Journal of Experimental Biology and Agricultural Sciences, December - 2023; Volume – 11(6) page 964 – 975

Journal of Experimental Biology and Agricultural Sciences

http://www.jebas.org

ISSN No. 2320 – 8694

Assessing the Migration of BPA and Phthalic Acid from Take-out Food Containers: Implications for Health and Environmental Sustainability in India

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Received – October 12, 2023; Revision – December 16, 2023; Accepted – December 26, 2023
Available Online – December 31, 2023

DOI: http://dx.doi.org/10.18006/2023.11(6).964.975

KEYWORDS

Take-out foods

Plastic packaging

Bisphenol A

Phthalic acid

Health risks

ABSTRACT

The research investigates the escalating consumption of take-out food in India and the associated health risks stemming from the extensive use of plastic packaging. Through a comprehensive nationwide online survey, the study delved into dietary preferences, frequency of take-out food consumption, delivery service timing, and the types of packaging commonly encountered by Indian consumers. To address these concerns, the research team developed an analytical method to detect Bisphenol A (BPA) and Phthalic acid migration from food-contact materials (FCMs) into various food simulants. The investigation revealed that prolonged exposure to elevated temperatures led to increased migration of BPA and Phthalic acid, particularly in polyethylene pouches using 3% acetic acid as a food simulant, with the highest concentrations observed after 45 minutes of exposure. Additionally, a microbial bioassay demonstrated the mutagenic potential of migrated plasticizers, showcasing significant effects in mammalian systems, particularly under metabolic activation. The study underscores the substantial health risks associated with plastic packaging in take-out food, emphasizing potential implications for consumer health and calling for more extensive research and considerations regarding food packaging materials.

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Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

Production and Hosting by Horizon Publisher India [HPI] (http://www.horizonpublisherindia.in/).

1 Introduction

Fast food refers to food and drinks sold immediately after purchase, either at the food outlet or elsewhere. Take-out food consumption has increased significantly due to the popularity of mobile ordering and delivery apps. The online food delivery sector in the United States generated up to USD 17.5 billion in revenue in 2018, while in China, it generated USD 34.7 billion (Han et al. 2021). India's restaurant industry gross merchandise value reached USD 2.7 billion in 2019 from USD 300 million in 2016 (Samuel Anbu Selvan and Andrew 2021). The take-out food business is expanding quickly due to the affordability and convenience of choosing food from the comfort of home, and the age group from 15 to 34 accounts for the majority of online orders (Gallego-Schmid et al. 2019). This trend has been observed globally, and the convenience and affordability of take-out food have led to its increased popularity among consumers.

The COVID-19 pandemic has significantly impacted the food industry, with food services restricted to take-out to comply with public health and safety regulations and using personal reusable items prohibited (Molloy et al. 2022). As a result, online meal delivery services have become increasingly popular, as individuals were compelled to stay indoors to curb the spread of the virus. The pandemic has highlighted the need for contactless and hygienic food delivery options, leading to a surge in online food delivery services.

The choice of materials for take-out food containers is an essential environmental consideration in the food industry. The production and use of single-use takeaway containers, made from plastic materials and polymers like styrofoam and polyethylene, have significant environmental impacts. Plastic is the most commonly used material in take-out food containers, with polypropylene accounting for more than 60% of plastic containers. Styrofoam comprises 10 to 40% of take-out containers, while paper makes up more than 20% of the containers (Han et al. 2021). Single-use polypropylene containers have the lowest potential for recycling, contribute significantly to environmental pollution, and result in global warming. Chemical additives such as plasticizers, stabilizers, and flame retardants are commonly used in plastic and paper packaging to achieve the desired product qualities (Struzina et al. 2022). Recycled plastics and paper used to make take-out containers to reduce costs may contain harmful substances such as phthalates, plasticizers, and photoinitiators (Blanco-Zubiaguirre et al. 2021). Therefore, there is a need for more sustainable and environmentally friendly takeaway container options to minimize the adverse impact of single-use takeaway containers on the environment and human health.

Using synthetic plasticizers like bisphenol A (BPA) and phthalic acid esters (PAEs) in food and beverage packaging leads to human exposure when these plasticizers migrate from containers into the contents. Figure 1 illustrates the circumstances that result in the release of BPA and PAEs from plastic containers into foods and the potential impacts on human health. BPA is an endocrine disruptor that has a weak binding affinity for estrogen receptors (Hafezi and Abdel-Rahman 2019), while PAEs are plasticizers used for improving plasticity, flexibility, softness, and elasticity (Caldeirão et al. 2021). BPA and PAEs have been detected in food samples and are considered endocrine-disrupting chemicals with anti-androgenic properties. Even though BPA is mainly used in polypropylene and polycarbonate-based products, the occurrence of BPA and other bisphenol analogs (BPs) in polystyrene-made plastic products, such

