European Journal of Nutrition & Food Safety

12(11): 104-113, 2020; Article no.EJNFS.63582 ISSN: 2347-5641

Heavy Metal (Pb, Cd, Hg and As) Content of Some Pineapples Juice Produced in Informal Market in Abidjan, Côte d'Ivoire

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Authors' contributions

This work was carried out in collaboration among all authors. Author YAAAD designed the study, author SS performed the analysis and author YAAAD wrote the first draft of the manuscript. Authors YAAAD, LG, SA and HGB supervised the study and analysed the data. All the authors managed the literature search writing of the final manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJNFS/2020/v12i1130326 *Editor(s):* (1) Dr. Adetunji Charles Oluwaseun , Edo University, Nigeria. *Reviewers:* (1) Benti Ofga Jaleta, Ethiopian Biodiversity Institute, Ethiopia. (2) Khalisanni Khalid, Malaysian Agricultural Research and Development Institute (MARDI), Malaysia. Complete Peer review History: http://www.sdiarticle4.com/review-history/63582

Original Research Article

Received 27 September 2020 Accepted 02 December 2020 Published 19 December 2020

ABSTRACT

Aims: The objective of this study was to determine the level of micropolluants contamination (mercury, lead, cadmium, arsenic) handcrafted pineapple juices, packaged in recovery bottles and sold on the outskirts of the city of Abidjan.

Study Design: Samples were collected from street vendors in four districts of Abidjan city (Marcory, Treichville, Cocody and Koumassi).

Place and Duration of Study: The study was conducted at the Central Food Hygiene Laboratory and Agribusiness between December 2019 and June 2020.

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Methodology: 32 samples collected were analyzed by atomic absorption spectrophotometer.

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Results: It appears that traces of the few metallic micropollutters were found in pineapple juices at varying rates with sometimes exceeding the maximum values recommended for lead in 75% of the pineapple juices analyzed. Considering the municipalities, 100% of the juices collected from vendors in the commune of Cocody have arsenic concentrations exceeding the maximum regulatory values. Overall, Daily Exposure Doses (DDE) (0.024 10⁻⁴ mg/kg bw of mercury, 0.345 $10⁴$ mg/kg bw of lead, 0.001 10⁻³ mg/kg pc of cadmium and 0.5 10⁻⁴ mg/kg bw of arsenic) are lower than the Previsional Tolerable Dose (PTD).

Conclusion: Therefore, can the risk be eliminated for the general population? While the concentrations of metallic micropolluants found in pineapple juices remain below the thresholds for mercury and cadmium, those of lead and arsenic are high, increasing the risk of adverse effects.

Keywords: Pineapple juice; cadmium; lead; mercury; arsenic; exposure risks.

1. INTRODUCTION

Fruits play a vital role in human health and wellbeing, especially in their ability to prevent essential nutrients deficiencies, but above all recommended to reduce the risk of several diseases [1]. In Côte d'Ivoire, as in developing countries, in order to meet the dietary requirements (healthy, balanced and diverse food) of a growing population, the marketing of fresh fruit or processed by artisanal methods is increasingly developing [2,3]. In Abidjan, pineapple (*Ananas comosus*) is sold in fresh markets or processed into juice. According to FIRCA [4], Côte d'Ivoire is the largest pineapple exporter of the African continent. Exports account for 14% of world production (25 million tons, in 2018). Unfortunately, the issue of hygienic quality is one of the factors limiting the market penetration of fruits and products from their transformation at the western level [5]. Their high-water content combined with the 4^{fh} range technique makes all these products vulnerable to microbiological and physical-chemical agents [6].
The availability, proximity and relatively The availability, proximity and relatively affordable price of these foodstuffs attract all categories of people to Abidjan. Consumers are often more interested in the convenience of these products than in safety and hygiene issues [7]. The system of transfer of metals from soils to plants is a way that could explain the presence of metal elements of soils in leaves, stems and fruits and the risk of contamination of the food chain [8]. In 1980, some reported cases of respiratory disease in women aged 35 to 75, living around copper smelters, as well as contamination of surrounding fruits and vegetables [9]. Indeed, the level of food contamination by trace elements caused by the state to both natural and anthropogenic sources. The sources of Micropolluant Trace Elements (MTEs) affect also their habitats [10]. The consumption of contaminated organs would

cause serious health toxicity problems. Lead (Pb), cadmium (Cd), arsenic (As) and mercury (Hg) are considered as strict and toxic contaminants to living things [11,12,13,14].

