



# Bisphenol A in Indian Take-Out Soups: Compliance, Implications and Sustainable Solutions

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## ABSTRACT

This research investigates the migration of Bisphenol A (BPA) from packaging containers into take-out vegetable soups and premixed tomato soups through three replicate studies. The samples underwent extraction using solid-phase extraction (SPE) cartridges, followed by separation on a C18 column. BPA concentrations in the soups were assessed at 15, 30, and 45-minute intervals, consistently revealing undetectable levels (<LOQ). Plastic packaging samples, known for BPA utilization in production, remained below the Specific Migration Limit (SML) set at 0.5 mg.kg<sup>-1</sup>, irrespective of material type or contact conditions. These results, conforming to EC regulations, suggest that food-contact materials (FCMs) in the Indian market pose no apparent health hazards during initial use. The absence of detectable BPA levels is attributed to the limited time-temperature relationship during the study. However, caution is warranted as BPA migration can occur with repeated use, emphasizing the importance of considering material quality and intended use of FCMs. The study underscores the significance of understanding BPA leaching under varied conditions, necessitating further research to explore long-term implications. Overall, the findings provide valuable insights for regulators, manufacturers, and consumers, contributing to the ongoing discourse on food safety and using plastic materials in food packaging.

## INTRODUCTION

Bisphenol A (BPA) is prominently featured among the most commonly utilized substances in contemporary industrial practices, serving as a fundamental constituent in the composition of epoxy resins and plastic containers. Its prevalence is attributable to its robust and transparent physical properties (Sharma et al. 2023). In 2020, the projected production of polycarbonate plastics (PC) in the United States amounted to roughly one million metric tons. PC boasts diverse applications, owing to its attributes such

as heat resistance, lightweight construction with exceptional durability, transparency, impact resistance, and inherent flame retardancy. These qualities are harmoniously balanced, contributing to the continuous growth of PCs and the exploration of novel, innovative applications. Consequently, its production has been experiencing an annual increase of approximately 4% (Koizumi et al. 2023). However, extensive investigations have identified BPA as a major environmental pollutant capable of permeating soil and water systems, with the potential to enter the food chain over an extended period. According to research findings, individuals' primary avenue

of bisphenol exposure is consuming food and beverages contaminated with BPA (Hahladakis et al. 2023). BPA can potentially permeate from plastic containers into the associated food or beverage, especially under conditions involving elevated temperatures or acidic substances (Dey et al. 2021, Kumar et al. 2023b). This phenomenon raises concerns about human exposure to BPA, which is associated with adverse health issues, including elevated anxiety levels, modified exploratory behavior, diminished social interaction, heightened aggression, and impairments in spatial or recognition learning and memory (Bakoyiannis et al. 2021). The BPA migration process encompasses both physical and chemical migration (Khalili et al. 2023). Physical migration results from the diffusion of residual BPA monomers after manufacturing. Conversely, chemical migration occurs due to BPA release from the polymer surface, predominantly due to hydrolysis. Hence, the presence of BPA in food items can be ascribed to either the migration of residual BPA or the hydrolysis of the polymers.

The European Chemical Agency (ECHA) has classified BPA as a highly toxic chemical, and therefore, many regulatory authorities have set specific limits on its usage and migration. However, in 2009, the World Health Organization (WHO) concluded that imposing public limits on BPA is unnecessary. In the United States, due to its potential hazards, the Food and Drug Administration (FDA) withdrew its approval for using BPA in packaging materials for baby formula and feeding bottles in 2014. Similar restrictions have been implemented by the European Union (EU) and Canada. The United States Environmental Protection Agency (USEPA) has determined that a toxic level of BPA is up to  $50 \mu\text{g}\cdot\text{kg}^{-1}$  of body weight per day but advises caution as lower amounts may not be entirely safe (EU - COM 2014/015, 2014).

Furthermore, the European Commission (EC) has established a Specific Migration Limit (SML) for BPA in foods at  $0.6 \mu\text{g}\cdot\text{g}^{-1}$  of the food substance. The Indian government banned manufacturing, importing, and selling PC baby bottles containing BPA in 2010, citing concerns about potential health effects on infants. However, there are currently no specific regulations or bans on using BPA in other food-contact materials (FCMs), such as epoxy resins and polystyrene containers.

