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Research Article



Ochratoxin Assay, Proximate and Mineral Composition of Commercially Packaged Fruit Juices Sold in Owerri, IMO State, Nigeria.

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ABSTRACT

The ochratoxin presence, proximate and mineral composition of 21 packaged single and multiple fruit juices sold in Owerri metropolis, Nigeria were assessed. The ochratoxin assay was carried out using an ochracard test kit. Results from the analysis showed that the moisture content of the juices ranged from 85.04 to 90.16%, ash content from 0.01 to 0.24%, the carbohydrate content from 9.80 to 14.4%, the pH values ranged from 2.95 to 3.77, the protein content from 0.32 to 1.48%, vitamin C from 4.90 to 36.00mg/100ml, the vitamin A from traces to 0.12 mg/100ml. Fibre and fat contents were in trace amounts. The mineral contents ranged from 65 to 234 mg/l for iron, 20 to 180 mg/l for calcium, 30 to 171 mg/l for sodium, 18 to 255 mg/l for potassium and 12 to 201 mg/l for magnesium. Nine (9) out of the 21 samples were positive for ochratoxin and the contamination rate was 42.9% for the fruit juices. The proximate and mineral content analyses were done using standard chemical methods. The analysis of variance (ANOVA) test was employed to test the equality of the different fruit juices in terms of different mineral and proximate contents assayed in the samples.

Keywords: Ochratoxin, Mineral Content, Proximate Composition, Analysis of Variance.

INTRODUCTION

Fruit juices are the unfermented but fermentable liquid obtained from fresh, ripe and healthy fruits. It can be produced using a single type of fruit or mixed fruits¹. The major component of fruit juice is water. The other constituent is carbohydrate which comprises sucrose, fructose, glucose and sorbitol. Juices are fat free, nutrient dense beverages rich in vitamins, minerals and naturally occurring polynutrients which offer health and therapeutic benefits. Fruit juices promote detoxification in the human body². The presence of mycotoxins in food is however often overlooked due to public and manufacturer ignorance about their existence, the absence of adequate regulatory mechanisms, dumping of below grade food products in developing countries and the introduction of contaminated commodities in the human food chain during chronic food shortages³. This study therefore evaluates the Ochratoxin presence, proximate and mineral composition of commercially packaged fruit juices sold in Owerri Metropolis.

MATERIALS AND METHODS

COLLECTION OF SAMPLES

The fruit juice samples used were collected from different retail shops, supermarkets, markets and road side retailers in Owerri Metropolis. The fruit juice samples were made up of 11 mixed and 10 single fruit juices respectively. The expiry dates and batch numbers were noted and the packs were ensured to be intact. Each

sample was put into a sterile nylon bag, properly labelled and taken to the laboratory for analysis.

Proximate Analyses

The parameters tested were pH, ash values, moisture content, fibre, fat, crude protein, vitamin C, vitamin A, iron, calcium, magnesium, potassium, carbohydrate and energy value. They were done according to methods in⁴⁻⁸. The determination of pH was done with a uniscope pH meter (Hanna instruments) previously standardized. The muffle method was used in ash determination. The moisture content was done using the oven method, crude fibre content by the weeden gravimetric method, soxhlet fat extraction method for fat determination, crude protein by the Kjeldahl method and total available carbohydrate using the colorimetry refractometry method. Vitamin C was determined using the visual titration method, Vitamin A by the spectrophotometric method and energy value by methods used by^{4,5}. The samples were analysed in duplicates and the values recorded as the mean.

Determination of Minerals

The minerals determined were iron, calcium, magnesium, potassium and sodium according to the methods in^{4,6,8}. Iron content was by thiocyanate method, calcium and magnesium content by ethylene diaminetetraacetic acid (disodium) EDTA, while the flame photometry method was used to determine the potassium and sodium content.



Ochratoxin Assay and Test Principle

Ochratoxin test in packaged fruit juice samples was carried out using an Ochracard test kit according to the manufacturer's instructions (R-Bio Pharm Rhone Ltd, Glasgow, Scotland). The Principle of the kit is based on monoclonal antibody that act against a single specific antigen and be produced by a single clone of B cells that could be maintained indefinitely in the laboratory which is highly specific and sensitive.

