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Impact of consumer behavior on furan and furan-derivative exposure during coffee consumption. A comparison between brewing methods and drinking preferences

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Chemical compounds:

Furan (PubChem CID: 8029); 2-methylfuran (PubChem CID: 10797); 3-methylfuran (PubChem CID: 13587); 2,3-dimethylfuran (PubChem CID: 34337); 2,5-dimethylfuran (PubChem CID: 12266); Furan-d_4 (PubChem CID: 2733420)

Abstract

This study examined the influence of consumer behavior on furan, 2-methylfuran, 3-methylfuran, 2,5-dimethylfuran and 2,3-dimethylfuran exposure in coffee. Coffees brewed using a filter, fully automatic, capsule machine or reconstituted instant coffee were found to have a significant different cup concentrations of furan derivatives. Coffee brewed with the fully automatic machine contained the highest furan and furan derivative concentrations (99.05 μg/L furan, 263.91 μg/L 2-methylfuran, 13.15 μg/L 3-methylfuran and 8.44 μg/L 2,5-dimethylfuran) whereas soluble coffee did not contain detectable levels, thereby contributing least to a consumer's dietary exposure. Furan and furan derivative concentrations were found to decrease significantly upon cooling, reducing consumer exposure by 8.0-17.2 % on average once the coffee reached drinking temperature 55-60 °C, in ceramic cups. Serving coffee in a ceramic or disposable cup were found to influence the cooling dynamics of the coffee but did not statistically influence the consumers exposure at a given temperature.

1. Introduction

Furan, 2-methylfuran, 3-methylfuran, 2,5-dimethylfuran and 2,3-dimethylfuran, hereafter collectively called furan derivatives, **Table S1**, have been known to be present in coffee since the 1960s and 1970s (Maga & Katz, 1979; Stoffelsma, Sipma, Kettenes, & Pypker, 1968). Nevertheless, it was only since the mid-nineteen nineties, when the International Agency for Research on Cancer (IARC, 1995) classified furan as type 2B, *possibly carcinogenic to humans*, that determining consumer's exposure became necessary. Ubiquitously present in thermally processed foods, furan exposure studies revealed that coffee contributes most significantly to an adult's dietary exposure (Fromberg, Mariotti, Pedreschi, Fagt, & Granby, 2014; Mariotti, Granby, Rozowski, & Pedreschi, 2013; Scholl, Scippo, De Pauw, Eppe, & Saegerman, 2012; Waizenegger, Winkler, Kuballa, Ruge, Kersting, Alexy, et al., 2012). Moreover, coffee is one of the only foods known where 2-methylfuran levels consistently exceed those of furan (Becalski, Hayward, Krakalovich, Pelletier, Roscoe, & Vavasour, 2010), revealing that coffee also significantly contributes to a consumer's dietary exposure to 2-methylfuran. Methylfurans appear to be metabolized, at least in part, in a similar manner to furan, resulting in highly reactive intermediates leading to a similar toxicity (Becalski, Hayward, Krakalovich, Pelletier, Roscoe, & Vavasour, 2010). Due to a shared metabolic pathway, methylfurans are considered to contribute to furan toxicity (Becalski, Hayward, Krakalovich, Pelletier, Roscoe, & Vavasour, 2010). Despite coffee being a significant dietary source of furan derivatives, in 2016 the IARC completed their reassessment on the potentially carcinogenic effects of coffee, reclassifying it as type 3, ”*not classifiable as to its carcinogenicity to humans”,* based on insufficient evidence to justify coffee's previous classification as type 2B, "*possibly carcinogenic"* (IARC, 2016).

