Journal of Dry Zone Agriculture, 2020, 6 (2): 68 - 78
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ISSN 2012 – 8673

Analytical Method to Determine the Migrated Polymers from Edible Oils Adulterated with Polyethylene Terephthalate to Deep Fried Cassava Chips

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Abstract: Non-branded deep fried manioc chips were evaluated by following a newly developed and validated method to determine the migration of Terephthalic acid (TPA) and Isophthalic acid (IPA) from the frying oils adulterated with pet bottles to make the snacks crisp and long last. Reporting method is comprised with soxhlet extraction of oils from deep fried manioc chip samples, solvent extraction of TPA and IPA using centrifuged oil samples, sample preparation for High Performance Liquid Chromatography (HPLC) injection and HPLC analysis of samples along with the Terephthalic and Isopthalic acid standard series. The percentage recovery range for TPA and IPA was in between 80-120% for manioc chip samples fried in edible oils incorporated with PET bottles. The results showed that the specific migration of TPA and IPA into the manioc chip samples conform to European Union legislation that identifies specific migration limits (SML). The intra day assays for TPA and IPA were expressed as Relative Standard Deviation (RSD) 5.123% and 2.015% respectively while inter-day precision assays for TPA and IPA were expressed as relative standard deviation (RSD) 2.089% and 2.105% respectively. Results highlighted that the data is tightly clustered around the mean with a good precision. Analytical curves for TPA and IPA were found to be linear over a wide concentration range (6ppb – 120ppb) with a correlation coefficient of 0.998 for TPA and 0.999 for IPA. The lower limit of quantification was determined to be 6 ppb, with a relative standard deviation lower than 10%. TPA and IPA in sample are well separated from each other and from the background oil. The study shows that the method presented in the methodology can be used as a suitable method for analytical determination of Terephtahlic acid and Isophthalic acid in deep fried crispy snacks like manioc chips with high precision and accuracy.

Keywords: Edible oils, Manioc chips, Isophthalic acid, Migration, Terphthalic acid

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Introduction

Application of plastics in food packaging has largely increased during the last decades because of their availability and the enormous variety of these materials. In the last few decades, the simple long-chain polymer, polyethylene terephthalate (PET) has become one of the most common packaging polymers. Polyethylene terephthalate (PET) is a plastic material that has found increasing applications within the packaging field. It is a simple long-chain polymer, and its chemical inertness together with its physical properties has made it particularly suitable for food packaging applications. PET is a long-chain polymer that belongs to the generic family of polyesters (Brody and Marsh, 1997). PET has become a packaging material for many food products, particularly beverages. Being an inert material with good physical properties and glass-like transparency makes PET a good option for water packaging. Food Packaging is a rapidly evolving field. Besides keeping foods safe from contamination and retaining the nutritional properties and sensory characteristics of foods, packaging provides additional features that are important to consumers. Potential influence of these substances on product safety and quality remains in question as migrated compounds exceed the Maximum Allowable Limits in foods. Monomers and additives used in packaging materials are safety concern as they can be migrated from the package into food, creating hazardous conditions. The toxicity effects of isophthalic acid (IPA) and terephthalic acid, the migrants from PET packaging into foods has been demonstrated by many investigations. The EFSA fixed levels of Isophthalic acid (IPA) and Terephthalic acid (TPA) migration into foods from plastics of 5 and 7.5 mg kg^{-1} of food, respectively (Regulation 2011/10/EU).

Incorporation of PET bottles to edible oils which are being using for deep frying of crispy Snacks has become a strategy for crispy snack manufacturers to make the snacks crisper and long last. The tendency of monomer and oligomer migration increases when a plastic is exposed to high temperatures during thermal processing. Thus, Long term consumption of foods comprised with plastic migrant results mutations in cell structures which drives towards occurring of cancers in human bodies.

Hence, the objective of this experiment was to develop and validate an accurate method to quantify the migration of TPA and IPA from PET bottles to edible oils during deep frying of manioc chips and the quantification of TPA and IPA contamination in locally available non branded crispy snack samples in order to ensure the safety of consumption.

Materials and Methods

Experimental Location

Experiments were conducted at the

laboratories of National Institute of Post-Harvest Management, Anuradhapura.

Sample Preparation and Experimental Treatments

Commercially available deep fried manioc chip samples were purchased from the retail shops distributed island wide. The special focus was to collect non-branded deep fried manioc chip samples from small scale sellers/ manufacturers. Special attention was given to small scale sellers and manufactures because they have very limited access for the food safety and food sanitation measures as well as regulations imposed on food manufacturing and selling in order to ensure consumer safety. One hundred and fifty Small scale manufactured non branded deep fried manioc chip samples were drawn island wide and prepared thirty composite samples by mixing them. Analytical standards of TPA and IPA were purchased from Sigma Aldrich, USA. HPLC gradient-grade water, acetonitrile, and methanol (HPLC grade) solvents were purchased from Sigma Aldrich, USA. In this study method development and validation for analytical determination of migration of terephthalic acid and isophthalic acid into deep fried manioc chips have being conducted.