Sample Preparation

Fifty millilitre (50ml) was weighed into a 1litre capacity solvent resistant blender jar. Hundred millilitre (100ml) of 100% methanol was added and blended at high speed for 1minute. Hundred millilitre (100ml) of 1% sodium bicarbonate was also added and blended at high speed for 2 minutes. The sample was filtered through whatman NO. 113. Twelve millilitre (12ml) of filtrate was diluted with 12ml of PBS (Phosphate Buffered Saline). Ten millilitre (10ml) of the diluted filtrate was passed through the immuno affinity column. The diluted filtrate was then passed through the immuno affinity column at a flow rate of 2ml per minute. The column was washed by passing 5ml of PBS through it at a flow rate of approximately 5ml

per minute. Air was passed through the column to remove residual liquid. The toxin was eluted from the column at a rate of 1drop per second using 1ml of 100% methanol and collect the elute in a suitable collection vial. Back flushing was recommended. Two millilitre (2ml) of sample diluent buffer was passed through the column and collected in the same collection tube as the eluate to give a final volume of 3ml.

Test Procedure

The test kit was removed from the refrigerator and left for 30 minutes at room temperature. Five hundred microlitre (500µl) of cleaned sample was applied onto the port and allowed to pass through the membrane for 5 minutes. 100µl of ready to use conjugate (red label) was applied and allowed to pass through the membrane. Once passed through the membrane, 100µl of wash buffer (green label) was applied. The wash buffer was allowed to pass through the membrane and wiped around the port with a paper tissue. Hundred microlitre (100µl) substrate (blue label) was then applied to the membrane and allowed for the colour to develop for 5minutes. After 5minutes, 100µl of stop solution (yellow label) was applied and results read immediately.

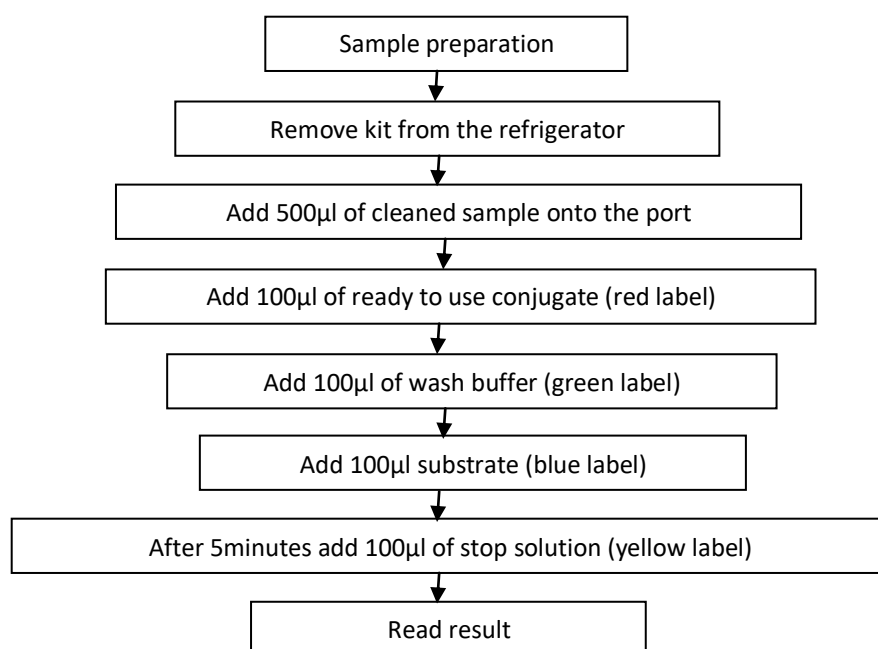


Figure 1: Flowchart for Ochratoxin assay in packaged fruit juices Source: [9]

DATA ANALYSIS

Testing For Equality of the Fruit Juices

This analysis dealt with testing for equality of the fruit juices with respect to the constituent attributes contained in them, their proximate and mineral contents. The ANOVA test technique was employed to accomplish the task. With respect to each of the constituent attributes contained in the juices, the test null and alternative

hypotheses were stated in the form: $H_0: \mu_{FACB} = \mu_{DO} = \dots = \mu_{FUO}$

$H_1: \mu_i \neq 0, \text{ for all } i, i = FACB, DO, \dots,$

FUO (this implies that at least one of the fruit juices was significantly different). The test rejects the null hypothesis in each case when the p-value is less than or equal to the α -value (which in this study has been chosen to be 5% $\sum \alpha = 0.05$).