Initially absent in green coffee beans, furan derivatives are generated upon roasting from the thermal degradation of endogenous components (Becalski & Seaman, 2005; Limacher, Kerler, Davidek, Schmalzried, & Blank, 2008; Locas & Yaylayan, 2004; Van Lancker, Adams, Owczarek-Fendor, De Meulenaer, & De Kimpe, 2011; Yaylayan, 2006). Model studies conducted under simulated roasting conditions indicate that furan and its derivatives originate from similar precursors in separate but parallel pathways (Limacher, Kerler, Davidek, Schmalzried, & Blank, 2008). Furan was found to preferentially form directly from carbohydrate degradation (Limacher, Kerler, Davidek, Schmalzried, & Blank, 2008; Van Lancker, Adams, Owczarek-Fendor, De Meulenaer, & De Kimpe, 2011), where arabinose, the most liable carbohydrate moiety within green coffee beans (Bradbury, 2008), served as a particularly efficient precursor (Limacher, Kerler, Davidek, Schmalzried, & Blank, 2008). Whereas 2-methylfuran forms predominantly from the condensation of carbohydrate moieties generated during the Maillard reaction (Limacher, Kerler, Davidek, Schmalzried, & Blank, 2008), origins of 3-methyl-, 2,5-dimethyl- and 2,3-dimethyl-furan have yet to be established.

The concentrations generated during roasting are not directly predictive of the consumers' exposure. Instead, concentrations of furans in the cup will depend upon the coffee's composition, processing and brewing method, amongst numerous other factors (Altaki, Santos, & Galceran, 2011; La Pera, Liberatore, Avellone, Fanara, Dugo, & Agozzino, 2009; Mariotti, Granby, Rozowski, & Pedreschi, 2013; Morehouse, Nyman, Mcneal, Dinovi, & Perfetti, 2008; Waizenegger, et al., 2012). Furan has been found to decrease by approximately 90% from bean to cup (Guenther, Hoenicke, Biesterveld, Gerhard-Rieben, & Lantz, 2010), due to its high volatility, reflective of its low boiling point, 32 °C. Little information, however, is available regarding cup concentrations of furan's higher boiling methyl derivatives (see **Table S1**). Initial furan derivative concentrations within freshly brewed coffee may be decreased further during cooling, as suggested by Guenther (Guenther, 2012), reducing consumers' exposure.

Previous studies demonstrate varying degrees of furan loss upon coffee cooling. Goldmann *et al.* (Goldmann, Perisset, Scanlan, & Stadler, 2005) as well as Zoller and associates (Zoller, Sager, & Reinhard, 2007) observed that consumer furan exposure was reduced by approximately 45-50% when the coffee was cooled for an hour, whereas Guenther *et al.* (Guenther, Hoenicke, Biesterveld, Gerhard-Rieben, & Lantz, 2010) reported a 10% loss within the same time period. Moreover, Mesìas and Morales (Mesias & Morales, 2014) findings suggest that furan loss is also dependent upon stirring before consumption. Their findings demonstrate that passive cooling is 10% more efficient than brief manual stirring, which decreases furan content by 64%, whereas continuous mechanical stirring will reduce furan exposure by 94% within five minutes (Mesias & Morales, 2014). Han and colleagues (Han, Kim, & Lee, 2017) observed a 2-22% decrease in furan over five minutes, reporting furan loss was dependent on coffee type, water temperature, storage temperature and the presence or absence of a lid. These authors observed that while furan loss was greatest from coffees cooled without a lid, using a higher temperature to prepare or store the coffee led to higher furan levels within the freshly brewed cup (Han, Kim, & Lee, 2017). The higher furan levels found within canned coffee stored at 60 °C were offset by the greater rate of furan loss upon can opening, resulting in approximately equivalent furan exposure between the two storage temperatures after five minutes (Han, Kim, & Lee, 2017). Interestingly, their results demonstrate that preparing instant coffee at a higher temperature, 100 °C instead of 85 °C, led to higher concentrations, suggesting *in situ* formation of furan (Han, Kim, & Lee, 2017).

The present study aims to investigate consumer exposure to furan and its methyl derivatives resulting from typical coffee preparation and drinking behaviour. Furan loss over cooling was assessed in four typical coffee brews, including fully automatic, filter, instant and capsule systems, which were prepared in either a ceramic or lidded disposable cup.

2. Materials and Methods

2.1. Chemicals

The following chemicals were used without further purification: furan (≥99%), d_4-furan (≥99%), 2,5-dimethyl furan (99%), 2,3-dimethyl furan (99%) purchased from Sigma-Aldrich (Schnelldorf, Germany); 3-methylfuran (>98%) purchased from Thermo Fisher Scientific (Geel, Belgium); methanol (≥99.9%) purchased from Honeywell (Bucharest, Romania). Commercially available 100% Arabica blend medium roasted Tchibo coffee as well as Cafissimo capsule "strong" coffee 100% Arabica (Hamburg, Germany). Nescafé Gold Blend instant coffee (United Kingdom).

