applied sciences

MDPI

Article

Styrene Migration from Polystyrene for Food Contact: A Case Study on the Processing Chain of Yoghurt Pots

Valeria Guazzotti *, Veronika Hendrich, Anita Gruner, Dominik Fiedler, Angela Störmer and Frank Welle

Fraunhofer Institute for Process Engineering and Packaging, IVV., Giggenhauser Straße 35, 85354 Freising, Germany; anita.gruner@ivv.fraunhofer.de (A.G.); dominik.fiedler@ivv.fraunhofer.de (D.F.); angela.stoermer@ivv.fraunhofer.de (A.S.); frank.welle@ivv.fraunhofer.de (F.W.)

* Correspondence: valeria.guazzotti@ivv.fraunhofer.de

Abstract: A precautionary specific migration limit of 40 µg/kg for styrene from food contact materials is currently under evaluation in Europe. In the ongoing discussion about applicable methods to demonstrate compliance, testing with food instead of simulants or total transfer calculations has been proposed. In this study, the residual styrene levels in high-impact polystyrene (HIPS) blended with general-purpose polystyrene (GPPS) were determined at several levels of the processing chain of yoghurt pots (from pellets to sheets, then to pots). The styrene migration from extruded sheets and thermoformed pots was analyzed in food simulants at 10 days/20 °C and 40 °C, as well as in yoghurt after storage at 8 °C after 50 days. The obtained results show that the residual styrene content in the PS materials correlates with migration. However, the migration of styrene was far below the total transfer assumption. Styrene migration from the sheets into food simulants and into yoghurt exceeded that migrating from the pots. Styrene migration into food simulants in 20% ethanol at 10 days/20 °C and 40 °C and, more clearly, in 50% ethanol at 10 days/40 °C, was higher than that into yoghurt tested on the best-before date. Styrene migration from PS pots into yoghurt after storage at 8 °C for 50 days reached up to 15 µg/kg (applying the EU cube model), which would be conservatively covered by testing the PS pots with 20% ethanol at 10 days/20 °C.

Keywords: styrene; migration; polystyrene; food packaging; compliance testing

check for updates

Citation: Guazzotti, V.; Hendrich, V.; Gruner, A.; Fiedler, D.; Störmer, A.; Welle, F. Styrene Migration from Polystyrene for Food Contact: A Case Study on the Processing Chain of Yoghurt Pots. *Appl. Sci.* **2024**, *14*, 9056. https://doi.org/10.3390/app14199056

Academic Editors: Marco Iammarino and Emmanouil Tsochatzis

Received: 17 May 2024
Revised: 23 July 2024
Accepted: 26 September 2024
Published: 7 October 2024

Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

The production of a food contact material (FCM) typically passes through numerous processing stages. In Europe, the compliance of FCMs with the applicable legal requirements must be assured at each stage of the supply chain. Plastics Regulation (EU) No. 10/2011 (for plastics used in applications with food contact) requires a written declaration of compliance (DoC), which is to be provided at all stages of the supply chain excluding the retail stage [1]. Therefore, each manufacturer must declare compliance with respect to their responsibility and role in the supply chain. Communication through the production chain is essential, clarifying what work has been performed and what tasks are delegated to actors further down the supply chain [2]. The compliance of FCMs can be verified by migration testing. The above Plastics Regulation sets a maximum overall migration limit (OML) and specific migration limits (SMLs) for toxicologically relevant substances. Furthermore, it describes the required testing procedure [1]. Migration testing in food legally prevails. However, migration is usually tested with 'simulants', which are used to represent defined food categories. The migration testing is performed under standardized time/temperature conditions, chosen depending on the intended specific food use and the maximum shelf-life of packaged food. Yogurts and similar dairy products are usually stored at refrigerated temperatures, with shelf-lives of about 40 to 50 days. The official simulant for milk products is 50% ethanol (simulant D2). The contact condition of 10 days at 40 °C represents long-term storage for more than 30 days in refrigerated conditions, according to Regulation (EC) No 10/2011, Annex V.2.1.4 [1].

High-impact polystyrene (HIPS) is used for packaging fresh dairy products like yoghurt, buttermilk, puddings, and dairy desserts in small cups, pots, or tubs [3]. Crystal polystyrene (or general-purpose polystyrene, GPPS) is converted to HIPS by adding impact modifiers like butadiene rubber in order to improve the flexibility of the materials. Commercial HIPS grades are usually blended further with additional GPPS at the stage of the production of sheets for dairy applications. The final cups are directly thermoformed from sheets at the creamery in form–fill–seal (FFS) packaging machines, with subsequent filling of the product and sealing while in-line; alternatively, dairy manufacturers buy preformed cups from packaging material converters and then only have to fill and seal them [4].

Polystyrene contains residues of styrene monomer, which partially migrate from the food contact article into the foodstuff. A precautionary specific migration limit (SML) for styrene of 40 μg/kg has been recently proposed by the EU Commission [5]. The European Food Safety Authority (EFSA) is currently evaluating the available studies to assess the genotoxicity potential of styrene after oral exposure to foods in plastic FCMs [6].

The residual levels in the PS material, the characteristics of the polymer, and food properties such as fat content, as well as the contact temperature and the duration of storage of the packed food are the main factors influencing the migration of styrene [7–15]. The latest research reveals that when testing PS materials with food simulants under the conditions defined by Regulation (EC) No 10/2011, there is a risk of obtaining overly high migration results for styrene that are not comparable to the level of migration seen into real food [16–19]. In a previous study [20], we found that the levels of styrene migrating into different refrigerated dairy products (packed in PS pots) ranged from 2.8 μg/kg to 22.4 μg/kg; in contrast, when PS pots were tested with food simulants comprising 10%, 20%, and 50% ethanol for 10 days at 40 °C, the migration results were higher and, in most cases, exceeded 40 μg/kg. The explanation of such findings was that ethanol/water mixtures cause (especially at elevated temperatures) the swelling of PS. Only using 10% or 20% ethanol at a lower time and temperature (i.e., 10 days at 20 °C) was revealed to be adequate to simulate styrene migration in food products stored under refrigerated conditions (e.g., yoghurt).