In recent years, there has been much discussion regarding the use of BPA in food-grade plastic. Some studies suggest that low levels of exposure to BPA are unlikely to cause harm, while other research links BPA exposure to various health issues, including developmental and reproductive problems, cancer, obesity, and diabetes (Sharma et al. 2023). Additionally, its capacity to migrate

from FCMs into food matrices and the environment poses human and environmental health risks. FCMs, constructed from postconsumer materials, are particularly at high risk of containing these compounds. The assessment of post-consumer recycled feedstocks in FCMs is mandatory, and the careful selection of a suitable detection method is essential for compliance with relevant regulations, ensuring the comprehensive evaluation of human and environmental safety (Tumu et al. 2023). The amount of BPA released into food from FCMs depends on various factors such as material type, preparation time, environment, and nature of the food. According to recent studies by (Kumar et al. 2023a), the storage temperature and storage time of bottled water in polyethylene terephthalate (PET) bottles can have an impact on the release of BPA. The brand of the PET bottle can influence the degree of BPA release, possibly due to raw material contamination during the manufacturing or recycling process. Higher temperatures and longer storage durations have been observed to correlate with increased release of BPA (Khanniri et al. 2023). Additionally, the diffusivity of BPA is found to escalate with elevated storage temperatures (Ahmad et al. 2023). These findings suggest that factors such as temperature and storage duration play crucial roles in the release dynamics of BPA from packaging materials.

The significance of this study is underscored by several factors, given that BPA is a widely employed chemical found in various products, including plastic containers, food packaging, and epoxy resin. The potential for BPA to leach into the environment from these products raises concerns about its ability to pose health hazards. This has heightened apprehensions about the potential health consequences of BPA exposure, particularly in susceptible populations such as pregnant women and children. Considering that India is among the largest producers and consumers of plastic products globally, coupled with a swiftly expanding population and economy, BPA exposure levels in India are anticipated to increase.

## MATERIALS AND METHODS

### Sample Collection

A quantity of 200 mL of vegetable soup designated for take-out from local food establishments was distributed equally (30 mL) across six distinct types of containers. These containers were labeled as follows: BN1 for polycarbonate (PC) plastics, BN2 for polystyrene (PS) plastics, BN3 for polypropylene (PP) plastics, BN4 for polyethylene (PE) pouches, BN5 for aluminum foils, and BN6 for areca leaf containers (Fig. 1). The containers were procured from the local market. The investigation focused on examining the