RESULTS



Table 1: Mean Proximate Composition of the Fruit Juice Samples

Sample Code	Moisture Content (%)	Ash (%)	Carbohydrate (%)	pH	Protein (%)	Fibre (%)	Fat (%)	Vitamin C (mg/100ml)	Vitamin A (mg/100ml)	Energy Value (K Joule/gram)
SFF	87.07±0.03	0.12±0.00	14.40±0.04	2.95±0.03	1.27±0.01	Trace	Trace	10.00±0.28	Trace	251
SFC	89.07±0.01	0.24±0.00	12.50±0.04	3.67±0.01	1.36±0.01	"	"	18.33±0.01	0.02	223
SFD	89.21±0.03	0.19±0.00	12.40±0.71	3.77±0.03	1.14±0.03	"	"	30.00±0.14	0.03	217
SFE	88.65±0.01	0.09±0.00	12.90±0.03	3.00±0.01	1.28±0.01	"	"	7.40±0.06	Trace	228
SFA	90.16±0.03	0.16±0.01	10.60±0.01	3.68±0.01	1.48±0.01	"	"	5.60±0.04	Trace	194
SFB	88.89±0.01	0.06±0.00	12.20±0.01	3.00±0.03	1.40±0.04	"	"	33.50±0.03	Trace	219
SFG	88.07±0.04	0.13±0.00	10.20±0.07	3.70±0.01	1.34±0.06	"	"	5.30±0.01	Trace	185
SFH	89.01±0.01	0.11±0.00	9.80±0.01	3.40±0.03	1.10±0.03	"	"	4.90±0.04	0.02	175
SFI	85.04±0.01	0.12±0.00	13.20±0.01	3.10±0.00	1.23±0.03	"	"	8.00±0.03	Trace	232
MFF	89.37±0.01	0.13±0.00	10.40±0.01	3.61±0.01	1.04±0.03	"	"	26.67±0.01	Trace	184
MFE	88.66±0.01	0.154±0.00	12.40±0.03	3.39±0.01	1.13±0.03	"	"	20.10±0.09	Trace	217
MFB	85.72±0.03	0.16±0.00	13.40±0.01	3.52±0.01	0.97±0.01	"	"	16.00±0.28	Trace	230
MFA	88.26±0.03	0.01±0.00	12.50±0.01	3.67±0.01	0.98±0.01	"	"	28.40±0.03	Trace	216
MFG	88.06±0.01	0.08±0.00	12.80±0.01	2.98±0.01	1.25±0.01	"	"	18.00±0.06	Trace	226
MFI	86.71±0.01	0.24±0.00	13.50±0.00	3.45±0.03	0.87±0.01	"	"	15.00±0.07	Trace	230
MFJ	89.23±0.01	0.08±0.00	12.50±0.06	3.45±0.01	0.95±0.03	"	"	7.20±0.01	Trace	216
MFK	88.21±0.04	0.13±0.00	13.05±0.01	3.20±0.03	0.89±0.04	"	"	36.00±0.04	Trace	223
MFH	89.01±0.01	0.09±0.00	12.50±0.03	3.25±0.01	0.32±0.01	"	"	20.12±0.03	Trace	205
MFD	87.96±0.01	0.15±0.00	11.20±0.04	3.64±0.01	1.45±0.04	"	"	13.28±0.04	0.11	203
MFC	86.97±0.01	0.06±0.00	12.80±0.03	2.96±0.03	1.33±0.03	"	"	5.20±0.01	0.02	227
SFJ	89.36±0.01	0.124±0.00	10.40±0.01	3.61±0.0141	1.04±0.03	"	"	26.67±0.01	0.12	184

Results of the proximate analysis and mineral content of the fruit juices are recorded in tables 1 and 2. It showed that the moisture content was within the range of 85.04% to 90.16%. The samples recorded ash content in the range of 0.01% to 0.24%. Sample MFI had the highest value of 0.24%, while sample MFA had the lowest value of 0.01% (Table 1). The carbohydrate content was within the range 9.8% to 14.40%. Sample SFF had the highest value of 14.40%, while sample SFH had the lowest value (Table 1). The Table also showed that the pH values of the fruit

juices ranged between 2.95 to 3.77. Sample SFD had the highest pH value of 3.77, while sample SFF had the lowest pH value of 2.95. The protein content range from 0.32% to 1.48%. Sample SFA had the highest value of 1.48%, while sample MFH had the lowest value of 0.32%. Fifteen (15) fruit juices samples had traces of vitamin A while the remaining six (6) had values ranging from 0.02mg/100ml to 0.12mg/100ml. Sample SFJ had the highest value of 0.12mg/100ml while SFC, SFH, and MFC had the lowest values of 0.02mg/100ml.