Instrumentation and Chromatographic Conditions

The method was performed on a HPLC system (Ultimate 3000, Thermo scientific, Germany) equipped with an Agilent G1311A quaternary pump and Agilent G1315A diode array detector. The determinations were performed with UV-Vis detector set at 242nm.The column was a Knauer C18 AQ column (250 mm, 5 µm particle diameter and 4.6 mm internal diameter). The column temperature was kept at 30 $^{\circ}$C using an Agilent G1316A oven. The two mobile phases used for gradient HPLC elution were (A) H_2O buffered with 0.1 trifluoroacetic acid/acetonitrile (90:10, v/v) and (B) H_2O buffered with 0.1 trifluoroacetic acid/ acetonitrile (60:40, v/v) with the following proportions: A–B 90–10, 83–17, 75–25 and 60–40 at 0, 3, 6 and 12 min, respectively. The mobile phases were filtered through a 0.45 µm millipore membrane filter (model-FN 2545) and degassed with helium for 15 minutes before use. The flow rate was 1mLmin^{-1} and the volume of injection was 10µL (Khaneghah *et al*., 2013).

Standards and Calibration Curves

A mixed stock standard solution of 1000 ngmL^{-1} was prepared from TPA and IPA that were dissolved in methanol and was stored in the dark at refrigerator temperature (4 $^{\circ}$C). Calibration standard solution series was prepared on the day of use at concentrations of 6, 12, 24, 60 and 120 ngmL^{-1} and calibration graphs were plotted using these concentrations of standard solutions.

The detection limit was defined as the concentration corresponding to a peak

height three times the baseline noise level. Recovery studies were carried out by spiking selected samples of oils with the blended standard solution (mix of TPA and IPA) at three concentrations (240, 750 and 1000 μg kg^{-1}). The spiked samples, as well as the controls, were analysed in triplicate experiments. Recovery rates (percent) were calculated by comparing peak area in the chromatogram with the peak area calculated from the standard calibration curves.

Oil Extraction from Manioc Chip Samples

Thirty homogeneous, composite samples were prepared from one hundred and fifty manioc chip samples drawn from island wide in order to reduce the time consumption of the procedure. Three replicates of each composite sample were analysed. Soxhlet Extractor was used for oil extraction with petroleum ether as the solvent and each sample was kept for 5 hours for oil extraction.

Extraction of Migrated Compounds

A mixture of methanol (1 mL), chloroform (3 mL) and NaOH (1 mL) was used for the extraction of migrated monomers from 1 g of oil sample. Centrifugation was done in order to extract migrated monomers into oils. After centrifugation (Hermle.Z326K Laborteknik GmbH, Germany) at 6000 rpm and at −3 °C for 20 min, the separated samples were analysed by HPLC.

Statistical Analysis

Statistical analysis was done with SPSS version 16.0 (SPSS Inc., Chicago, IL, USA) and Minitab version 16 (Minitab Inc., State College, PA, USA). The standard calibration curves were plotted using Excel 2016 (Microsoft, USA). The significance level was $P<0.05$.

Method Validation

Validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. Validation is a formal and systematic way to demonstrate suitability of the method to provide useful data to ensure that the process or the method gives satisfactory and consistent results within the scope of the process. The analytical methods refer to the way of performing the analysis. Linearity, accuracy, precision, limit of quantification and specificity were measured in order to validate the method.

Results and Discussion

Terephthalic acid and isophthalic acid are the two common migratable monomers in polyethylene terephthalate (PET). If the manioc chips are adulterated with PET bottles these two compounds should be detected during the experiments. In this study, a HPLC method for quantitative analysis of terephthalic acid (TPA) and isophthalic acid (IPA) in deep fried manioc chips was developed and validated. Simultaneous analysis of all island samples was done by using the validated method.

Accuracy

Accuracy can be expressed as the percent recovery of known amounts of TPA and IPA, added to a sample and it is one of the most important parameters of an analytical methodology. The recoveries were determined by adding known amounts of the TPA and IPA reference substances (6 ppb, 24 ppb and 120 ppb) to the oil sample which is previously tested for zero levels of TPA and IPA. The results presented in the following table (Table 1) refer to the average of triplicates for each concentration. Since the percentage recoveries remain in between 80 - 120% for each concentration, it can be concluded that the results are in good agreement with acceptable values for the validation of an analytical procedure (Britain, 1998).