The scope of the present work was to evaluate the residual levels and the migration of styrene from intermediate and final food contact PS materials into food simulants and yoghurt. The analyses were carried out at several levels of the processing chain of yoghurt pots: (1) content of styrene in GPPS and HIPS pellets; (2) content of styrene in the extruded PS sheets; (3) content of styrene in the PS pots after thermoforming the sheets; (4) migration of styrene into food simulants after contact with the sheets and the pots; (5) migration of styrene in yoghurt after contact with the sheets and the pots. The same source of HIPS and GPPS was used for the materials employed in each of the steps, from pellets via sheets to pots, which enabled direct comparisons to be made.

2. Materials and Methods

2.1. Sample Materials

The study was initiated and financially supported by the Styrenics Steering Committee of Plastics Europe (Plastics Europe AISBL, Rue Belliard 40, 1040 Brussels, Belgium), which represents the relevant manufacturers of styrene polymers. According to the guidelines of pre-competitive studies within the framework of Plastics Europe, the trade names of the polymers used may not be mentioned. The companies involved in the Styrenics Steering Committee selected representative materials for the yoghurt sector in this study. Five types of pellets were provided. These included three grades of general-purpose polystyrene (GPPS-1, GPPS-2, GPPS-3) and two grades of high-impact polystyrene (HIPS-1, HIPS-2). Their physical properties were as follows: the Vicat softening temperature was approx. 100 °C and the melt volume rate ranged from 3 cm^3/10 min (GPPS-2) to 12 cm^3/10 min (GPPS-3); the Charpy notched impact strength was approx. 12 kJ/m^2 (HIPS-2). Additionally, three types of extruded sheets (PS-S-11, PS-S-22, PS-S-32) and three types of thermoformed pots (PS-P-11, PS-P-22, PS-P-32) were provided. The sheets were produced

using a 50:50 blend of the GPPS and HIPS pellets (the typical composition of PS pots for food contact); from the sheets, the pots were thermoformed by two different converters. The number codes refer to the GPPS and HIPS grades (see Table 1). The sheets had a thickness of approx. 1000 µm and an area weight of approx. 10 g/dm^2. The pots had a thickness ranging from 150 to 300 µm (depending on the pot type and the local measurement point on the wall of the cup) and an area weight ranging from 1.6 to 3.2 g/dm^2. Regarding the age of the samples, they are representative materials within the current supply chain. After receipt in the laboratory, all the materials were stored refrigerated (5 °C) until analysis.

Table 1. Content of styrene monomer in the investigated PS sample materials.

Sample Name	Description	Styrene Residual Content ± SD [µg/g]
GPPS-1	GPPS pellets grade 1	373 ± 5
GPPS-2	GPPS pellets grade 2	407 ± 8
GPPS-3	GPPS pellets grade 3	505 ± 15
HIPS-1	HIPS pellets grade 1	248 ± 3
HIPS-2	HIPS pellets grade 2	312 ± 3
PS-S-11	PS sheets made with GPPS-1 and HIPS-1 (converter A)	286 ± 2
PS-S-22	PS sheets made with GPPS-2 and HIPS-2 (converter B)	381 ± 5
PS-S-32	PS sheets made with GPPS-3 and HIPS-2 (converter B)	412 ± 4
PS-P-11	PS pots made from PS-S-11 (converter A)	262 ± 6
PS-P-22	PS pots made from PS-S-22 (converter B)	376 ± 4
PS-P-32	PS pots made from PS-S-32 (converter B)	394 ± 5

SD: standard deviation of triplicate analysis.

2.2. Concentration of Styrene Monomer in the Sample Materials

Gas chromatography with flame ionization detection (GC-FID) was used to determine the residual levels of styrene monomer in the sample materials after statical extraction in acetone, as described in a previous publication [20]. Triplicate experiments were performed. An Agilent 6890 GC/FID system (Agilent Technologies, Santa Clara, CA, USA) equipped with a DB 1 column was used. The styrene was quantified through external calibration using a reference standard (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan); additionally, to check the stability and the performance of the GC analysis, di-(tert-butyl)-hydroxyanisol (BHA) (Fluka, Seelze, Germany) and Tinuvin 234 (Ciba, Basel, Switzerland) were used as internal standards. The exhaustiveness of the extraction and recovery of the whole analysis procedure was checked by spiking styrene on PS sheets before extraction. The recovery percentages for styrene ranged between 88% and 108%.

2.3. Specific Migration of Styrene Monomer from PS Pots and Sheets into Food Simulants

Determination of the specific migration of styrene monomer into food simulants was achieved using the thermoformed PS pots and the extruded sheets.

Migration testing was carried out by the total immersion of the test specimen in pressure-resistant stainless-steel cells under the conditions of 10 days/40 °C in the 50% ethanol food simulant. Additionally, migration experiments were performed under the conditions of 10 days/40 °C and 10 days/20 °C in the 20% ethanol food simulant. Each test was carried out in triplicate. The test specimens were made into strips, with dimensions of 2.5 cm × 10 cm, cut from the pots and the sheet samples. These strips were placed in stainless steel migration cells containing 80 mL of food simulant, minimizing the remaining headspace. The applied surface/volume ratio in the migration experiments corresponded to the conventional EU cube model of 6 dm^2/kg. The total surface (0.5 dm^2) in the total immersion test was calculated by considering both sides of the test specimens, excluding the area of the cut edges. This choice was made by taking into account that PS is a low-diffusive polymer and migration is independent of both sides, in accordance with the draft "Technical guidelines for compliance testing" [21].

Static headspace GC, coupled with FID, was applied to determine the migrated amount of styrene in the simulants. An Agilent 6890 system (Santa Clara, CA, USA) equipped with a ZB 624 column was used, under the same conditions as those described in a previous publication [20]. The quantification of styrene was carried out by external calibration in 20% ethanol using m-xylene (CAS: 108-38-3; Sigma-Aldrich, Saint Louis, MO, USA) as the internal standard. Independent triplicate migration experiments and triplicate measurements of each migration solution were performed.

The HS-GC-FID method used for the analysis of food simulants has a detection limit of 1.3 µg/kg in 20% ethanol and of 3.2 µg/kg in 50% ethanol.