2.2. **Standards preparation**

Furan standard containing furan (40 μL), 2-methylfuran (120 μL), 3-methylfuran (10 μL), 2,3-dimethylfuran (10 μL) and 2,5-dimethylfuran (10 μL) were prepared fresh daily in 20 mL headspace vial as a methanol stock solution. 250 μL of the furan derivative stock solution was then added through the septum into a 20 mL headspace vial containing 20 mL of Milli-Q water. d_4-furan internal standard was prepared according to the Food and Drug Administration (USFDA) guidelines (USFDA, 2004). Limit of quantitation (LOQ) and limit of detection (LOD) were calculated according to the following equations for each compound, in each matrix, and are included in the supplementary material (**Table S2**).

$$LOD = (3 \times SD)/slope \ (ng/mL)$$

$$LOQ = (10 \times SD)/slope \ (ng/mL)$$

2.3. Sample preparation

Coffee was received approximately one week after roasting and immediately stored at -20 °C. A single 1 kg bag was thawed every day for two hours before use to achieve room temperature.

2.3.1. Whole bean furan content: Whole beans were kept at -20 °C before grinding. Coffee was ground at three grind sizes on Mahlkönig EK43/1 (Hamburg, Germany), 3 (D[3,4] 327 ± 4 µm), 6 (D[3,4] 540 ± 1 µm) and 9 (D[3,4] 760 ± 11 µm). Standard addition was performed on two water to coffee mixtures, 0.05 g coffee in 4.95 mL water or 0.1 g coffee in 4.9 mL water. The coffee, vials and water were kept at 4 °C during weighing.

2.3.2. Filter Coffee: A Moccamaster KBG 741 AO (Amerongen, Netherlands) was used for the purpose of this study. Coffee was ground on a Mahlkönig EK43/1 (Hamburg, Germany) using a grind size typical for filter coffee (grinder setting 9 D[3,4] (766 ± 12 µm)). Five grams of coffee were used to clean the grinder before the brewing sample was ground. Within three minutes of grinding, 48 ± 0.29 g of coffee was weighed into a No. 4 Moccamaster filter (Amerongen, Netherlands) resting within the respective machine's brewing basket. The basket was then returned to the respective machine that had been previously filled with 900 mL of water (Total hardness: 80 ppm $CaCO_3$. Alkalinity: 45 ppm $CaCO_3$). Brewing was immediately commenced after replacement of the brewing basket and allowed to complete to the last drop before the carafe was removed from the hotplate. The full brewing time was 4 min 48 ± 18 s. 125 ± 2.25 g of coffee was poured into the respective container (ceramic cup or disposable cup) before a temperature sample was taken. A fresh pot of coffee was brewed for every temperature measurement.

2.3.3. Fully Automatic: A Schaerer Coffee Art Plus (Zuchwil, Switzerland) was used, programmed and calibrated to deliver 125 g of brew within an 18.9 ± 0.3 s extraction time from 9 g (particle size D[3,4] (366 ± 6 µm)) of coffee. An unmeasured cup of coffee was brewed before each series in order to rinse and heat up the system.

2.3.4. Capsule: Cafissimo Classic coffee machines (Wallisellen, Switzerland) were used in the current study to brew the capsule coffees. Average weight of the coffees was 125 ± 2 g with an extraction time of 41.4 ± 0.2 s. A blank capsule was prepared before each series of experiments to heat up the system.

2.3.5. Instant coffee: 2 g of instant coffee was dissolved in 125 g of 100 °C water.

2.3.6. ***Sampling:*** Sampling was identical, irrespective of the brewing method. Once the coffee was within the disposable cup (Verpackungsteam GmbH, Rohrbach, Germany) or ceramic cup (Cucina & Travola Prima, Migros, Switzerland) the temperature was immediately monitored by a testo 735 thermocouple (Germany) placed within the cup 2 cm above the bottom.