Table 1: Analytical recovery of TPA and IPA mixed standard solution added to sample

Amount Added (ppb)	Recovery			
	TPA		IPA	
	ppb	Percentage	ppb	Percentage
6.00	4.744 ± 0.069	81.843 ± 1.469	5.142 ± 0.1671	85.7 ± 1.768
24.00	22.138 ± 0.1714	92.250 ± 0.703	22.998 ± 0.804	95.825 ± 0.983
120.00	112.04 ± 0.4514	93.372 ± 0.376	115.01 ± 0.795	95.841 ± 0.921

*ppb – parts per billion, TPA- Terephthalic Acid, IPA- Isophthalic Acid
Each value represents mean ± S.D of three replicates

Precision

The precision refers to the variability of the results in repeated analyses of the sample under identical experimental conditions. The method was validated by evaluating the intra- and inter-day precision. The precision was calculated from an average of six determinations of a homogeneous sample (USP, 2004). The intra day assays for TPA and IPA were expressed as Relative Standard Deviation (RSD) 5.123% and 2.015% respectively while inter-day precision assays for TPA and IPA were expressed as relative standard deviation (RSD) 2.089% and 2.105%, respectively, indicating that the data is tightly clustered around the mean and method presents a good precision (Brittain, 1998). The detailed precision data are shown at Table 2.

Table 2: Analysis of intra- and inter-day precision assays

Theoretical concentration	Concentration							
	Intraday TPA		Inter day TPA		Intraday IPA		Inter day I	
	ppb	%	ppb	%	ppb	%	ppb	%
7.0	5.7401	82.00	5.8321	83.31	5.8301	83.28	5.8211	83.15
7.0	6.1520	87.88	5.7511	82.15	5.9269	84.67	5.9123	84.46
7.0	5.7188	81.69	5.8200	83.14	5.9831	85.47	5.8272	83.24
7.0	5.8697	83.85	5.9820	85.45	5.7854	82.64	5.8113	83.01
7.0	5.9596	85.13	6.2712	89.58	5.7454	82.07	6.1120	87.31
7.0	6.2814	89.73	6.1230	87.47	5.8282	83.26	6.0230	86.04
Average	5.8869		5.9632		5.7998		5.9178	
Standard Deviation (ppb)	0.3016		0.1246		0.1169		0.1246	
Relative Standard Deviation (%)	5.123		2.089		2.015		2.105	

*ppb – parts per billion, TPA- Terephthalic Acid, IPA- Isophthalic Acid, %-Percentage

Linearity

The analytical curves for TPA acid and IPA acid standards were constructed by plotting the area under the curve (AUC) of the main peak versus migrant concentrations (|Table 3, 4, 5, and 6) It was found to be linear over a wide concentration range (6ppb – 120ppb) with a correlation coefficient of 0.998 for TPA and 0.999 for IPA. The straight line equations obtained from the experimental results were found to be:

$y = 0.029 - 0.121 \longrightarrow$ Equation for TPA

$y = 0.086 - 0.229 \longrightarrow$ Equation for IPA

The data were validated by analysis of variance, which demonstrated significant linear regression and non-significant deviation from linearity ($P < 0.05$). The Relative Standard Deviation of the slope and of the intercept of the three lines obtained for TPA standard were 12.1 % and 2.9%, respectively. The RSD of the slope and of the intercept of the three lines obtained for IPA standard were 22.9% and 8.6 % respectively.

Thus, this HPLC method can be considered to show adequate linearity in the concentration range (6 ppb-120 ppb) for quantitative analysis of TPA and IPA under the experimental conditions described.

Table 3: Linearity for TPA

Model summary				
Model	**R**	**R Square**	**Adjusted R Square**	**Std. Error of the Estimate**
1	.999[a]	.998	.998	.0666575

Table 4: Linearity for IPA

Model Summary			
R	**R Square**	**Adjusted R Square**	**Std. Error of the Estimate**
.999[a]	.999	.998	.1651860

Table 5: Coefficients for TPA

		Unstandardized Coefficients		**Standardized Coefficients**			**95% Confidence Interval for B**	
Model		**B**	**Std. Error**	**Beta**	**t**	**Sig.**	**Lower Bound**	**Upper Bound**
1	(Constant)	-.121	.043		-2.798	.068	-.259	.017
	concentration	.029	.001	.999	40.731	.000	.027	.031

a. Dependent Variable: MAU

Table 6: Coefficients for IPA

		Unstandardized Coefficients		**Standardized Coefficients**			**95% Confidence Interval for B**	
Model		**B**	**Std. Error**	**Beta**	**t**	**Sig.**	**Lower Bound**	**Upper Bound**
1	(Constant)	-.229	.107		-2.135	.122	-.570	.112
	concentration	.086	.002	.999	48.981	.000	.080	.091

a. Dependent Variable: MAU

Limit of Quantification

The lower limit of quantification was determined to be 6 ppb, with a relative standard deviation lower than 10%.

