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Assessment of different physicochemical parameters of leachates from two locally unbranded yogurt containers in Erbil City, Kurdistan Region, Iraq

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Abstract

Exposure of humans to toxic chemicals can lead to significant health risks. This study evaluates the physicochemical parameters in the leachates of two local yogurt containers purchased from Erbil City, Iraq, comprising white plastic (WPC) and aluminum containers (ALC). Acetic acid, distilled water, ethanol, lactic acid, sodium carbonate, and sodium chloride at varying concentrations were examined as effective simulants for assessing leaching from the two containers (1.0 cm² container/1.0 mL simulant). Leaching experiments were conducted under three different conditions, including Refrigerated/First Condition (1stC, 4±1°C for 72 hours), Ambient/Second Condition (2ndC, 25±2°C for 24 hours), and Elevated/Third Condition (3rdC, 60±2°C for 2 hours). After leaching, many tests, such as the change in physical state, pH measurement, estimation of leached oxidizable materials, UV absorbing materials (UV-AMs), weight loss (W_L), and heavy metals (HMs) level, were examined for the obtained leachates. The observations showed that there was a significant change on the ALC specimens' surface. Most of the analyzed HMs were found in levels below the permissible limits (1000 µg/L), except the content of Pb was found above this limit in the ALC leachates using 5% sodium carbonate. The results showed that the maximum migration level of oxidizable matters and UV-AMs were observed in the leachates of ALC (3rdC, 5% sodium carbonate) and WPC (1stC and 2ndC, lactic acid), respectively. The W_L was additionally recorded at a high level in many kinds of local yogurts. Results exceeded many international guidelines and clearly confirm that the continuous use of the ALC and WPC may contribute significantly to the daily intake of toxic chemicals and can pose a significant health hazard.

Introduction

It is well-known, that aluminum (Al) is a naturally occurring element that is found in different types of matrices, including food, soil, and water (Ochmański and Barabasz, 2000). The primary way that people are exposed to Al is through their diet including vegetables, beverages, cereals and items made from them (Soni *et al.*, 2001). In the food industry, Al is broadly utilized to make containers and packaging that come into touch with food, and it is known that there is a risk when Al leaks from the packaging into the food. Therefore, another significant source of this metal that raises the intake of Al ingested through food is the usage of Al containers and foils (Fermo *et al.*, 2020; Ali and Ali, 2023).

As a result, the toxicity of Al to humans has been debated over the past few decades (Alabi and Adeoluwa, 2020; Ali and Ali, 2023). High levels of Al have been detected in the brain tissue of people suffering from dialysis encephalopathy, Parkinson's disease, and Alzheimer's disease. Because Al accumulates up in the liver, brain, and bones, it is considered neurotoxic. There had also been reports of Al toxicity, mainly in the elderly and in those with renal failure (Exley and Korchazhkina, 2001; Soni et al., 2001). Following that, in 2008, the European Food Safety Authority (EFSA) carried out a thorough investigation and published a comprehensive report on the safety of dietary Al use. The World Health Organization (WHO) and EFSA revised and combined report announced in 2008, a provisional tolerance weekly intake (PTWI) of 1.0 mg of Al per kilograms body weight per week (1.0 mg-Al/kg bw/w) was suggested and verified in 2011. The acceptable dosage was therefore not more than 70 mg-Al/week (10 mg-Al/day) for a person weighing 70 kg (EFSA, 2008; EFSA, 2011). The Belgian adult population's estimated daily intake of Al, for instance, was determined to be 0.030 mg-Al/kg bw/day (Fekete *et al.*, 2013), or 21% of the approved PTWI, which was established in 2008 (EFSA, 2008; EFSA, 2011) and later validated by German research project in 2016 (Merkel, 2016).

Specific release limits (SRL) for metals that can come into contact with food were approved by the European Council (EC) in 2013. This resolution specifies that the maximum amount of Al that can be released into food is 5.00 mg-Al/kg food (0.50 mg-Al/100 g food). The SRL specifies, the maximal quantity of different metal ions may be shifted from a well-defined surface of the contact material to any kinds of the food or simulant medium (European Council, 2013).

Since the outbreak of global COVID-19 pandemic, disposable plastic and paper containers have been utilized more broadly and with higher rate, producing significant environmental contamination (Parashar and Hait, 2021). The use of disposable paper and plastic as a convenient food containers have been increased in our daily life due to the rapid development of the fast and takeout food industries (Zeng *et al.*, 2023).

In the food packaging area, plastics are widely utilized and come into direct contact with food items (Shrinithivihahshini and Mahamuni, 2024). To improve the performance and versatility of plastics, a wide range of additives are added including metals (Ncube *et al.*, 2020). These additives possess mobility and like to transfer into the foods content under the effect of many physicochemical factors. Thereby contaminating the food with the risk toxic health hazard to the consumer can be happen (Lahimer *et al.*, 2017; Al-Malaika *et al.*, 2017). Physical reasons may lead some plastics to leach toxins into food during preparation or storage (Shrinithivihahshini and Mahamuni, 2024). Because metals can enter food content during preparation or storage, trace metal contamination of packing materials is a major concern (Shamloo *et al.*, 2024).

Disposable food containers may release a variety of toxic chemicals and harmful substances due to presenting high temperature (Ranjan *et al.*, 2021). A number of variables, such as pH, salinity, temperature, food fat content, and exposure duration might influence the release of chemical components by food foils and containers (Al Juhaiman, 2012; Inan-Eroglu *et al.*, 2018; Fermo et al., 2020; Ali and Ali, 2023).

The measurement of heavy metals (HMs) in food container and packaging materials, especially aluminium and plastics, offers many challenges because of the complexity of the containers matrix and the extremely low levels of content in which the toxic elements are found (Puligundla and Ko, 2013; Parkar and Rakesh, 2014; Khan and Khan, 2015). Numerous analytical methods have been established for measuring HMs in foods, nutritional supplements, and food packaging samples (Mukherjee *et al.*, 2023).

The HMs are dangerous and can cause many health effects with prolonged exposure or ingestion; some of them are well known as carcinogens by many world agencies for toxic substances. Additionally, long-term oral exposure and excessive consumption of HMs in foodstuffs can cause to diseases such as birth defects, lung, breast, liver, skin, bladder, and kidney cancer, tumor formation, gastrointestinal damage, acute renal failure, *etc*. (Nguyen *et al.*, 2023).

In recent years, toxic HMs were detected in many analyzed samples including occupational workplace (KhalidJalal *et al.*, 2020), children's toy (Jalal *et al.*, 2020), drinking water (KhalidAli *et al.*, 2020), foods (Ali *et al.*, 2020; Almashhadany *et al.*, 2020), and workers blood (KhalidHamadamin *et al.*, 2020) in the Erbil City, Kurdistan Region, (KRG), Iraq. For instance, each of the Pb (393.05 mg/kg) and Cd (21.47 mg/kg) contents were determined with a high level in commonly used yogurts containers made from Al and exceeded the permissible world limit (100.00 mg/kg by weight for the sum of content levels of HMs) in the Erbil city (Khalid *et al.*, 2024).

This study aims to validate and apply an advanced analytical method appropriate for the assessment of different physicochemical parameters of the leachates of the locally made yogurts container used in the Erbil city, Iraq. The results can provide a reliable procedure to estimate of leached HMs, oxidizable materials, UV-absorbing materials, and global migration residues in widely used plastic and Al containers for local yogurt.