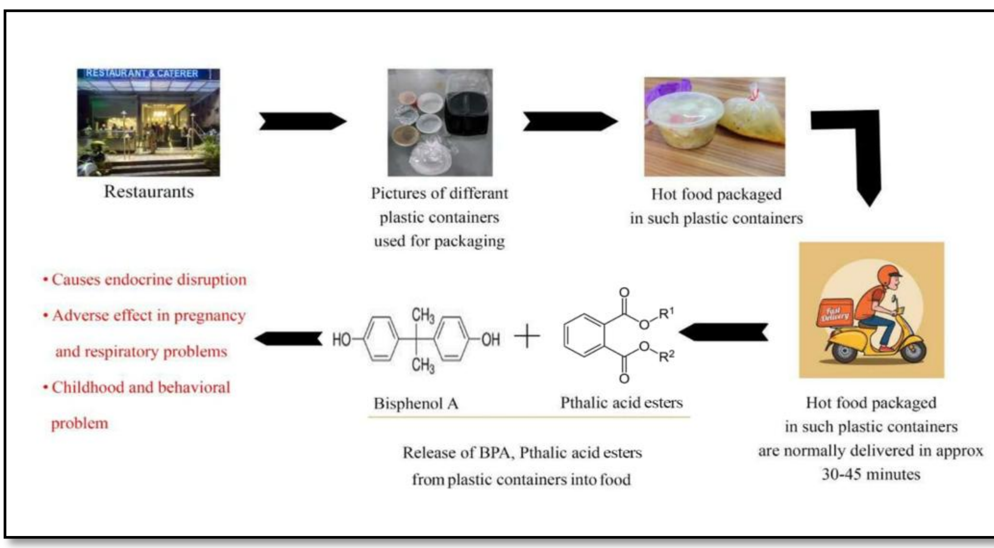


Figure 1 The series of events that result in the release of BPA and PAEs from plastic containers into foods and the potential implications on human health

as styrofoam, has also been reported (Zhao et al. 2023). Further, Wang et al. (2021) found that the migration of PAEs from packaging to food is influenced by the cooking method, with higher amounts of PAEs detected in Chinese foods cooked through methods like pan-frying, deep-frying, stir-frying, steaming, and boiling compared to simpler cooked foods consumed in US and Europe.

There is a need for more research in India on migrating BPA and PAEs from food-contact materials (FCMs) used for packaging take-out foods. Although some studies have been conducted in China regarding the migration of BPA and PAEs, it is essential to note that India, a tropical country with various hot and spicy cuisines, uses different packaging materials. Therefore, it is necessary to investigate how various packaging materials would interact with the hot and spicy foods consumed in India to understand the migration of plasticizers like BPA and PAEs from packaging materials into take-out foods. This study aims to address this research gap by using a questionnaire model to quantify BPA and Phthalic acid migration from FCMs used for packaging take-out foods commonly consumed by the Indian population. The study is the only one in India that quantifies the migration of BPA and Phthalic acid from various FCMs at different time intervals required for delivery. The research adheres to the recommended food simulants by the Bureau of Indian Standards (BIS) for migration detection. Finally, the study evaluates the potential mutagenicity of the leached plasticizers using the Ames test.

2 Materials and methods

2.1 Sampling

A nationwide online survey was conducted to understand the eating habits of people who consume takeaway food. The survey collected information about the Indian population's eating habits and used it to assess health risks to the population. The questionnaire asked general questions about the respondents, such as their gender, age, occupation, and area of residence. The age categories used were based on the USEPA classification of age groups. Participants were then asked about their regular food choices, how often they ate takeaway food, their taste preferences, average delivery times, and the packaging materials of the food. The detailed results of the survey are listed in Table 1. A total of 450 valid questionnaires were collected; most respondents were college students, company staff, workers, and freelancers. We purchased disposable containers online and selected ten containers of each type, dividing them into six main categories: polycarbonate plastics (PC), polypropylene containers (PP), polystyrene trays (PS), polyethylene pouches (PE), aluminum foils, and paper-pulp-based containers. We randomly selected three to four containers from each category for use in the experiment, resulting in 105 containers. The food container material was coded as FCM-X based on its manufacturing material. (Figure 2).