2.4. Concentration of Styrene Monomer in Yoghurt (Migration Kinetics)

Migration kinetics (from both sheets and pots) were measured at 8 °C by total immersion in yoghurt using glass vials. Five time points were measured (i.e., 10, 20, 30, 40, and 50 days of storage). Natural yoghurt (3.5% fat content) that had not been in previous contact with styrene-based materials (500 g packaged in PP) was used and was preserved by mixing with 0.1% sodium azide in order to avoid mold growth during the migration experiment. Test specimens with dimensions of 3.8 cm × 1.6 cm (0.12 dm^2 total surface, considering both sides and excluding the edges) were cut from the pots and the sheet samples and placed in glass vials containing 20 g of yoghurt. The applied surface/volume ratio in the migration experiments corresponded to the conventional EU cube model of 6 dm^2/kg. Each migration test series was carried out in triplicate. At each contact time point, the test specimens were removed from the vials, and the remaining yoghurt clinging to the samples was scraped off into the vials using a metal spatula. The vials containing yoghurt were stored at 5 °C until analysis.

The concentration of styrene in yoghurt was measured by dynamic headspace (purge and trap) gas chromatography coupled with mass spectrometry (Thermo GC Trace Ultra MS-System Thermo Scientific ISQ 7000, with VSP 4000 Autosampler, IMT, Moosbach, Germany), using a previous published method [20]. After mixing the yoghurt samples with water (HPLC grade) and spiking them with styrene d-8 (CAS 19361-62-7) (Sigma-Aldrich, Saint Louis, MO, USA), the analyses were performed with a VSP Autosampler (40 °C), a Peltier water trap, and a cooled Tenax® trap. The GC column was a Restek RXI-624Sil MS. Quantification was obtained by monitoring the ions at *m*/*z* 104 for styrene and *m*/*z* 112 for styrene d-8. The calibration in yoghurt (using the same yoghurt as for the migration experiment) was prepared for external quantification at six levels, diluted from a stock solution prepared in dimethylacetamide. Additionally, independently prepared control standards (of a known spiked amount) in yoghurt were analyzed in parallel to the samples to check the recovery. Triplicate migration experiments and duplicate measurements were performed. The detection limit of the P&T-GC-MS in yoghurt was 0.4 µg/kg.

2.5. Characterization of the Sample Materials by Scanning Electron Microscopy

The surface morphology of the specimens was studied using a field emission SEM JEOL JSM-7200F (JEOL Ltd., Tokyo, Japan) with an acceleration voltage of 5 kV. To avoid charging effects in the SEM analysis, the prepared surfaces were sputtered in advance with a thin gold layer, using argon plasma at 5 kV and 20 mA for 1 min. The sample was placed into a vacuum chamber and rinsed with a gentle stream of argon to a final pressure of 0.016 kPa. The plasma was then ignited for 1 min. After introducing the sputtered polymer samples into the high-vacuum chamber of the SEM, the working distance was set to 10 mm. Magnification up to a factor of 50,000× established the surface qualities; finally, the nanoscaled gold layer could be obtained.

3. Results

The contents of styrene monomer in the investigated PS sample materials (pellets, extruded sheets, and thermoformed pots) are summarized in Table 1.

The migration rates of styrene into food simulants and into yoghurt under different time/temperature conditions from the extruded sheets (PS-S-11, PS-S-22, PS-S-32) and the thermoformed pots (PS-P-11, PS-P-22, PS-P-32) are depicted in Figure 1.

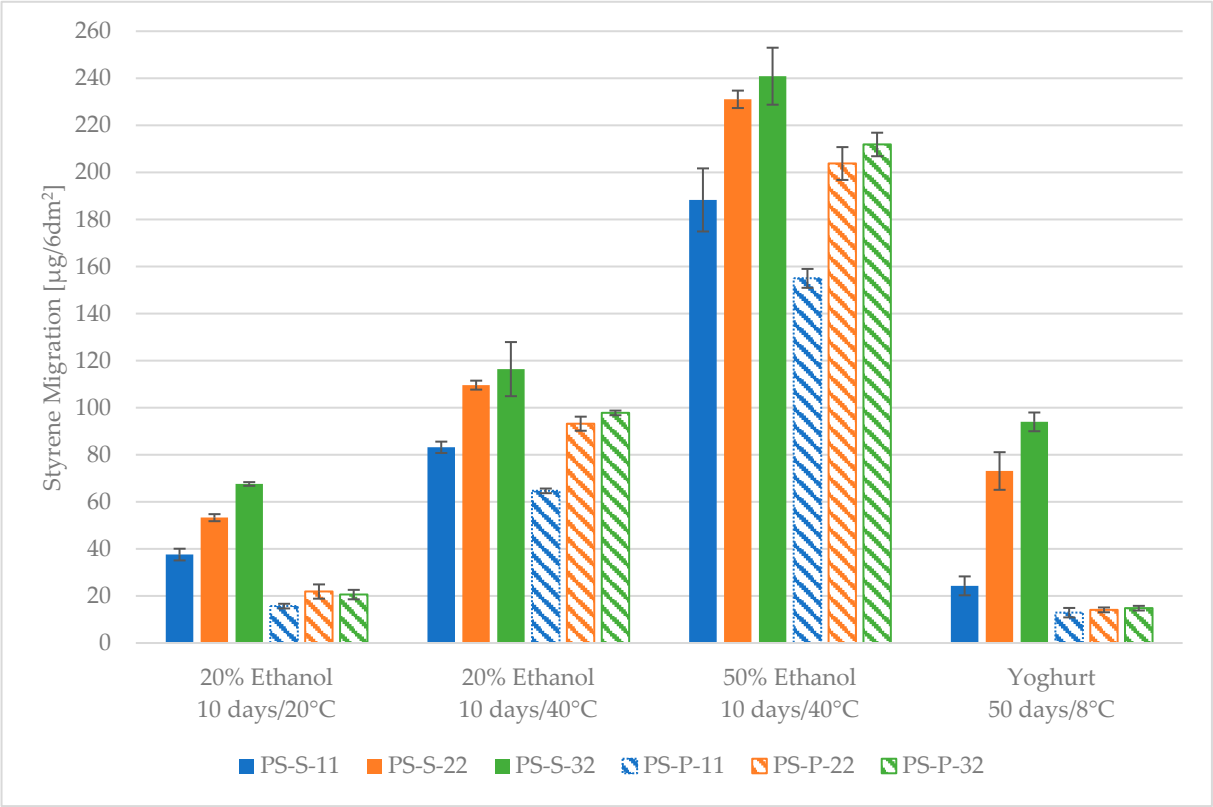


Figure 1. Specific migration of styrene into food simulants from the extruded sheets (solid bars) and the thermoformed pots (dashed bars).