Materials and Methods

Chemicals and instruments

All the chemicals used in this work were of analytical reagent grade (AG), including concentrated sulfuric acid (H_2SO_4 97%), nitric acid (HNO_3 65%), acetic acid, AAc (CH_3COOH 99.99%), ethanol (CH_3CH_2OH 99.99%), sodium thiosulphate pentahydrate ($Na_2S_2O_3.5H_2O$ 99.5%), potassium iodide (KI 99.5%), sodium chloride (NaCl 99.5%) Scharlau, Extra Pure, Spain), and lactic acid, LAc (≥ 90%, including $CH_3CHOHCOOH$ 75% and lactide 15%, BDH, Laboratory Reagent, England). Many other AG chemicals, such as sodium carbonate (Na_2CO_3 99.5%, Honeywell Riedel-de Haën, Germany), potassium permanganate ($KMnO_4$ 99%, Fluka, Germany), and starch indicator, were also used during this study. Seven different local yogurt samples were also used as a real simulating medium. Distilled water (DW) was applied as a neutral simulant medium and also used during washing glassware, sample preparation, and dilution when required.

A classical digestion heater (serial flask heater, Gerhardt, GmbH, Kleve, KI26, Germany) with a digestive Kjeldahl flask was used during sample digestion. An inductively coupled plasma optical emission spectrometer (ICP-OES, Spectro Arcos FHS22, GmbH, Kleve, Germany) was then used for metal analysis. Shimadzu UV-visible double beam spectrophotometer (UV-1800, Japan) was utilized for spectrophotometric measurements of leachates. An electronic sensitive balance (± 0.0001 from Mettler Toledo, AB104-S, Scientific Balance, USA), a bench-top digital multi-parameter (pH Meter, PHS-550, China), a drying oven (Huanghua Faithful Instrument, WHL Series 65B, 10–300 °C, China), and a home refrigerator were also used wherever required.

Questionnaire and survey

Two types of yogurt containers (YC) were selected (Table 1), specifically white plastic (WPC) and Al containers (ALC), widely used for the storage and marketing of fresh yogurt products by local cattle herders in the Erbil city, Kurdistan Region-Iraq (Khalid *et al*., 2024). Prior to leaching experiments, a set of formal assessment questionnaires was prepared and directly requested from participating parents in the city of Erbil, KRG. A survey was conducted to collect information about the participants' profiles, views on local yogurt containers, yogurt type preferences, daily consumption of local yogurt products, kinds and uses of its containers, and much more.

Sample collection and preparation

The WPC (Figure 1, A1) and ALC (Figure 1, B1) samples were purchased from different local markets in the Erbil city, KRG-Iraq. In a worst case, there were no labels and no prior information notice on the purchased samples. The containers were cut into square specimens (Figures 1, A2 and B2) of dimensions 5 x 5 cm (with ~ 0.5 mm for ALC and 1 mm for WPC thickness). The average exposed area of each YC plates (YCP) was 50 cm^2/specimen sheet for both sides (Exposed sheets surface area = 5×5 cm × 2 (both sides) = 50 cm^2/specimen sheet, Table 1). Prior to each experiment, the ALC and WPC specimen sheets were well washed thoroughly with detergent and DW, finally dried in an oven, cooled and kept till leaching experiments.

Simulating solvents

Based on the nature of food, five different chemical solutions with varying levels, including 3% acetic acid (AAc, v/v, pH ≈ 2.5 & 4), 8% ethanol (v/v, pH ≈ 7), distilled water (DW, pH ≈ 7), 0.9% sodium chloride (w/v, pH ≈ 7), and 5% sodium carbonate (w/v, pH ≈ 11.5), were freshly prepared and used as simulating solvents (United States Pharmacopeia, 1995; IS, 1998). In this study, two different pH media of lactic acid (LAc) solution (including LAc-1 (v/v), pH ≈ 2.5, and LAc-2, pH ≈ 4.0) were additionally proposed as new extra simulating solvents (Table 1). These solvents were then kept and individually applied for assessing leaching of the two examined YC.

Yogurt samples

Different local yogurt samples, including two each of cow (Cow-1 & 2), goat (Goat-1 & 2), sheep (Sheep-1 & 2), and one buffalo (Buffalo-1) yogurt products, were freshly collected and used in this study (Table 1). These samples (4.32-4.65 pH range) were also used as a real simulant medium for evaluation and application of the two examined containers, with parallel sets of yogurt simulants, without samples, as a basal control.

Simulating and leaching conditions

Before the leaching process, all used glassware's were well cleaned, washed with diluted nitric acid, and then distilled water to prevent contamination. The examined YC (ALC and WPC) sheets were individually immersed in 200 mL of each simulating solvent in a sterile beaker in a ratio of 1.0 cm^2 YC/1.0 mL solvent. It means that four sheets of the same YC samples (4 sheets × 50 cm^2 per specimen for both sides = 200 cm^2, an average dimension for both sides) were separately exposed in 200 mL of each proposed simulant solvent under three different leaching conditions (Figure 1, A3 and B3). The samples were individually kept at each of 4±1°C for 72 hrs. (Refrigerated/First Condition, 1stC), 25±2°C for 24 hrs. (Ambient/Second Condition, 2ndC), and 60±2°C for 2 hrs. (Elevated/Third Condition, 3rdC) during the leaching process (Table 1). For basal control/blank, parallel sets having simulating solvents only were also run under the three identical conditions. It means that a blank test was also done in parallel for each of the simulating solvents under the three applied identical conditions. After leaching process, the simulant solvents were finally separated from the examined YC specimens and then labeled and kept both of them till final analysis (United States Pharmacopeia, 1995; IS, 1998).

Tests performed

After leaching, different tests (Table 1) such as estimating levels of leached HMs, pH change, oxidizable materials, UV absorbing materials, and global migration residues of the examined YC sheets were well studied according to the Bureau of Indian Standards, IS No 9845: 1998 guideline (IS, 1998; Nasibullah *et al.*, 2014). These tests were done in Analytical Labs and Scientific Research Center - Salahaddin University-Erbil.

Estimation of leached heavy metals

After the leaching process, 150 mL of the leachate solution was quantitatively taken and individually added to the digestive Kjeldahl flasks. The sample solution was then treated and digested with concentrated nitric acid. The analysis was done in a fuming chamber utilizing a classical digestion heater until finalizing the digestion step. After cooling, the clear solution obtained from the digested samples was quantitatively transferred, scaled down to 15 mL using 0.1 N diluted nitric acid, and then labeled before being analyzed. The quantitative metal analysis was next done in triplicate for each of the leachates extracted from the 1stC and 3rdC using the ICP-OES instrument. Ten HMs levels, including Al (Al), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), lead (Pb), and zinc (Zn), were detected in the digested leachates. Leaching and digestion steps were also repeated three times for each sample and blank solution in the same condition. Finally, the estimated contents of the ten HMs were detected in triplicate, and the results are also presented in μg/L [parts per billion, ppb, as mean ± standard deviation (SD)]. The level of the HMs should not be more than 1000 μg/L (Cd should be lesser and not be more than 100 μg/L) according to many regulatory agencies (United States Pharmacopeia, 1995; IS, 1998).

pH measurement

The pH of the examined simulation was also recorded before and after each of the leaching tests. The change of pH ($\pm\Delta pH$) value was then calculated for each of the leaching processes (Mohammad *et al.*, 2011).

Estimation of leached oxidizable material

Oxidizable matters are also introduced as antioxidants, which are used for protecting containers by reacting with the atmospheric oxygen. The level of these matters can be evaluated by titration of the leachate with standard sodium thiosulfate solution and compared to the results achieved with blanks. At first, 20 mL of the examined extract was added into an Erlenmeyer flask that included a mixture of 20 mL of 0.01 N potassium permanganate and 1 mL of 2 N sulfuric acid. The solution was then boiled for about three minutes and allowed to cool. The mixture was finally titrated with 0.01 N standard sodium thiosulfate solution after adding 0.1 g of potassium iodide and a few drops of the starch solution as an indicator. A blank was also titrated and done in parallel. The difference in the recorded volume of the standard consumed in the titration of the examined leachate and blank provided the measure of oxidizable matter according to the Indian Pharmacopoeia Guideline 1996 (Nasibullah *et al*., 2014).

