Toxicology Reports 7 (2020) 288–295

Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

Risk assessment of trihalomethanes exposure by consumption of IV gamma products: Evidences from a Portuguese regional survey

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ARTICLE INFO

Keywords:
Trihalomethanes
Food safety
Exposure
Chlorine disinfection
Ready-to-eat vegetables
IV gamma products

ABSTRACT

Due to the current demand for healthier food, the IV gamma products industry has grown over the years. Additionally, it is well known that chlorine is a disinfectant largely used in this industry. However, the risk assessment associated with the formation of carcinogenic by-products from this halogenated disinfection has not been widely studied. The aim of this study was to assess the trihalomethanes (THMs) exposure through the consumption of IV gamma products, more specifically, salad mixes, and potential health risk in Portuguese population using a deterministic method. The quantification of THMs in salad mixes from the most representative Portuguese brands was performed by gas chromatography coupled to mass spectrometry (GC–MS). The THMs exposures were estimated by combining the THMs concentration data with a ready-to-eat salad mix consumption survey (case study applied in the Lisboa e Vale do Tejo area; n = 271). The concentration of THMs in all samples was below the limit of quantification of the equipment (1 μg/L), and this limit was used for the evaluation of the exposure. It was found that it is very small for the consumption pattern of these products in the studied population categories, suggesting that there is no risk for consumers due to the halogenated disinfection process. The highest estimated daily exposure was $7{,}65X10^{-5}$ mg/ kg bw/ week, which corresponds to a person who consumes such products daily.

1. Introduction

Due to modern lifestyle and consumer preference for fresh, healthy, and ready-to-eat foods, the production of IV gamma products is a growing segment of food industry. Packed ready-to-eat vegetables are included in this group and, although they are minimally processed, they need to be subjected to a disinfection process aiming to reduce microbial cross-contaminations, thus assuring a safety product for consumers. Among the available disinfection processes, chlorine is known for its efficacy against a large spectrum of microorganisms and, due to its low cost, has been widely adopted in the disinfection of fresh-cut vegetables [1]. However, this chemical process needs to be held carefully. Effectively, the side reactions that lead to disinfection by-products (DBPs) consume a part of the sanitizers, leaving less chlorine available to inactivate pathogens. Consequently, it is used an excessive chlorine dosing to compensate the loss of chlorine efficacy [2]. The high concentration of chlorine, through the reaction with organic matter, enhances the levels of toxic DBPs such as trihalomethanes (THMs), chloramines, and haloacetic acids (HAAs), among others [3–5]. Effectively, due to their potential carcinogenic effects, they can be regarded as a threat to human health [2] and, for this reason, chlorine has been prohibited in some European countries [6,7]. Alternative strategies and the use of combined methods to replace and/or to reduce the use of chlorine, as well as to minimize the formation of DBPs were recently reviewed [4,7] and some of these alternative strategies can be applied in the fresh-cut food industry.

Among DBPs, trihalomethanes, namely, trichloromethane ($CHCl_3$), bromodichloromethane ($CHCl_2Br$), dibromochloromethane ($CHClBr_2$), and tribromomethane ($CHBr_3$) can be found in tap water, vegetables, and in a great variety of other foods [6,8–12]. DBPs represent a greater concern for consumers´ exposure because they remain bound within food biopolymers and can be liberated during digestion [3]. Four THMs and six HAAs are regulated by several countries in drinking waters, but no regulation for these DBPs has been established in foods [11]. Maximum contaminant levels for the sum of four THMs (80 μg/L) or five HAAs (60 μg/L) in drinking water has been set by US Environmental Protection Agency, while in Europe, THMs (100 μg/L) are the only class of DBPs regulated by the European Union Drinking Water Regulations [9,13]. Therefore, the Food and Agriculture Organization of the United Nations recommends more research based on the occurrence of DBPs in different food categories. Scarce data are available regarding the presence of THMs in edible products in general, and in ready-to-eat

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https://doi.org/10.1016/j.toxrep.2020.01.019
Received 23 October 2019; Received in revised form 28 January 2020; Accepted 29 January 2020
Available online 30 January 2020

vegetables in particular. Consequently, studies on risk assessment for the consumption of these products are mandatory [6]. In this context, one of the objectives of the present work is to evaluate the presence of THMs produced during the chlorinated disinfection process in several samples of packed salad mixes commercialized in Região de Lisboa e Vale do Tejo, Portugal. For this purpose, a hyphenated technique based on headspace-solid phase microextraction and gas chromatography coupled to mass spectrometry (HS-SPME/GC–MS) was used. Crossing analytical data with consumption behavior obtained through surveys' results will allow a preliminary assessment of consumer's exposure to THMs by consumption of IV gamma products. As to our knowledge, this is the first report concerning the evaluation of THMs content in ready-to-eat salads commercialized in Portugal.

