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Evaluation and health risk assessment of phthalates in Okpa (cow pea pudding) packaged and cooked with polyethene bags in Nsukka, Enugu state, South-East Nigeria

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Abstract
Environmental toxicants enter the body via ingestion, inhalation or dermal absorption. Food is one of the major ways by which these toxicants get into the body. Food packaging has evolved in so many ways that materials made with plastics and its additives (Phthalates) are now used. Phthalates are compounds used to make plastics to enhance its functionality. Some have been associated with some health hazards such as endocrine dysfunction, reproductive problems, skin irritations and cancer. The current study was performed to evaluate the risk associated with consuming phthalates in okpa (cow pea pudding). The phthalate quantification and health risks were evaluated using gas chromatography mass-spectrometry (GC–MS) and models adopted by environmental protection agency (EPA) respectively. The Phthalate identified in okpa were diethyl phthalate (DEP), di-n-Butyl phthalate (DBP), benzylbutyl phthalate (BBP), di-iso-butyl phthalate (DiBP), and di (2-ethylhexyl)phthalate DEHP. The total concentration of phthalate detected was 0.0653 mg/kg with DEP as the highest (0.0196 ± 0.000 mg/kg) and BBP as the least (0.0077 ± 0.001 mg.kg). The highest THQ evaluated was DiBP for both adults (2.84E−1) and children (1.42E−1). The THI for adults and children was 3.35E−1 and 3.01E−1 respectively. The THQ and THI values obtained for adults and children were all less than 1 implying that it is safe. The carcinogenic risk (CR) evaluated for adults and children were 7.23E−6 and 3.61E−5. These values obtained for the THQ, THI and CR all together were within the safe limits stipulated by USEPA, FAO and WHO. However, it is still pertinent to continuously monitor the level of phthalates that may migrate into okpa because red oil content variations may positively impact on its leaching ability.

Keywords Phthalates · Risk assessment · Okpa · Food · Polyethene materials and environmental toxicants

1 Introduction

Environmental toxicants have grown since industrialization and population boom. They are toxic agents people come in contact with in their daily activities. These environmental toxicants range from heavy metals, exhaust particles, particulate matter polychlorinated biphenyls (PCBs) and phthalates. These toxicants can be grouped as allergens, neurotoxins, carcinogens, mutagenes, teratogens and hormonal diruptors. They have been detected in a plethora of products and can enter the body via inhalation, ingestion (food and drink consumption) and dermal absorption (Skin contact).

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Food packaging has been in existence since early civilization prior to the current forms of packaging. They have been in form of skin hides and plant materials [1].

Some of these old forms still co-exist with the new form of packaging in Nigeria. This can be seen in the packaging of a food delicacy called Okpa with dry banana leaves or plantain leaves in the South Eastern part of Nigeria and the packaging of Ofada rice also with plant materials usually in the South Western part of Nigeria [2]. In recent times, packaging have evolved to the use of materials containing polyethylene and plasticizers. This can be attributed to the exponential population growth, the increased rate of road side food consumption and to make these food readily available for consumption [1, 3]. The use of polyethylene to package both raw and readymade foods has continuously increased in this part of the world for ease. However, it has generated a lot of health questions and concerns regarding food safety and possible health risks. These packaging materials also contain compounds such as phthalates. These phthalates are used to enhance the mechanical properties (transparency, durability and flexibility) of the material.

Pthalates and its esters are synthetic compounds incorporated into packaging material to enhance its functionality. They are also called plasticizers which are loosely bound to the material and this makes it possible for them to emigrate into the food being packaged [4]. A number of phthalates have been identified in foods with high fat content such as sea food (fish) and oil where they can be absorbed [5]. They have also been detected in water and liquid food samples packaged with plastics. Their presence in these food substances can be affected by some conditions such as temperature and duration of contact (contact time) [6, 7]. They have been implicated in diseases such as hormonal imbalance (where they act as endocrine disruptors) under development and abnormalities of a growing fetus (teratogenic), DNA mutations (mutagenic) and cancer (where they act as carcinogens) [8, 9] Since the ingestion is usually the most common route of exposure, some countries have meticulously tried to monitor them. The maximum allowable intake to prevent health hazards were stipulated to be 0.05 mg/kg bw/day and 0.01 mg/kg for Diethylhexyl phthalate (DEHP) and di-*n*-butyl phthalate (DBP) respectively by the European Food safety Authorities (EFSA) [10] while European Union stipulated 1.50 mg/kg bw/day and 0.30 mg/kg bw/day for DEHP and DBP respectively. However, the EFSA suggested and aggregate allowable intake of DEHP, DEP, BBP, DBP and DiNP ranges from 0.9–7.2 and 1.6–11.7 µg/kg bw per day for mean and high consumers, respectively. [10, 11].