Estimation of UV-absorbing materials

UV-absorbing materials (UV-AMs) are substances that have strong UV-absorption capacity in the wavelength range of 200 to 400 nm and can provide characteristic absorption peaks in the UV region. Nowadays, many additive and filler materials have been used as UV-light protection and added into the food packaging materials during the synthesis of containers to keep them from degradation by lights (Roy *et al.*, 2024). Following the simulation process, leachates were investigated for the assessment of the migration of UV-AMs from the two investigated YCs (Nasibullah *et al.*, 2014). The absorption profile of the obtained leachates can be assessed and considered by spectral scanning from 220 to 400 nm utilizing a UV-visible spectrophotometer. Results were shown as the difference in optical density (OD) achieved from the leachates and the blanks and should not be more than OD–0.3 (Nasibullah *et al*., 2014).

Estimation of global migration residues

In the present work, a weight loss (W_L) test for the two types of YC sheets was also suggested by estimating of global migration residues. The W_L test was successfully applied on each of the ALC and WPC specimens in a ratio of 1.0 cm^2 YC per 1.0 mL simulant solvents using direct and indirect methods, respectively. Each of the LAc-2 and AAc-2 (pH ≈ 4.0) solutions were suggested and examined as effective simulating medium (Al Juhaiman, 2010; Nasibullah et al., 2014).

Prior to leaching, the ALC specimens (50 cm^2/specimen sheet for both sides) were weighed accurately in a scientific balance (±0.0001) and then individually immersed in the two proposed acid solvents (200 cm^2 ALC specimens per 200 mL simulant). All leaching experiments were independently done in the acid solutions and continued at the three conditions. After leaching, the ALC specimens were separated, immersed, and cleaned in a hot cleaning solution of CrO_3 and H_3PO_4 mixture at 80°C for a step is needed for removing the reaction products on the examined specimen surface. After that, the specimens were well washed with detergents, DW, and then acetone. Finally, the ALC plates were dried in an oven, cooled and were accurately reweighed again in order to measure the differences before and after the leaching. The W_L level (as milligrams of YC per 100 mL of simulant) can be directly found for the examined ALC specimens [Eq. 1] after subtracting the controls/blanks value (Al Juhaiman, 2010). It means that, a blank set shall also be carried out without the sample, and the calculation of the final W_L equal to the obtained W_L in mg minus the blank value.

The W_L test for the WPC specimens was also applied using an indirect method (Nasibullah *et al*., 2014). In this method, the overall global migration residues of the WPC that are not volatile up to 95°C can also be measured after the leaching. Following the simulation, the leaching solvents were separated from the WPC plates and kept in constant pre-weighted containers for evaporation till dryness (90°C, 24 hrs. in oven). The container was then cooled after the evaporation and accurately weighed again. Finally, the difference in the weight recorded was taken as the measure of the W_L level and global migration residue expressed as mg-WPC/100 mL of the examined simulant (Nasibullah *et al*., 2014). Level of migration residues and the W_L value for the examined plastic container should not be more than 5.0 mg/100 mL of extract, according to Bureau of Indian Standards guidelines, BIS No. 9845 (IS, 1998). The tests were accurately performed in triplicate, and blank is also required for finding the final W_L value [Eq. 1].

Final W_L = Obtained W_L - Blank value [Eq. 1]

Where, W_L is the final weight loss (mg) or the difference in the container weight after leaching (ΔW) and subtracting of blanks value (IS, 1998).
The recorded final W_L values can then be used for calculating the YCs corrosion rate (C_R) (IS, 1998; Al Juhaiman, 2010). The C_R values are expected to be different using each of the three conditions (1stC, 2ndC, and 3rdC) and can be obtained applying Eq. 2:

$C_R = W_L/(A \times T)$ [Eq. 2]

Where, W_L is a final weight loss value (ΔW as mg) after leaching and subtracting of blanks value; A is used for the exposed surface area (cm^2), and T is the immersion time (h) of the two examined YC specimen in simulants.

Real samples application
Different kinds of local yogurt were additionally proposed and used as a real simulation medium for leaching experiments. The W_L level can be directly found for the ALC plates and then used for recording the intake amount of the ALC (as mg of the Al-ALC intake per person per day, mg-Al/person.day) using seven different local yogurt samples as real simulant against the control one. The W_L values can then be compared with the acceptable Al level approved by the WHO/EFSA (the Al PTWI of 1.0 mg/kg bw/w) and the EC (the Al SRL of 0.50 mg/100 g of food/food simulant) guidelines (EFSA, 2008; EFSA, 2011; European Council, 2013). The $\pm\Delta pH$ value was also recorded for the examined yogurts after finishing the process.

Statistical analysis
Microsoft Excel 2016 and GraphPad Prism 8 software programs were utilized for statistical analysis and graphing scientific data. Statistical analysis was employed using Student's t-test, and the significance level was set to 0.05. A p-value less than 0.05 is considered as significant. Results were calculated and presented as average with SD and below the detection limit (BDL) of triplicate measurements (n=3).

Results and Discussion
In this study, many physicochemical parameters were thoroughly examined for the two commonly used yogurt containers in Erbil city, KRG, Iraq. The assessments were accurately conducted by contacting and leaching the two YC specimens with many simulating solutions, including 3% AAc, 8% ethanol, distilled water, 0.9% sodium chloride, 5% sodium carbonate and LAc under varying conditions.

Assessment questionnaire for parents' profiles and lifestyles
Before the leaching experiments, a significant questionnaire, mainly on the daily intake of local yogurt products and using the two kinds of YC, was directly requested from a hundred participating parents (Kurdish, 35% male and 65% female) in Erbil city. Detailed information on the attended participants is shown in *Supplementary Table 1* (as mean ± SD and %), including gender, family members, yogurt-consuming routine, favorable kinds, and availability of the products at breakfast. Much data related to participating families' lifestyle, such as storing products after buying and preferred types of containers, is exhibited in *Supplementary Table 1*.

In this study, results exhibited that the average number of the participating family (mean ± SD) was equal to 4.7 ± 1.6 members/family (range = 2-8 persons). Results verified that consuming local yogurt is more favorable than imported products. Data showed that the average yogurt consumption is 3250 g/family.week. It means that the average yogurt consumption is 650 g/person.week when assuming that each family has 5 members. Thus, the average local yogurt consumption per person is around 93 g/day (*Supplementary Table 1*). According to the survey data, the final yogurt intake is assumed to be about 100 g/day.person (or 100 mL/day person because yogurts' density = 1.01±0.02 g/mL when n = 7) due to consuming extra yogurt in some seasons.

Change in physical state

After the leaching process, the evaluation of the physical state change is focused on in this study. According to the regulatory agencies, the results of the physical state, odor, and clarity of the obtained leachates of the examined container should not change after preparation of leachates. It is also important for the leachates to be clear, odorless, and colorless (Puligundla and Ko, 2013). In this study, the observations showed that there was no change in the physical state of the WPC surface after the leaching process. The leachates of all the examined samples were also clear, odorless, and colorless. However, the observations showed that there was a significant structural failure, roughness, and color change on the surface of the examined ALC specimens after finishing the leaching experiments (Figure 2).

Estimation of heavy metals

Recorded data in the present study confirms that the locally WPC and ALC used in Erbil city contain many HMs such as Al, Cd, Co, Cu, Fe, Mn, Ni, Pb, and Zn. These HMs were detected from the analysis of leachates obtained from different solvents, including 3% AAc, 8% ethanol, distilled water, 0.9% sodium chloride, 5% sodium carbonate and LAc under different conditions. The results of the analysis of leachates presented that both of the YC samples were found to contain the selected HMs in varying levels.