2. Materials and methods

2.1. Trihalomethanes analysis

THMs determinations were performed at Laboratório de Análises Industriais do Instituto Superior Técnico (LAIST), Lisboa, Portugal, by headspace solid-phase microextraction and gas chromatography coupled to mass spectrometry (HS-SPME/GC–MS).

2.1.1. Chemicals

Analytical grade reagents and solvents were used. The stock solutions of trihalomethanes (trichloromethane, bromodichloromethane, dibromochloromethane and bromoform) in methanol (1 g/L) were prepared from standard pure solutions obtained from Sigma Aldrich (Barcelona, Spain), and stored at −18 °C. Working solutions were prepared daily by appropriate dilutions of the stock solutions. Sodium sulfate, used to maximize THMs extraction, was purchased from Merck (Darmstadt, Germany).

2.1.2. Samples

Samples of mixed salads containing coriander, purple and green lettuce, from five well-known Portuguese brands, were purchased at local supermarkets in the Região de Lisboa e Vale do Tejo. The samples were transported to the laboratory in isothermic bags and refrigerated at 4 ± 1 °C. Individual salad packages were opened immediately before analysis and crushed in a mortar. Three replicates were made with aliquots (~1 g) of each sample from five different suppliers

2.1.3. Headspace solid phase microextraction

For headspace solid phase microextraction, a 100 μm thick polydimethylsiloxane (PDMS), 57300-U fiber (Supelco, Bellefort, PA, USA) was used. An aliquot (~1 g) of minced salad mix was weighed in a 20 mL glass vial (10 mL headspace/10 mL suspension) supplied with anhydrous Na_2SO_4 (1 g) and mineral water (10 mL). Then, vials were sealed with the SPME fiber, and stirred (1000 rpm) for 20 min, at room temperature. Then, the fiber was removed and immediately introduced in the GC injector.

2.1.4. Instrumentation

The determination of THMs was performed on a ThermoQuest (Trace GC 2000) gas chromatograph (GC) coupled to a ThermoQuest (TraceMS) quadropole mass spectrometer (MS) (ThermoQuest Corporation, Austin, TX, USA). Separations were performed on a DB-624 capillary column (30 m ×0.320 mm I.D. × 1.8 μm film thickness) with the stationary phase composed of 6 % cyanopropylphenyl / 94 % dimethylpolysiloxan (Agilent Technologies). Samples were introduced on the GC equipment by manual injection. Injection was done in splitless mode, and helium was used as carrier gas (1.0 mL/min).

The initial temperature of the GC oven was set to 50 °C for 3 min, followed by an increase of 8 °C/min to 90 °C, held for 4 min, and an increase of 6 °C/min to 150 °C.

The mass spectrometer operated in the following conditions: without solvent delay; transfer line temperature, 220 °C; ion source temperature, 230 °C; electron impact ionization mode at 70 eV. Quantification of THMs was performed in selected ion monitoring (SIM) mode and data acquisition was performed with Xcalibur software (ThermoQuest).

2.1.5. THMs quantification

Calibration curves were constructed for each trihalomethane (1 − 20 μg/L, being correlation coefficient values greater than 0.997. The limit of quantification LOQ of the equipment was 1 μg/L. HS-SPME conditions fiber type, temperature, stirring, and exposure time, as well as GC/MS parameters, were previously optimized for the determination of THMs in water in LAIST routine analysis. The performance of the method was assessed according to ISO/IEC 17025 recommendations. All the assays were performed under controlled room temperature 24 ± 2 °C.

2.2. Dietary intake of THMs

2.2.1. Food consumption questionnaires

2.2.1.1. Questionnaire design. As mentioned previously, one of the objectives of the work was to know the consumption pattern of some of the most relevant IV gamma products (namely ready-to-eat washed, sliced and packed for salad, for soup and fruits) in the supermarkets under study. In this way, it was possible to estimate the preliminary assessment of consumer's exposure to THMs by consumption of IV gamma products. On the other hand, with the application of this consumer survey it was possible to design the consumer profile, as well as to know some of the habits associated with these products.

The questionnaire was conceived to assess individual consumer preference and consumption patterns for the population of the Região de Lisboa e Vale do Tejo. Its design was structured based on ready-to-eat washed, sliced and packed for salad, for soup and fruits. The experience of previous questionnaires on consumption patterns and frequencies of similar products [14,15], such as fresh fruits or vegetables, was used to design a model. However, given the purpose of the above-mentioned questionnaire, it required that preference questions be introduce with reference to gamma IV products (namely, vegetables or fruit that have been washed, disinfected, cut, packaged and ready for consumption). The meal quantities and the personal features (gender, age, body mass index, purchasing power according to residence, education level and monthly income) parts were also adapted to the Portuguese reality. Accordingly, four different parts were included: (1) personal features of the respondents; (2) general products preferences; (3) consumption frequencies through the time of year (i.e., during the hottest period - May to October or in the coldest period of the year - November to April) and (4) usual disinfection treatments. The questionnaire was designed to ensure the principles of anonymity and confidentiality, and the data collected were used exclusively for the present study. In addition, in order to guarantee a higher level of participation and sincerity, a compromise had to be reached between accuracy and simplicity. Thus, the number of questions was limited and required a careful choice of questions and wording.