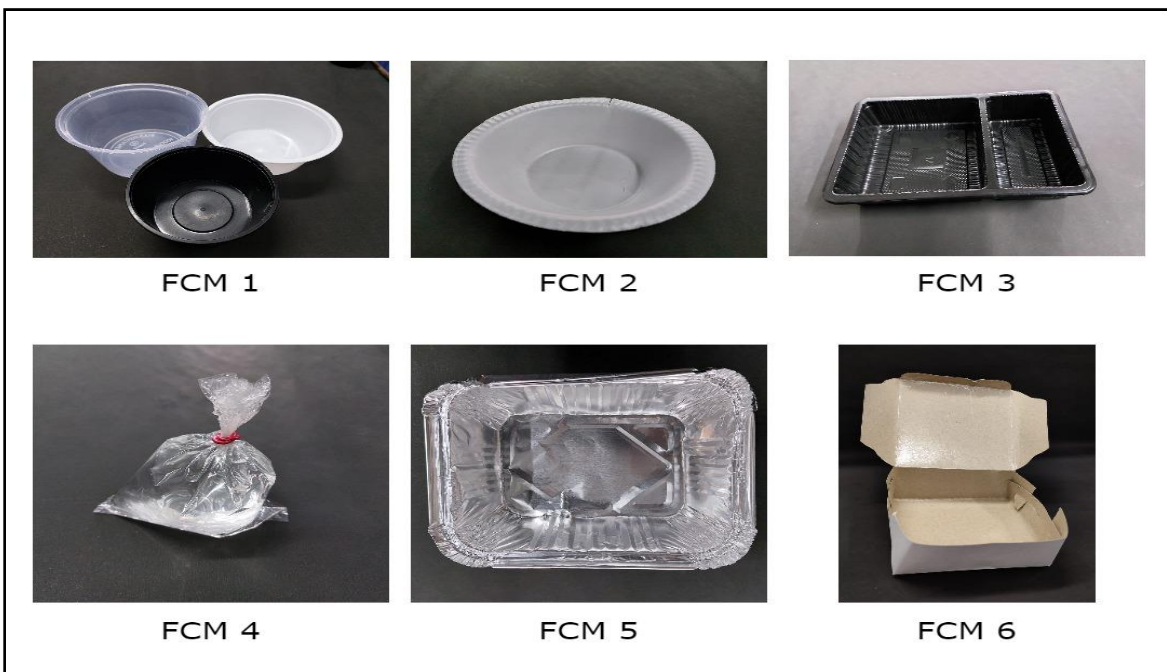


Figure 2 Design and material code FCM -X classifies six distinct food container types. These include FCM 1: rigid, transparent polycarbonate containers (2-5 mm thick), FCM 2: circular white polystyrene containers (0.3-5 mm thick), FCM 3: square polypropylene trays (1-5 mm thick), FCM 4: clear polyethylene pouches (25 and 50 µm thick), FCM 5: rectangular aluminum foil containers (30-50 µm thick), and FCM 6: degradable paper pulp containers with a high-density polyethylene inner lining (0.03 mm thick)

Table 1 The questionnaire details are presented in a table format

Characteristic	Categories	Sub total		Frequency of take-out food				Food preference				Delivery time (minutes)			
		n	%	<1/w	1/w	2/w	>2/w	PF	DF	SF	ST	<15	15-30	30-45	>45
Gender	Male	251	54.56	133 (52.98%)	89 (35.45%)	19 (7.57%)	10 (3.98%)	101 (40.23%)	62 (24.70%)	25 (9.97%)	63 (25.09%)	62 (24.70%)	81 (32.27%)	98 (39.04%)	10 (3.98%)
	Female	209	45.43	97 (46.41%)	83 (39.71%)	16 (7.65%)	13 (6.22%)	79 (37.79%)	48 (22.96%)	22 (10.52%)	60 (28.70%)	37 (17.70%)	62 (29.67%)	91 (43.54%)	19 (9.09%)
Age (years)	<17	16	3.47	11 (68.75%)	5 (31.25%)	0 (0.00%)	0 (0.00 %)	1 (6.25 %)	5 (31.25%)	5 (31.25%)	5 (31.25%)	8 (50%)	3 (18.75%)	5 (31.25%)	0 (0.00 %)
	17-22	194	42.17	131 (67.52%)	43 (22.16%)	9 (4.63%)	11 (5.67 %)	77 (39.69%)	48 (24.74%)	17 (8.76%)	52 (26.80%)	31 (15.97%)	79 (40.20%)	67 (34.53%)	17 (8.76%)
	23-28	109	23.69	71 (65.13%)	23 (21.10%)	13 (11.9%)	2 (1.83%)	52 (47.70%)	20 (18.34%)	18 (16.51%)	19 (17.43%)	21 (19.26%)	20 (18.34%)	59 (54.12%)	9 (8.25%)
	28-35	85	18.47	43 (50.58%)	22 (25.88%)	10 (11.76%)	10 (11.76%)	48 (56.47%)	19 (22.35%)	15 (17.64%)	3 (3.52%)	2 (2.35%)	27 (31.76%)	46 (54.11%)	10 (11.76%)
	35-40	11	2.39	5 (45.45%)	5 (45.45%)	1 (9.09%)	0 (0.00%)	3 (20 %)	3 (27.27%)	0 (0.00%)	5 (45.45%)	2 (18.18%)	4 (36.37%)	5 (45.45%)	0 (0.00%)
	>40	45	9.78	17 (37.37%)	23 (51.11%)	5 (11.11%)	0 (0.00%)	14 (31.11%)	10 (22.22%)	11 (24.44%)	10 (22.22%)	13 (28.89%)	28 (62.22%)	4 (8.89%)	0 (0.00%)
State of Residence	Eastern India	396	86.08	226 (57.07%)	110 (27.77%)	33 (8.33%)	27 (6.81%)	161 (40.65%)	89 (22.47%)	45 (11.36%)	101 (25.50%)	60 (15.15%)	105 (26.51%)	207 (52.27%)	24 (6.06%)
	Western India	10	2.17	5 (50%)	5 (50%)	0 (0.00%)	0 (0.00%)	6 (60%)	2 (20%)	1 (10%)	1 (10%)	4 (40%)	5 (50%)	1 (10%)	0 (0.00%)
	Northern India	47	10.21	26 (55.13%)	16 (34.04%)	5 (10.63%)	0 (0.00%)	14 (29.78%)	17 (36.17%)	10 (21.27%)	6 (12.76%)	15 (31.90%)	20 (42.56%)	12 (25.53%)	0 (0.00%)
	Southern India	7	1.52	3 (42.85%)	3 (42.85%)	1 (14.28%)	0 (0.00%)	1 (14.28%)	3 (42.85%)	0 (0.00%)	3 (42.85%)	1 (14.28%)	2 (28.57%)	5 (71.42%)	0 (0.00%)

The first number signifies quantity, while the number in brackets denotes the percentage. The frequency of take-out food consumption is denoted as <1/w (less than once a week), 1/w (once a week), 2/w (twice a week), and >2/w (more than twice a week). Food preference abbreviations are PF (pan-fried), DF (deep-fried), SF (stir-fried), and ST (steamed)

Table 2 Classification of foods and selection of simulants (Park et al., 2018).