It is known that the level of each HM should not be more than 1000 µg/L (Cd should be lesser and not be more than 100 µg/L) according to USP, IP, BIS, MFDSK and many other regulatory agencies (United States Pharmacopeia, 1995; IS, 1998; MFDSK, 2021). After leaching of the ALC specimens (1stC and 2ndC, *Supplementary Table 2*), most of the selected HMs were found in levels below the permissible limits according to the announced regulatory agencies, including chromium. However, the mean content of Pb was found above the allowed limit (1000 µg/L) in the ALC specimens using 5 % sodium carbonate, and equal to 1534.4 µg/L and 1407.9 µg/L levels in the 1stC and 2ndC, respectively. Cadmium content did not exceed the permitted level (100 µg/L) by the announced legislations (United States Pharmacopeia, 1995; IS, 1998; MFDSK, 2021). Meanwhile, the highest levels of Cd (82.67±7.8 µg/L in the 1stC and 81.62±8.1 µg/L levels in the 2ndC), which were near the permissible limit (100 µg/L), were also detected in the leachates of the ALC specimens in the case of using 5 % sodium carbonate (*Supplementary Table 2*).

In terms of metal analysis in the WPC leachates, most of the HMs were found in levels below the world's permissible limits. Both the Cr and Cd levels were also BDL in all the examined WPC leachates (*Supplementary Table 3*).

Among the examined HMs in the WPC using the 1stC, the total leached metals load (TLML) level (µg/L) was acceptable and follows the sequence 247.59 µg/L (AAc-1) > 233.3 µg/L (LAc-2) > 223.7 µg/L (LAc-1) > 112.1 µg/L (5 % sodium carbonate) > 90.26 µg/L (8 % ethanol) > 70.94 µg/L (0.9 % sodium chloride) > 11.23 µg/L (DW). However, the TLML levels for the 3rdC were totally low in comparison with those levels via the 1stC (*Supplementary Table 3*, Figure 3a).

Among the studied HMs in the 1stC, the TLML (µg/L) in the ALC follows the sequence 2105.2 µg/L (5 % sodium carbonate) > 206.6 µg/L (AAc-1) > 187.3 µg/L (0.9 % sodium chloride) > 152.4 µg/L (8 %

Ethanol) > 143.5 µg/L (DW) > 121.6 µg/L (LAc-1) > 44.2 µg/L (LAc-2). However, the TLML for the 3rdC was different and follows the sequence 1967.6 µg/L (5 % sodium carbonate) > 229.3 µg/L (0.9 % sodium chloride) > 214.7 µg/L (AAc-1) > 198.4 µg/L (LAc-1) > 151.2 143.5 µg/L (DW) > 105.1 µg/L (8 % Ethanol) > 64.2 µg/L (LAc-2) (*Supplementary Table 2* and Figure 3b). Recorded results (Figure 3) indicate that most of the leached HMs and Al levels were bound to pH-dependent charges.

Leaching of Al from the ALC was also examined under the two different conditions, including the 1stC and 3rdC (Figure 3c). The results indicate that very low and high pH values were found to increase the leaching of Al levels from the examined ALC specimens. Recorded results showed that the leached level of Al (µg/L) for the ALC (Figure 3c) in the proposed simulants follows the order: 1192.4 × 10^3 µg/L (5 % sodium carbonate, pH ≈ 12.5) > 29400 µg/L (LAc-1, pH ≈ 2.5) > 23700 µg/L (AAc-1, pH ≈ 2.5) > 7900 µg/L (0.9 % sodium chloride, pH ≈ 7) > 5200 µg/L (LAc-2, pH ≈ 4.0) > 600 µg/L (8 % Ethanol, pH ≈ 7) > 500 µg/L (DW, pH ≈ 7) for the 1stC. However, the leached Al contnets level were higher in the 3rdC and follow the pattern 1475.3 × 10^3 µg/L (5 % sodium carbonate, pH ≈ 12.5) > 68800 µg/L (AAc-1, pH ≈ 2.5) > 62900 µg/L (LAc-1, pH ≈ 2.5) > 9300 µg/L (0.9 % sodium chloride, pH ≈ 7) > 6500 µg/L (DW, pH ≈ 7) > 5000 µg/L (LAc-2, pH ≈ 4.0) > 4700 µg/L (8 % Ethanol, pH ≈ 7).

The results were then compared with the SRL for metals including Al metal (5.00 mg-Al/kg food or 5000 µg-Al/L simulant) which can come into contact with food were approved by the European Council guideline (European Council, 2013). Recorded data confirmed that the levels of the leached Al in all the examined leachates were higher than the EC permissible value (5000 µg-Al/L simulant for the SRL) excepting ALC leachates after using 8 % ethanol, where the content of Al was found below the allowed limit.

Measuring of pH

Results showed that the maximum change in the pH of the whole examined leachates (*Supplementary Table 4*) was recorded for ALC samples in the 2ndC except for the 5 % sodium carbonate. However, the maximum change in the pH of all leachates was recorded for WPC samples in the 3rdC, excepting of the sodium carbonate. Results verified that the change in the pH of the leachate of 8 % ethanol was greater than that change in all other obtained leachates for all conditions compared to the controls. The maximum change in the pH of the leachates was noted for each of the WPC and ALC leaching process due to using neutral simulating solvents, including the DW, 8 % Ethanol, and 0.9 % sodium chloride.

Estimation of oxidizable matters

The recorded data revealed that migration of oxidizable matter was detected with varying levels in each of the obtained leachates (*Supplementary Table 4*). As a result, the required volume of the standard (0.01 N sodium thiosulphate titrant) is different (ranging between 0.2-5.1 mL) to react with 20 mL of the leachates obtained from the leaching analysis of the two yogurt containers.

At 60±2°C for 2 hours of conditioning (3rdC), the results showed that the maximum migration level of oxidizable matter was observed in the leachates obtained from the ALC and WPC specimens in the 5% sodium carbonate medium (5.1 and 2.7 mL, respectively, difference in the recorded volume of sodium thiosulphate consumed) compared with the other simulating solvents.

Estimation of the UV absorbing materials

Migration of the UV-AMs was only investigated on the WPC leachates using each of the LAc and AAc (pH ≈ 2.5 and 4.0, *Supplementary Table 4*) solvents. Measuring UV absorbance for the leachates was used as optical density (OD) for estimating these materials. Data verified that the maximum migration of UV-AMs value was in the examined leachates of LAc-1 and equal to OD–0.481 and OD–0.750 after applying the 1stC, and 2ndC, respectively. At 60±2°C for 2 hours (3rdC), the migration values were low and ranged between OD–0.013 in the LAc-1 to OD–0.077 in the AAc-2 leachates. Meanwhile, at 45±2°C for 24 hours (2ndC), high migration levels for the UV-AMs, including OD–0.200 and OD–0.281 values, were also recorded after using the LAc-2 (pH ≈ 4.06).

Estimation of global migration residue

The test of global migration residue, or W_L, has been recommended by many national and international regulatory agencies (IS, 1998; EFSA, 2008; EFSA, 2011) and is important since some of the additives are toxic in food containers (Ammar *et al.*, 2023; Vincoff *et al.*, 2024). In this study, the W_L test was successfully applied on each of the ALC and WPC specimens using direct and indirect methods, respectively. The W_L value was determined in 100 mL of extract and shown as mg-YC/100 mL.

The W_L value can be calculated indirectly via measuring the dried global migration residues of the examined WPC (Nasibullah *et al.*, 2014). The net weight of the WPC precipitate was obtained after finishing the leaching process and drying the residual solvent (Figure 4a). The obtained W_L value for the WPC specimens can also be represented as the 'estimated' WPC intake and compared with the BIS limitation guideline. The recorded results verified that the W_L levels for the WPC specimens in the two simulating solvents (Figure 4b) were lower than the standard BIS permissible limit, *i.e.*, 5.0 mg/100 mL of extract (IS, 1998).