2.2.1.2. Data collection. Survey data were collected through questionnaires in Região de Lisboa e Vale do Tejo (whose population size is 3 077 534) during the first half of 2018. The questionnaire was pre-tested to check their general understanding and expected response time (n = 12). Minor adjustments were then made to the final questionnaire. A sample of 274 participants (over 18 years old) was determined using the most conservative estimate for a single proportion (0.5), a confidence level of 95 % and a margin of error of 5.0 %.

2.2.2. Exposure assessment

Considering the consumption questionnaire and the consumption differences between peoples, three consumption scenarios were used

Table 1
Carcinogenicity classification by IARC and Reference Dose of THMs [29].

Compound	Carcinogenicity	RfD
chloroform ($CHCl_3$)	B-2	0.07 mg/kg/week
dichlorobromomethane ($CHCl_2Br$)	B-2	0.14 mg/kg/week
bromoform ($CHBr_3$)	B-2	0.14 mg/kg/week
chlorodibromomethane ($CHClBr_2$)	C	0.14 mg/kg/week

B-2: Possibly carcinogenic in humans - limited evidence in humans and lack of evidence in laboratory animals.
C: Possibly human carcinogenic.

Table 2
Estimation of TMHs exposure and risk assessment.

Scenarios	Consumption (kg/ week)	Estimated weekly intake (mg/kg bw)[a]	HQ
I	0.008	$1.09X10^{-5}$	0.0002
II	0.024	$3.28X10^{-5}$	0.0005
III	0.560	$7.65X1065X10^{-5}$	0.0011

[a] Calculation based on the average body weight of consumers (see 3.2) =73.18 kg.

Table 3
Gender, age, body mass index, purchasing power (classification according to residence), education level and monthly income of survey respondents.

Baseline Characteristics	Survey respondents (%)
Gender	
Man	47.8
Woman	52.2
Age	
18-30	9.6
31-36	16.7
37-40	21.9
41-49	25.2
50-64	19.6
65+	7.0
Body mass índex[1]	
< 18,49	1.5
18,50-24,99	51.1
25,00-29,99	35.2
30,00-34,99	9.3
35,00 +	3.0
Purchasing power (classification according to residence)[2]	
Low	23
Intermediate	44.1
High	33.0
Education	
Basic	2.2
Secondary	21.1
Higher	70.7
Net monthly income of the household	
< 600€	6.3
601€-1200€	31.1
1201€-3000€	48.5
3000€ +	14.1

[1] The classes for body mass index were created according to the National Health Program [30], in order to allow a coherent discussion of the results.
[2] The classes were created according to data from the National Institute of Statistics [31], and the residence can be aggregated by purchasing power per capita.

for adults based on average data on food consumption obtained (see 2.2.1).

The estimation of individual exposure to THMs by an adult resident of the analysed area, depending on scenario, was calculated using a deterministic approach multiplying mean values of THMs concentrations (or the limit of quantification (LOQ) of the analytical equipment) by mean ingestion data and dividing by adult's average weight [16]. Exposures are expressed in mg/ kg bw/ week.

Table 4
General results (%) of the survey into consumption preferences and frequencies of ready-to-eat washed, sliced and packed vegetables or fruits (raw).

Parameters			Survey respondents (%)[1]
Preference		Salad	66.5
		Soup	10.0
		Fruit	23.5
Frequency	May to October	≤3 x/ week	
		Salad	82.2
		Soup	91.8
		Fruit	57.1
		> 3 x/ week	
		Salad	17.8
		Soup	8.2
		Fruit	42.9
	December to April	≤3 x/ week	
		Salad	83.0
		Soup	87.1
		Fruit	58.8
		> 3 x/ week	
		Salad	17.0
		Soup	12.9
		Fruit	41.3
Disinfection mode	With tablets	Salad	2.3
		Soup	1.9
		Fruit	2.4
	With water	Salad	41.9
		Soup	53.4
		Fruit	48.2
	Do not wash or disinfect	Salad	55.8
		Soup	44.7
		Fruit	49.4

[1] All respondents who did not respond to this question were not considered in the analysis.

2.2.3. Risk characterization

THMs are carcinogenic products, classified by IARC, being chloroform the most representative one, for which a Reference Dose (RfD) of 0.07 mg/kg/week has been established (Table 1).