Food stimulant	Description	Food types
Distilled water or water of equivalent quality	Aqueous, non-acidic foods (pH>5) without fat	Honey, mineral water, sugar syrups molasses, skimmed milk, sweets, etc
3% acetic acid	Aqueous, acidic foods (pH≤5) without fat	Fruit juices, squashes, vinegar, jams, jellies, carbonated beverages, processed vegetables, preparation of soups, broths, sauces etc.
10% ethanol	Alcoholic beverages having alcohol concentration less than 10 percent	Beer and some pharmaceutical syrups
50% ethanol	Alcoholic beverages having alcohol concentration above 10 percent	Wine, brandy, whiskey, arrack and other alcoholic drinks
n-heptane	Oils, fats, and processed dry foods with surface fat or volatile oil	Vegetable oils, ghee, cocoa butter, lards, biscuits, spice powder, snacks and savoury.

2.2 Reagents, standards and food simulants

BPA and Phalic acid (99% pure) were purchased from SRL (Sisco Research Laboratories), India. Methanol, ethanol, acetic acid, and n-heptane (HPLC grade) were purchased from Merck. Water (HPLC grade) for chromatography was also purchased from Merck. A stock solution of BPA and Phthalic acid was prepared by dissolving 10 mg of each substance in 100 ml of methanol and stored in dark amber bottles. Standard working solutions of five different concentrations of 0.05, 0.2, 0.5, 1, and 5 µg/ml were prepared by dilution with water. The migration was determined using five different simulants (Table 2) as per the standards laid down by BIS (IS 9845:1998) (Park et al. 2018).

2.3 Migration test

All take-out FCMs which were labeled as per the FCM-X series (FCM-1: PC, FCM-2: PS, FCM-3: PP, FCM-4: PE, FCM-5: Aluminum foil, and FCM-6: Paper-pulp) were rinsed with distilled water (25-30ºC) to remove extraneous materials before the migration analysis. The food simulants were preheated before the migration analysis to provide the general conditions for actual analysis. Water, 3% acetic acid, 10% and 50% ethanol were preheated at 70ºC and n-heptane for 25ºC (Park et al. 2018). All the containers and pouches were filled to their capacity with the preheated simulants and lids closed. In the case of pouches, air was excluded before sealing it. The containers and pouches were exposed to 70ºC maintained in a hot air oven for 15, 30, and 45 minutes, respectively. After exposure for the specified duration, the contents were transferred immediately into clean Pyrex beakers and were allowed to cool to room temperature. After cooling, the simulants were filtered through a 0.22 µm PVDF sterile syringe filter and were injected into the HPLC-UV for BPA and Phthalic acid analysis.

2.4 BPA and Phthalic acid determination

BPA and Phthalic acid in the simulants were analyzed by HPLC-UV using the protocol explained by Park et al. (2018) operating at 210 nm with a BioSuite C18 column (Table 3). The mobile phase was an isocratic elution of 30% trifluoroacetic acid (TFA) in HPLC grade water and 70% acetonitrile (ACN) at a flow rate of 1ml min^{-1}.

2.5 Ames Salmonella assay

Salmonella typhimurium strains TA 98 and TA 100 were obtained from Microbial-type culture collection (MTCC) in Chandigarh, India, to perform the top agar method of Ames *Salmonella* mutagenicity test. These histidine auxotrophic strains are typically used to detect chemical mutagens and assess their mutagenic potential (Zainol et al. 2021). *S. typhimurium* cultures (MTCC 1251 and MTCC 1252) were grown for 10 hours and used as the starter culture for the assay. 100 µl of the starter culture was mixed with 100 µl food stimulants, which showed considerable levels of

Table 3 Analytical parameters and conditions for BPA and Phthalic acid concentration determination in food simulants by HPLC-UV.

Substance detected	Parameter	Conditions
BPA and Phthalic acid	Column	Biosuite C18 PA-A (500 Å, 7 µm, 4.6 mm x 150 mm)
	Detector	UV (210nm)
	Pump	Agilent 1290 Infinity II
	Mobile phase	30% TFA in HPLC grade water + 70% ACN
	Injection volume	50 µl using Hamilton syringe
	Flow rate	1 ml min^{-1}
	Run time	8 minutes

BPA and Phthalic acid in the HPLC-UV detection method (concentration above quantification limit) along with 500 µl of S9 liver pooled fraction (for metabolic activation assays) or 500 µl of phosphate buffer (in control set for assays without metabolic activation). The solution was added to 2 ml molten top agar (0.6%) tubes seeded with histidine and then vortexed gently and poured on sterile Glucose Minimal Agar (1.5%) plates. The plates were incubated for 48 hours at 37ºC (Nepalia et al. 2018). After 48 hours, the revertant colonies on the test (with food simulants) and control plates (without food simulants) were counted. The experimental samples (food simulants) were tested with and without metabolic activation with S9 liver, pooled fraction (Sigma-Aldrich S2067) for their mutagenic potential.