The relevant exemplary chromatograms are available in the Supplementary Materials for the detection of styrene with: (a) GC-FID (in the acetone extracts of the PS samples), (b) HS GC-FID (in 20% and 50% food simulants), and (c) P&T-GC-MS (in yoghurt).

All migration experiments in food simulants and in yoghurt were carried out as independent triplicates. The migration from extruded sheets and thermoformed pots was determined in the yoghurt after storage at 8 °C for 10, 20, 30, 40, and 50 days. The mean values and standard deviations of all the measurements are reported in Table 2. Migration values are given in µg/kg (food), which correspond to µg/6 dm^2 according to the EU cube model (where 1 kg of food is in contact with 6 dm^2 of packaging), and which were used for the migration contact experiments in this study.

In Table 3, the relative migration of styrene is given, expressed as a percentage; it was calculated by dividing the mass of styrene found in the food (simulant) by the initial mass determined in the sample material.

Table 2. Migration of styrene in food simulants and in yoghurt under different testing conditions.

Food (Simulant)	Testing Conditions	Styrene Migration [µg/kg] [1] ± SD					
		PS-S-11	PS-S-22	PS-S-32	PS-P-11	PS-P-22	PS-P-32
20% Ethanol	10 days/20 °C	38 ± 3	53 ± 2	68 ± 1	16 ± 1	22 ± 3	21 ± 2
20% Ethanol	10 days/40 °C	83 ± 2	110 ± 2	116 ± 12	65 ± 1	93 ± 3	98 ± 1
50% Ethanol	10 days/40 °C	188 ± 13	231 ± 4	241 ± 12	155 ± 4	204 ± 7	212 ± 5
Yoghurt	10 days/8 °C	17 ± 1	22 ± 3	23 ± 5	5 ± 1	4 ± 1	7 ± 1
Yoghurt	20 days/8 °C	23 ± 2	39 ± 8	38 ± 3	7 ± 1	5 ± 1	10 ± 1
Yoghurt	30 days/8 °C	21 ± 3	53 ± 4	64 ± 1	9 ± 1	12 ± 1	7 ± 1
Yoghurt	40 days/8 °C	24 ± 3	53 ± 3	59 ± 10	9 ± 1	7 ± 1	11 ± 1
Yoghurt	50 days/8 °C	24 ± 4	73 ± 8	94 ± 4	13 ± 2	14 ± 1	15 ± 1

[1] The experiment was performed by applying the EU cube model of 6 dm^2/kg.

Table 3. Relative migration of styrene as a percentage.

Food (Simulant)	Testing Conditions	Relative Migration of Styrene [%] [1]					
		PS-S-11	PS-S-22	PS-S-32	PS-P-11	PS-P-22	PS-P-32
20% Ethanol	10 days/20 °C	0.2	0.2	0.3	0.3	0.6	0.5
20% Ethanol	10 days/40 °C	0.4	0.5	0.5	1.3	2.6	2.6
50% Ethanol	10 days/40 °C	1.0	1.1	1.0	3.1	5.7	5.6
Yoghurt	10 days/8 °C	0.1	0.1	0.1	0.1	0.1	0.2
Yoghurt	20 days/8 °C	0.1	0.2	0.2	0.1	0.2	0.3
Yoghurt	30 days/8 °C	0.1	0.2	0.3	0.2	0.3	0.2
Yoghurt	40 days/8 °C	0.1	0.2	0.2	0.2	0.2	0.3
Yoghurt	50 days/8 °C	0.1	0.3	0.4	0.3	0.4	0.4

[1] Calculated by dividing the mass of styrene found in the food (simulant) by the initial mass determined in the sample material (considering the area weight of each sample material).

4. Discussion

The residual content of styrene monomer in the PS sample materials (pellets, sheets, and pots) ranged from 248 to 505 µg/g of polymer. These results are consistent with the previous reported values for various food-grade non-expanded PS resins [22–24]. Three different grades of GPPS (GPPS-1, GPPS-2, GPPS-3) and two grades of HIPS (HIPS-1, HIPS-2) in the form of granules were analyzed in this study. The concentrations of styrene in the GPPS pellets were found to be slightly higher than those in the HIPS pellets. However, when also considering the data available in the literature, GPPS does not generally show a higher residual content of styrene compared to HIPS. The differences may be related to the different polymer grades used.

The pellets were converted to sheets by extrusion, blending a 50:50 mixture of GPPS/HIPS pellets, which is a typical composition of PS pots for food contact. Three types of extruded sheets were produced: PS-S-11 (blending GPPS-1 and HIPS-1), PS-S-22 (blending GPPS-2 and HIPS-2), and PS-S-32 (blending GPPS-3 and HIPS-2). The residual content of styrene monomer found in the extruded sheets reflects their composition. In fact, blending the GPPS and the HIPS grades with higher styrene residuals also resulted in higher styrene residual content in the sheets manufactured therefrom and vice versa. The sheets were further processed to create three types of pots (PS-P-11, PS-P-22, and PS-P-32) by thermoforming. The residual styrene contents in both the granulate and sheet were similar, at the most slightly decreasing during thermoforming. A decrease of approx. 10% in the styrene content was found in pot PS-P-11, obtained from sheet PS-S-11; however, in the other pots PS-P-22 and PS-P-32, it corresponded only to 1% and 4%, respectively, which can be regarded as insignificant. Previous studies involving the processing of PS granules into either cups or containers for dairy products support the evidence that there is a general tendency for residual styrene levels to decrease during thermoforming, which reflects and depends upon the temperature and vacuum conditions applied during processing [25].

The migration of styrene in food simulants and in yoghurt was tested using extruded sheets and thermoformed pots made of PS (using three types of pots manufactured from the corresponding sheets).

For compliance testing according to the EU Food Contact Regulation [1], standardized experimental conditions and proper food simulants must be chosen, depending on the food contact applications and time/temperature conditions of use.

During the industrial production and filling of stirred yoghurt, the temperature at the point of contact with the PS containers does not exceed 20 °C (in the first hours); the subsequent storage usually takes place below 5 °C. The common shelf life given by producers is about 40–50 days [19]. For household refrigerators, 8 °C is a representatively conservative temperature. These filling, fermentation, and storage conditions are tested using the standardized condition of 10 days/40 °C, which also covers hot-fill conditions according to Regulation 10/2011 (Annex V, chapter 2.1.4 (b)).