Heavy metals are metals that are shown to be harmful and toxic to human and animal body [14]. Those metals are a major public health problem related to micropolluant elements belonging to the class of health hazards through human and animal feed such as mycotoxins and microorganisms, pesticides and Polycyclic Aromatic Hydrocarbons (PAHs) [15]. Faced to this situation, and considering the protection of consumers, the European Commission [16] introduced a regulation setting the maximum limits for toxic microelements residues for fruits of 0.5 mg/kg for mercury, 0.2 mg/kg for arsenic and 0.05 mg/kg for cadmium and lead heavy metals that are a major public health problem related to micropolluant elements belonging to the class of health hazards through human and animal feed [17]. Systematic food control should ensure their safety. The aim of this study is to discover the level of contamination of pineapple juices by artisanal processes in Abidjan, Côte d'Ivoire, by metal micropollutors. Estimating the level of contamination by metal micropolluants contained in juices makes it possible to characterize pineapple juices produced near roads. Pineapple juice is, according to a study conducted in Côte d'Ivoire in 2019, the main pineapple-based product, destined for the local market in Côte d'Ivoire [18].

2. MATERIALS AND METHODS

2.1 Sampling and Sample Collection

Sampling was achieved in accordance with the Regulation N° 333/2007of the European Commission [19]. The organic material consisted of pineapple fruit juices. The sampling took place in four (4) municipalities in the city of Abidjan: Marcory, Treichville, Cocody and Koumassi (Fig. 1). The choice of municipalities was made according to the following criteria: accessibility, population density, their proximity to markets, the availability of raw materials. In each municipality selected, the samples consisted of buying from each seller selected for the study, a sample consisting of pineapple juice (Ananas comosus) packaged in plastic bottles of 1 liter. The samples were stored in insulated containers containing refrigerant packs and sent to the Central Laboratory for Food Hygiene and Agribusiness (LCHAI) to assess their level of contamination with toxic metal trace elements. The collection of samples took place over two months: July and August 2019, at a rate of four (4) samples per week per commune. A total of thirty-two (32) pineapple juice samples were collected for this study.

2.2 Metallic Polluant Toxic Analysis

2.2.1 Apparatus and conditions of quantification of heavy metals

An Atomic Absorption Spectrophotometer (AAS, type VARIAN SPECTRAA 110) with GTA 110 furnace was used for the determination of cadmium, arsenic and lead. Regarding the

determination of mercury, the AAS was equipped with a VGA77 vaporization unit in the presence of a solution of 10% tin-II chloride previously prepared with 37% fuming hydrochloric acid. Nitrogen was used as a vector gas for the analysis. The operating conditions of the AAS device are shown in Table 1. The different determinations were made from calibration rights established in five (5) points for each metal to be studied. The tests were taken in triples. For each set of analyses, a digestion white and a known concentration reference sample were analyzed to ensure quality control of the results. The operating conditions of the atomic absorption spectrophotometer are presented in Table 1.

2.2.2 Validation of analytical method of determining metal micropolluants

The analyses were carried out according to validated operating procedures at the Central Laboratory for Food Hygiene and Agri-Industry at LANADA. Table 2 presents the results of validation of the method of determining micropolluants (cadmium, lead, arsenic, mercury) in a plant matrix [20]. The method of validation was conducted by using the method of the French Association for Standardization (NFV03- 110/1998).

Fig. 1. Places of collection of pineapple juice samples

	Cadmium	Lead	Mercury	Arsenic
Current intensity (mA)				
Width of the slot (mm)	0.5		0.5	0,5
Wavelength (nm)	228.8	217	253.7	193.7
Coefficient of variation (%)				
Integration time (seconds)		G		
Number of repetitions				

Table 1. Operating conditions of the atomic absorption spectrophotometer

2.2.3 Method of metal micropolluants mineralization

An Aliquot of 0.5 g homogenate of each sample was heat mineralized with 7 ml of concentrated nitric acid (65%) and 1 ml of hydrogen peroxide (35%) using a microdigest for 20 min [21]. The mineralizate was reduced with a 10% chloride tin II solution previously prepared with 37% steaming hydrochloric acid for the mercury [22]. The resulted mineralised was then diluted in high quality ultrapure water and investigated with Atomic Absorption Spectrophotometer.