Okpa (cow pea pudding) is a local food that originated from and within Nsukka senatorial zone, Enugu state, Nigeria. It has now made its way to other parts of Nigeria. It is usually prepared with Bambara nut which is rich in protein (can be up to 47% with an average value above 23%). It is also rich in minerals and vitamins [12]. Due to its nutritive value, it contributes immensely to the dietary intake of vitamin A and protein to school children [13]. Okpa is usually prepared with the milled form of the Bambara nut. It is usually made by milling and sieving with a mesh sizes of 0.5 mm. it is sieved at least three times before the flour can be used to prepare Okpa. This food is sold by street vendors freshly prepared in the morning hours, afternoon and early hours of the night. This is why it is fondly called "Okpa di oku" (meaning hot Okpa) in our dialect. It is consumed by all especially the low income earners. It is also one of the major source of staple food recommended for diabetic patients. Due to its availability and how affordable it is, the students of The University of Nigeria Nsukka (UNN) had a saying that goes "Okpa, saving lives since 1960". The earlier innovation of this food usually used plantain leaves to package them but as time passed they have been packaged with polyethylene materials (bags) and materials made of tin as seen in Fig. 1 below. The processes involved in making Okpa requires mixing the Bambara nut flour with just pepper, salt, oil and warm water. It requires boiling with the package material for at least 45 min to 1 h depending on the sizes you have packed. This food usually contains a reasonable amount of red oil which enhances migration of phthalates form the materials in to the food. Considering the rate of consumption of this food in the South-Eastern part of Nigeria and Nigeria at large, it is important to evaluate the health risk of consumption of phthalate via okpa.

1.1 Study location

The study was conducted in Nsukka between March and April 2022. Google forms were used to distribute questionnaire to the community to obtain important details such age, gender, weight, height and residence. They also filled in the data such as okpa consumption frequency, duration of consumption, the type of okpa they consume (that is polyethylene packaged, those packaged in banana leave and or tin cup package okpa) in other to find out how often they consume phthalates that maybe detected in okpa.

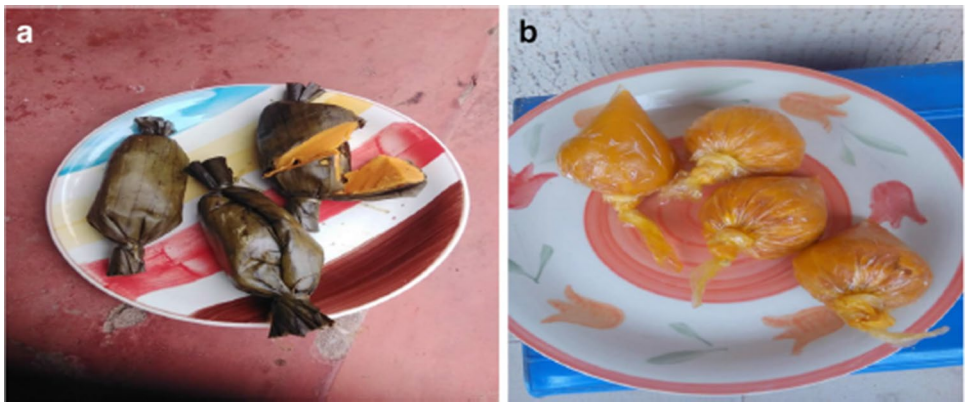

Fig. 1 The different packaging materials 2used for Okpa [2]

2 Materials and methods

2.1 Collection of materials

The materials used for this study were cowpea seeds, transparent polythene, red oil, salt and red pepper purchased from Ogige Market, in Nsukka Local government Area of Enugu State.

2.2 Chemicals and reagents

The phthalate ester standards, other chemicals and solvents were purchased from Sigma-Aldrich USA. The phthalate ester standards were dissolved in 100 µg/L of ethyl acetate and stored in the refrigerator (4 °C) for calibration purposes. The glass wares were assiduously washed with detergents and distilled water and dried. Furthermore, it was rinsed with n-hexane just for precision before use.