Hazard quotient (HQ), i.e. the ratio of the expected exposure and the RfD for THMs at which no adverse effects are expected, was considered to assess the health risk by ingestion of food. A ratio lower than 1 indicates a tolerable exposure level and a value equal or higher than 1 indicates an adverse health effect [16,17].

2.3. Statistical analysis

Differences in baseline characteristics (purchasing power according to residence, age, gender, body mass index, education level and monthly income) according to IV gamma products consumption preferences (namely, ready-to-eat for salads, soup and fruit) were tested using chi-square test for independence. All requirements to the chi-square test for independence were validated. However, where these were not met, the analysis was performed using exact tests in order to obtain accurate results [18,19].

In order to evaluate whether the frequency of consumption between the two periods under analysis (i.e., during the hottest period - May to October or in the coldest period of the year - November to April) covaries the Spearman's rank-order correlation coefficient was used. Kruskal-Wallis test was carried out to analyze the overall distribution of respondents (as a function of baseline characteristics) according to consumption frequencies through the time of year (i.e., May to October or November to April). The use of the Kruskal-Wallis test showed to be appropriate since it allows comparing distributions of two or more at least ordinal variables observed in two or more independent samples.

Table 5
Baseline characteristics of the participants as a function of preference of ready-to-eat washed, sliced and packed vegetables or fruits (raw) consume.

	n[1]	Preference of consumption (%) of ready-to-eat washed, sliced and packed vegetables or fruits (raw)			
Gender		Salad	Soup	Fruits	p-value
Man	137	30.0	3.8	13.5	0.162
Woman	123	36.5	6.2	10.0	
Age					
18-30	26	6.9	0.8	2.3	0.717
31-36	45	10.0	1.9	5.4	
37-40	58	13.8	3.5	5.0	
41-49	66	16.9	2.3	6.2	
50-64	51	14.6	1.5	3.5	
65+	14	4.2	0.0	1.2	
Body mass index					
≤18,49	3	0.8	0.4	0.0	0.743
18,50-24,99	135	33.1	6.2	12.7	
25,00-29,99	93	24.6	2.3	8.8	
30,00-34,99	22	6.2	0.8	1.5	
35+	7	1.9	0.4	0.4	
Purchasing power (classification according to residence)					
Low	61	15.0	2.7	5.8	0.455
Intermediate	114	31.5	4.2	8.1	
High	85	20.0	3.1	9.6	
Education					
Basic	4	1.6	0.0	0.0	0.610
Secondary	55	14.6	2.8	4.9	
Higher	188	49.8	7.3	19.0	
Net monthly income of the household					
< 600€	15	3.5	1.2	1.2	0.005**
601€-1200€	81	19.6	4.2	7.3	
1201€-3000€	128	36.5	4.2	8.5	
3000€ +	36	6.9	0.4	6.5	

[1] All respondents who did not respond to this question were not considered in the analysis.
** The correlation (χ^2 test or exact test) is significant at the 0.01 level.

Table 6
Spearman's Rank-Order correlation coefficient for the frequency of consumption of ready-to-eat washed, sliced and packed vegetables or fruits (raw) between the two periods.

		December to April		
		Salad	Soup	Fruit
May to October	Salad	0.813**	0.433**	0.260**
	Soup	0.424**	0.949**	0.562**
	Fruit	0.265**	0.564**	0.953**

** The correlation is significant at the 0.01 level.

The difference of means between pairs was resolved by using confidence intervals in a Dunn test. To analyze the frequency of consumption in relation to the gender, the nonparametric test of Mann-Whitney was used. Level of significance was set for p-value < 0.05. Analyses were carried out using IBM SPSS Statistics 25.

3. Results and discussion

3.1. Occurrence of THMs

Dietary intake constitutes one of the main ways of exposure to harmful contaminants like heavy metals [20], alkenylbenzenes [21], organochlorine pesticides [22], sulfites [23] and THMs [24,25], among others.

In the present work, the use of HD-SPME coupled to GC–MS proved to be a suitable techniquefor the extraction and identification of THMs from the samples under study, due to their easy handling, fast execution, and sensitivity. Concentrations were calculated based on the *m/z* ratios of trichloromethane (*m/z* 83, *m/z* 85), bromodichloromethane (*m/z* 83, *m/z* 85), dibromochloromethane (*m/z* 127, *m/z* 129), and tribromomethane (*m/z* 173, *m/z* 175), being ions at *m/z* 83, *m/z* 127, *m/z* 173 used for quantification purposes.

It was verified that THMs concentration in all the analyzed samples were below the limit of quantification (LOQ) of the equipment. The average concentration of each THM (< 10 μg/kg) was lower than the maximum contaminant level for these compounds in potable water established by the Portuguese and European Union legislation, which is 100 μg/L for the sum of the four THMs [26]. So, in a first approach, it seems that a rational consumption of those salad mixes do not represent a health risk to consumers of Região de Lisboa e Vale do Tejo.