Cooling studies refer to a series of studies performed on all brewing methods. These studies investigate the effect of cooling temperature after brewing on the content of furans within the coffee cup. Samples were taken from independently brewed coffees at specific temperatures within the range 75 - 50 °C for black coffee. The temperature was monitored with the thermocouple for the cooling studies until the desired temperature was obtained, 75, 70, 65, 60, 55 or 50 °C. Ambient temperature was stable at 22 °C. Once the desired temperature was achieved, a 5 mL Supelco gastight syringe, previously rinsed with cold water, was used to aliquot 5 mL of sample into a previously cooled 20 mL Gerstel headspace vial (Sursee, Switzerland). The vial was then immediately closed and stored on ice until further handling. This procedure aimed at minimizing furan loss between sampling and measurements. The densities of these coffees were also monitored in order to ensure that 5 mL aliquots were approximately equivalent to 5 g samples normally taken for coffee.

Quantification: residual coffee was used as a matrix for the calibration curve background, which entailed allowing it to cool to 35 °C. The temperature of the background was controlled to promote compositional consistency of the matrix between runs (days and bags of coffee). Once the coffee had cooled to 35 °C, it was transferred to a Duran Schott bottle and cooled to 4 °C to ensure furan retention in the matrix before further handling.

Once the coffee had undergone additional cooling in the fridge for an hour, a standard addition curve was generated according to the FDA protocol for furan(USFDA, 2004). Standard addition curves were plotted using peak area ratios at characteristic retention times for 2-methylfuran (m/z 82/72), 3-methylfuran (82/72) and 2,5-dimethylfuran (96/72) with R^2 value > 0.98, against their respective concentrations. From the standard addition curve, the background furan derivative concentration was determined. The background concentration of these furans were then added to the known content, used to generate the standard addition curve, in order to generate the calibration curve (concentration in ng/mL versus their respective m/z ratios) within the coffee matrix. The calibration curve was then used to quantitate the furan concentrations within the cooling series.

2.4. Headspace Gas chromatography-Mass spectrometry (HS-GC/MS):

A Gerstel MultiPurpose Sampler (Sursee, Switzerland) Agilent 7890A gas chromatograph coupled to an Agilent 5975C inert XL quadrupole detector (Delaware, USA) was used for HS-GC/MS analysis. Samples were incubated at 60 °C for 30 minutes with continual agitation, 10 s *on* and 1 s *off*. One milliliter of sample was collected from the headspace vial and delivered at a rate of 0.5 mL/s into a splitless inlet heated to 240 °C. The initial temperature of the column was set at 50 ˚C, increasing to 225 ˚C at a rate of 10 ˚C/min, which was held for 12.5 min. A constant flow of 1.7 mL/min was used during the analysis. The transferline temperature was held at 225 ˚C. The ion source was maintained at 230 ˚C. The mass range analysed was 35–150 amu. The column was a Supel-Q PLOT column (30 m length, 0.32 mm i.d., and 0.15 µL film thickness). The identity and purity of the chromatographic peaks were determined by comparison with commercially available standards as well as using NIST V 2.0 and MSD ChemStation software.

2.5. Statistical Analysis

RStudio (Version 1.0.152, 2017) was used to run Shapiro-Wild normality tests, studentized Breusch-Pagan tests, Scheirer Ray Hare tests as well as Dunn's tests to determine whether brewing method, cooling temperature and serving vessel had a significant influence on furan, 2-methylfuran, 3-methylfuran and 2,5-dimethylfuran concentration or percent loss, for a confidence interval of 95%.

3. Results and Discussion

Coffee used in the current study was 100% Arabica medium roasted in a single batch, with the exception of the soluble coffee that came from a commercially available product. A portion of the whole beans were processed and packaged separately for capsule coffee. Once the coffee was received, within two weeks of roasting, the coffee was stored at -20 °C for the duration of the study.