Nevertheless, the W_L value was directly recorded (Figure 4b) for the ALC specimens by finding the differences in the weight before and after the leaching (Al Juhaiman, 2010). The estimated W_L levels (average±SD) for the examined ALC in the AAc-2 medium were accurately recorded and ordered as follows; 10.35±0.9 mg/100mL for 2ndC > 3.9±0.1 mg/100mL for 3rdC > 3.8±0.1 mg/100mL for 1stC. However, recorded data confirmed that the W_L levels (average±SD) for the ALC in the LAc-2 medium were lower than the detected levels in the AAc-2 medium and ordered as follows; 6.0±0.6 mg/100mL for 2ndC > 3.5±0.1 mg/100mL for 3rdC > 3.5±0.3 mg/100mL for 1stC.

The recorded W_L values can be then used for finding the two containers corrosion rate (C_R) level (Al Juhaiman, 2010). The C_R values expected to be different using different conditions and can be calculated using the equation 2 in this study.

The LAc-2 solvent used in the 2ndC, is taken as an example for calculating the C_R level of the ALC plates, and the ALC leaching per hour (under the 2ndC, 25±2°C for 24h hrs.) will be equivalent to [Eqs. 2-4]:

$$C_R = W_L/(A \times T) \quad \text{[Eq. 2]}$$

$$C_R = \frac{6.0 \text{ mg of the ALC}}{100 \text{ cm}^2 \text{ of the ALC} \times 24 \text{ hrs leaching}} \quad \text{[Eq. 3]}$$

$C_R = 2.5 \times 10^{-3}$ mg/cm². h for the ALC plate in the LAc-2 under the 2ndC (Al Juhaiman, 2010) [Eq. 4]

Where, in the 2ndC, the value of A = 100 cm^2 of the ALC per 100 mL of the LAc-2 solvent, T = 24 hours during the leaching experiment, and the average W_L = 6.0 mg of the ALC (Figure 4b).

Summarized data of the C_R values for the two examined YC specimens (shown as mg-YC/cm².h) were found using the two simulating media, including the LAc-2 and AAc-2 solvents and shown in Figure 4c. In terms of corrosion, the results verified that the C_R levels for the ALC in the AAc-2 medium were higher than those levels reported in the LAc-2. The $C_R \times 10^3$ level (mg-ALC/cm². h) in the AAc-2 medium was ordered as 19.3 (3rdC), 4.3 (2ndC), and 0.5 (1stC), while its level in the LAc-2 was lower and ordered as 17.3 (3rdC), 2.5 (2ndC), and 0.5 (1stC). Data confirmed that the obtained C_R levels for the WPC plates were lower than those levels recorded for the ALC specimens in the two examined acidic medium for all conditions (Figure 4c).

Applications

In this study, both the LAc-2 and AAc-2 were mainly used for assessing the two YC plates because their pH level (pH≈4.0) is close to most of yogurt's pH range (Matela *et al.*, 2019). The LAc-2 is additionally known as a main component, responsible for the yogurt acidity, introduced as the most important acid and predominant among various organic acids detected in the yogurt samples (Costa and Junior, 2015; Costa *et al.*, 2016). Though in smaller amounts than lactic acid, acetic acid is also present in yogurts composition (Li *et al.*, 2019). Many kinds of local yogurt samples (ranging; pH 4.32-4.65) were also used as a real simulating medium for examining the W_L test of the two examined YC plates and pH change of the medium.

The change of the pH value (ΔpH) for each of the LAc-2 and many examined yogurt samples was accurately recorded at the beginning and after finishing of the leaching experiments (*Supplementary Table 4*, Figure 5a and b). Results showed that the ΔpH value of the LAc-2 solution included the ALC specimens (~0.14 for the 1stC, 0.25 for the 2ndC, and 0.2 for the 3rdC in *Supplementary Table 4*) were nearly within the range of those values (~0.06-0.17 for the 1stC, 0.07-0.23 for the 2ndC, and 0.09-0.25 for the 3rdC in Figure 5a) recorded for the whole yogurt samples after leaching. The experiment was also done for the WPC plates in the selected media. In this case, the obtained results verified that the ΔpH value for the LAc-2 solvent (*Supplementary Table 4*) was also within the recorded ΔpH ranges of the used yogurt samples (Figure 5b) after finishing leaching experiments.

The W_L test was also used for assessing of ALC specimen leachate in each of the proposed acids (Figure 4b) and local yogurts (as mg-ALC/100 mL of acid or yogurt). The W_L value was firstly recorded in each of the two examined acids and yogurt samples. The attained W_L value then used for finding the 'estimated' milligrams of the ALC intake per person per day (mg-ALC/person.day, Figure 5c) in many kinds of local yogurt samples.

The obtained results were compared with the WHO and EFSA permissible limit (PTWI) announced in 2008. A 70 kg adult can consume 70.0 mg-Al/week for the rest of their life, while a 14 kg child can consume 14.0 mg (EFSA, 2008; EFSA, 2011). It means that the allowable limit value for an adult weighing 70 kg and a child weighing 14 kg would be 10.0 and 2.0 milligrams of Al per day (mg-Al/person.day as Provisional Tolerance Daily Intake, PTDI), respectively.

Another interesting point to mention was the 'estimated' Al-ALC intake level by the Kurdish *via* yogurt consumption. The test was also done using different kinds of local yogurts as a real simulating medium (Figure 5c). The Al intake level is only calculated for consumers who eat yogurts inside the ALC because this container is more preferable by most of the Kurdish (93%, *Supplementary Table 1*). The 'estimated' Al-ALC intake level was determined in 100 grams of yogurt (Figure 5c) because the final yogurt intake is assumed to be about 100 g-yogurt/day.person by Kurdish according to the study survey (*Supplementary Table 1*). The 'estimated' Al-ALC levels intake by Kurdish people were then compared with the PTDI permissible limit.

Obtained results (W_L levels) in many kinds of yogurt samples (Figure 5c) were mainly evaluated with the world permissible limit (10.0 mg-Al/person.day as PTDI for 70 kg adults). In the case of all conditions, all samples (Figure 5c) showed the W_L values below the PTDI permissible limit for adults *i.e.*, 10.0 mg-Al/person.day (EFSA, 2008; EFSA, 2011).

However, recorded results (Figure 5c) in all yogurt samples verified that the 'estimated' Al-ALC intake was always higher than the allowable PTDI limit for children (2.0 mg-Al/d for a 14 kg child BW) announced by the guidelines (EFSA, 2008; EFSA, 2011). This indicating that a well defined subgroup of the population might be at risk. It is challenging to find out what effects the long-term intake of limited quantities may have, such as that which happens because of repeated ingestion of small quantities of Al metal or its compound through the daily diet. That is why, many researches have also been documented on the many sources of Al in order to plan the minimizing of the quantities ingested (Crisponi *et al.*,

2013; Hardisson *et al.*, 2017). Those who are most at risk for Al exposure per kg bw/w are children, who typically consume more food than adults relative to their body weight (EFSA, 2008).
Another noticeable point to mention is the SRL for Al announced by the EC in 2013. Recorded results (Figure 5c) confirmed that the 'estimated' Al-ALC intake level (mg-Al/person.day via 100 gm yogurt consumption by the Kurdish) were totally higher than the declared SRL for Al by the EC (0.50 mg-Al/100 g food). It means that every Kurdish is at risk in terms of Al intake via consuming yogurt. It can be daily happen due to exceeding the SRL for Al intake permitted by the EC legislations (European Council, 2013).
The attained results in this research clearly exhibit that these two types of containers include toxic chemicals that can be easily leached into yogurt under different conditions. A detailed analysis of the two containers showed the presence of global migration residue, toxic HMs, and UV-AMs in significant levels, some of which were above world permissible limits. Cadmium, which belongs to the group of toxic and carcinogenic HMs (Charkiewicz *et al.*, 2023), was present in high amount in the ALCs composition. The obtained results confirmed that the WPC could be seen as a safer YC in terms of leaching chemicals. The results also indicate that the ALC, including yogurt products, have been widely used by Kurdish people and may contribute significantly to the daily intake of toxic chemicals and high doses of Al into their diet and body through yogurts' consumption. Al leaching is dependent on a number of factors, including temperature, pH, salinity, and dietary composition, according to the literature (Al Zubaidy *et al.*, 2011). Using ALC can leach high quantities of Al into the local yogurt in the Erbil city, which increase the amounts of Al to high dosages and could be unsafe to children health, the elderly and especially people with kidney problems (Niu, 2018). That is why it is preferable to avoid using these two types of containers and safer to use a recent alternative container, such as glass (Maske *et al.*, 2024). Additionally, it is advisable for families to transfer yogurts to their own glass container directly after purchasing and store them till complete yogurts consumption.