2.2.4 Estimated intake of metal micropolluants from the consumption of pineapple juice

The intake of metal micropolluants was estimated from tropical fruit juice consumption data in Côte d'Ivoire described by the Trade and Regional Integration Support Program (PACIR, 2013). The annual consumption of fresh pineapple fruit juice per head is lower than that of many producing countries, it is barely one (1) kg, or 2.74 g per day. The daily mode of consumption of an adult, sub-Saharan African was considered and the intake was calculated according Equation 1:

$$
ADI=CxQ \qquad (1)
$$

With, ADI: Average daily intake of TTE (mg/kg/d). C: Concentration for each of the metal elements found in pineapple juices (mg/kg).

Q: Daily amount of pineapple juice consumed per person in Côte d'Ivoire.

2.2.5 Characterization of risk associated with exposure of metal micropolluants

The daily dose of exposure (DDE) is the dose of substance received by the body related to the weight of the individual and the number of days of the whole life (Anonyme 2). The DDE for the substances ingested is obtained by Equation 2:

DDE
$$
(mg.kg^{-1}.w^{-1}.d^{-1}) = \frac{C_m \times Q_i}{P} \times \frac{T \times F}{T_m}
$$
 (2)

With, DDE: Daily Dose of Exposure; Cm: Average concentration of TTE in juice (mg/kg); Qi: Amount of food consumed by an individual per day (kg), P: Average body mass of target /kg (60kg) (CODEX ALIMENTARIUS, 2014), T:Exposure time (year), F:frequency of exposure (days/year) and Tm: Average exposure time period (days).

With TTE non-carcinogenic substance (mercury, lead, cadmium, arsenic), the Hazard Ratio (HR) is the ratio between the dose weekly exposure (DWE) by ingestion of the substance to the Previsionnal Tolerable Weekly Dose (PTWD). The HR is obtained by the following formula:

$$
HR = \frac{DWE}{PTWE}
$$
 (3)

This ratio allowed us to conclude only on the potential appearance of adverse effects and not on their importance. The Previsional Tolerable Weekly Dose are those described by OMS, N°505 /1972 et CODEX STAN 193-1995. PTWD represents the maximum amount of substance that an individual can ingest daily/weekly for a lifetime without incurring a risk to his health (Table 3).

Indeed if:

HR < 1 means that the exposed population is theoretically safe, i.e. this exposed population is not likely to develop the health effects studied.

HR > 1 means that the toxic effect can be declared without being possible to predict the probability of this event occurring.

2.2.6 Data analysis

The results were used based on averages, standard deviations and variances. The statistical tests and graphs were done using a computer software of statistical analysis called grapPhad.Prism.V5.01. The data was analyzed with ANOVA One Way and the non-parametric TUKEY test was used to compare the variance of values between them. The difference between two variances was significant, at p < 0.05.

	Cadmium	Lead	Mercury	Arsenic
Coefficient of determination (R^2)	0.997	0.998	0.998	0.998
Limit of detection (LD) (μ g/l)	0.03	1.85	2.92	0.05
Limit of quantification (LQ) (μ g/l)	0.07	6.52	3.32	0,166
Repetability (CV%)	3.32	2.11	2.74	3.56
Reproductibility (CV%)	3.98	3.28	3.33	4.82
Recovery rate (%)	102.78±3.7	104.31 ± 4.3	97.72 ± 5.1	95.81 ± 2.6

Table 2. Results of validation of the method of determination of micropolluants in a plant matrix

Table 3. Previsionnal tolerable weekly dose (PTWD) for non-carcinogenic effects

Metal	Maximum limit value (mg/kg) (RgIt /UE)	PTWD (mg/kg b.w.W)
Mercury	0.5	0.005
Lead	0.05	0.025
Cadmium	0.05	0.007
Arsenic	0.2	0.015