3.1. Content of furans within dry coffee

Initial concentrations of furans demonstrated a clear loss during processing. Whole beans contained the highest content of furans, ranging from 17.25 mg of 2-methylfuran to 0.83 mg of 3-methylfuran per kilogram of coffee, whereas less than half these concentrations were found within capsule and instant coffees, **Table 1.**

188 **Table 1:** Furan derivative concentrations within dry coffee samples

Product	Concentration in mg/kg							
	Furan	**2-methylfuran**	**3-methylfuran**	**2-ethylfuran**	**2-pentylfuran**	**2,5-dimethylfuran**	**2,3-dimethylfuran**	**Reference**
Current Study								
Whole beans	6.67	17.25	0.83	n.d.	n.d.	1.07	<LOD	a
Capsules	2.14	5.22	<LOD	n.d.	n.d.	0.48	<LOD	a
Instant	0.74	2.50	0.27	n.d.	n.d.	0.22	<LOD	a
EFSA								
Roasted bean	2.16-11.00	-	-	-	-	-	-	b
Roasted ground	0.48-6.90	-	-	-	-	-	-	b
Not specified	1.04-6.59	-	-	-	-	-	-	b
Instant	0.06-2.20	-	-	-	-	-	-	b
Whole beans	2.50-3.90	2.40-6.60	-	-	-	-	-	c
Whole beans	3.51-6.10	-	-	-	-	-	-	d
Ground coffee	0.72-2.80	4.89-13.10	0.03-0.62	-	-	-	-	e
Ground coffee	1.40-2.20	1.10-4.30	-	-	-	-	-	c
Coffee	0.05-2.82	0.12-5.98	-	<0.10	<LOD	0.03-0.47	-	f
Decaffeinated	1.64-3.45	6.57-13.50	0.27-0.63	-	-	-	-	e
Cartridge	2.11-2.66	9.44-13.10	0.43-0.56	-	-	-	-	e
Instant	0.40-1.50	0.40-2.40	-	-	-	-	-	c
Instant	0.05-0.74	0.20-6.20	0.01-0.27	-	-	-	-	e
Instant decaffeinated	0.03-0.90	0.13-6.15	0.01-0.21	-	-	-	-	e

Pads	1.40-2.00	1.50-2.10	-	-	-	-	-	c

[a] – current study; [b] –P25 to maximum values taken from **Table 5** of the EFSA, 2011 report (European Food Safety, 2011); [c] – (Test, 2004); [d] – (Kuballa, Stier, & Strichow, 2005); [e] – (Becalski, Halldorson, Hayward, & Roscoe, 2016); [f] – (Fromberg, Mariotti, Pedreschi, Fagt, & Granby, 2014); n.d. – not determined

Guenther and colleagues(Guenther, Hoenicke, Biesterveld, Gerhard-Rieben, & Lantz, 2010) observed a relationship between furan concentration and roast degree, ranging from 2.00 to7.00 mg furan/kg coffee, suggesting that the coffee used in the present study is of a darker roast than intended. Nevertheless, Guenther *et al*. (Guenther, Hoenicke, Biesterveld, Gerhard-Rieben, & Lantz, 2010) demonstrated that a coffee's furan concentration is dependent not only on the roast profile, but also the green beans used, both of which were different between the Guenther and associates' (Guenther, Hoenicke, Biesterveld, Gerhard-Rieben, & Lantz, 2010) and the current study.

The furan concentrations obtained in the present study for whole beans (6.67 mg/kg), capsules (2.14 mg/kg) and instant coffee (0.74 mg/kg) all fall within the ranges reported by EFSA (European Food Safety, 2011) for Roasted bean (2.16-11.00 mg/kg), not specified (1.04-6.59 mg/kg) and instant coffee (0.06-2.20 mg/kg), respectively.

3.2. Content of furans within freshly brewed coffee

Using the concentration of furans within the whole bean, a theoretical concentration was calculated based on the quantity of coffee used in the brewing method and the coffee yield, also known as brew (**Table S3**). This theoretical value is representative of a 100% extraction of the furan derivatives from the coffee grounds, based on the solubilities presented in the EFSA report (Knutsen, Alexander, Barregård, Bignami, Brüschweiler, Ceccatelli, et al., 2017), as well as 100% retention of these compounds within the coffee brew.

