Microplastics Leaching in Local Candies, Pickles and Yogurt Packed in Plastic Containers

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ABSTRACT

Plastics degrade into nano plastic or microplastic. Microplastics (MPs) leached from plastic packaging to food affect the human health. In this study overall migration of different plastics packaging were determined by using IS 9845: 1998 method. Food simulant 3% acetic acid (simulant B) for aqueous, acidic food (pH≤ 5) without fat at 40°C/10 days and n-heptane (simulant D) for edible fatty foods at 38°C/0.5 hour were used as per Indian Standards IS - 9845-1998. The Overall migration in Local candies (Titaura) ranged from 4.5158 to 20.4331 mg/kg in food simulant 3% acetic acid (simulant B) and by using n-heptane (simulant D) it ranged from 12.2400 to 41.1066 mg/kg. Similarly in Pickles migration of microplastics ranged from 51.16 to 58.56 mg/kg in simulant B and in case of simulant D it ranged from 7.2266 to 58.6266 mg/kg. The overall migration of microplastics in Yogurt samples were found to be 3.0186 to 19.2093 mg/kg using simulant B and with the use of simulant D, it ranged from 7.2266 to 58.6266 mg/kg. The variation in overall migration in different simulants indicate the risk of increase in microplastic leaching depending on the product condition like pH, temperature, contact time and nature of products. Coliform (TC), test for E. coli, and staphylococcus aureus for Local candies were carried out according to American Public Health Association (APHA), which showed no growth of microorganism.

Key words: Overall migration, microplastics, simulant, plastic, acetic acid, n-heptane

INTRODUCTION

Plastic is a polymer having different physical and chemical properties. Plastic has properties like lightweight, durability, easy to excess, low cost, versatility and many other properties because of which it is used widely [1]. It is used for different purposes, from household to large industrial scale. Different types of plastic used in different fields like in automobiles, medicine, buildings, aerospace, machinery, agriculture, packaging, etc., due to this large applicability plastic production increases day by day [2]. Packaging protect food from contamination, preserving its integrity, maintenance of hygienic conditions. Packaging make foods and drinks safe and protect their quality [3]. Food and drink products packed in plastic containers will be in contact with the plastics throughout their shelf life. Normally plastics used for the packaging are polyvinylchloride (PVC), high density polyethylene(HDPE), low density polyethylene (LDPE), polyethyleneterephthalate (PET), polypropylene(PP), etc. Polymerization of ethylene glycol and dimethyl terephthalate through polycondensation gives polyethylene terephthalate (PET). These kind of plastics are used in the beverage
and food packaging due to their good temperature stability and good water stability [4]. High density polyethylene (HDPE) is thicker, stronger more brittle, less flexible and a good barrier to gases and moisture. Waterproof packages can be made from HDPE. HDPE have high puncture and tear resistance. Milk bottles, ice-cream containers, etc. made from this kind of plastic [5]. Low density polyethylene (LDPE) is inert, order free, heat sealable and shrinks when heated. It is relatively permeable to oxygen, good moisture barrier, and poor odor barrier, less expensive than other plastic films. This kind of plastics are used for bags, squeeze bottles, etc., also used for hot food packaging [6]. Polypropylene is a clear glossy film, less barrier to moisture, odors and gases. PP is a kind of plastic which is not affected by humidity. It is used to pack snack foods, ice-cream, microwave dishes, etc. Addition polymerization of styrene is source of polystyrene. Polystyrene films are brittle, transparent and hard. Used for packaging of ice cream, coffee, yogurt, as plastic cutlery and fruit juice bottles. Polyvinyl chloride is a copolymer of vinyl chloride and vinylidene chloride. It is heat shrinkable, barrier to moisture, gases, aromas, fatty products, oil products and heat sealable. PVC is used in thin film packaging, aroma and flavor sensitive food packaging, etc. [5].