According to Cardador and Gallego [9,10] in low processed vegetables, THMs may not be detected once they could volatilize before they reach the consumer. However, these authors report the presence of this group of compounds in canned (0.3 − 25 μg/kg) and in frozen (1.2 − 30 μg/kg) vegetables. On the other hand, Coroneo and co-workers [6] determined the levels of THMs in different types of ready-to-eat vegetables washed with chlorinated water and, in 115 analyzed samples, the average value of total THMs was equal to 76.7 ng/g, being the highest percentage due to chloroform. Also, Huang and Battermann [12] investigated the sorption behavior of THMs on foods, including vegetables, during rinsing and cooking. They concluded that, among vegetables, THMs´ levels, at 25 °C, presented a great variability ($CHCl_3$: 213–774 ng/g; $CHCl_2Br$:53 to 609 ng/g; $CHClBr_2$:150 to 845 ng/g), highlighting the role of food as an exposure source. Loan and collaborators [27] reported the formation of 3-chlorotyrosine, another halogenated contaminant, in ready-to-eat vegetables due to hypochlorite treatment. Combining consumption data of ready-to-eat vegetables by Spanish and Belgian consumers, a quantitative exposure assessment was performed. The authors concluded that 3-chlorotyrosine exposure via lettuce mixes could be regarded as a public health concern, in particular in higher consuming populations.

These results evidenced that vegetables´ chlorinated disinfection should be optimized in order to minimize the risk for consumers. Therefore, the search for alternative methods of disinfection continues to be a true challenge [7] and mitigation strategies to reduce DBPs levels are mandatory [26]. New chemical and physical post-harvest treatments e.g. ozone, electrolyzed water, high pressure processing, among others, were recently described [1,28]. According to these authors, it is possible to achieve similar efficacy as chlorine without the production of undesirable DBPs or compromising the quality of ready-to-eat vegetables.

3.2. Consumption data

The survey into the IV gamma products (namely, ready-to-eat for salads, soup and fruit) consumption preferences and patterns in the Região de Lisboa e Vale do Tejo population was successfully completed and 270 valid completed questionnaires were reached. There were some deviations in the age (only 7.0 % of individuals older than 65 years), body mass index (only 1.5 % of individuals were below 18.5 and 12.3 % were above 30.0), monthly income (only 6.3 % of individuals were in the lower category, that is below 600 €) and education level (more than 75 % with higher education) distributions (Table 3). Indeed, these deviations may be at least partially attributed to the difficulty to involve individuals for the importance of contributing to this type of study. Despite these disadvantages, the survey results were considered relevant and the total number of responses was analyzed and characterized. In addition, no studies are yet known to investigate the consumption of this type of products, so the analysis becomes even more relevant (although it has a preliminary character that cannot be discarded). Concerning ready-to-eat for salads, soup and fruit

Table 7
General results (%) of the survey into consumption frequencies of ready-to-eat washed, sliced and packed vegetables or fruits (raw) for the period between December and April.

Consumption Frequency	Never (%) Salad	≤ 3 x/week (%)	> 3 x/week (%)	p-value	Never (%) Soup	≤ 3 x/week (%)	> 3 x/week (%)	p-value	Never (%) Fruit	≤ 3 x/week (%)	> 3 x/week (%)	p-value
Gender												
Man	10.0	30.7	7.0	0.612	20.4	23.7	3.7	0.345	14.8	20.0	13.0	0.009**
Woman	11.5	34.4	6.3		25.2	23.7	3.3		25.9	14.8	11.5	
Age												
18-30	1.9	6.7	1.1	0.125	4.1	5.2	0.4	0.518	3.7	4.8	1.1	0.662
31-36	3.3	12.2	1.1		8.9	6.3	1.5		7.0	4.4	5.2	
37-40	4.8	15.2	1.9		10.4	10.0	1.5		9.6	8.5	3.7	
41-49	4.8	14.4	5.9		8.9	14.1	2.2		9.3	7.4	8.5	
50-64	3.7	13.0	3.0		10.0	8.1	1.5		8.1	7.4	4.1	
65+	3.0	3.7	0.4		3.3	3.7	0.0		3.0	2.2	1.9	
Body mass índex												
< 18,49	0.7	0.7	0.0	0.511	1.1	0.4	0.0	0.490	1.1	0.4	0.0	0.215
18,50-24,99	9.6	34.4	7.0		20.7	27.0	3.3		20.7	16.7	13.7	
25,00-29,99	8.1	21.9	5.2		17.0	15.6	2.6		12.6	13.3	9.3	
30,00-34,99	1.9	6.7	0.7		4.8	3.7	0.7		4.8	3.3	1.1	
35,00 +	1.1	1.5	0.4		1.9	0.7	0.4		1.5	1.1	0.4	
Purchasing power (classification according to residence)												
Low	3.0	16.3	3.7	0.053	9.3	11.1	2.6	0.089	7.8	7.8	7.4	0.017*
Intermediate	12.2	27.8	4.1		22.6	20.4	1.1		21.5	15.2	7.4	
High	6.3	21.1	5.6		13.7	15.9	3.3		11.5	11.9	9.6	
Education												
Basic	0.8	0.8	0.8	0.972	1.6	0.8	0.0	0.412	1.6	0.8	0.0	0.274
Secondary	5.1	13.8	3.5		9.1	11.8	1.6		8.3	9.1	5.1	
Higher	15.0	50.8	9.4		35.0	34.6	5.5		29.9	25.6	19.7	
Net monthly income of the household												
< 600€	2.2	3.0	1.1	0.435	2.2	3.3	0.7	0.547	3.0	1.5	1.9	0.568
601€-1200€	6.3	22.2	2.6		12.6	17.0	1.5		12.2	11.5	7.4	
1201€-3000€	9.3	31.1	8.1		22.6	23.3	2.6		20.7	17.4	10.4	
3000€ +	3.7	8.9	1.5		8.1	3.7	2.2		4.8	4.4	4.8	