Conclusions

This study documented the exposure of humans to toxic chemicals through widely used local yogurt containers in Erbil city. The results revealed that both the WPC and ALC leachates were found to include many HMs s, oxidizable materials, and migrated residue material in varying levels. The recorded results clearly specify that the utilization of the ALC container may contribute significantly to the daily intake of toxic chemicals and high doses of Al through yogurt consumption. The continuous use of these containers may cause many human health consequences due to their high leachability in terms of toxic chemicals. In the meantime, because some consumer groups, especially children, are at risk, efforts must be made to monitor and notify the Kurdish population. Therefore, it is preferable for consumers to avoid using these types of containers and at least transfer yogurt into glass containers at home directly after purchase and store it until consumption. Also, the manufacturers have to use safe alternative yogurt containers to provide safe products and protect consumers' health.

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Online supplementary material:

Supplementary Table 1. Detailed information on the attending Kurdish parents' profiles, lifestyles, and yogurt intake.

Supplementary Table 2. Leached levels of some HMs (µg/L) for a 100 cm^2 sheet of ALC for both sides per 100 mL simulant.

Supplementary Table 3. Leached levels of some HMs (µg/L) for a 100 cm^2 sheet of WPC for both sides per 100 mL simulant.

Supplementary Table 4. Recorded data including change in the pH, optical density, and oxidizable matter level for different leachates obtained after leaching ALC and PC sheets in different conditions.

Table 1. Summarized information on the collected yogurt container, specimens preparation, simulating solvents, yogurt samples, simulating and leaching conditions, and many performed test during this study

Collected containers information and preparing specimens	
Types of yogurt containers	White plastic container (WPC) and Aluminum container (ALC)
Number of the collected samples	Collecting about 25 containers for each kind in different places
Available size of the containers	Two specific sizes were available for each kinds of the containers including 1 Kg and 2 Kg volume.
Cutting and preparing YC sheets	5 x 5 cm dimensions for each sheet
The exposed area of each plate	50 cm^2/specimen sheet for both sides
Simulating solvents	
Types, pH and levels of the prepared simulating solvents	3% Acetic acid (AAc, v/v, pH ≈ 2.5 and pH ≈ 4) 8% Ethanol (v/v, pH ≈ 7) Distilled water (DW, pH ≈ 7) 0.9% Sodium chloride (w/v, pH ≈ 7) 5% Sodium carbonate (w/v, pH ≈ 11.5) Lactic acid (LAc, v/v, pH ≈ 2.5, and pH ≈ 4.0)
Collected yogurt samples	
Kinds, numbers and symbols of the collected and examined yogurt samples during this study	Two samples of cow yogurt (Cow-1 & Cow-1) Two samples of goat yogurt (Goat-1 & Goat-2) Two samples of sheep yogurt (Sheep-1 & Sheep-2) Only one sample of buffalo yogurt (Buffalo-1)
Simulating ratio and proposed leaching conditions	
Ratio of the examined YC plate dimension per simulant volume	1.0 cm^2 YC/1.0 mL simulant
The three proposed leaching conditions	4±1 °C for 72 hrs. (Refrigerated/First Condition, 1stC) 25±2 °C, 24 hrs. (Ambient/Second Condition, 2ndC) 60±2 °C for 2 hrs. (Elevated/Third Condition, 3rdC)
Tests performed	
After leaching experiments, types of performed tests	Leached HMs pH Measurement Leached Oxidizable Materials UV-Absorbing Materials Global Migration Residues Real Samples Application

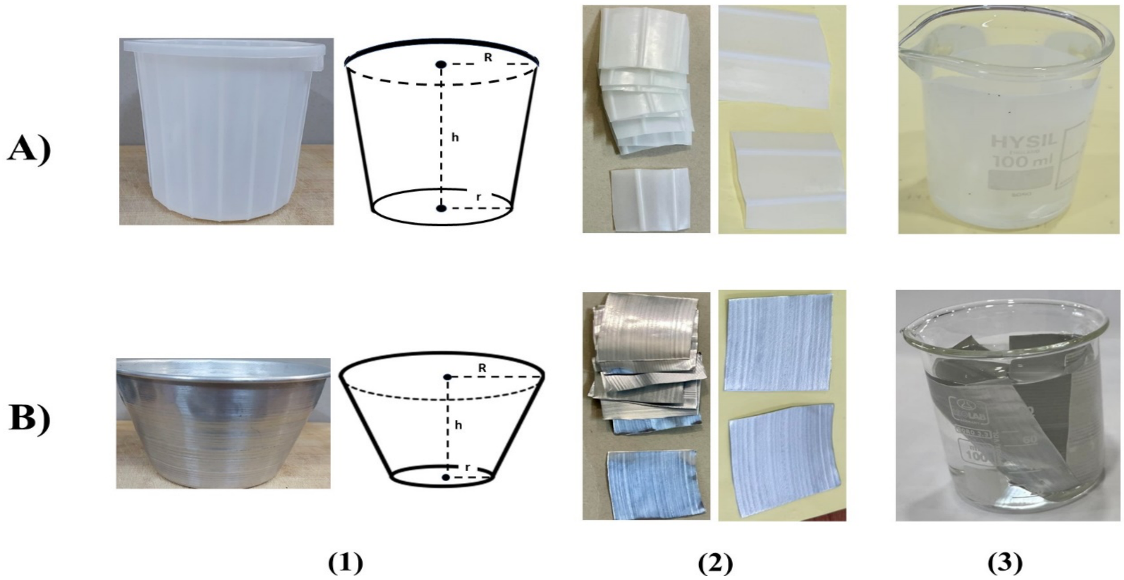

Figure 1. Examples of the 1) two commonly used local YC in Erbil city, 2) prepared square specimens' dimensions (5 x 5 cm) with 50 cm^2 per specimen sheet for both sides, and 3) leaching process with a ratio of 1.0 cm^2 YC/1.0 mL simulant solvent for the two examined containers including (a) WPC, and (b) ALC.