3. RESULTS AND DISCUSSION

3.1 Determination of Micropollutant's Levels in Pineapple Juices

The average concentrations of Toxic Traces Elements (TTE) in the juices according to the municipalities are reported in Table 4. The reported levels for mercury and cadmium, for all samples, are in line with the maximum values defined by requlations. There is neverless, defined by regulations. compared to the average cadmium concentration found in juices from the town of Marcory, a significant difference ($p = 0.0001$) with the levels of concentrations found in juices from pineapples from Cocody, Koumassi, Treicheville. The mercury contents in the juices regardless of the municipalities selected are all below the regulatory criteria (0.0075 mg/kg). At the level of the commune of Cocody, Koumassi, Treichville, the cadmium (0.005±0.003 mg/kg) and arsenic (0.157±0.093 mg/kg) contents reported are lower than the average contents found in the juices coming from the juices coming from the commune of Marcory. For these two metals, there is a very significant difference between the values of the three communes and those coming from Marcory. Overall, in the samples analyzed, there is a significant difference between the average content reported and the regulatory criteria. The data show that the average level of lead contamination of pineapple juices is above the maximum limits set by EU regulation N°420/2011 of the Commission of 29 April 2011 amending the regulation (EC) N°1881/2006 setting maximum levels for certain contaminants in foodstuffs (0.05 mg/kg) and concern 75% of pineapple juices analyzed.

Analysis of the various pineapple juice samples revealed the presence of trace metal elements: mercury, lead, cadmium, arsenic. The average concentrations reported vary depending on the municipality
and the metal measured. The highest and the metal measured. average concentration is lead followed immediately by arsenic. And any biological, physical or chemical agent that may have a "harmful effect on health" is a danger [23]. In science life/domain, their concentration relative to the body's dry matter is below 0.01% and has an essential function for the health of humans and all living organisms [24]. In pineapple juices, the reported concentrations of toxic trace elements (TTE) are less than 0.01%. However, the results of their work have shown that plants capture TTE in their environment but very little information about their metabolisms is available to date. These (TTE), despite their low concentration in juices are considered non-essential. These plants then contribute to the release of potentially dangerous foreign substances into a living organism through consumption through the products of their transformations. With regulatory standards and values, data on maximum levels for certain contaminants in foodstuffs are available. To assess the level of contamination of a plant organ, the simplest approach is to compare the results of the analysis of a plant sample with the maximum values defined by the different texts [25]. In the analyzed pineapple juices, the concentrations found for lead are at least twenty times higher than the maximum value defined by the regulation.

Table 4. The metal concentration (mg/kg) of pineapple juices in the present study

*The significance levels are expressed by: *= p< 0.05; ** = p< 0.001; *** = p< 0.0001*

In 2017, Mohamed stated in this work that the development of a living organism is largely governed by the substances it draws from its environment. However, the increase in human activities with industrialization has disrupted these balances by massive releases of contaminants, such as organic compounds and metal trace elements. The first origin of the presence of TTE in a soil is natural: alteration of the parent rock. Secondarily, TTE can also be brought by wind or rain in the form of dust or aerosols, by runoff with concentrations of up to 50 g/kg [26]. These amounts vary depending on the type of metal and result in varying concentrations in plants described as sensitive. So, the metal trace element content in the fruit is highly dependent on soil quality [27]. Also, differences in concentration can be explained by the stage of fruit development compared to fruit picking and lead accumulates more in plants [28].

A research office, on the quality of pineapple juice, reminds that the pineapple production system has a real impact on the quality of the juice produced. In Benin, pineapple is grown on degraded land where land and demographic pressures are high with a production system heavily dependent on chemical fertilizers and processing products [29]. This office also describes practices that can bring TTE to soils such as copper salts, lead arsenate, calcium amendments, sludge and compost, atmospheric fallout, mineral fertilizers and animal droppings. The high levels of lead and arsenic in some of the juices tested may explain the agricultural practices applied to them. The processing of fruit in Côte d'Ivoire remains confidential at present. Indeed, the players of artisanal processing in Abidjan source from the pineapple production areas that are the east of the Comoé River mainly (Bonoua, Adiaké and Aboisso) and the localities of Dabou, Tiassalé, Azaguié, Agboville. Processed pineapple products are mainly destined for the domestic market because in the face of the international market, the exacting standards of quality and food safety required are met [18].