preferences, the universe of respondents clearly prefer salad (66.5 %) to soup (10.0 %) or fruit (23.5 %) (Table 4). In fact, the percentage of respondents who showed a preferential consumption of soup or fruit is quite low (for the general baseline characteristics; Table 5). The only statistically significant relationship was observed for monthly income (p-value < 0.01; Table 5). With respect to consumption frequency results, frequencies are always higher for consumption till to three times a week for both periods under analysis (Table 4). Respondents who indicate that they consume this type of product more than three times a week clearly decrease (for all three products) in both periods. Notwithstanding this pattern, there is superiority for salad and fruit consumption for the period between December and April when compared to the rest of the year. The opposite is observed for soup consumption (Table 4). In addition, consumption frequency results for the three products under study showed that there is a significant correlation between the two periods of the year (May to October *versus* December to April; Table 6). In this way, there is a pattern associated with the time of the year. Therefore, who chooses to consume this type of products, does it in a uniform way, whether it is a hotter or colder period of the year. Thus, it is possible to conclude that the time of the year does not assume a differentiating role to characterize the consumption of the IV gamma products under analysis. This result is mainly due to the non-seasonality of these products as they are always available to consumers regardless of the time of year. The performed survey results were also subjected to an additional statistical analysis, which addressed the dependence of the IV gamma products consumption frequencies on relevant personal factors, such as, gender, age, body mass index, purchasing power according to residence, education level and monthly income. Regarding gender, there are significant differences between consumption frequencies concerning fruit (p-value < 0.01 regardless of the time of year; Tables 7 and 8). Men consume more fruit than women and this result is achieved, whether for consumptions lower or higher than three times a week (Tables 7 and 8). Contrastingly, the pattern reverses when we observe the non-consumption results, where the weight of women who do not consume fruit (regardless the time of the year) is always higher. For other questionnaire items (salad and soup), no difference was found. Concerning purchasing power according to residence, fruit frequency consumption also showed statistically significant differences (p-value < 0.05 regardless of the time of year; Tables 7 and 8). Thus, the results showed that the differences are between the intermediate level, when compared with the lower level. Moreover, the achieved pattern showed that the frequencies are always higher for the intermediate level, for consumption till to three times/week or cases where this consumption is not an option (Tables 7 and 8). Notwithstanding the nonexistence of statistically significant differences in the frequency of consumption, when compared to the higher level, the results indicated that the highest consumption of fruit (i.e. more than three times/week) underlies this category. Similarly, for other questionnaire items (salad and soup), no difference was found. For the remaining baseline characteristics (age, body mass index, education level and monthly income), no statistically significant differences were observed (Tables 7 and 8). With respect to the disinfection mode, it was found that the use of disinfection tablets is not relevant for the ready-to-eat salad (2.3 %), soup (1.9) and fruit (2.4) (Table 4). The option to only use water or not to apply any method of sanitation is clearly the most important for participants (Table 4). Regarding the relationship between the consumption frequency and the disinfection mode of the products under study, it was found that the use of disinfection tablets is not significant (Table 9). For salad, there was a statistically significant relationship between frequency of consumption and mode of disinfection, which was accentuated by the absence of disinfection between December and April (p-value < 0.05; Table 9). Nevertheless, a similar trend is observed for the warmer period of the year, it was not significant. However, it is important to note that the tendency to wash

Table 8
General results (%) of the survey into consumption frequencies of ready-to-eat washed, sliced and packed vegetables or fruits (raw) for the period between May and October.