2.6 Calculations

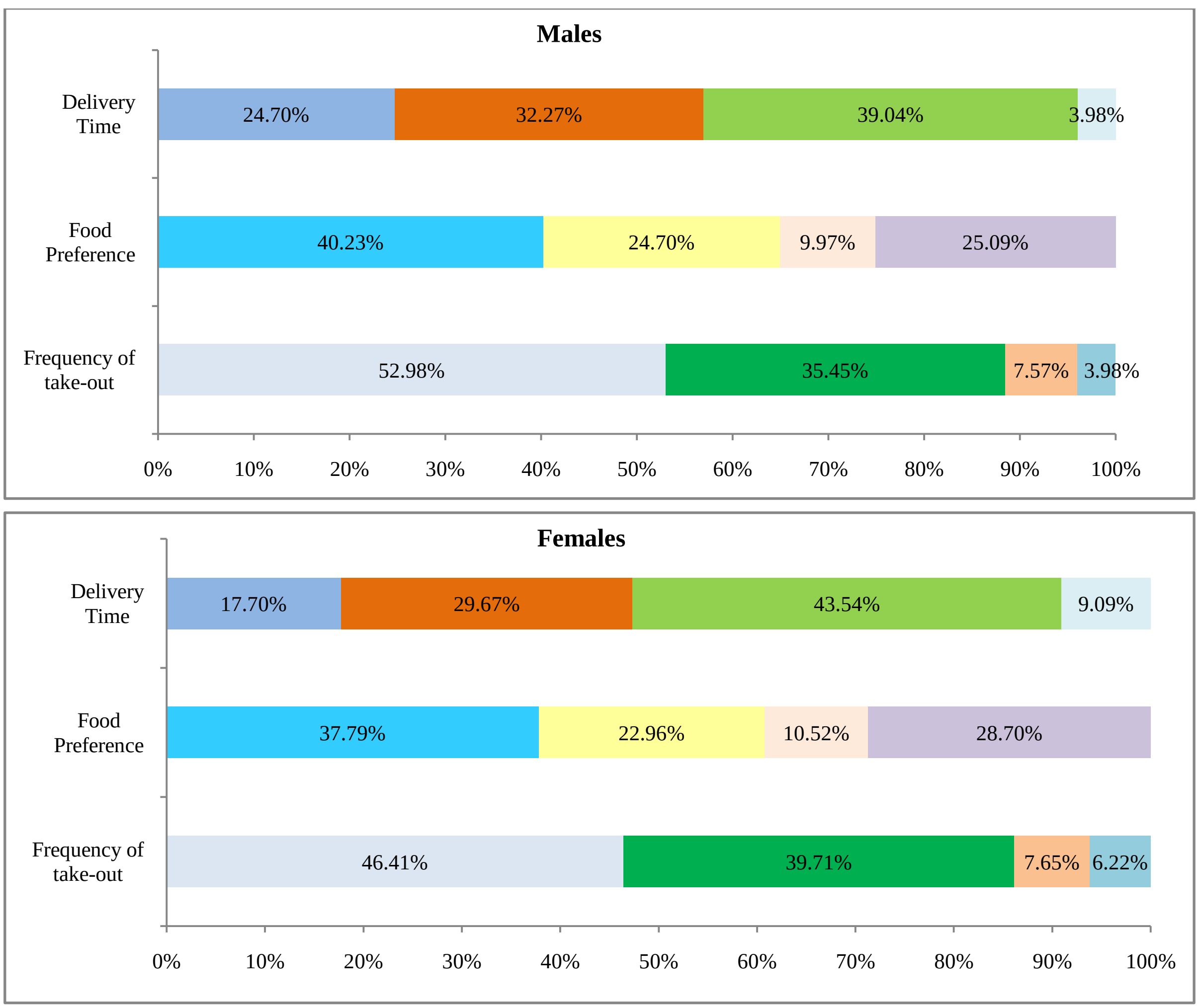
2.6.1 Non-statistical analysis

The assay results are presented in terms of mutagenicity ratio (MR) with standard deviation (Samiei et al. 2015). The MR is calculated by dividing the number of reverse mutation colonies on sample-treated plates (x) by the number of reverse mutation colonies in the negative control plate (x_0). The two-fold rule is followed, which means that if the reproductive doubling of spontaneous reversion rate (MR ≥ 2) occurs in the sample at one or two doses, it is considered a positive response (Nepalia et al. 2018).

3 Results and discussion

3.1 Results of the questionnaire

Within the sample of 450 respondents, 251 were male and 209 were female. They reported their long-term residence in various regions of India, including Eastern, Western, Northern, and Southern areas. Because the number of respondents in the Western and Southern regions was less than 20, they were excluded from the statistical analysis. Drawing upon the prevalent trends and insights gathered from questionnaire data, it is discernible that the age group from 17 to 28 represents a considerable share of online take-out food orders. As indicated in Figure 3A shows that 52.98%



Delivery Time (mins)	<15		15-30		30-45		>45	
Food Preference	PF		DF		SF		ST	
Frequency oftake-out	<1/w		1/w		2/w		>2/w	

Figure 3AThe colored bars in the graph illustrate the percentage distribution of preferences between male and female respondents about the responses they gave in the questionnaire when asked about the frequency of take-out food, food choices, and the average delivery time for take-out food.