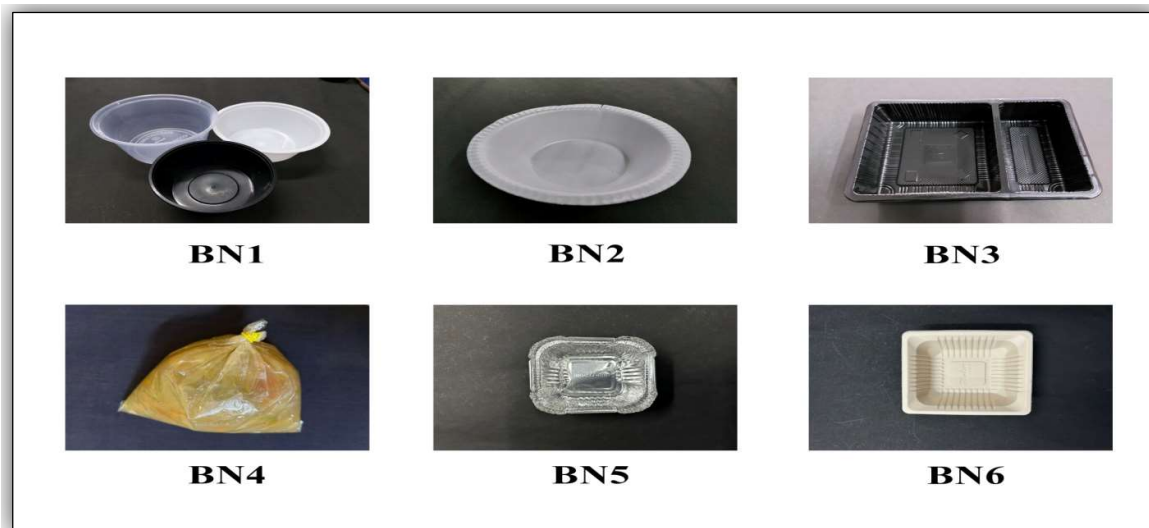


Fig. 1: BPA leaching in take-out soups from various packaging containers, with material codes assigned as: BN1 for polycarbonate (PC) plastics, BN2 for polystyrene (PS) plastics, BN3 for polypropylene (PP) plastics, BN4 for polyethylene (PE) pouches, BN5 for aluminum foils, and BN6 for areca leaf containers.

potential migration of BPA from the packaging containers into the vegetable soup over durations of 15, 30, and 45 min. To facilitate comparison, 18.6 g of premix tomato soup sample was dissolved in 200 mL of hot water and distributed, with 30 mL allocated to each of the same six containers for parallel analysis of BPA leaching.

### Sample Temperature and pH

The soup samples, upon being packaged in the containers, were subjected to heating at a temperature of 70°C in an oven, in accordance with the temperatures commonly encountered in food establishments. Subsequently, the pH of the sample was measured using a calibrated pH meter (Systronics type 335). The pH of the vegetable soup was found to be 6.12, and the premix tomato soup was 6.91.

### Reagents and Chemicals

BPA (purity  $\geq 99\%$ ) was purchased from Sigma-Aldrich (Bengaluru, India). Acetonitrile (ACN) and methanol were procured from Merck (Mumbai, India). Water was supplied using a Milli-Q Ultrapure water purification system (Millipore, Bedford, MA, USA). Solid phase extraction (SPE) (Oasis MAX) cartridge (30 mg.mL<sup>-1</sup>) was purchased from Waters (MA, USA).

### Stock Solutions and Standard Solutions

The stock solution of BPA (100 mg.L<sup>-1</sup>) was prepared in methanol in dark amber bottles and stored at 4°C before use. Further dilution of the stock solution with water guides to all

standard working solutions of five different concentrations of 0.05, 0.2, 0.5, 1, and 5 µg.mL<sup>-1</sup>.

### Sample Preparation

The extraction methodology outlined by (Liao & Kannan 2013, Kaushik et al. 2009) was adhered to for this study. Each soup sample, amounting to 30 mL each, was precisely measured and subsequently transferred into the six distinct types of packaging containers, each with a capacity of 150 mL. Subsequently, they were spiked with 0.5 mg.L<sup>-1</sup> of the internal standard BPA. Extraction was done twice using ACN (5 mL each), employing a shaker for 60 minutes. The resulting sample mixture underwent centrifugation at 5000 g for 5 min, and the obtained extract was combined. This combined extract underwent evaporation to near dryness under a nitrogen stream at 50°C, and the resulting extraction residue was reconstituted in 1 mL of 100% ACN (v/v). The extract was purified using solid-phase extraction (SPE) through passage through an Oasis MAX cartridge.

### Migration Analysis

Agilent 1290 Infinity II HPLC System with Biosuite C18 column (500 Å, 7 µm, 4.6 mm x 150 mm) fitted with a UV detector set at 217 nm. The mobile phase comprises 30% TFA in HPLC-grade water and 70% ACN. The injection volume was 50 µL using a Hamilton syringe, the mobile phase flow rate was 1 mL.min<sup>-1</sup>, and the run time was set for 8 min. The conditions for HPLC are given in detail in Table 1.

Table 1: Analytical parameters and conditions for BPA determination by HPLC-UV.

Substance detected	Parameter	Conditions
BPA	Column	Biosuite C18 PA-A (500 Å, 7 µm, 4.6 mm × 150 mm)
	Detector	UV (217nm)
	Pump	Agilent 1290 Infinity II
	Mobile phase	30% TFA in HPLC grade water + 70% ACN
	Injection volume	50 µl using a Hamilton syringe
	Flow rate	1 mL min <sup>-1</sup>
	Run time	8 minutes

## Method Validation

Soup samples were spiked using the BPA with concentrations of 0.5 mg.L<sup>-1</sup> before the studies (Fig. 2). The results were repeated thrice after one week. The average recovery of BPA in spiked samples was in the range of 89-119%. Quantification of BPA was performed using regression equations (regression coefficient, R<sup>2</sup> > 0.99) generated from a 5-point calibration standard at concentrations ranging from 0.05 to 5 µg.mL<sup>-1</sup>. The limit of quantization (LOQ) detected for BPA was 0.3 ppm.