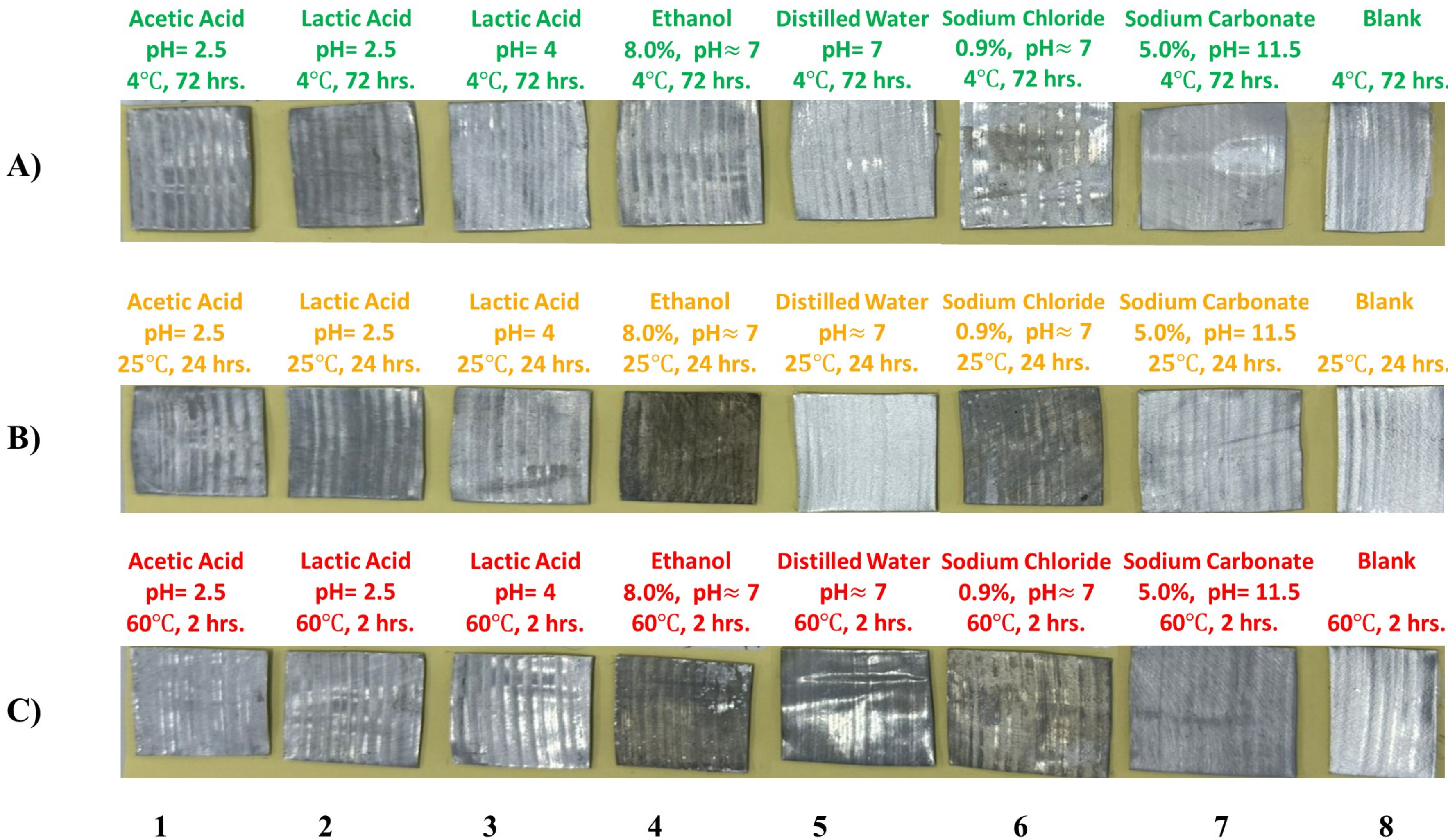

Figure 2. Observations surface of the examined ALC specimens after leaching experiments in different media including; 1) Acetic acid (pH ≈ 2.5), 2) LAc-1 (pH ≈ 2.5), 3) LAc-2 (pH ≈ 4.0), 4) 8% Ethanol (pH ≈ 7), 5) Distilled Water (pH ≈ 7), 6) 0.9% sodium chloride (pH ≈ 7), and 7) 5% sodium carbonate (pH ≈ 11.5) and 8) Blank, for each of the a) 1stC, b) 2ndC, and c) 3rdC experiments.

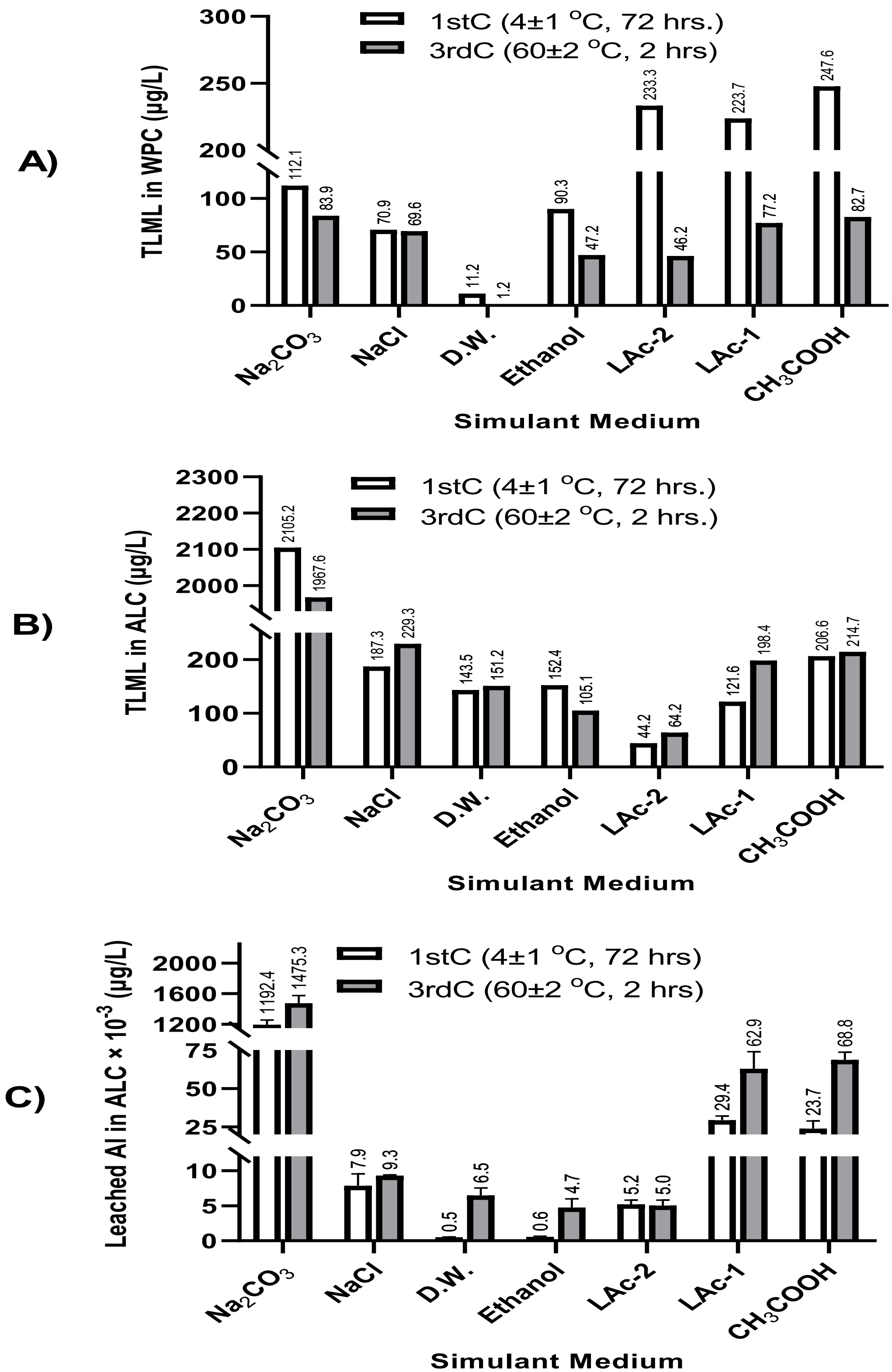

Figure 3. Content (µg/L) of the: a) TLML in the WPC, b) TLML in the ALC specimens, and c) leached Al level in the ALC using different simulating solutions