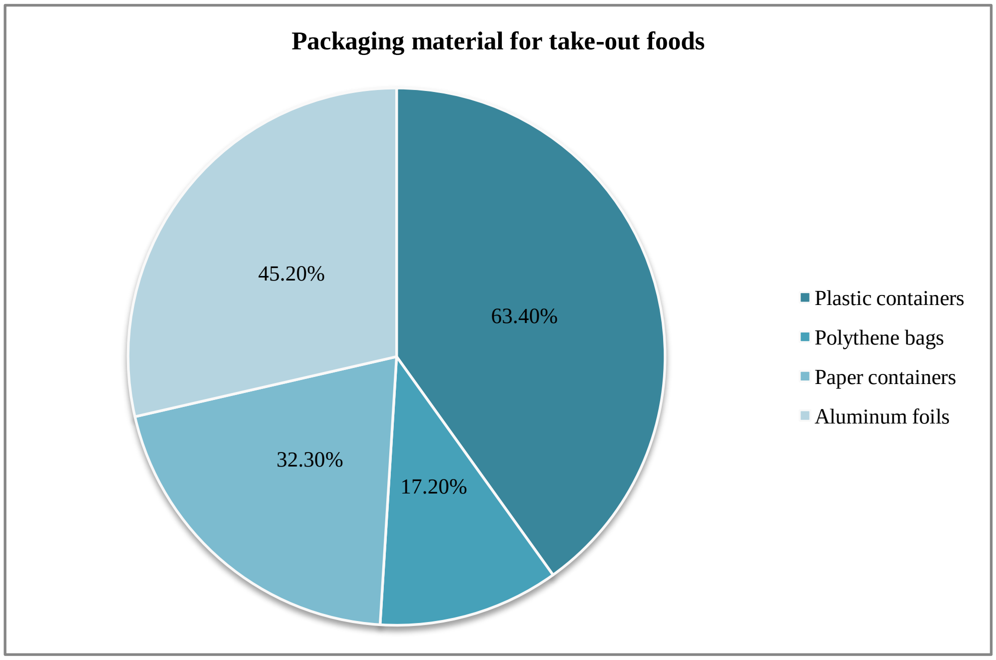


Figure 3B The pie chart displays the percentage breakdown of packaging materials used for take-out food based on survey respondents

of male respondents reported ordering take-out food less than once a week, while 35.45% ordered once a week, 7.57% ordered twice a week, and 3.98% ordered more than twice a week. Furthermore, the majority of male respondents preferred their take-out food to be pan-fried (40.23%), followed by steamed (25.09%), deep-fried (24.70%), and stir-fried (9.97%). Similar results were observed among female respondents, with 46.41% ordering take-out food less than once a week, 39.71% once a week, 7.65% twice a week, and only 6.22% more than twice a week. Female respondents also demonstrated a preference for pan-fried (37.79%) and steamed (28.70%) take-out food, followed by deep-fried (22.96%) and stir-fried (10.52%) options.

As many take-out foods are typically packaged in various containers, the questionnaire included inquiries regarding the take-out food packaging and the average delivery duration. Findings in Figure 3B shows that the most commonly used packaging method for take-out food was directly packed into plastic containers, followed by aluminum foils, paper-pulp containers, and PE pouches. In addition, most respondents reported that the average delivery time for their take-out food ranged from 30 to 45 minutes.

3.2 HPLC-UV method validation

The analytical methodology employed for detecting BPA and Phthalic acid has been rigorously validated using various parameters, including linearity, limit of detection (LOD), limit of quantification (LOQ), recovery, and precision (AOAC 2016). Linearity was assessed by determining the correlation coefficient (R^2) obtained from the calibration curve, constructed using five standard concentrations (0.05, 0.2, 0.5, 1, and 5 µg/ml) for both BPA and Phthalic acid. R^2 for BPA was > 0.99, and for Phthalic acid $R^2 > 0.994$, emphasizing the strong linearity in the calibration curves for these analytes. The LOD and LOQ were calculated employing the formulas 3.3 σ/S and 10 σ/S, respectively, where σ represents the standard deviation of the response, and S signifies the slope of the calibration curve. As reported in Table 4, the validation parameters LOD and LOQ values for BPA were determined to be 0.475 and 1.44 µg/ml, respectively, while for Phthalic acid, the corresponding values were 0.251 and 0.763 µg/ml. In order to assess recovery, experiments were conducted by introducing standard solutions into samples in triplicate. The average recovery percentages for BPA and Phthalic acid ranged from 74.98% to 125.39% and from 78.60% to 123.57%,

Table 4 Recovery and precision detection of BPA and Phthalic acid in spiked food simulants

Substance	Spiked level (mg/L)	Water		3% acetic acid		10% ethanol		50% ethanol		n-heptane	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
BPA	0.05	96.35	3.60	74.98	1.78	110.67	5.22	123.37	1.82	87.94	0.90
	0.5	118.88	2.57	88.89	2.88	99.60	0.76	115.74	4.10	104.39	4.77
	5	125.39	6.45	117.01	1.30	103.76	2.01	117.64	4.33	97.70	1.58
Phthalic acid	0.05	101.31	6.72	82.90	0.78	78.60	2.71	92.13	3.01	123.57	5.26
	0.5	97.60	2.55	100.69	8.93	102.22	3.35	98.90	6.25	100.10	3.76
	5	102.67	4.89	105.75	4.78	87.98	2.18	83.92	1.01	111.07	7.10

respectively. Precision was gauged by calculating the relative standard deviations (RSD %) of the obtained recovery values.