2.3 Preparation of pulverised cow pea

A known quantity 5 kg worth of cowpea seeds were purchased and pulverised into smooth powder form using a mechanical grinder. The grinding was repeated three times to ensure homogeneity, and then the smooth powder was sieved with mesh of 0.5 mm pore size for fine sized particles to pass through. This was transported to the laboratory for the preparation of the pudding.

2.3.1 Preparation of cow pea pudding (Okpa)

A known quantity, 3 kg of the pulverised cow pea sample was weighed and put in a bowl. The salt (10 g was added which may differ based on taste) pepper (3 g depending on how spicy you want it) and red oil (20 g it is usually more compared to any other ingredient to be added) were added into the pulverised cow pea and gradually stirred with warm water (800 mL was used in this case) and clean hands until a preferred homogeneity is obtained (not too thick and not too watery). The warm water was added little by little and stirred at every addition. The achieved homogeneity was transferred into transparent polythene bags (an average of 300 g) each and put in an already boiling water (1 L) and boiled for about 45 min (i.e. till it's ready).

Boil till slightly water warm and pour the water little by little into a bowl containing the mixture of flour and ingredients. At each stage stir very well.

Repeat the process until you achieve a moderate concentration of solution (not too thick, not too watery). Then dispatch into a container or nylon and cook till done as seen in Fig. 2a–f. Allow to cool, and measure the dry weight of the sample.

2.4 Determination of phthalates in Okpa

2.4.1 Preparation of sample for phthalate determination

This was done according to the method described in Ayamba [1]. The Fig. 2e is the control sample (just after mixing and packaging) while the Fig. 2f is the Test sample (cooked and readymade).

2.5 Extraction

A known quantity (10 mL) of acetonitrile was added to 2 g of meshed Okpa. The mixture was shaken vigorously (vortexed) for about 60 s and centrifuged at 3000 rpm for 5 min. After the centrifugation, 6 mL of the sample extract was taken for clean up.

2.6 Clean up

The 6 mL acetonitrile extract was added into a centrifuge tube already containing 150 mg of a sorbent (primary secondary amines used for solid-phase extraction) and 900 mg $MgSO_4$. It was vortexed for 60 s and then centrifuged at 3000 rpm for 5 min. Thereafter, 4 mL is taken out of the 6 mL clear sample and transferred into another flask where a mixture of 40 µL of 1% formic acid in acetonitrile was added and concentrated using rotary evaporator. The 2 mL of ethyl acetate was added to the dried sample and ultra-sonication was performed for 60 s.