For yoghurt (ref. food category N. 07.02, shown in Table 2 of Annex III) the simulant D1 (50% ethanol) is specified and was used for the study. Simulant B, for the acidic character of yoghurts, was not considered in this study as the solubility of styrene is worse in 3% acidic acid compared to yoghurt or ethanol–water mixtures. Styrene, with a Log $K_{o/w}$ of 2.95, dissolves only slightly in water. However, it is soluble in alcohol. In the scientific literature, it has also been reported that testing styrene migration from PS by employing aqueous food simulants may lead to underestimation of the migration processes occurring in real food products [9–13].

As it has already been shown in previous studies that 50% ethanol swells the surface and leads to exaggerated migration values in relation to real foods [17], in the present study, 20% ethanol for 10 days/40 °C and for 10 days/20 °C were tested additionally as less severe simulants. According to Regulation 10/2011 (Annex III), 20% ethanol (food simulant C) should be used for alcoholic foods with an alcohol content of up to 20% and for those foods containing a relevant amount of organic ingredients that render the food more lipophilic, while 50% ethanol (food simulant D1) should be used for alcoholic foods with an alcohol content of above 20% and for oil-in-water emulsions, including milk products (Annex III, Tables 2 and 3 of the Regulation).

However, for some substances listed in Annex I, additional rules are given (Table 1, column 11) that should be respected in situations where there is a risk of non-compliance. Among these, the notes on the verification of compliance N.22 state that "when used in contact with non-alcoholic foods for which Table 2 of Annex III assigns food simulant D1, food simulant C (20% ethanol) shall be used for verification of compliance instead of food simulant D1". This recommendation followed an EFSA Scientific Opinion [26], in which it was noted when the compliance of polyethylene furanoate (PEF) polymer is verified with 50% ethanol, interactions (i.e., swelling) between this food simulant and the plastic can occur, which would not take place in real food contact applications. A similar situation might be stated for polystyrene. As expected, the migration results from the tested food simulants and from the yoghurt confirm that the migration of styrene depends on its residual content in the PS materials, with migration from grades with higher styrene residuals resulting in higher migration and vice versa. Moreover, as previously reported, the migration of styrene depends on the ethanol content of the food simulant and the temperature used [13,15,20,24,27]. This applies to both pots and sheets. However, the level of migration from the extruded sheets was found to be clearly higher than that from the thermoformed pots (solid bars versus dashed bars in Figure 1). This confirms our findings in a previous study [20]. The migration of styrene in 50% ethanol from the sheets ranged between 188 and 241 µg/6 dm^2 and from the pots between 155 and 212 µg/6 dm^2. In 20% ethanol tested at 10 days/40 °C, it ranged between 83 and 116 µg/6 dm^2 for the sheets and between 65 and 98 µg/6 dm^2 for the pots. The migrations in 50% and 20% ethanol, tested at 10 days/40 °C, from the pots were only 12–22% lower than those of the corresponding sheets (produced with identical PS grades). The migration levels in 20% ethanol, tested at 10 days/20 °C, ranged between 38 and 68 µg/6 dm^2 from the sheets and between 16 and

22 µg/6 dm^2 from the pots. This means that in these conditions, the migration from the pots is approx. 58–70% lower than that of the corresponding sheets. The styrene migration in food simulants exceeded the predicted precautionary SML for styrene of 40 µg/kg for all the tested sheets. When testing the pots with food simulants, the results exceeded this limit, with the exception of the conditions for 20% ethanol of 10 days/20 °C.

Similarly, the migration of styrene in yoghurt from the extruded sheets was found to be higher than that from the thermoformed pots. The migration from pot PS-P-11 was half of the one obtained from sheet PS-S-11. The migration from the other pots, PS-P-22 and PS-P-32, was 80% lower than that in the corresponding sheets (produced with the identical PS grades). At a temperature of 8 °C, the migration in yoghurt from the sheets with the higher styrene residual content (PS-S-22 and PS-S-32) already exceeded 40 µg/6 dm^2 after 20 days of storage, reaching 73 µg/6 dm^2 and 94 µg/6 dm^2 after 50 days. It was only from the sheet with the lowest residual styrene content (PS-S-11) that migration did not exceed this limit. Additionally, only for this sheet, the migration seemed not to increase over time (in contrast to the other two sheets; see Figure 2). This finding might be due to surface effects that were possibly caused by the converting process during the manufacture of this sheet, which might have affected the surface of the material and caused a different migration behavior compared to the other two sheets. When testing the pots with yoghurt, migration remained below or at 15 µg/6 dm^2, even after 50 days of storage at 8 °C. All the pots complied with the SML of 40 µg/kg that has been proposed for styrene. Additionally, as found for the sheet with the lower residual styrene levels (PS-S-11), migration from the pots seemed not to considerably increase over time. After 30 and 40 days, respectively, the migration of styrene from pots PS-P-32 and PS-P-22 into yoghurt seemed slightly to decrease, but considering the low levels measured, this could be due to the uncertainty of the analytical method.