3.3 Migration of BPA

Maximum BPA was leached from packaging containers when 3% acetic acid was used as a food stimulant. The level of BPA migration increased when the exposure time was increased from 15, 30 and 45 minutes. Maximum BPA migration was observed for PE pouches (47.07 µg/ml) followed by aluminum foils (46.67 µg/ml), PP trays (45.98 µg/ml), and PS containers (45.59 µg/ml) when preheated food simulants were exposed in them for 45 minutes. When 50% ethanol was used as a food stimulant, PE pouches (3.36 µg/ml) and aluminum foils (1.74 µg/ml) showed increased BPA leaching only at 45 minutes of exposure time. Similarly, Cao et al. (2021) reported BPA migration from PC samples into aqueous or fatty foods when food simulants such as 4% acetic acid (12-113 µg/kg), 10% ethanol (14-407 µg/kg), and olive oil (1-30 µg/kg) was used. Park et al. (2018) observed BPA migration from different PC samples when heated with varying simulants of food such as water (83.7 µg/L), 4% acetic acid (40.9 µg/L), 50% ethanol (54.3 µg/L), and n-heptane (142 µg/L). Interestingly, paper-pulp-based containers showed BPA migration (1.66 µg/ml) for 10% ethanol when exposed for 45 minutes, which is attributed to the presence of hydrophobic inner plastic film lining, mostly made of PE and sometimes co-polymer alternatives. Similar results were reported by (Ranjan et al. 2021), where they observed that disposable paper cups commonly used to consume hot beverages (85-90ºC) are normally laminated with hydrophobic high-density polyethylene (HDPE) lining. At such a high temperature, when exposed for 15 minutes, it causes deterioration of the hydrophobic film. It causes the leaching of microplastics ($102 + 21.1 \times 10^6$ microplastic particles/ml) and ions like fluoride, chloride, sulfate, and nitrate into the liquid. However, no leaching of BPA from packaging containers was observed when water, 10% ethanol, and n-heptane were used as food simulants. The concentrations shown in Table 5 are positive mean values for each packaging sample and food stimulant. According to these results, the choice of food stimulant is an essential factor for BPA migration, as migration levels vary depending on the choice. Other than the choice of food stimulant, the exposure time of the preheated food simulants with different packaging materials also plays a vital role in BPA migration. The greater the time of exposure, the greater the level of migration.

3.4 Migration of Phthalic acid

Concentrations of migrated Phthalic acid from different FCMs are shown in Table 6. When water and n-heptane were used as food stimulants, none of the simulants extracted from different FCMs showed Phthalic acid concentration above the LOQ level (0.763 µg/ml). After exposure to 3% acetic acid for 15, 30, and 45 minutes, Phthalic acid was detected in all food contact materials stimulants. The highest Phthalic acid concentration was recorded for PE pouches (50.75 µg/ml), closely followed by paper-pulp containers (50.31 µg/ml) and PP trays (49.59 µg/ml) after 45 minutes of exposure. With 50% ethanol migrated, Phthalic acid was detected only in paper-pulp containers when exposed for 45 minutes. Phthalic acid was detected in food simulants extracted from PE pouches, paper-pulp containers, aluminum foils, and PC containers after 45 minutes of exposure to 10% ethanol.

3.5 Risk impact assessment

The Ames assay was performed to assess the mutagenicity of the migration of plasticizers into food simulants from different FCMs commonly used in packaging. As indicated in Table 7, the results show that few simulants extracted were significantly mutagenic. Thus, a risk impact assessment becomes pertinent as this is a matter of great concern, as mutagenesis is a critical component of carcinogenesis. When 3% acetic acid was used as a simulant (without S9 exposure), most of the tested FCMs demonstrated mutagenicity ratios below 2, while only a few samples demonstrated mutagenicity ratios greater than 2, mainly after 45 minutes of exposure. PS-based FCMs were the most mutagenic, exhibiting mutagenicity ratios greater than 2 for both *S. typhimurium* strains (TA 98 and TA 100) tested after 30 and 45 minutes of exposure. This was followed by aluminum foils, which

Table 5 Concentration (µg/ml) of BPA in different food simulants extracted from different food-contact materials and analyzed by HPLC-UV

Samples	Water			3% acetic acid			10% ethanol			50% ethanol			n-heptane		
	15 mins	30 mins	45 mins	15 mins	30 mins	45 mins	15 mins	30 mins	45 mins	15 mins	30 mins	45 mins	15 mins	30 mins	45 mins
PC containers	ND	ND	ND	18.67±0.05	30.76±0.04	35.53±0.05	ND	ND	ND	ND	ND	ND	ND	ND	ND
PS containers	ND	ND	ND	33.14±0.06	40.71±0.05	45.59±0.04	ND	ND	ND	ND	ND	ND	ND	ND	ND
PP trays	ND	ND	ND	28.98±0.06	34.79±0.04	45.98±0.05	ND	ND	ND	ND	ND	ND	ND	ND	ND
PE pouches	ND	ND	ND	29.59±0.06	32.34±0.05	47.07±0.04	ND	ND	ND	ND	ND	3.36±0.05	ND	ND	ND
Aluminium foils	ND	ND	ND	29.48±0.06	39.09±0.05	46.67±0.04	ND	ND	ND	ND	ND	1.74±0.05	ND	ND	ND
Paper-pulp containers	ND	ND	ND	30.86±0.05	34.04±0.05	38.96±0.04	ND	ND	1.66±0.05	ND	ND	ND	ND	ND	ND

ND: Not Detected or below LOQ (1.44 µg/ml)

Table 6 Concentration (µg/ml) of Phthalic acid in different food simulants extracted from different food-contact materials and analyzed by HPLC-UV

Samples	Water			3% acetic acid			10% ethanol			50% ethanol			n-heptane		
	15 mins	30 mins	45 mins	15 mins	30 mins	45 mins	15 mins	30 mins	45 mins	15 mins	30 mins	45 mins	15 mins	30 mins	45 mins
PC containers	ND	ND	ND	19.89±0.06	33.02±0.05	38.21±0.06	ND	ND	1.08±0.05	ND	ND	ND	ND	ND	ND
PS containers	ND	ND	ND	35.62±0.05	43.84±0.05	49.14±0.04	ND	ND	ND	ND	ND	ND	ND	ND	ND
PP trays	ND	ND	ND	31.09±0.05	37.40±0.06	49.59±0.04	ND	ND	ND	ND	ND	ND	ND	ND	ND
PE pouches	ND	ND	ND	31.75±0.06	34.74±0.04	50.75±0.05	ND	ND	3.25±0.04	ND	ND	ND	ND	ND	ND
Aluminium foils	ND	ND	ND	33.13±0.06	36.59±0.05	41.94±0.04	ND	ND	1.53±0.05	ND	ND	ND	ND	ND	ND
Paper-pulp containers	ND	ND	ND	31.64±0.06	42.07±0.05	50.31±0.05	ND	ND	1.66±0.05	ND	ND	1.40±0.04	ND	ND	ND