## Statistical Analysis

ANOVA statistical analysis was used to compare BPA leaching in different packaging containers at different time points using R statistical software. The p-values for both the containers factor and the time factor are very low (p < 0.05), indicating that both container type and time significantly affect BPA leaching. The interaction between containers and time also has a very low p-value, suggesting that the effect of time on BPA leaching differs among the packaging containers. Overall, these results suggest significant differences in BPA leaching among the packaging containers and across different time points, as well as significant interactions between container type and time.

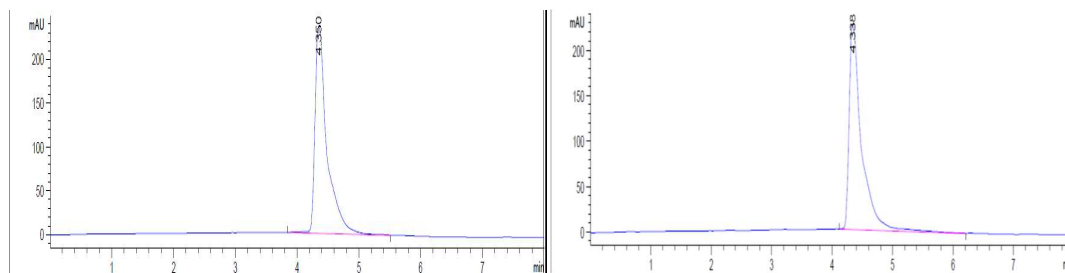
Table 2: Concentration (ppm) of BPA in take-out vegetable soup samples and premix tomato samples from packaging containers at different time intervals.

Packaging containers	Concentration of BPA					
	Take-out vegetable soup samples			Premix tomato soup samples		
	15 mins	30 mins	45 mins	15 mins	30 mins	45 mins
BN1	0.10	0.14	0.20	0.11	0.15	0.22
BN2	0.17	0.24	0.25	0.18	0.27	0.30
BN3	0.15	0.19	0.22	0.10	0.17	0.20
BN4	0.19	0.23	0.28	0.23	0.28	0.29
BN5	0.19	0.24	0.27	0.14	0.20	0.22
BN6	0.03	0.09	0.11	0.08	0.11	0.17

## RESULTS AND DISCUSSION

The investigation involved three replicate studies to determine how BPA migrates into take-out vegetable soup and premixed tomato soup from various packaging containers. Results from BPA concentration assessments in the vegetable soup samples and in premix tomato soup samples at time intervals of 15, 30, and 45 min consistently revealed concentrations below LOQ levels, as indicated in Table 2.

Although the levels of BPA found in both the take-out vegetable soup and premixed tomato samples were below LOQ, there was an observed increase in BPA concentration with extended packaging durations from 15 to 30 and 45 minutes. The vegetable soup packaged in PE pouches had the highest migration level of BPA, followed by those in aluminum foils and PS containers. On the other hand, minimal migration of BPA was observed in vegetable soup packaged in areca leaf containers. In the premixed tomato soup samples, the highest BPA migration was noted in PS containers, followed by PE pouches and aluminum foils. Similarly, the lowest migration of BPA, as observed in the vegetable soup, was noted for areca leaf containers, as indicated in Fig. 3.

Fig. 2: HPLC chromatograms for take-out soups with and without spiking with 0.5 mg.L<sup>-1</sup> BPA.