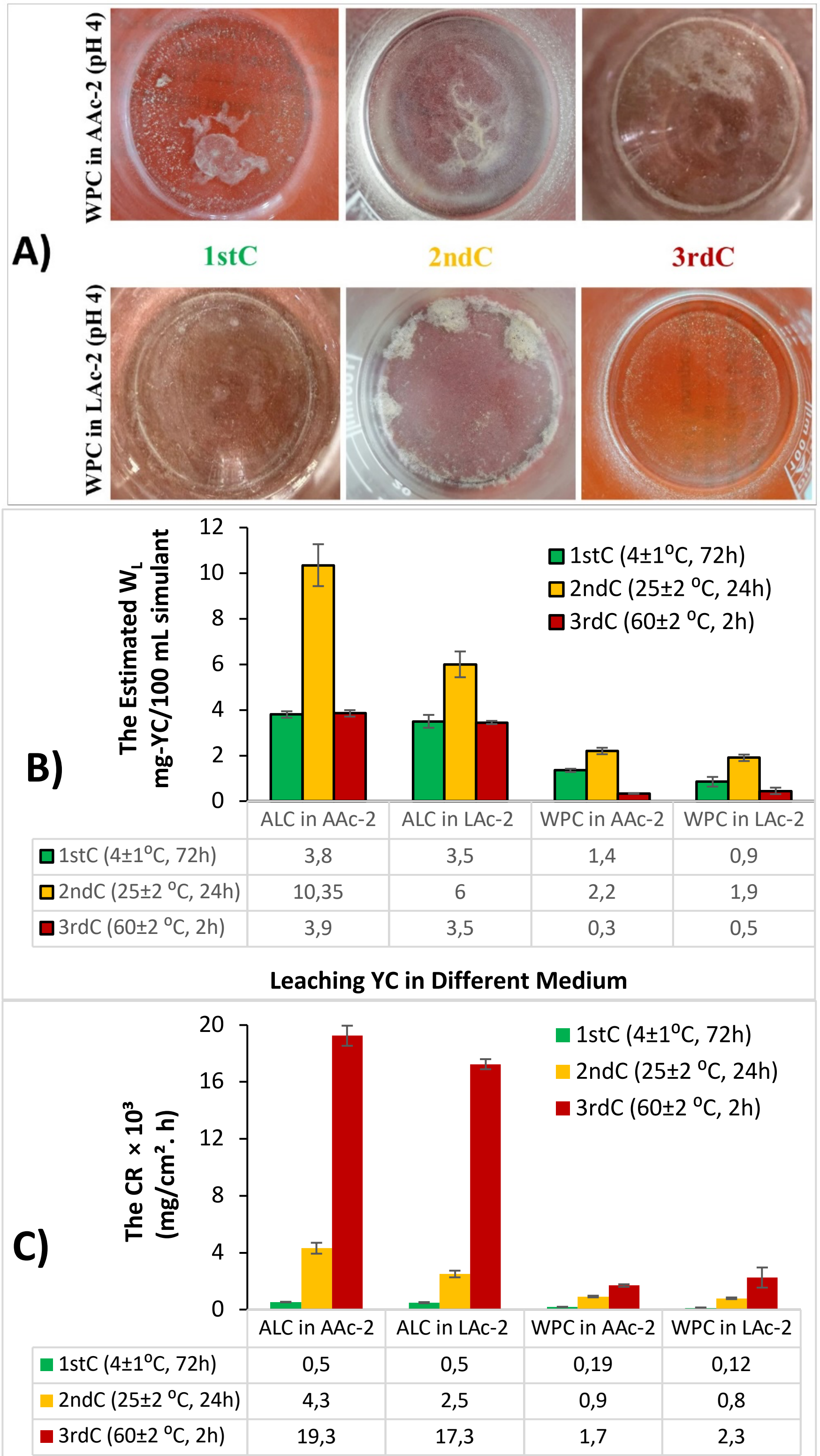

Figure 4. Results of the a) migrated residual precipitate of the WPC after drying, b) 'estimated' W_L level (mg-YC/100 mL simulant of the LAc-2 and AAc-2, and c) levels of the corrosion rate (C_R) for the two YC plates

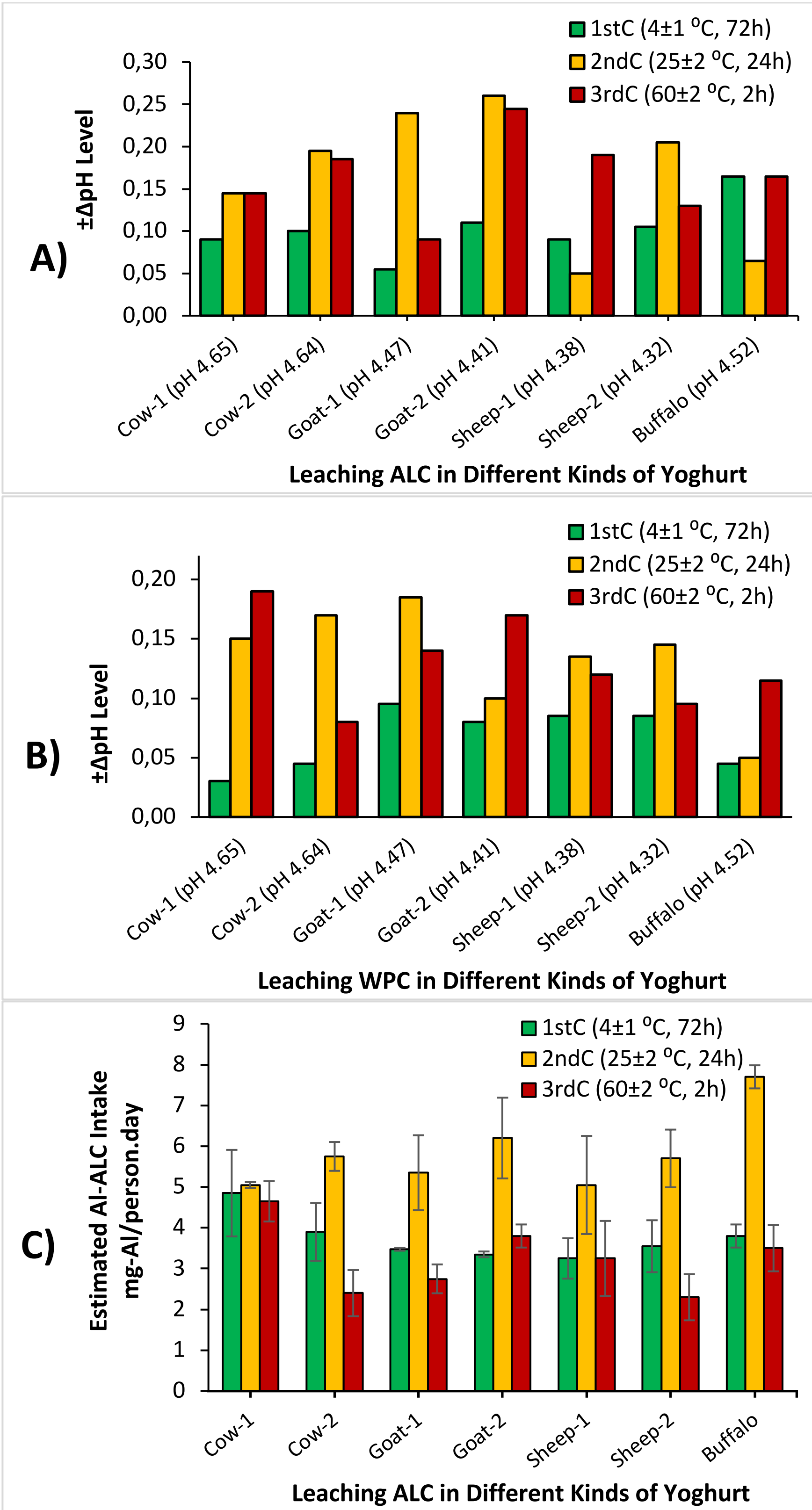

Figure 5. Results of the a) recorded ΔpH level after immersing and leaching each of the ALC, and b) WPC specimens, and c) 'estimated' Al-ALC levels intake (mg-Al/person.day) in many kinds of local yogurt samples