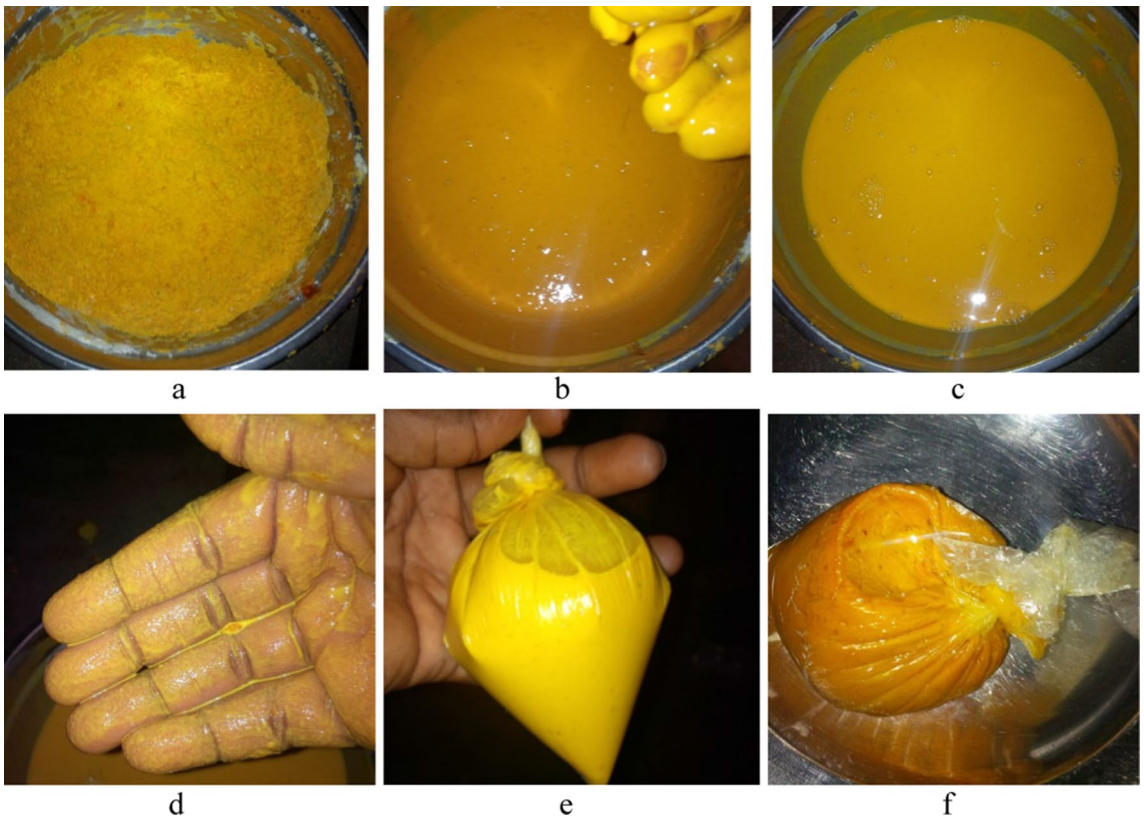

Fig. 2 The process of making Okpa (cowpea pudding). **a** Pulverised cowpea mixed with oil, salt and pepper. **b** Mixing the mixture with warm water to attain a desired consistency. **c**, **d** Desired consistency check. **e** Packaging the acquired and desired consistency of the cowpea puddiing (control). **f** The cooked and ready to eat cow pea pudding (Test sample)

2.7 Standard preparation

A known quantity (10 g) of the stock standard solution was dissolved with 10 mL ethyl acetate in a 50 mL beaker. This was further transferred into a 250 mL flask while the remnants from the 50 mL beaker were rinsed with ethyl acetate into the 250 ml volumetric flask. The flask was made up to 100 mL with ethyl acetate. This gives a 10 mg/mL or 100 mg/L of the mixed bulk standard. From this content, 0.1 mg/mL was transferred into a 250 mL volumetric flask and made up to 100 mL with ethyl acetate to yield 100 µg/L. The standard calibration was done for 1, 5, 10 and 20 µg/L and stored in the fridge at 5 °C until analysis. The limits of detection (LOD) and limits of quantification (LOQ) of the individual.

substances were: 0.0002 µg/mL and 0.0006 µg/mL (BBP); 0.0001 µg/mL and 0.0003 µg/mL (DBP); 0.0004 µg/mL and 0.0012 µg/mL (DEHP); 0.0002 ug/mL and 0.0006 ug/mL (DEP); and 0.0005 ug/mL; 0.00015 ug/mL (DiBP).

2.8 Recovery analysis

This was done to evaluate the accuracy of the extracted sample and the procedure taken for analysis. The available phthalates (10 µg/L) was added to a known quantity of the test sample (spiked sample), vortexed vigorously and stored till dawn. Furthermore, an equal volume of test sample was prepared without the addition of phthalates (without spiking it). This sample was also kept till dawn (6 h) after which both spiked and unspiked samples were extracted and phthalate content evaluated. The percentage recovery was calculated using the following equation

$$R\% = \frac{x - y}{z} \times 100$$

where R is the percentage recovery, X is the concentration of phthalates in the spiked sample, Y is the concentration of the phthalates in the unspiked sample while Z is the concentration used for spiking.

2.9 Phthalate determination in Okpa with gas chromatography-mass spectrometer

The Gas chromatograph used was Gas Chromatograph-Buck M910 scientific gas chromatography equipped with a single quadruple mass spectrometer. The mass column has a thickness of 0.25 µm film and internal diameter of 30 m × 0.25 mm. the injector was programmed as follows; the injector and initial column temperature was set at 290 °C and 50 °C respectively and held for 60 s. The first ramp: was from 50 to 280 °C. at a rate of 30 °C for another minute, It was also held at 310 °C for 4 min. The splitless injecton was used to analyze the samples. The gas carrier was helium at 1 mL/min (i.e. for constant flow).

2.10 Phthalates quantification

The levels of Phthalates were estimated using the peak area strategy. The peak areas extrapolated were those that their retention time corresponded with that of the standards on their calibration curves to obtain the concentration. Although, phthalates which shows poor defined peaks were recorded as not detected (ND).

The Phthalate concentration were determined with the equation below

$$RF = \frac{Peak\ areas\ of\ pthalate\ in\ sample}{peak\ area\ of\ standard}$$

where RF stands for response factor. The peak area can also be the concentration.