Specificity

HPLC chromatogram for sample loaded with TPA and IPA is shown in Figure 1. The results obtained from the chromatograms indicate that the retention times of the two compounds were between 11 and 15 min and peaks of solvents were eluted in about 1–2 min. Run time for the analysis of TPA and IPA was 20 min. TPA and IPA in sample are well separated from each other and from the background oil (Table 7 and 8) Following chromatogram illustrates that the extraction solvent did not critically interfere with the detection of the monomers.

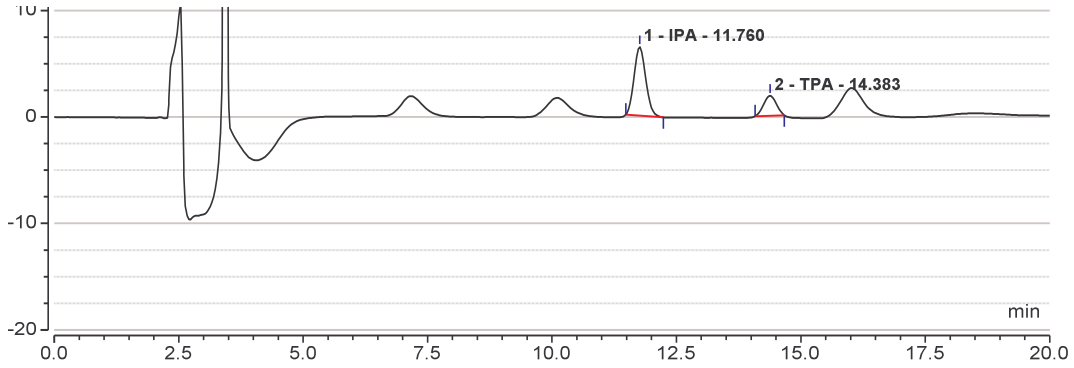

Figure 1: HPLC chromatogram for sample loaded with TPA and IPA

HPLC Analysis of All Island Samples

None of the samples collected island wide shows a positive result for TPA and /or IPA from HPLC Analysis.

Table 7: TPA Contamination for samples drawn island wide (30 composite samples prepared from 150 samples)

No	Injection name	Ret.Time min UV_VIS_1 IPA	Area mAU*min UV_VIS_1 IPA	Height mAU UV_VIS_1 IPA	Amount ppb UV_VIS_1 IPA	Real Area % UV_VIS_1 IPA	Peak type UV_VIS_1 IPA
1	Sample 1	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
2	Sample 2	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
3	Sample 3	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
4	Sample 4	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
5	Sample 5	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
6	Sample 6	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
7	Sample 7	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
8	Sample 8	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
9	Sample 9	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
10	Sample 10	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
11	Sample 11	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
12	Sample 12	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
13	Sample 13	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
14	Sample 14	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
15	Sample 15	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
16	Sample 16	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
17	Sample 17	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
18	Sample 18	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
19	Sample 19	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
20	Sample 20	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
21	Sample 21	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
22	Sample 22	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
23	Sample 23	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
24	Sample 24	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
25	Sample 25	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
26	Sample 26	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
27	Sample 27	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
28	Sample 28	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
29	Sample 29	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
30	Sample 30	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

Table 8: IPA Contamination for samples drawn island wide (30 composite samples prepared from 150 samples)

No	Injection name	Ret.Time min UV_VIS_1 IPA	Area mAU*min UV_VIS_1 IPA	Height mAU UV_VIS_1 IPA	Amount ppb UV_VIS_1 IPA	Real Area % UV_VIS_1 IPA	Peak type UV_VIS_1 IPA
1	Sample 1	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
2	Sample 2	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
3	Sample 3	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
4	Sample 4	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
5	Sample 5	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
6	Sample 6	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
7	Sample 7	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
8	Sample 8	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
9	Sample 9	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
10	Sample 10	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
11	Sample 11	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
12	Sample 12	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
13	Sample 13	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
14	Sample 14	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
15	Sample 15	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
16	Sample 16	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
17	Sample 17	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
18	Sample 18	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
19	Sample 19	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
20	Sample 20	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
21	Sample 21	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
22	Sample 22	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
23	Sample 23	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
24	Sample 24	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
25	Sample 25	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
26	Sample 26	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
27	Sample 27	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
28	Sample 28	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
29	Sample 29	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
30	Sample 30	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

Conclusion

The results obtained from the study show that the HPLC method presented, can be considered as a suitable method for the identification and quantification of terphthalic acid and is ophthalic acid migrated to edible oils used for deep frying of manioc chips. None of the sample drawn island wide were contaminated with TPA or IPA. Thus, they are generally safe for the consumption.

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