Table 2: Mineral Content of Fruit Juice Samples

Sample Code	Fe (mg/l)	Ca (mg/l)	Na (mg/l)	K (mg/l)	Mg (mg/l)
MFA	94	25	165	180	69
MFB	166	120	30	60	18
MFC	69	83	122	95	57
MFD	150	159	124	220	35
MFE	136	160	120	40	30
MFF	141	35	84	70	26
MFG	79	20	30	25	14
MFH	75	89	150	90	45
MFI	160	125	50	55	20
MFJ	80	30	40	25	15
MFK	65	55	35	80	15
SFA	176	180	170	255	200
SFB	71	58	32	86	18
SFC	234	170	140	100	40
SFD	201	50	134	86	28
SFE	87	24	32	18	12
SFF	140	75	50	50	25
SFG	169	175	160	200	190
SFH	172	179	171	230	201
SFI	150	70	40	45	23
SFJ	140	35	84	70	26

KEYS:

Fe = Iron

Ca = Calcium

Na = Sodium

K = Potassium

Mg = Magnesium

Table 2 showed the mineral contents of the fruit juice samples. It shows that the iron content ranged from 65mg/l to 234mg/l. Calcium content in the fruit juices ranged from 20mg/l to 180mg/l, Sodium content from 30mg/l to 171mg/l. SFH recorded the highest value of 171mg/l, while sample MFB and sample MFG had the lowest value of 30 mg/l. Potassium in the fruit juices ranged from 18mg/l to 255mg/l. Sample SFA had the highest value of 255mg/l, while sample SFE had the lowest value of 18mg/l. Magnesium in the fruit juices ranged from 12mg/l to 201mg/l. Sample SFH had the

highest value of 201mg/l, while sample SFE had the lowest value of 12mg/l (Table 2).

Ochratoxin analysis in the different fruit juices is presented in Table 3. The results obtained showed Ochratoxin presence in only nine (9) samples (5 multiple and 4 single fruit juices) while Ochratoxin was not detected in 12 fruit juices samples (6 multiple and 6 single fruit juices). This translated to 9(42.9%) for Ochratoxin presence for single and multiple fruit juices, and 12(57.1%) for Ochratoxin absence in single and multiple fruit juices.



Table 3: Percentage Occurrence of Ochratoxin in Multiple and Single Fruit Juices

Sample Code	Ochratoxin
MFA	+
MFB	-
MFC	-
MFD	+
MFE	+
MFF	-
MFG	+
MFH	+
MFI	-
MFJ	-
MFK	-
SFA	+
SFB	-
SFC	-
SFD	-
SFE	-
SFF	+
SFG	-
SFH	+
SFI	-
SFJ	+
Presence percentage	9 (42.9%)

KEYS:

MF = Multiple Fruit Juice

SF = Single Fruit Juice

+ = Presence of Ochratoxin

- = Absence of Ochratoxin

DISCUSSION

Fruit juices are well recognized for their nutritive value, mineral and vitamin content. They are beverages that are consumed for their thirst quenching properties, stimulating effect and medicinal values. The proximate and mineral contents investigated include moisture content, Ash values, carbohydrate, pH, protein, fibre, fat, vitamin A, iron, calcium, sodium, potassium and magnesium. The fruit juice samples investigated showed high moisture content that ranged from 85.04% to 90.16%¹⁰ in their work on packaged fruit juices sold in Onitsha, Nigeria reported high moisture content in the range of 92.38% to 82.14%¹¹ in their work on industrially processed fruit juices reported moisture content in the range of 89.98% to 85.80%. High moisture encourages growth of most bacteria and fungi¹² stated that fruit juices are made up of high moisture content and these has been found to promote growth of yeast and bacteria. The pH of the samples varied from 2.95 to 3.77. Several authors have reported a low pH in packaged fruit juices¹³