2.11 Health risk assessment

This was examined to ascertain the feasibility of developing health complications from ingesting Okpa made with transparent polyethylene bags. This can be done using the blue print and some values stipulated by USEPA as seen in Table 1.

Table 1 The oral reference dose (RFD), tolerable daily intake (TDI) and cancer slope factor (CSF)

Phthalates	Oral reference dose (mg/kg bw/day)	Tolerable daily intake (max allowable limit) (mg/kg bw/day)	Cancer slope factor (mg/kg-day)
BBP	0.2 [16]	0.5 [19]	
DBP	0.1 [17]	0.01 [20]	
DEHP	0.02 [18]	0.05 [21]	0.014
DIBP	0.02 [3]	0.14	
DEP	0.8	0.5	
DiNP		0.15	
DiDP		0.15	

Where *BBP* Benzyl butyl phthalate, *DBP* Di-*n*-Butyl phthalate, *DEHP* Di (2-ethylhexyl) phthalate, *DIBP* Di-isobutyl phthalate, *DiNP* Di-isononyl phthalate, *DiDP* Di-isodecyl phthalate

2.12 Determination of the estimated daily intake (EDI) of phthalates in Okpa

The essence of evaluating EDI is to be informed on the specific intake of phthalates from the okpa and juxtapose it with the acceptable tolerable limits. This can be done with the module below and expressed in mg/kg body weight/day

$$EDI = \frac{Cp \times OIR \times EF \times ED}{BW \times AT}$$

where EDI is the estimated daily intake (mg/kg day^{-1}), *Cp* is the Phthalate concentration in okpa (mg/kg), OIR is the Okpa ingestion rate (3.29 g/day^{-1}), EF is the exposure frequency (365 days year^{-1}), ED is the exposure duration (year) (for children: ED = 6, for adults: ED = 70), BW is the body weight (children: BW = 14 kg, adults: BW = 70 kg), AT is the average lifespan (children: AT = 2190 days, adults: AT = 25,550 days).

2.13 Determination of the Hazard quotient and index

The non-carcinogenic health risk also known as the hazard quotient associated with consuming okpa (cow pea pudding) samples was evaluated using the hazard quotient strategy expressed in mg/kg/day. This method is based on dividing the concentration of ingested phthalates through okpa by the stipulated reference dose. This was developed by USEPA. It is also important to note that a hazard quotient values above 1 (> 1) suggests a menace to human health but below 1 (< 1), it is termed safe to consume over a life time. This does not necessarily mean that the consumers is suffering from adverse health effects by consuming okpa but gives an insight towards the risks associated with consuming them [14]

The hazard index (HI) is the gross risk that an individual may possibly encounter by consuming all the phthalates in okpa. That is the total risk from consuming all the phthalates in the food. This was determined by summing the hazard quotient of all the detected phthalates in okpa. HI < 1 is considered to be acceptable. However, HI > 1 indicates that adverse health effects may occur [15].

$$HQ = \frac{EDI}{RFD}$$

$$HI = \sum_{i=1}^{n} \frac{EDI}{RFD} or(HQ)$$

Here HQ stands for hazard quotient, HI stands for hazard index, RFD stands for reference dose, n stands for the number of phthalates detected while I stands for the individual phthalate.

2.14 Cancer risk

The product of the cancer slope factor (CSF) and the estimated daily intake (EDI) gives rise to the cancer risk. The probability of developing cancer over a period of lifetime is called the incremental lifetime cancer risk (ILCR) and can be evaluated as seen in equation below. The value obtained for determining cancer risk that are within 1.0×10^{-6} to 1.0×10^{-4} are in the acceptable range [14]. The aggregation of ILCR informs on the risk index (RI)

$$\mathrm{CR} = \mathrm{EDI(mg/kg/day)} \times \mathrm{CSF(mg/kg/day)}^{-1}.$$

where EDI represents estimated daily intake and ICSF represents ingestion carcinogenic slope factor. The risk index is given in the formula below

$$RI = \sum_{i=1}^{n} ILCR$$

Here n is the number of phthalates and 'i' is the individual phthalates.

