongated	*Rhodotorula mucilaginosa*
	Oval	*Candida rugosa*
Pasteurized pineapple juice.	Oval	*Candida kruesei*
	Round	*Saccharomyces cerevisiae 1*
	Oval	*Candida glabrata.*
Clarified pasteurized banana juice.	Cylindrical	*Candida pelliculosa*
	Oval	*Candida boidinii*
	Abundant small conidia, ovoid arising in ball like groups on short right angled branches.	*Trichoderma reesii.*
	Conidial heads columnar, conidiophores smooth and short. Vesicles flat shaped phialides present, metulae absent. Conidia small, globose, rough.	*Aspergillus fumigatus*
	Penicilli diverticulate with small, smooth ellipsoidal. Cleistothecia are seldom produced.	*Talaromyces thermophilus.*
Pasteurized orange juice.	Conidial heads round or large. Conidiophores colourless to brown, smooth, vesicles present, foot cells and metulae are usually present.	*Aspergillus niger*
	Long conidiophores, coarsely roughened. Vesicles round, phialides and metulae present. Foot cells present,elliptical present.	*Aspergillus flavus*

Table 4 Biochemical Characterization on Spoilage Bacteria Isolated from Fruit Juice Samples

API.	Identified Spoilage Micro-organisms	Glucose	Fructose	Lactose	Mannose	Sucrose	Citrate utilization	Voges proskaeur	Gelatin hydrolysis	Ortho nitrophenol	Arginine dihydrolase	Lysine	Ornithine decarboxylase	H_2S production	Urease activity
20E/50CH	*Bacillus coagulans*	+	-	+	-	+	-`	+	+	+	+	-	-	-	-
	Bacillus amyloliquefasciens	+	+	+	+	+	-	+	+	+	+	-	+	-	+
	Bacillus licheniformis	+	+	-	+	-	+	+	+	+	+	-	-	-	-
	Bacillus non-reactive	-	-	-	-	-	+	+	+	+	+	+	+	-	+
	Bacillus megatarum	+	-	+	+	-	-	+	+	+	-	-	-	-	-
	Brevibacillus agri	-	-	-	+	-	+	+	+	-	+	+	+	+	+
	Corynebacterium ovis	+	+	-	+	-	+	+	-	+	+	+	+	-	+
	Corynebacterium murium	+	-	-	+	+	+	+	+	+	+	+	+	-	+
Staph	*Staphylococcus aureus*	+	+	+	+	+	NA	+	NA	NA	+	NA	NA	NA	+
	Micrococcus varians	+	+	-	-	+	NA	-	NA	NA	+	NA	NA	NA	-
	Micrococcus luteus	+	-	-	-	-	NA	+	NA	NA	-	NA	NA	NA	+

and fungi respectively isolated from juice extracts. Tables 10 showed effect of re-introduced spoilage isolates on freshly produced pineapple juice. The figures 1 and 2 showed analytical profile index kit for biochemical tests of isolates. *Bacillus* spp., *Corynebacterium* spp., *Staphylococcus* sp., *Micrococcus* spp., *Candida* spp., *Saccharomyces spp., and Aspergillus* spp. are amongst the microorganisms isolated from the samples and also used to determine their effect on freshly produced pineapple juice

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Table 5 Biochemical Characterization of Spoilage Yeast Isolated From Fruit Juice Samples

Isolates	**API 20C AUX.**																		
	Glucose	Glycerol	2KG	Arabinose	Xylose	Adonitol	Xylitol	Galactose	Inositol	Sorbitol	Methyl-αD-Glucopyranoside	N-Acetyl-Glucosamine	Celiobiose	Lactose	Maltose	Sucrose	Trehalose	Melezitose	Raffinose
Candida krusei	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
Candida glabrata	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
Candida pelliculosa	+	+	-	+	+	-	+	+	-	+	+	-	+	-	+	+	+	+	+
Candida boidinii	+	-	-	+	+	-	+	+	-	+	+	+	-	-	-	-	_	-	-
Saccharomyces cerevisiae 1	+	+	-	-	-	-	-	+	-	-	-	-	-	-	+	+	-	-	+
Candida rugosa	+	-	-	+	+	-	-	+	-	+	-	+	-	-	-	-	-	-	-

Table 6 Effect of Clarified Carbonated Pineapple Juice on Spoilage Microorganisms

Microbial isolate	Colour	Turbidity	Flocculation	Total Titratable Acidity	Total Dissolve H_2S	pH
Control	Orange	-	-	0.67	0.47	2.90
Micrococcus luteus	Whitish	++	-	0.33	0.05	2.90
Bacillus licheniformis	Whitish	++	+	0.31	0.11	2.88
Bacillus amyloliquefasciens	Whitish	+++	+	0.33	0.16	2.51
Staphylococcus aureus	Orange	+	-	0.46	0.32	2.87
Bacillus coagulans	Orange	-	-	0.56	0.16	2.93
Bacillus megatarium	whitish	++	-	0.59	0.11	2.88
Brevibacillus agri	whitish	+++	-	0.38	0.08	2.87
Bacillus non-reactive	whitish	+++	+++	0.61	0.06	2.87
Corynebacterium murium	whitish	++	+	0.44	0.11	2.91
Corynebacterium ovis	Orange	+	-	0.44	0.16	2.90
Talaromyces thermophilus	whitish	++	+	0.46	0.17	2.91
Rodotorula mucilaginosa	Orange	-	-	0.49	0.13	2.92
Aspergillus niger	Orange	-	-	0.44	0.11	2.90
Aspergillus flavus	Orange	-	-	0.44	0.12	2.89
Candida rugosa	Orange	-	-	0.58	0.11	2.90
Saccharomyces cerevisae	Orange	-	-	0.41	0.10	2.88
Aspergillus fumigates	whitish	+	+	0.38	0.48	2.90
Candida pelliculosa	Orange	-	-	0.42	0.17	2.89
Candida glabrata	whitish	++	-	0.55	0.13	2.91
Candida kruesei	Orange	-	-	0.59	0.10	2.92

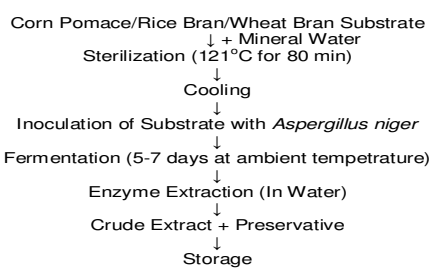

Figure 1 Flow Diagram for Pectinase Enzyme Production

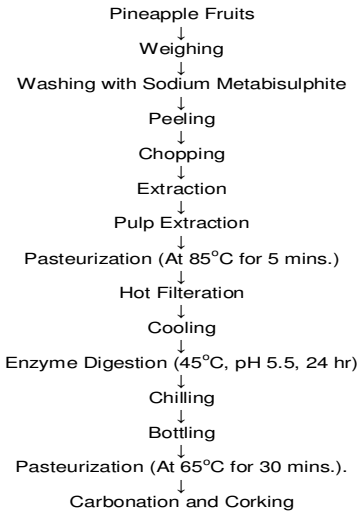

Figure 2 Flow chart for preparation of carbonated fruit juice

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