ND: Not Detected or below LOQ (0.763 µg/ml)

Table 7 Mutagenicity ratios (MR) for *Salmonella typhimurium* strains TA98 and TA100, both with and without S9 metabolic activation

Food-contact materials	Exposure time (mins)	Mutagenicity ratio TA98		Mutagenicity ratio TA100	
Food simulant used: 3% acetic acid					
		Without S9	With S9	Without S9	With S9
PC containers	15	1.13±0.06	2.00±0.30	1.57±0.03	2.33±0.46
	30	1.31±0.11	3.42±0.30	1.78±0.09	4.83±0.93
	45	1.63±0.12	4.14±0.51	3.67±0.17	6.42±0.06
PS trays	15	1.36±0.06	2.42±0.20	1.71±0.04	3.67±0.47
	30	1.81±0.09	5.14±0.09	2.07±0.22	5.75±0.30
	45	3.85±0.18	8.09±0.33	3.92±0.04	7.91±0.58
PP containers	15	1.09±0.10	2.42±0.90	1.42±0.21	2.40±0.19
	30	1.22±0.10	4.28±0.21	1.57±0.05	4.67±0.24
	45	1.63±0.09	4.71±0.38	1.92±0.05	8.83±0.07
PE pouches	15	1.04±0.08	1.42±0.09	1.85±0.22	4.33±0.25
	30	1.18±0.15	2.57±0.09	2.42±0.25	5.21±0.32
	45	1.45±0.07	3.28±0.20	2.71±0.07	5.98±0.20
Aluminium foils	15	1.22±0.35	2.39±0.41	1.35±0.04	3.00±0.47
	30	1.90±0.46	3.07±0.03	1.71±0.28	4.67±0.47
	45	2.04±0.11	7.85±0.64	2.03±0.05	4.83±0.21
Paper-pulp containers	15	1.09±0.01	1.14±0.07	1.35±0.04	2.33±0.93
	30	1.13±0.09	2.28±0.11	2.07±0.25	4.50±0.70
	45	1.95±0.10	4.59±0.25	2.28±0.19	7.34±0.47
Food simulant used: 50% ethanol					
PE pouches	45	1.40±0.09	2.76±0.16	1.08±0.11	2.17±0.09
Aluminium foils	45	1.13±0.16	3.67±0.33	1.43±0.08	2.04±0.21
Paper-pulp containers	45	1.03±0.12	1.85±0.10	1.29±0.08	1.96±0.15
Food simulant used: 10% ethanol					
PC container	45	1.10±0.14	2.08±0.16	1.42±0.21	2.28±.11
PE pouches	45	1.58±0.08	2.56±0.09	1.35±0.04	1.98±0.30
Aluminium foils	45	1.70±0.16	2.66±0.10	1.57±0.09	2.42±0.90
Paper-pulp containers	45	1.04±0.06	1.54±0.09	1.18±0.11	1.95±0.01

Values showing mutagenicity ratio ≥ 2 indicates significant genotoxicity.

showed mutagenicity ratios greater than 2 for both strains only after 45 minutes of exposure. PE pouches and Paper-pulp containers showed mutagenicity ratios greater than 2 for TA 100 strain only after 30 and 45 minutes of exposure. PC containers demonstrated mutagenicity ratios more significant than two only after 45 minutes of exposure. When food simulants were used for 50% and 10% ethanol (without S9 exposure), no tested FCMs demonstrated a mutagenicity ratio greater than 2. The addition of the S9 mix increased the number of induced revertants, thereby increasing the mutagenicity ratio. Among the tested FCMs, when 3% acetic acid was used as a simulant, the highest mutagenicity ratio recorded with the S9 mix for TA 98 strain was for PS containers after 45 minutes of exposure, and for TA 100 was for PP containers after 45 minutes of exposure. When using 50% ethanol and 10% ethanol with an S9 mix, the highest mutagenicity ratio was noted for PE pouches and aluminum foils after 45 minutes of exposure, respectively.

Conclusion

The research findings underscore the prevalent preference for hot and spicy pan-fried take-out foods among the Indian population, particularly among individuals aged 17-28, consumed at least once a week. These foods, commonly packaged in non-biodegradable plastic containers, are associated with increased migration of harmful substances like BPA and Phthalic acid, exacerbated by high-flame cooking methods and acetic acid infusion. The study, involving HPLC-UV analysis on various FCMs, highlighted higher migration levels of these substances in 3% acetic acid food simulants, particularly within delivery durations of 15 to 45 minutes. The Ames test indicated significant mutagenicity in specific samples, with effects escalating upon metabolic activation, suggesting adverse impacts on mammalian systems. These outcomes provide crucial insights for safety regulators and risk evaluators to assess health risks associated with these food containers, prompting further consideration for in vivo assays to deepen the understanding of plasticizers' effects on mammalian health.

Disclosure statement

No potential conflicts of interest are reported by the authors.

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