3 Results and discussion

3.1 Population studies

The population used for this study were 1227 in number of which most were male (78.85%) with an average age of 34 years and a range of 18 to 50 years as shown in Table 2. The body mass index (BMI) of the subjects showed that almost half (47.91%) of the population had normal BMI (18.9–22.2) while those that are underweight (16.2–17.1) were about 28.6%. The population of those overweight (25.2–27.8) were 18.2% while the obese people (30.4–31.2) were 5.29%. A total of seven people (0.6%) reported that they don't consume okpa. The population studies for the consumption pattern of okpa was carried out using the 1220 people that reported that they consumed it. From this population, those that

Table 2 Consumption pattern of okpa packaged with polyethene material

Data	Population (n = 1220)	Percentage (%)
Number of times (meals)/day		
1	1100	90.16
2	100	8.19
3	None	–
Number of days in a week		
1–2	474	38.85
3–4	762	62.45
5–6	84	6.88
Everyday	14	1.14
Period of consumption		
2–4 years	88	7.21
5–7 years	86	7.04
8–10 years	246	20.163
> 10 years	694	63.93
Quantity consumed amount (weight)		
100 Naira (165 ± 23 g)	458	38.16
200 Naira (300 ± 21 g)	734	61.17
300 Naira	8	0.67
> 300 Naira		

Vendors mostly sell packaged okpa for 100 and 200 naira only. If you consume more than 200 naira, you only have to buy an extra of 100 or 200 naira depending on the quantity the individual consumes

Table 3 Percentage recovery and response factor of identified phthalates

Phthalates	Percentage recovery (%)	Response factor (RF)
DEP	96.08	0.039
BBP	98.44	0.015
DBP	97.04	0.029
DiBP	97.48	0.025
DEHP	76.4	0.023

Table 4 Phthalate in Okpa concentration in mg/kg

	Control	Test sample
DEP	BDL	0.0196 ± 0.000
BBP	0.0025 ± 0.001	0.0077 ± 0.001
DBP	BDL	0.0149 ± 0.001
DiBP	0.0109 ± 0.001	0.0121 ± 0.001
DEHP	BDL	0.0110 ± 0.000
DiNP	BDL	BDL
DiDP	BDL	BDL

don't consume okpa more than once (1 meal/day) in a day were 90.163%, those that could eat it twice in a day (2 meal/day) were 8.19%. The consumption rate for 1–2 times in a week was 38.85%, 3–4 times in a week was 62.45%, 5–6 times in a week was 6.88% while those that consumed it every day was 1.14%. Most of the population (63.93%) reported that the consumed okpa with in transparent polyethylene bags for more than 10 years. Those who have consumed okpa in transparent Polyethylene bags between 8–10 years were 20.16% while those who consumed between 2–7 years were within the range of 7.04 to 7.21%. A higher number of the population (61.17%) consume about 300 ± 21 g. Out of these population, 20 returned invalid forms (those who filled the forms wrongly).

3.2 Recovery analysis

The sensitivity and reproducibility of the method showed that they were within the guidelines. The guidelines stipulated that percentage recovery within 70–120% (EU, 2008) is efficient and has high accuracy with reproducibility as shown in Table 3. The percentage recovery for the identified phthalates ranged from 76.40–98.44%.

3.3 Phthalate level in Okpa in µg/g

A total of five phthalates were identified in the test sample (okpa or cowpea pudding) as seen in Table 4. The phthalates identified in both control and test sample were low molecular weight phthalates. There are two different phthalates that were detected in the control sample and they were BBP and DiBP. Those identified in the test samples were DEP, BBP, DBP, DiBP and DEHP. They all ranged from 0.0196 to 0.0077 mg/kg. The highest concentration of phthalate detected was DEP (0.0196 mg/kg). The lowest level of phthalates detected was BBP which was 0.0077 mg/kg and still didn't exceed the TDI. The level of detected Phthalates were in the following order: DEP > DBP > DiBP > DEHP > BBP. The DBP detected was 0.0149 mg/kg which slightly exceeded the TDI (0.01 mg/kg) for food consumption. Phthalates such as DiNP and DiDP were not detected. The total concentration of phthalate detected was 0.0653 mg/kg.