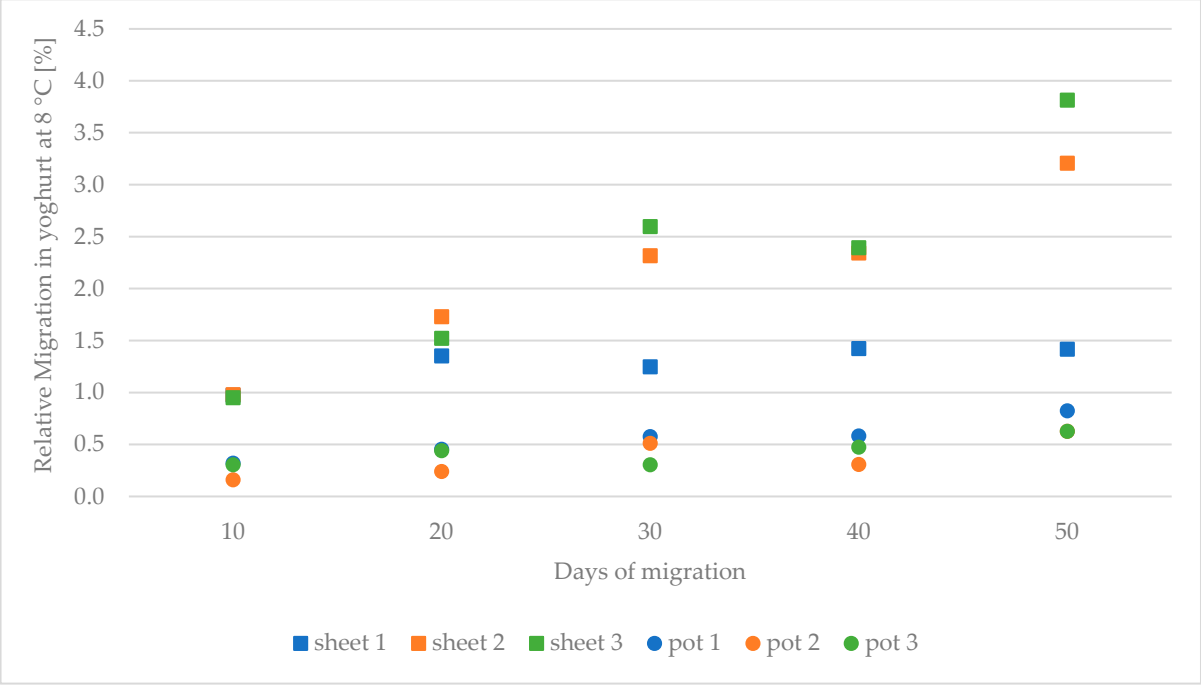


Figure 2. Relative migration of styrene in yoghurt at 8 °C (area weight normalized to 1 g/dm^2).

In Figure 2, the relative migration of styrene in yoghurt was depicted after storage at 8 °C for 10, 20, 30, 40, and 50 days. In this figure, to allow for comparisons, the percentage values have been recalculated with an area weight normalized to 1 g/dm^2 for all the sample materials.

The styrene levels measured in yoghurt within this study are in general agreement (only slightly higher) with previously published data on refrigerated dairy products packed

in PS pots taken from the supermarket [19,20]. In these studies, using natural stirred yoghurt products with 3.5% fat content analyzed on their best-before date (BBD), the styrene levels ranged from 5.2 μg/6 dm^2 to 11 μg/6 dm^2. In different milk desserts with a fat content of 0.2–15%, on their BBD, migration values ranged from 2.8 μg/6 dm^2 to 10 μg/6 dm^2. It should be noted that in the present study, for experimental reasons, strips cut from the pots were used for the migration contact. These strips were totally immersed in yoghurt within a glass vial with minimal headspace. In this experimental design, the edges of the PS material were in contact with the yoghurt, and the losses of styrene were minimized. This does not reflect the real-use contact conditions of pots filled with yoghurt but can be considered the worst case.

Polystyrene is a low-diffusive polymer. Therefore, the migration of styrene from sheets of approximately 1000 μm in thickness and pots of approximately 150 μm–300 μm in thickness should be similar from a theoretical migration point of view. From both types, only a minor percentage of the initial residual styrene content was transferred to the simulants or yoghurt (Table 3). Therefore, both thicknesses can be regarded as infinite for migration. In Figure 2, the migration of styrene is normalized to equal material thickness (area weight) and equal styrene concentration (expressed as a percentage of the initial amount) for better comparison. The reasons for the higher styrene migration from the extruded sheets compared to the thermoformed pots are not clear. The minimally different residual contents of styrene that were determined in the sheets and in the pots (manufactured from the corresponding sheets) do not explain these findings. The sheets with greater thickness have more cut edges relative to the surface area, compared to the pots. More precisely, the area of the sheet-edges was approx. 2.5 cm^2 (with simulants) and 1.1 cm^2 (with yoghurt); the area of the pot-edges was approx. 0.5 cm^2 (with simulants) and 0.2 cm^2 (with yoghurt). In all the cases, the area of the cut edges was less than 10% of the surface area. The "edge effect" is known, especially for some polymers like PS, to influence the migration extent when testing by total immersion [22]. Cut edges have different migration properties compared to the smooth surfaces of the sheets or the pots. Another explanation might be differences in the depletion of styrene in the near-surface region of the sheets and the pots during production, which were not equalized during storage before the experiment. The pots were manufactured by thermoforming. This process involves heating the extruded sheet to the point of thermal deformability and then forming it using a mold by means of vacuum or air pressure to obtain the desired pot shape. Previous studies [22,23,28] have already postulated that some physical treatments such as injection molding, extrusion, and thermoforming could lead to a deviation from the expected diffusion-controlled (Fickian) migration process. In thermoforming, the polymer chains become more orientated compared to the sheet, which improves the barrier property of the PS and might partly explain the lower migration of styrene from the pots. Scanning electron microscopy (SEM) images of the surfaces of the pots and sheets are shown in the Supplementary Materials. The pictures of the sheets show some surface damage (probably barrel scratches) parallel to the extrusion direction. Such scratches are less evident in the microstructures of the pots. An elongation of the polymer matrix around spherical or elliptical holes takes place, following the direction of thermoforming. However, the SEM pictures did not allow for a clear interpretation of differences in polymer orientation or material porosity. However, other authors [29] investigated HIPS yoghurt pots using transmission electron microscopy (TEM) and could clearly see the stretching of both the polymer matrix and the embedded rubber particles when using different processing conditions. Higher temperatures and production rates during thermoforming lead to higher degrees of stretching, which result in an increase in the mechanical quality (i.e., strength and stiffness) of the products.

The relative migration of styrene from the tested PS materials (calculated by dividing the mass of styrene found in the food simulant by the initial mass determined in the sample material) ranged from 1% to 6% in 50% ethanol samples tested at 40 °C. That recorded in yoghurt stored for 50 days at 8 °C ranged from 0.1% to 0.4%, which agrees with the

previously reported values [19,20]. These values demonstrate that actual styrene migration in food (and also in simulants) is far below the total transfer of the residual monomer level in the PS materials.

The styrene migration levels measured in yoghurt from the PS pots were lower than those from the same pots in the food simulants. Styrene migration from PS pots into yoghurt after storage at 8 °C for 50 days reached up to 15 µg/kg (when applying the EU cube model), which would be conservatively covered by testing the PS pots with 20% ethanol at 10 days/20 °C. A significantly higher migration was found with the 20% ethanol and 50% ethanol food simulants when tested for 10 days at 40 °C. These values ranged from 65 to 98 µg/kg (20% ethanol) and from 155 to 212 µg/kg (50% ethanol), respectively, at the surface-to-volume ratio of the EU cube model. In previous studies [16–18], the swelling effect caused by ethanol/water mixtures at elevated temperatures on the different styrene polymers was related to the major overestimation of styrene migration. Other authors [15] have already shown that the level of styrene migration after 24 h at 40 °C in 3.6% fat milk correlates approximately with that seen in 15% ethanol. In a previous study [26], the styrene concentration found in 3.5% fat milk was found to be comparable to that in 50% ethanol. However, in that study, the experiments were carried out by article-filling and storage at 30 °C for 10 days in pots sealed with aluminum lids. Eventually, the losses of styrene from the 50% ethanol solution were higher during migration contact with this experimental design, although 50% ethanol was less critical in terms of losses than 10% or 20% ethanol. However, a major difference is that the samples were stored under gentle movement conditions, which might increase the migration of styrene into the liquid milk compared to semi-solid yoghurt in static contact.