Leaching of chemical compound from packaging material to food is called migration. Migration is a diffusion process which is followed by Fick’s law of diffusion. Migration takes place by two ways-direct transfer or gas transfer phase [7]. Multilayer films are used for packaging. Compounds from these films migrate easily to the foods by diffusion and partition process. This process of migrating chemical compounds from external surface of printed plastic to food is called set-off [8]. Although plastic is very useful product for humankind, it also has many demerits which affect our life directly or indirectly. Because of different physical and chemical activities plastic leaches or migrate chemicals into the content that packed in that plastic containers. Plastic is chemically inert but due to presence of various additives, it shows some reaction and migration occurs. Food and beverages can be aggressive and sometimes they react with materials come in contact. In this situation certain compounds from packaging materials migrate to food substances [3]. Different factors that affect the migration are: temperature, time, condition of food packed, type of food, type and properties of packaging materials, etc. [5]. Plastics degrade into Nano plastic or Microplastic. This term microplastic attracted people’s attention in 2004 from an article published by Thompson [1]. Microplastics are molecular form of plastics. The term microplastic is attracting our attention due to the wise use of plastic in almost all types of food packaging. Microplastics are particles of different shape and dimensions between 1 µm to 5 mm. Microplastics are insoluble in water [9]. Directly or indirectly microplastics are impacting our environment, human and animal life. Microplastic if present in soil, then it destroys the productivity of soil. MP is a threat to aquatic and terrestrial environment [10]. MPs are found in tissues, stomach, intestine of aquatic animals [11]. Due to photo-oxidation process plastic material breaks into smaller particles like micro or Nano plastics [12]. Microplastics are of two types present in the environment – Primary & Secondary microplastics. Primary microplastics can directly enter the environment. Primary MPs are used for various purposes by human for cosmetics, industrial use, etc. [13]. Primary MPs are plastic pellets, fibers, personal care and cleaning products, beads, etc. [14]. The main source of the secondary microplastic is the degradation of macroplastic by aging. Microplastic which originally manufactured to small size are primary MPs while MPs fragmented from larger plastic particles are secondary [15].

For the overall migration analysis Indian standard: IS 9845:1998 gives different simulants and test conditions for different type of foods. The choice of simulant depends upon the type of food products to be packed and time-temperature condition also depends upon the use of food products. IS 9845:1998 categorizes the food products into seven categories which have different simulants and test conditions. This categorization is based on the pH and fat content of the food products. Microplastic if present in soil then it destroys the productivity of soil. [10]. MPs are found in tissues, stomach, intestine of aquatic
animals[11]. In the present study, simulant B (3% acetic acid) and simulant D (n-heptane) were used for the test samples. 60 mg/L, 60 mg/kg or 60 ppm is the maximum limit given by EU or ISO for the microplastics leaching into the food and beverages. If there is presence of color in migrating substance, then that is not suitable for food contact material even though the extractive value is within the limit [16]. Different types of foodborne diseases can be spread from micro-organisms like *E. coli*, *Staphylococcus aureus*, etc. [17] For general public health safety of food is very necessary parameter however there is very less data available for microbiological test of food products that are specially consumed by children.[18]

**MATERIALS AND METHODS**

Test Method: IS 9845:1998 was used for the determination of overall migration of different plastic packaging materials. All the regents/chemicals used were of Analytical grade. All the laboratory works were carried out at Zest laboratory and Research Center (P) Ltd, Balkot-2, Surabinayak Municipality, Bhaktapur, Nepal.

**PREPARATION OF 3% ACETIC ACID**

3 mL glacial acetic acid of analytical grade was added to 97 mL purified water for the formation of 100 mL 3% acetic acid.

**DISTILLATION OF N-HEPTANE**

250 mL of n-heptane was taken in a 500 mL round bottom flask for distillation. Electrical hot plate was used as heating source. During this process cold water was passed through condenser for cooling of vapor and slowly the vapor passing through condenser gets condensed and condensate/distillate is collected in the receiving flask.

**CONJUGATIVE WEIGHT DETERMINATION OF BEAKERS**

Conjugative weight of the beakers which were used for the research work is very important. For this, fifteen beakers of 100 mL capacity were selected, marked then cleaned. After cleaning beakers were kept in oven at 100°C for two hours, after two hours beakers were taken out from oven and put inside the desiccator for cooling. Weight of beakers were taken after cooling. This process was repeated till the conjugative weight was obtained. This procedure of conjugative weight determination was done for all the samples separately.

**SELECTION OF SAMPLE**

Samples were collected from local market of Kathmandu valley. Selection of sample was based on the type of product, plastic, products that remain in plastic container for long time, low pH sample, local manufacturing products, and products widely used by children and teenagers and taken to the laboratory. Triplicate samples representing the same batch number were selected. 12 pouches of Local candies (Titaura), 12 pouches of Pickles and 12 containers of Yogurt of four different brands were selected for the analysis.

**PREPARATION OF TEST SPECIMEN**

Containers used for the test were rinsed three times with distilled water to avoid the extraneous material.

**PROCEDURE**

At first, pH of each sample was measured. Foods from the containers/pouches selected for the test were removed and rinsed with distilled water three times to remove extraneous material. Then plastic containers/pouches were filled to their nominal capacity with their simulant and closed. To avoid bubbles sonication was done. In case of the plastic pouches, air was removed as much as possible before sealing. Then filled containers/pouches were exposed to the specified temperature-maintained oven (FCE-3000 serials, Faithful) for the specified duration of time. After exposer for the specified duration, samples were taken out from oven and contents were transferred to clean beakers along with three washing with small amount of fresh simulant. This procedure was applied for all the samples.