3.2 Evaluation of Exposure to Metal Micropolluants by Consuming Pineapple Juice

Table 5 presents the health risk to the consumer
of prineapple iuice containing metal of pineapple juice containing metal micropolluants through the daily exposure dose (DDE) and the hazard quotient at the rate of

previsional tolerable dose per day. The contact time of the juice contaminated by TTE with the consumer's body by taking food is four (4) hours per day. Daily intakes (DI) were estimated for a 60 kg adult male consuming 2.74 g of pineapple juice for the different of metal micropolluants found in the juice. The results of laboratory showed that the level of metal micropolluants intakes changes with the concentration of metal micropolluants in the matrix while the amount absorbed by the consumer is constant. In fact, these daily intakes range from 14.10-5 mg/kg for cadmium to 1.10-5 mg/kg for lead. The concentration is above the maximum regulatory concentration for lead (1.101 mg/kg). Weekly Exposure consumption of pineapple juice range from 1.10- 5 mg/kg for cadmium to 3.10-3 mg/kg for arsenic. The risk of adverse effects on the lead exposed consumer through the consumption of pineapple juice is the likelihood of adverse effects. In our study, the risk of consuming pineapple juice contaminated primarily with arsenic (0.2), lead (0.048), cadmium (0.0014) and mercury (0.004). The ratio is less than one. Nevertheless, the daily exposure dose of each contaminating metal is lower than the previsional tarelable weekly dose (PTWD) regardless of daily intake (Fig. 2). The risk associated with arsenic from the consumption of pineapple juice represents 79% of the overall risk.

Globally, FAO apparent consumption data from the WHO program shows an average fruit consumption of 95 g/d for Africans [30]. In Côte d'Ivoire, the average consumption per person reported by PACIR is very low. The average TTE intake provided by individual consumption of 2.74 g per day is lower than the EDI. However, the overall analysis of pineapple juices shows that, in order of importance, the risk associated with lead and arsenic, contribute to 86% and 13% respectively of the probability of adverse effects in the consumer. As for the Hg and the Cd, the percentage is very low (less than 1%).

In 2004, research showed that with more than 50% daily intake of a ETT per food, the later posing a risk for consumption [24]. However, arsenic, with an average concentration in pineapple juices below the maximum value, represents 62% of the risk. The source of contamination is probably the transfer of pollutants to plants through soil [8], agricultural practices or the atmosphere is more concentrated in juices at any sample site. Indeed, the work on the evaluation of the

Fig. 2. Importance of each metal studied to become a hazard in human being

population's exposure to metals through the consumption of animal products [28] and those on the validation of the method of determining heavy metals in plant products consumed in Côte d'Ivoire [19] recorded high lead concentrations in both plant and animal dies. In general, plant products (fruits and vegetables) accumulate lead and the contribution by plants of metal trace elements is more important with plants than with animal products. However, weekly exposure doses are all below the PTWD. Whatever the metal, the hazard quotients are all less than 1, reflecting a low risk. Would the low level of fruit consumption and production in our study area explain the nature of the consumer's risk of exposure? However, it is desirable that the calculation of the bioconcentration factor be made to better describe the transfer of metal micropollutant's from the biotope to organisms.

4. CONCLUSION

The study found that hand-crafted pineapple juices contain trace metal elements: mercury,

publiation's exposure to metals through the lead, cadmium and arsenic. The research was a
population's exposure to method of determining The concentrations of these metal trace
avey metals in plant products (28) and those done by atomic absorption spectrophotometry. The concentrations of these metal trace elements in the juices vary depending on the metal and the municipalities. Lead has been reported in pineapple juices beyond the maximum levels defined by the regulation. Juices are essential for the well-being of consumers, but the presence of trace metal elements are dangers that cause consumers to be exposed to the risks associated with their consumption. Even if the risk is low, the accumulation of elements in a living organism can cause adverse effects on the health of the consumer following long-term ingestion. To better assess the level of soil and plant transfer, it would be essential to assess concentrations at soil level and then at the level of the fruit. Thus, to declare that a fruit juice is of quality, having good processing technology is not enough. cadmium and arsenic. The research was
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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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> *Peer-review history: The peer review history for this paper can be accessed here: http://www.sdiarticle4.com/review-history/63582*