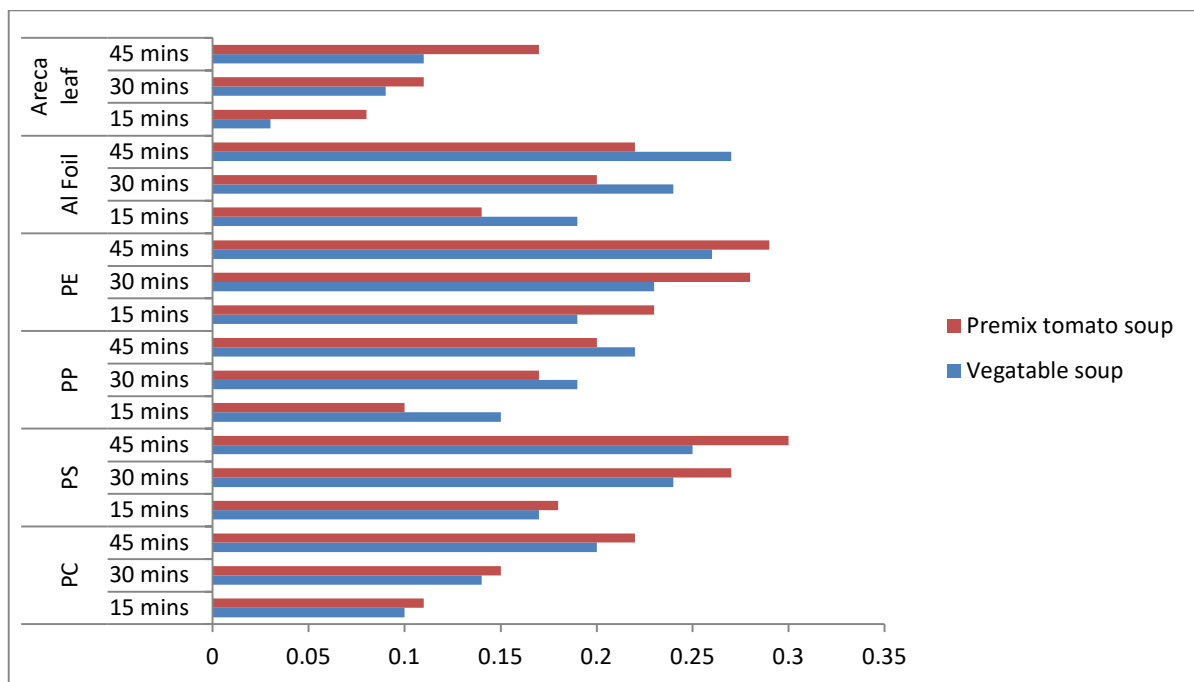


Fig. 3: Horizontal bar graphs showing the concentration of BPA leaching in take-out vegetable soup and premix tomato soup samples over time from different packaging containers.

The packaging container samples, tested and known for their utilization of BPA in production, exhibited BPA levels within the method's LOQ (0.3 ppm) for all tested soup samples. These levels were consistently below the SML for foodstuff, set at  $0.5 \text{ mg.kg}^{-1}$  by EC (Krivohlavek et al. 2023), irrespective of the packaging material type or the temperature and duration of contact with the food during migration tests. It is worth noting that our analysis was confined to BPA only, in adherence to the requirements of EU Directives. Upon initial examination, these findings suggest that packaging materials used in the Indian market conform to the permissible concentrations, posing no apparent health hazards regardless of the material composition. Consistent findings were reported by Baviera et al. (2023), indicating that the migration levels of BPA from the walls of PC bottles remained within acceptable limits. Even under extreme conditions, such as exposure to high temperatures in conjunction with washing scenarios involving detergents and the use of solid alkalis (which contribute to the degradation of PC material), the observed BPA contamination did not approach levels corresponding to the Tolerable Daily Intake (TDI) of  $4 \text{ } \mu\text{g.kg}^{-1}$  per body weight (bw) per day. These findings suggest that BPA migration does not occur during the initial use of FCMs. However, reports indicate that BPA migration into foods can occur with repeated use (Khalili et al. 2023). Therefore, how FCMs are utilized is crucial. Moreover, BPA migration is influenced by the