Consumption Frequency	Never (%) Salad	≤ 3 x/week (%)	> 3 x/week (%)	p-value	Never (%) Soup	≤ 3 x/week (%)	> 3 x/week (%)	p-value	Never (%) Fruit	≤ 3 x/week (%)	> 3 x/week (%)	p-value
Gender												
Man	9.3	32.6	5.9	0.498	20.7	25.6	1.5	0.685	14.1	19.6	14.1	0.005**
Woman	9.6	34.1	8.5		24.8	24.4	3.0		25.6	14.8	11.9	
Age												
18-30	1.5	6.7	1.5	0.456	4.4	5.2	0.4	0.274	3.7	4.4	1.5	0.730
31-36	3.3	11.9	1.5		8.9	7.8	0.0		6.3	5.2	5.2	
37-40	4.4	14.1	3.3		10.7	10.7	0.4		9.6	7.8	4.4	
41-49	3.7	17.4	4.1		8.9	14.1	2.2		9.3	7.4	8.5	
50-64	3.0	13.7	3.0		9.6	8.5	1.5		8.1	7.0	4.4	
65+	3.0	3.0	1.1		3.3	3.7	0.0		2.6	2.6	1.9	
Body mass índex												
< 18,49	0.7	0.7	0.0	0.511	1.1	0.4	0.0	0.446	1.1	0.4	0.0	0.140
18,50-24,99	8.5	35.9	6.7		20.7	28.1	2.2		19.3	17.8	14.1	
25,00-29,99	7.0	22.6	5.6		17.0	16.7	1.5		13.0	11.9	10.4	
30,00-34,99	1.9	6.3	1.1		4.8	4.1	0.4		5.2	3.0	1.1	
35,00 +	0.7	1.1	1.1		1.9	0.7	0.4		1.1	1.5	0.4	
Purchasing power (classification according to residence)												
Low	3.0	16.7	3.3	0.430	9.3	12.6	1.1	0.277	7.8	7.0	8.1	0.016*
Intermediate	10.0	28.1	5.9		22.6	19.6	1.9		21.1	14.8	8.1	
High	5.9	21.9	5.2		13.7	17.8	1.5		10.7	12.6	9.6	
Education												
Basic	0.8	0.8	0.8	0.621	1.6	0.8	0.0	0.317	1.6	0.8	0.0	0.211
Secondary	4.7	15.4	2.4		9.1	11.8	1.6		9.1	7.9	5.5	
Higher	13.0	50.8	11.4		35.4	37	2.5		28.0	26.4	20.9	
Net monthly income of the household												
< 600€	1.9	3.7	0.7	0.109	2.2	3.3	0.7	0.266	3.0	1.5	1.9	0.387
601€-1200€	5.6	22.6	3.0		13.0	17.0	1.1		11.9	11.1	8.1	
1201€-3000€	8.1	30.4	10.0		22.2	24.1	2.2		20.7	16.7	11.1	
3000€ +	3.3	10.0	0.7		8.1	5.6	0.4		4.1	5.2	4.8	

Table 9
General results (%) of the survey into consumption frequencies and disinfection mode of ready-to-eat washed, sliced and packed vegetables or fruits (raw) for the two periods under analysis.

Consumption Frequency	n[1]	≤ 3 x/week (%)	> 3 x/week (%)	p-value	≤ 3 x/week (%)	> 3 x/week (%)	p-value
Salad		May to October			December to April		
Disinfection mode							
With tablets	5	2.3	0.0	0.114	2.4	0.0	0.039*
With water	87	35.7	5.2		37.3	4.3	
Do not wash or disinfect	121	43.7	13.1		43.1	12.9	
Soup		May to October			December to April		
Disinfection mode							
With tablets	3	2.1	0.0	0.511	2.1	0.0	0.821
With water	80	52.1	3.5		49.0	7.6	
Do not wash or disinfect	61	37.5	4.9		35.9	5.5	
Fruit		May to October			December to April		
Disinfection mode							
With tablets	3	1.9	0.0	< 0.001**	1.9	0.0	< 0.001**
With water	74	15.3	31.8		18.5	29.9	
Do not wash or disinfect	80	38.2	12.7		37.6	12.1	

[1] All respondents who did not respond to this question were not considered in the analysis; also all respondents who stated that "never consume" were excluded from the analysis.
* The correlation (exact test) is significant at the 0.05 level.
** The correlation (exact test) is significant at the 0.01 level.

these products does not fall to those who consume them more often a week. This may be an indicator of consumer confidence in this type of products. Also for the fruit a significant tendency was observed between the mode of disinfection and the frequency of consumption (p-value < 0.001; Table 9). In this case, this pattern is significant for both periods under analysis. Thus, by the results achieved the option to wash the fruit with water presented a greater influence for the most frequent consumers (> 3 times/week; Table 9). This pattern is similar for both periods. On the other hand, those who choose not to use any kind of disinfection are superior to consumers who consume less (≤3 times/week). For the remaining items, no statistically significant differences were observed. In summary, consumers have shown less confidence in the case of fruit compared to salad or soup.