The styrene migration levels measured from the extruded sheets in simulants and after 50 days of storage in yoghurt would exceed the foreseen precautionary SML for styrene of 40 µg/kg [5]. Even testing the sheets with yoghurt at 8 °C for more than 20 days shows non-compliance results. When sheets or pots are tested using 20% ethanol or 50% ethanol for 10 days at 40 °C, unrealistically high migration levels may be obtained (due to the strong interaction between this food simulants and PS), leading to the risk of falsely categorizing materials as non-compliant (exceeding the SML). Similarly, testing the extruded sheets using 20% ethanol for 10 days at 20 °C or with real food, such as yoghurt, is associated with a high risk of obtaining non-compliant results.

According to the results obtained in the present study, only testing the final article (the PS thermoformed pots) for 10 days at 20 °C with 20% ethanol would lead to migration levels comparable to or slightly higher than those found in food products, such as yoghurt, stored under refrigerated conditions. Testing intermediate materials such as extruded sheets and using other time/temperature conditions that appear to be too severe may also lead to significantly higher styrene migration levels. This means that the demonstration of compliance of FCMs with respect to styrene migration may be difficult (or uncertain) at an early production stage of the supply chain.

This case study shows that if the food contact regulations are moving in the general direction of keeping substances down to lower and lower levels, not only will the analytical identification/quantification of these substances be an issue, additionally, the processing conditions of FCMs may have a significant impact and (especially at low concentrations) increase the variability of the findings.

5. Conclusions

In the present study, the residual levels and the migration of styrene from intermediate and final food contact PS materials into food simulants and in yoghurt were assessed. Migration testing was performed with extruded sheets and thermoformed pots in 50% ethanol for 10 days at 40 °C and in 20% ethanol for 10 days at 40 °C and 20 °C, as well as in yoghurt after storage at 8 °C for 50 days. From an evaluation of the data obtained, the following conclusions can be drawn:

- The residual styrene content in the PS materials correlates with the migration values and is far below a total transfer assumption.
- Styrene migration levels, measured in food simulants and in yoghurt, from intermediates (i.e., extruded sheets) were higher than those found in the final materials (i.e., thermoformed pots) and exceeded the precautionary specific migration limit (SML) for styrene of 40 µg/kg, as proposed by the EU Commission [5].
- Styrene migration from pots, using conventional food simulants and under the test conditions specified in Regulation (EU) No. 10/2011 for refrigerated dairy products (50% ethanol for 10 days at 40 °C), overestimates the styrene migration values compared to its migration into food (yoghurt) under real-life storage conditions.
- Styrene migration from PS pots into yoghurt after storage at 8 °C for 50 days reached up to 15 µg/kg (applying the EU cube model), which would be conservatively covered by testing the PS pots with 20% ethanol at 10 days/20 °C.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app14199056/s1, Scanning electron microscopy (SEM) images of PS pots and sheets and exemplary chromatograms.

Author Contributions: Conceptualization, V.G.; data curation, V.G., A.G., D.F. and V.H.; formal analysis, A.G., V.H. and D.F.; funding acquisition, V.G., A.S. and F.W.; investigation, V.G., A.G., D.F. and V.H.; methodology, V.G.; project administration, V.G., A.S. and F.W.; supervision, A.S. and F.W.; validation, V.G. and A.S.; visualization, V.G., A.S. and F.W.; writing—original draft, V.G.; writing—review and editing, V.G., A.S. and F.W. All authors have read and agreed to the published version of the manuscript.

Funding: This research received financial support from the Styrenics Steering Committee of Plastics Europe.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data used to support the findings of this study can be made available by the corresponding author upon request.

Acknowledgments: The authors gratefully acknowledge financial support from the Styrenics Steering Committee of Plastics Europe. Thanks are due to Mark Pemberton (Systox Ltd.) for fruitful discussions and proofreading of the manuscript.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study, in the collection, analysis, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

References

1. European Commission. Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food. *Off. J. Eur. Union* **2011**, *12*, 1–89.
2. Grob, K.; Stocker, J.; Colwell, R. Assurance of compliance within the production chain of food contact materials by good manufacturing practice and documentation—Part 1: Legal background in Europe and compliance challenges. *Food Control* **2009**, *20*, 476–482. [CrossRef]
3. Mac Bean, R.D. Packaging and the shelf life of yoghurt. In *Food Packaging and Shelf Life: A Practical Guide*, 1st ed.; Robertson, G.L., Ed.; CRC Press: Boca Raton, FL, USA, 2009; pp. 143–156.
4. Form-Fill-Seal (FFS) vs. Fill-Seal (FS) Machines. Infographic. How to Choose a Yoghurt Packaging Machine. By Fabien Jego and Gilles Demare. Available online: https://www.bwpackaging.com/blog/post/blog/2021/09/10/form-fill-seal-(ffs)-vs-fill-seal-(fs)-machines (accessed on 12 February 2024).
5. European Commission's Directorate General for Health and Food Safety (DG SANTE) 2023. Presentation of the Working Group on Food Contact Materials, 9 February 2023. Available online: https://food.ec.europa.eu/system/files/2023-02/cs_fcm_wg_20230209_handout.pdf (accessed on 27 March 2022).
6. EFSA. Re-Assessment of the Risks to Public Health Related to the Presence of Styrene in Plastic Materials and Articles Intended to Come into Contact with Food. Available online: https://open.efsa.europa.eu/questions/EFSA-Q-2023-00365?search=styrene (accessed on 12 February 2024).