reported a low pH in their work on microbiology of commercially packaged fruit juices sold in Nigeria in the range of 3.20 to 4.50. Similar reports were given by¹¹ and¹⁴ on packaged fruit juices. Fruit juices have a low pH because they are comparatively rich in organic acid¹⁵ and limit the number and the type of bacteria that can survive or grow at this low pH. The low acidic pH of the fruit juices therefore greatly controls the growth of these microorganisms within the juices¹⁶.¹⁷ reported that growth rate of micro-organisms reduced in acidic medium but in basic medium the growth rate was increased. All the samples analysed contained different levels of vitamin C with the highest being 36.00mg/100ml and the lowest being 4.90mg/100ml. This result corroborates the works of¹³,¹⁸,¹⁹ who all reported the presence of ascorbic acid in packaged fruit juices. Many processors add ascorbic acid to their products to make up for processing losses²⁰. This could be the cause for the higher content of ascorbic acid in sample MFK. The protein values in the fruit juices were low and from 0.32% to 1.48%. This result agreed with²¹ who reported the presence of protein in the range



of 0.39% to 1.25% in their studies on proximate and mineral composition of packaged fruit juices sold in Nigeria. ¹¹ also reported the presence of protein in packaged fruit juices sold Bangladesh in the range of 0.001% to 0.66%. A considerable proportion of the protein content of fruits is insoluble and consequently remains in the pomace; therefore most fruit juices are very low in protein. ^{11, 22} reported that protein is generally less than 1 percent in fruits. The potassium content of the fruit juice samples analysed ranged from 18mg/l to 255mg/l which was the most abundant element in all the fruit juice samples analyzed. This was in agreement with the work of ²³ who reported that potassium occurred more than the other element found in the packaged fruit juice samples sold in Nigeria. The higher content of potassium that were detected in fruit juices may be due to that added during the production of this products. Many potassium compounds are added as sweeteners (acesulfame k) or preservatives (potassium bisulfite-E228, E212-potassium benzoate) which lead to an increase in the content of this macro- element in fruit juices over the baseline derived from the fruit ²⁴. The result of the iron content in the fruit juice samples analysed ranged from 65mg/l to 234mg/l. ²⁵ also reported high levels of iron in their study on levels of major and minor elements in commercial fruit juices available in Sabia. The Ochratoxin assay showed that nine (9) out of the 21 fruit juice samples analysed showed the presence of Ochratoxin. The fruit juices that showed presence of Ochratoxin were made of fruits of apple, grape, orange, pineapple, coconut, lemon, mandarin, lime, cherry, pear, black currant, guava, mango, passion, apricot and banana. Some of the fruit juice were made of only one type of fruit while others were made up of combination of fruits. ²⁶ has reported the occurrence of Ochratoxin A in different juices from Switzerland, Germany, Morocco and Brazil. They concluded that the most contaminated sample were grape juices. Similar results were previously reported by ²⁷ for grape juices from Spain and Germany. ²⁸ in their study of grape juices from Brazil, Chile and Argentina reported that Ochratoxin A was detected in 29% and 12.5% of the grape juice and pulp of frozen grape samples. The maximum level for Ochratoxin A for fruit juices set by the European Union is 2µg/kg. The screening level for the test was done at 5ppb (parts per billion). The maximum level set by the food and Agriculture organization is 5µg/kg (5.00 parts per billion). The presence of *Penicillium* species in packaged fruit juice samples examined in the present study could result in the production of toxic substances (mycotoxins), which could lead to health hazards ²⁹. The isolation of these organisms gives serious cause for concern because *Aspergillus* species is specifically known to produce mycotoxins ³⁰. The presence of Ochratoxin A in some of the fruit juice samples in this study calls for concern.

CONCLUSION

This work has shown that the locally available fruit juices contain safe levels of nutrients and minerals for human

consumption. The presence of Ochratoxin A in some packaged fruit juices sold in Nigeria calls for attention to carry out the test for presence of Ochratoxin A and other mycotoxins like patulin in fruit juice products consumed in Nigeria by the regulatory agencies e.g National Agency for Food, Drug and Administration and Control (NAFDAC) and the Standard Organization of Nigeria (SON) before they are certified for human consumption. Efforts should be made to improve public enlightenment of the nutritional and health benefits of adequate daily consumption of fruits and fruit juices. Mandatory daily fruit juice consumption programme is advised to be introduced in our primary and secondary schools. Government should encourage the establishment of small scale/collage fruit juice processing plants in the country. NAFDAC and SON should intensity their regulatory functions on fruit juice manufacturers to make sure they adhere to the required standard in fruit juice manufacturing. There should be more research in the area of mycotoxins in fruit juices and different foods in general. Government should also encourage research in these areas

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