3.3. Estimation of THMs exposure and risk assessment

The THMs exposure was estimated by combining the THMs concentration data (see 3.1) with the results obtained for consumption (see

3.2). Data on the average body weight of the population, obtained from the consumer survey conducted, where also considered. Considering that, 1 part of the salad mix corresponds to a value between 50 and 80 g, according to the guidelines in the guide for the establishment of portions for nutritional labeling [20] and with the objective of considering the worst consumption scenario, 1 portion was considered as 80 g of salad mix. In the survey, the given frequency of consumption possibilities include 'occasionally', 'up to 3 times a week', 7 times a week', 'more than 7 times a week'. Thus, for those who consume occasionally, it will be considered (in the worst case scenario) a consumption of 80 g of the salad mix per week and for those who consume 'up to 3 times a week' will be allocated a 240 g consumption of the salad mix per week and so on. Three scenarios were considered based on average estimated data through food consumption surveys (see 3.2): scenario I 0.08 kg /week, scenario II 0.24 kg/week and scenario III (worst scenario) 0.56 kg/week (Table 2).

Once the concentrations of THMs in all samples analyzed in the present work were below the LOQ (1 μg/L), this limit was used for the evaluation of consumer's exposure. The compared estimated intake was then with current RfD as shown in Table 2. Estimated total weekly intakes from selected food samples are shown in Table 2. The highest estimated daily exposure was $7{,}65X10^{-5}$ mg/kg bw/week, which corresponds to a person who consumes such products daily.

Considering the established RfD for TMHs of 0.07 mg/kg, the HQ was calculated (Table 2). It was found that it is very small for the consumption pattern of these products in the studied population categories, suggesting that there is no risk for consumers due to the halogenated disinfection process performed by the producers of the mixed salads here studied. However, more studies are needed through the analysis of a higher number of samples from more commercial brands, as well as the use of complementary detection methodologies.

At the present, there are no specific legal regulations for ready-to-eat salads, regarding the concentration of THMs and other undesirable by-products derived from disinfection procedures. Therefore, much attention has to be paid by national and international regulators to ensure the safety of IV gamma products, which preference is clearly growing among consumers.

4. Conclusions

Through the consumption survey of IV gamma products (namely, ready-to-eat washed, sliced and packed vegetables or fruits), it was possible to characterize the consumption pattern of the residents of Região de Lisboa e Vale do Tejo. Salad mix is the IV gamma product most consumed by respondents. The explanation for this may be associated with the fact that it is a very popular product in daily and regular Portuguese diet, as well as being a very affordable product in terms of availability as well as price. Also, this consumption does not vary throughout the year, which may be explained by the fact that these products are always available (not depending on the season associated with them as being favorable). Thus, it is possible to conclude that the time of the year does not assume a differentiating role to characterize the consumption of the IV gamma products under analysis. Concerning purchasing power according to residence, fruit frequency consumption results showed that the differences are between the intermediate level, when compared with the lower level. Moreover, the achieved pattern showed that the frequencies are always higher for the intermediate level, for consumption till to three times/week. Notwithstanding the nonexistence of statistically significant differences in the frequency of consumption, when compared to the higher level, the results indicated that the highest consumption of fruit (i.e. more than three times/week) underlies this category. It should be noted that, in general in the Portuguese commercial areas (namely, in the ones that were studied), the packaged fruits available are mango, pineapple, blueberries, raspberries, etc. Therefore, these packaged and ready for consumption fruits are more expensive, so it is expected that only those with high purchasing power will opt for these products. Finally, the most frequent form of consumption of the ready-to-eat washed, sliced and packed vegetables or fruits are without any washing or wash only with water. In addition, this preliminary study suggests that the industrial halogenated disinfection process of the analyzed samples allied to their consumption patterns do not represent a risk for consumers' health.

Author statement

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript. Furthermore, each author certifies that this material or similar material has not been and will not be submitted to or published in any other publication before its appearance in the Toxicology Reports.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Authors are very grateful to FCT (Fundação para a Ciência e a Tecnologia) for the financial support of this work through the projects UID/MAR/04292/2019, attributed to MARE - Marine and Environmental Sciences Centre, Politécnico de Leiria, Portugal. This work was also partially funded by the Integrated Programme of SR&TD "SmartBioR" (reference Centro-01-0145-FEDER-000018) co-funded by Centro 2020 program, Portugal2020, European Union, through the European Regional Development Fund. We also acknowledge Eng[a] Georgina Sarmento and Dr. Ana Cruz, from LAIST, for lab facilities and technical support related to THMs analysis.

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