quality of plastic containers. Sturdy plastics tend to leach lower amounts of BPA than softer plastics like polystyrene. The findings from our study had similar observations. Softer plastics such as PE pouches and PS containers were found to leach maximum amounts of BPA as they exhibit temperature and size-dependent leaching and were limited by molecular diffusion throughout the bulk polymer (Gulizia et al. 2023). Similarly, Zhao et al. (2022) reported high concentrations of BPA and bisphenol S (BPS) in plastic products made from polystyrene, such as white foam take-out containers (WFTOCs) from China. Compared to other plastic containers, PS exhibited the highest BPA migration due to the stronger plasticity induced by the foaming process, resulting in higher levels of plasticizers being used. Similarly, PE pouches displayed elevated levels of BPA due to their thinness ( $25\text{-}50 \text{ } \mu\text{m}$ ), which necessitates high plasticity requirements and, consequently, a higher content of plasticizers compared to sturdier opaque PC and PP containers. Interestingly, aluminum foils also demonstrated increased BPA levels, which could be attributed to incomplete polymerization. According to Deshwal et al. (2019), unpolymerized monomers such as BPA can leach from the finished product.

Several studies have underscored the influence of storage temperature and duration on the release of BPA. Consequently, elevating the temperature and prolonging

storage time facilitates the release of BPA (Mârşolea et al. 2023). Ajaj et al. (2021) reported a 1.9-fold increase of styrene monomers between the inaugural and fourth day of storage, succeeded by a subsequent elevation of 3.1fold from the initial to the tenth day. However, the tested soup samples exhibited insignificant BPA leaching levels, and this observation can be attributed to the time-temperature relationship. As indicated by Nepalia et al. (2018), an increase in temperature has been linked to elevated BPA migration. Since most of the tested soup samples were packaged at 70°C for a maximum duration of 45 minutes, which may not be conducive to significant BPA leaching, it is noteworthy that if the time duration had been longer, a higher concentration of BPA might have been noted, as reported by Li et al. (2024). Therefore, it is crucial to acknowledge that an escalation in temperature and prolonged storage duration heightens the risk of BPA migration.

Areca catechu leaf containers were found to exhibit the lowest levels of leached BPA. These findings align with the assertions made by Kora (2019), which describe areca leaves as rigid, dense, and heat-tolerant, resulting in plates and cups that are leak-proof, water-resistant, and odorless. Furthermore, areca leaf products are deemed suitable for freezing, microwaving, and oven heating. They are naturally biodegradable and compostable, considered safe for single use with moist food, and reusable multiple times with dry food. Similarly, Umamakeswari (2023) utilized the Technique for Order Preference by Similarity to the Ideal Solution (TOPSIS) method to evaluate four types of leaf plates (Bamboo, Siali, Palm leaf, and Bagasse) based on criteria such as durability, biodegradability, and thermal resistance. The study concluded that Siali leaf plates emerged as the optimal choice. This research collectively underscores the importance of assessing and regulating packaging materials for potential chemical migration, advocating for sustainable alternatives such as natural leaf-based products like Areca catechu or Siali plates for their eco-friendly attributes and suitability for various food handling scenarios.

## CONCLUSION

The three replicate studies conducted to investigate the migration of BPA into take-out vegetable soups and premix tomato soups from various packaging containers yielded consistent and reassuring results. The BPA concentration assessments at time intervals of 15, 30, and 45 minutes consistently revealed undetectable concentrations (<LOQ), indicating that the tested soup samples complied with permissible concentrations, as outlined by the EC regulations. The levels of BPA in packaging containers were consistently below the SML set at 0.5 mg.kg<sup>-1</sup> for all tested samples,

regardless of material type or contact conditions. These findings suggest that, in the Indian market, packaging containers conform to permissible concentrations, posing no apparent health hazards under normal use conditions. Notably, the absence of detectable BPA levels in the tested samples can be attributed to the limited time-temperature relationship during the study period. While these results are promising for initial use, it is essential to consider the potential for BPA migration with repeated use, as reported in previous studies. The observed variation in BPA leaching among different plastic materials underscores the importance of considering the material composition and the quality and intended use of FCMs. Further research is warranted to explore the long-term implications and potential risks associated with BPA migration under varied conditions of use and storage. This study emphasizes the significance of evaluating and regulating packaging materials to mitigate potential chemical migration risks. It advocates for the use of sustainable alternatives, such as natural leaf-based products, due to their eco-friendly properties and suitability for diverse food handling applications.

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