Among these pthalates, DEP was the most abundant. This finding is in contrast with the findings of Alp and Yelikaya [22] that evaluated phthalate ester migration from packaging materials. They observed that DEHP migrated the most. There are reports on previous studies on how the migration of phthalates into food can be determined by the properties of the food [23]. These properties includes the pH and the oil content of the food [24, 25]. Phthalates are hydrophobic in nature and foods that have a high content of fats and oil ncreases their rate of migration. A very low pH also favors the migration of phthalates into food. This is usually due to hydrolysis [26]. The control sample also contained some phthalates. The levels detected in the control could be as a result of unintentional contamination via handling or also preservation. This can also be seen in the study of Alp and Yerlikaya [22] where storage and time can introduce phthalates

into food. Usually these pulverized cow peas for making okpa are usually stored in polyethene bags and tied properly to prevent air from entering. The DEP identified in cow pea pudding slightly exceeded the tolerable limit. This compound is usually used as a plasticizer to make these materials more flexible [27]. Exposure to DEP have been considered to be non-lethal but can causes irritation to the skin and the eyes. Chronic and high exposure can also cause a damage to the nervous system [28]. However, there are works that have shown that DEP can exert some alterations on liver weight and histopatholoical and biochemical parameters of animals. It has also shown to have some mild male and female reproductive effects (androgen-independent male reproductive toxicity i.e. sperm effects, maternal organs gain weight, survival of the f1-offspring depending on the doses) on Wistar rats [27]. The BBP determined in this work were below the RFD. This is in line with the work of

Makkaew et al. [23] where BBP levels in plastic coated materials used for packaging was below the RFD. The BBP is usually metabolized from benzyl butyl phthalate to mono butyl phthalate (MBP) or mono benzyl phthalate (MBzP). These metabolites are the key compounds responsible for the health effects that may occur from consuming BBP. Some studies have shown that they can inhibit the estrogen function at high doses. It has also been implicated in the variations observed in the development of reproductive organs of new born boys [29]. Studies with animal models have shown decrease in the weight of the ovary and progesterone in the blood, post implantation embryonic loss and abnormal skeleton developments [30]. They have also shown to regulate the parathyroid hormone in animal models (rat) [29]. The DBP is a common plasticizer that has been used widely in many plastic materials such as some bowls and food wraps to make them soft and flexible [31]. The concentration detected in okpa was below the RFD. Di-n-butyl phthalate has not been implicated in any reproductive, developmental, or carcinogenic effects in humans. However studies have shown that they can affect the developing fetus and male testes in animals [32, 33]. Acute exposure can cause non carcinogenic health hazards such as skin and eye irritations while long term exposures may cause liver and kidney damage [34]. The level of DBP in this study is not in line with the study where DBP levels in polyethene food contact materials were far above the RFD [35]. The levels of DIBP observed in this study was also below the RFD. The DiBP has also been a type of plasticizer identified in this work. Studies have shown that they may be deleterious to the female reproductive system especially at concentration above the TDI for developmental effects (0.098 DiBP mg/kg/bw-day). In addition, no studies have shown that they may be carcinogenic [36]. However, there are so many studies on the hazards caused by DiBP on animals. Sub-chronic studies have shown that both female and male suffer from poor reproductive system development and even fetus development [37]. Di (2-ethylhexyl) phthalate (DEHP) had 0.0110 mg/kg. This compound is metabolized by humans to yield mono-(2-ethyl-5-hydroxyhexyl) phthalate (5OH-MEHP) and mono-(2-ethyl-5-carboxypentyl) phthalate (5cx-MEPP) as secondary metabolites [38]. Di (2-ethylhexyl) phthalate (DEHP) and its metabolites has been implicated in female fertility issues, alterations in the normal functioning of the placenta and preterm birth. These alterations in the normal function of the placenta has been associated with DNA methylation of the imprinted maternally expressed gene 3 (MEG-3 gene) of the placenta and placenta LINE-1, increased oxidative stress and a slow release of the HCG hormone in pregnant women [39]. Additionally, it has been implicated in diseases such as diabetes, cardiovascular diseases and obesity in women via the epigenetics mechanism [40]. United State Environmental Protection agency and European food safety authority stated that levels above 0.02 and 0.05 mg/kg can lead to swelling of the liver a phenomenon known as hepatomegaly and morphology alterations in testes that can lead to infertility [1, 41]. However, the levels of DEHP found in this study were below the RFD (0.02 mg/kg). This is in contrast with the work that observed a migration of 1.29 mg/kg of DEHP from polyethylene coated foods at 80 °C for 30 min [1]. It also varied with the findings of Makkaew et al. [23] who identified DEHP levels in plastic coated foods to be within the range of 0.020 to 0.046 mg/kg.