For Local candies (Titaura), two types of food simulants were used. Simulant B (3% acetic acid) and simulant D (n-heptane). First food simulant B was used for the triplicate samples of four different brands of local candies (Titaura). After removing the local candies from the pouches, the pouches were cleaned
with distilled water then food simulant was filled to nominal filled capacity in each pouch and was sealed by using sealer carefully. Pouches were exposed to oven at 40 °C for 10 days. After 10 days pouches were taken out from the oven and contents were transferred into clean beaker which conjugative weight was weighed already, along with three washings of the specimen with small quantity of the freshly prepared simulant.

Similar process of taking out the content from the pouches and cleaning with distilled water and filling up the pouches with simulant D were carried out for the Local candies. Then the pouches were exposed to oven at 38 °C for 30 minutes. After 30 minutes the pouches were taken out from the oven and simulant was transferred into a clean beaker along with three washings of the specimen with small quantity of the freshly prepared food simulant.

For Pickles samples, food simulants B and D were used same as in Local candies (Titaura). Same procedure was applied in Pickles as in Local candies for the test analysis.

For Yogurt samples, two different types of simulant B and D were used. The experiments were carried out similar to that of Local candies to obtain the conjugative weight of the extract. For all the samples blank were carried out by using same procedure.

**DETERMINATION OF AMOUNT OF EXTRACTIVE**

Content in the beakers weree vaporated to about 50-60 mL using heating plate (VELP, scientifica) then the beakers were placed in an oven at 100° C for an hour for complete dryness. After an hour, beakers were taken out from the oven and placed in desiccator for 30 minutes. When beakers were cooled down their conjugative weight were determined nearest 0.1 mg. Extractive was calculated in mg/kg.

For weighing, five-digit analytical balance (AUW220D, SHIMADZU) was used. In the same way blank was carried out. This procedure was same for the all samples.

Amount of extractive (Ex) = M/V * 1000 mg/L or ppm

Where M = mass of residue in mg minus blank value
V = total volume in mL of simulant used in each replicate.

Extractive resultant from n-heptane was divided by factor 5.

**MICROBIOLOGICAL ANALYSIS FOR LOCAL CANDIES (TITAUARA)**

Local candies (Titaura) samples were analyzed for total coliform (TC), test for *E. coli*, and test for *Staphylococcus aureus* according to American Public Health Association (APHA).

**Coliform test:** First sample was prepared in sterile beaker then medium Lauryl sulphate broth was prepared. 10 mL medium was transferred into test tubes and Durham tube was placed in inverted position to remove inner air and cotton plugged. Broth was sterilized at 121°C. 0.1g, 0.01g and 0.001g of sample was weighed and transferred into 3 test tubes of each using sterile spatula. One test tube having broth was taken as a control. All the test tubes were incubated at 37 °C for 24-48 hours. After completion of incubation, test tubes were observed for turbidity and gas production inside the Durham tube.

**E. coli and Staphylococcus aureus:** Soyabean casein digest broth was prepared (100 mL) and was sterilized at 121° C. After sterilization 10g of sample was transferred into sterilized medium at 37°C for 24 hours. For *E. coli* 10 mL MacConkey broth was prepared in each tube. Then previously prepared 1 mL soyabean cage digest medium containing sample was transferred. Tube was incubated at 44°C for 48 hours. After 48 hours no growth on the media was observed. For the *Staphylococcus aureus* Mannitol salt agar was prepared and sterilized at 121°C. One loopful of sample from previously prepared sample was taken and incubated at 37°C for 48 hours. No growth of organism was observed.
RESULTS AND DISCUSSION

Analysis of microplastics were done by using simulants, 3% acetic acid and n-heptane. From the analysis it was found that plastic pouches/containers of different brands leach microplastics but within the limit given by EU. Four different brands of Local candies, Pickles and Yogurt packed in plastic pouches and plastic containers leached microplastics within the limit given by EU/ISO. The study also shows that the overall migration of microplastics packed in plastic pouches are higher than in plastic containers. So, there is more risk in consuming food packed in plastic pouches than in plastic containers.

FROM LOCAL CANDIES (TITAURA)

Overall migration studies were carried out on different plastic pouches containing Local candies with 3% acetic acid for acidic food (pH<5) with fat at 40°C/10 days and n-heptane for fatty foods at 38°C/0.5 hr. Table. 1 and 2 show the amount of extractive in Local candies by using simulant B and D. With simulant B, amount of extractive ranges from 4.5158 to 20.4331 mg/kg with blank value 3.1176 mg/kg and by using simulant D, it ranges from 12.2400 to 41.1066 mg/kg with blank value 6.8000 mg/kg.