7. Khaksar, M.R.; Ghazi-Khansari, M. Determination of migration monomer styrene from GPPS (general purpose polystyrene) and HIPS (high impact polystyrene) cups to hot drinks. *Toxicol. Mech. Methods* **2009**, *19*, 257–261. [CrossRef] [PubMed]
8. Naziruddin, M.A.; Sulaiman, R.; Abdul Halim Lim, S.; Jinap, S.; Nurulhuda, K.; Sanny, M. The effect of fat contents and conditions of contact in actual use on styrene monomer migrated from general-purpose polystyrene into selected fatty dishes and beverage. *Food Packag. Shelf Life* **2020**, *23*, 100461. [CrossRef]
9. Till, D.E.; Ehntholt, D.J.; Reid, R.C.; Schwartz, P.S.; Sidman, K.R.; Schwope, A.D.; Whelan, R.H. Migration of styrene monomer from crystal polystyrene to foods and food simulating liquids. *Ind. Eng. Chem. Fundam.* **1982**, *21*, 161–168. [CrossRef]
10. Choi, J.O.; Jitsunari, F.; Asakawa, F.; Sun Lee, D. Migration of styrene monomer, dimers and trimers from polystyrene to food simulants. *Food Addit. Contam.* **2007**, *22*, 693–699. [CrossRef]
11. Amirshaghaghi, Z.; Emam Djomeh, Z.; Oromiehie, A. Studies of Migration of Styrene Monomer from Polystyrene Packaging into the Food Simulant. *Iran. J. Chem. Eng.* **2011**, *8*, 43–49. Available online: https://www.sid.ir/en/journal/ViewPaper.aspx?id=272009 (accessed on 17 June 2022).
12. Dawson, P.; Seydim, A.C.; Hirt, D. Styrene Monomer Migration from Expanded Polystyrene into Food Acids and Ethanol. *J. Food Res.* **2020**, *9*, 39. [CrossRef]
13. Ajaj, A.; J'Bari, S.; Ononogbo, A.; Buonocore, F.; Bear, J.C.; Mayes, A.G.; Morgan, H. An Insight into the Growing Concerns of Styrene Monomer and Poly(Styrene) Fragment Migration into Food and Drink Simulants from Poly(Styrene) Packaging. *Foods* **2021**, *10*, 1136. [CrossRef]
14. Linssen, J.P.H.; Reitsma, J.C.E.; Roozen, J.P. Polystyrene sheet composition and temperature as parameters for migration of styrene monomer into corn oil. *Pack. Technol. Sci.* **1992**, *5*, 217–222. [CrossRef]
15. Tawfik, M.S.; Huyghebaert, A. Polystyrene cups and containers: Styrene migration. *Food Addit. Contam.* **1998**, *15*, 592–599. [CrossRef] [PubMed]
16. Guazzotti, V.; Ebert, A.; Gruner, A.; Welle, F. Migration from acrylonitrile butadiene styrene (ABS) polymer: Swelling effect of food simulants compared to real foods. *J. Consum. Prot. Food Saf.* **2021**, *16*, 19–33. [CrossRef]
17. Guazzotti, V.; Gruner, A.; Juric, M.; Hendrich, V.; Störmer, A.; Welle, F. Migration Testing of GPPS and HIPS Polymers: Swelling Effect Caused by Food Simulants Compared to Real Foods. *Molecules* **2022**, *27*, 823. [CrossRef] [PubMed]
18. Welle, F. Diffusion Coefficients and Activation Energies of Diffusion of Organic Molecules in Polystyrene below and above Glass Transition Temperature. *Polymers* **2021**, *13*, 1317. [CrossRef]
19. Guazzotti, V.; Hendrich, V.; Gruner, A.; Fiedler, D.; Störmer, A.; Welle, F. Migration of Styrene in Yoghurt and Dairy Products Packaged in Polystyrene: Results from Market Samples. *Foods* **2022**, *11*, 2120. [CrossRef]
20. Guazzotti, V.; Hendrich, V.; Gruner, A.; Störmer, A.; Welle, F. Styrene Monomer Levels in Polystyrene-Packed Dairy Products from the Market versus Simulated Migration Testing. *Foods* **2023**, *12*, 2609. [CrossRef]
21. Hoekstra, E.J. *Technical Guidelines for Compliance Testing in the Framework of the Plastic FCM Regulation (EU) No 10/2011*; Draft for Stakeholder Consultation; Version 24/08/2016; Joint Research Center: Luxembourg, 2016.
22. Linssen, J.P.H.; Reitsma, J.C.E.; Roozen, J.P. Effect of sampling method on the level of styrene monomer migrated from polystyrene packaging material. *Pack. Technol. Sci.* **1991**, *4*, 171–175. [CrossRef]
23. Lickly, T.D.; Lehr, K.M.; Welsh, G.C. Migration of styrene from poly(styrene) foam food-contact articles. *Food Chem. Toxicol. Int. J. Publ. Br. Ind. Biol. Res. Assoc.* **1995**, *33*, 475–481. [CrossRef]
24. Paraskevopoulou, D.; Achilias, D.S.; Paraskevopoulou, A. Migration of styrene from plastic packaging based on polystyrene into food simulants. *Polym. Int.* **2012**, *61*, 141–148. [CrossRef]
25. MAFF. Survey of Styrene Levels in Food Contact Materials and in Foods. In *The Eleventh Report of the Steering Group on Food Surveillance*; Food Surveillance Paper No. 1983-11; Ministry of Agriculture, Fisheries and Food: London, UK, 1983.
26. EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF). Scientific Opinion on the safety assessment of the substance, furan-2,5-dicarboxylic acid, CAS No 3238-40-2, for use in food contact materials. *EFSA J.* **2014**, *12*, 3866. [CrossRef]
27. O'Neill, E.T.; Tuohy, J.J.; Franz, R. Comparison of milk and ethanol/water mixtures with respect to monostyrene migration from a polystyrene packaging material. *Int. Dairy J.* **1994**, *4*, 271–283. [CrossRef]
28. Murphy, P.G.; MacDonald, D.A.; Lickly, T.D. Styrene migration from general- purpose and high-impact polystyrene into food-simulating solvents. *Food. Chem. Toxicol.* **1992**, *30*, 225–232. [CrossRef]
29. Schade, C.; Renner, H.-J.; Heckmann, W. High impact polystyrene. In *Predictive Property Adjustments*; Translated from Kunststoffe 7/2010, Document Number: PE110453; Carl Hanser Verlag: Munich, Germany, 2010; pp. 60–64. Available online: https://en.kunststoffe.de/ (accessed on 16 April 2024).

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.