3.4 Health risk assessment

The Estimated daily intake (EDI) was used to determine the health risks associated with consuming okpa cooked with polyethylene materials. This evaluated the carcinogenic and non-carcinogenic health hazards as seen in Table 5. The EDI obtained in this work ranged from 4.60E−3 to 1.80E−3. The EDI showed that children will consume more of DEP (4.60E−3) compared to other phthalates while the adults will consume more of DiBP (5.68E−3). However children are usually prone to take in more of the phthalates as seen in the total EDI which is 1.53E−2. Adegunwa [3] also observed that children had a higher EDI when compared to adults however they consumed more DBP. The sequence at which this phthalates were ingested by consuming okpa was in the following order DEP > DBP > DiBP > DEHP > BBP.

The toxic hazard quotient (THQ) evaluated showed that none of the phthalates were above one (> 1) for both children and adults suggesting no adverse health implication. However, the phthalate with the highest THQ in adults and children was DiBP (2.84E−1) and (1.42E−1) respectively and this suggests that if there was to be any non-carcinogenic

Table 5 Health risk assessment

Pthalates	EDI		THQ		ILCR	
	Children	Adult	Children	Adult	Children	Adult
DEP	4.60E−3	9.21E−4	5.75E−3	1.15E−3	NA	NA
BBP	1.80E−3	3.61E−4	9.0E−3	1.80E−2	NA	NA
DBP	3.50E−3	7.00E−4	1.75E−2	7.00E−3	NA	NA
DiBP	2.84E−3	5.68E−3	1.42E−1	2.84E−1	NA	NA
DEHP	2.58E−3	5.17E−4	1.27E−1	2.58E−2	3.61E−5	7.23E−6
DiNP	NA	NA	NA	NA	NA	NA
DiDP	NA	NA	NA	NA		
Total	1.53E−2	8.18E−3	3.01E−1	3.35E−1	3.61E−5	7.23E−6

Hazard index for children = 3.01E−1

Hazard index for adults = 3.35E−1

NA is not applicable

Na is not available

health hazards associated with consuming okpa in adults or children, it may emanate from DiBP. The least THQ was seen in DEP for both adults and children. The hazard index noticed in adults (3.35E−1) was higher than children (3.01E−1). The toxic hazard index (THI) which is the health complication that may arise from consuming all the phthalates identified in okpa. The THI also uses the same scale of evaluation as the THQ were when the value is less than 1 (< 1), it is considered safe while when it is above 1 (> 1), it may lead to health hazards. The THI evaluated for this study showed that none was above 1 (> 1) suggesting that there may be no health hazards from consuming this okpa. However the THI obtained for adults (3.35E−1) was slightly higher than that of the children (3.01E−1). This may also mean that adults will be more susceptible to developing any health complication than children. This may happen if the levels of phthalates identified in okpa increased. The carcinogenic risk assessment was done since a carcinogenic phthalate was identified. Since DEHP has been the phthalate identified and associated with cancer it was used to evaluate the possibility of developing cancer over a period of lifetime. The cancer risk (CR) evaluated showed that both adults and children will be safe since their values were below 1E−3. Adults had 7.23E−6 while children had 3.61E−5 as their CR. This finding is in line with the works of Makkaew [23] who also found that it is safe to keep using plastic coated paper (polyethylene) for food packaging.

4 Conclusion

A total of 5 phthalate were identified in okpa and DEP had the highest amount (0.0196 mg/kg). The cumulative non carcinogenic risk was within the safe limit (THI < 1). The carcinogenic risk was also within the acceptable range. This suggests that at the moment it is safe to keep using polyethene bags to package okpa. However, it is still pertinent to continuously monitor the level of phthalates that may migrate into okpa because red oil content variations may positively impact on its leaching ability. This should be done in order to control and protect the safety of the consumers.

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Data availability All data generated or analyzed during this study are included in this published article and its supplementary information files.

Declarations

Ethics approval and consent to participate I confirm all relevant ethical guidelines have been followed, and any necessary IRB and/or ethics committee approvals have been obtained.

The details of the IRB/oversight body that provided approval or exemption for the research described are given below:
Ethical approval was obtained from the Faculty Research Ethics Committee, Faculty of Biological Sciences, University of Nigeria, Nsukka (Ethical approval number: FBSRA/UNN/23/0056). The research was carried out according to the guidelines of the ethics committee and the protocol was approved by FBSRA/UNN/23/0056) in accordance with the guidelines.
I confirm that all necessary participant informed consent has been obtained and the appropriate institutional forms have been archived, and that any patient/participant/sample identifiers included were not known to anyone (e.g., hospital staff, patients or participants themselves) outside the research group so cannot be used to identify individuals. Participants were all 18 and above.
I have followed all appropriate research reporting guidelines.

Competing interests The authors declare no competing interests.

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