FROM PICKLES POUCHES

Overall migration studies were carried out on different plastic pouches containing Pickles with 3% acetic acid for acidic food (pH<5) or with fat at 40°C/10 days and n-heptane for fatty foods at 38°C/0.5 hr. Table. 3 and 4 show the amount of extractive in Pickles pouches by using simulant B and D. With simulant B, amount of extractive ranges from 51.1600 to 58.5600 mg/kg with blank value 6.1600 mg/kg and by using simulant D it ranges from 7.2266 to 58.6266 mg/kg with blank value 2.2000 mg/kg in Yogurt samples.

FROM YOGURT CONTAINERS

Overall migration studies were carried out on different plastic containers with 3% acetic acid for acidic food (pH<5) or high fat and having high moisture content at 40°C/10 days and n-heptane for fatty foods at 38°C/0.5 hr. Table. 5 and 6 show the amount of extractive in Yogurt containers by using simulant B and D. By using simulant B, amount of extractive ranges from 3.0186 to 19.2093 mg/kg with blank value 4.56 mg/kg and with simulant D it ranges from 7.2266 to 58.6266 mg/kg with blank value 2.2000 mg/kg in Yogurt samples.

Microbiological test of Local candies (Titaura) was carried out. No growth on media was observed after incubation which showed the absence of coliform, *E. coli* and *Staphylococcus aureus* in Local candies.

<p>| Table.1 Overall migration from plastic pouch used for packing Local candies (Titaura) in 3% acetic acid |</p>
<table>
<thead>
<tr>
<th>Sample</th>
<th>Amount of simulant (mL)</th>
<th>Amount of extractive (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>150</td>
<td>4.5158</td>
</tr>
<tr>
<td>2</td>
<td>150</td>
<td>5.8935</td>
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<td>3</td>
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<td>8.7156</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>20.4331</td>
</tr>
</tbody>
</table>

<p>| Table.2 Overall migration from plastic pouch used for packing Local candies (Titaura) in n-heptane |</p>
<table>
<thead>
<tr>
<th>Sample</th>
<th>Amount of simulant (mL)</th>
<th>Amount of extractive (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>12.2400</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>13.7733</td>
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<tr>
<td>3</td>
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<td>20.5733</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>41.1066</td>
</tr>
</tbody>
</table>

<p>| Table.3 Overall migration from plastic pouch used for packing Pickles in 3% acetic acid |</p>
<table>
<thead>
<tr>
<th>Sample</th>
<th>Amount of simulant (mL)</th>
<th>Amount of extractive (mg/kg)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>130</td>
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<td>2.8480</td>
</tr>
<tr>
<td>4</td>
<td>130</td>
<td>12.7198</td>
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<p>| Table.4 Overall migration from plastic containers used for packing Pickles in n-heptane |</p>
<table>
<thead>
<tr>
<th>Sample</th>
<th>Amount of simulant (mL)</th>
<th>Amount of extractive (mg/kg)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>51.4933</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>58.5600</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>51.1600</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>53.5600</td>
</tr>
</tbody>
</table>
### Table 5. Overall migration from plastic containers used for packing Yogurt in 3% acetic acid

<table>
<thead>
<tr>
<th>Sample</th>
<th>Amount of simulant (mL)</th>
<th>Amount of extractive (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>200</td>
<td>9.3686</td>
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<td>2</td>
<td>200</td>
<td>3.0186</td>
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<td>3</td>
<td>100</td>
<td>8.3513</td>
</tr>
<tr>
<td>4</td>
<td>100</td>
<td>19.2093</td>
</tr>
</tbody>
</table>

### Table 6. Overall migration from plastic containers used for packing Yogurt in n-heptane

<table>
<thead>
<tr>
<th>Sample</th>
<th>Amount of simulant (mL)</th>
<th>Amount of extractive (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>24.8266</td>
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<tr>
<td>2</td>
<td>10</td>
<td>7.2266</td>
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<td>3</td>
<td>10</td>
<td>58.6266</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>45.2266</td>
</tr>
</tbody>
</table>

### CONCLUSION

As per EU standards, Limit for overall migration of microplastic from plastic containers used for food packaging is 60 mg/kg or 60 mg/L or 60 ppm. This study shows that migration of microplastic from plastic pouches /containers with food simulant n-heptane shows higher amount of migration than in food simulant 3% acetic acid in three different types of products like Local candies, Pickles and Yogurt samples. All the three types food samples tested for the overall migration of microplastic are within the limit specified by EU. Although they are within the limit, the consumption of these food regularly for long term is not good for health. Therefore, the food packed in the plastic containers and pouches are not encouraged to be consumed on a regular basis. Microbiological analysis of Local candies showed no growth of microorganism.

The current study was conducted in a small sample size of three different types of food items packed in plastic pouches and containers not similar in nature.

However, the variation in value of overall migration of microplastics clearly indicates the need of more studies in more varieties of items with larger size of samples.

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### REFERENCE