Filter and capsule coffees, **Table S3**, were found to have a furan extraction efficiency of 11.9 and 11.2% respectively, supporting Guenther and colleagues (Guenther, Hoenicke, Biesterveld, Gerhard-Rieben, & Lantz, 2010) as well as Kuballa *et al*.'s (Kuballa, Stier, & Strichow, 2005) findings for filter coffee where approximately 10% of furan found in the whole beans was found within the brew. Capsule coffees lose 65.7% of their furan during grinding and packaging of the whole bean in capsule processing, retaining only 34.3% of the concentration within the capsule. From the 34.3% retained within the capsule, 67.4% is lost during brewing, resulting in 32.6% of the furan content of the capsule coffee being found within the cup, or 11.2% of the furan content initially found in the whole beans. The loss between freshly ground coffee and capsule furan content is not attributed to the particle size, P[3,4] 492 µm, but rather to processing, seeing as 6.5 mg/kg (calculated from **Figure S1**), **Table 1**, would have been anticipated for this grind size.

Our findings support those of Kuballa's *et al.*'s (Kuballa, Stier, & Strichow, 2005), that brewing coffee with a fully automatic machine extracts furans more efficiently than filter methods. Nevertheless, our fully automatic method demonstrated a twofold higher extraction efficiency compared to filter methods in contrast to Kuballa's and colleagues' (Kuballa, Stier, & Strichow, 2005) fully automatic method that was often greater than three times as efficient to their filter systems.

Similar to the parent furan, approximately 78-88% of 2-methyl- and 3-methylfurans' theoretical cup concentration escapes during brewing, resulting in 12-22% remaining within the cup, **Table S3**. Release of 2,5-dimethylfuran is even more efficient, where only 6-11% of the concentration within the coffee beans is retained within the brew, **Table S3**. Regardless of the furan derivatives, coffees brewed with a fully automatic machine retains twice the furan content, of the original beans, than filter or capsule methods.

Initial comparison between levels of furans found within the current study and those previously reported within literature for various brewing methods are in agreement, **Table 2**. In the current set of experiments, freshly brewed black coffees were found to have a temperature of 75 °C when prepared with all brewing methods with the exception of capsule coffee prepared in a ceramic cup, which had an initial temperature of 65°C. Furan levels within freshly brewed coffee with a fully automatic (99.1 ± 6.4 µg/L), filter (47.3 ± 5.7 µg/L), or capsule machine (33.5 ± 3.0 µg/L), served in a ceramic cup, were all within the range reported by EFSA for roasted ground brew (0-228 µg/L), with a mean furan content between 39-42 µg/L (European Food Safety, 2011). The levels of furan reported herein are also consistent with those found by Altaki *et al.* (Altaki, Santos, & Galceran, 2011) for filter, (20-78 µg/L) and espresso machines (43-146 µg/L).

Altaki *et al.* (Altaki, Santos, & Galceran, 2011) report higher furan concentrations within capsule systems, 68-244 µg/L, than were found in the current study, 20-30 µg/L. This difference is partly due to Altaki and colleagues' (Altaki, Santos, & Galceran, 2011) use of 5.5 and 7 g of coffee to prepare 40 or 110 mL of coffee respectively, whereas in the current study, 6 g coffee was used to brew 125 mL of coffee. Calculating back to the furan content within the grinds for Altaki and associates (Altaki, Santos, & Galceran, 2011) capsule systems contain an average of 1.24 ± 0.30 mg furan/kg coffee, resulting in a concentration of 59 µg/L for the coffee ratio brewed in the current study. The higher theoretical concentration that would have been obtained using Altaki *et al.* (Altaki, Santos, & Galceran, 2011) capsule system suggests either that the capsule system used is twice as efficient at extracting furan than the system used in the current study, or that the concentration of furan in the capsule grinds was higher. If 1.24 mg/kg coffee represented 30% of the furan in the grinds, as observed in the current study, the capsule system used by Altaki *et al*. (Altaki, Santos, & Galceran, 2011) would have contained 5.58 mg furan/kg coffee. A higher furan retention within the capsule coffee would speak for the efficiency of the capsule manufacturing process and retention of the volatiles of the capsules examined by Altaki and colleagues (Altaki, Santos, & Galceran, 2011).

Furan derivatives were not detected in the instant coffee chosen for these series of experiments, resulting in the lowest exposure levels. The absence of furan is in agreement with levels of furan found within instant coffee according to EFSA (European Food Safety, 2011). The absence of furan in freshly brewed instant coffee allows for its exclusion from further investigation and discussion concerning the influence of the cooling and serving vessel.

Table 2: Furan derivative concentrations within brewed coffee

	Concentration in µg/L					
Product	**Furan**	**2-methylfuran**	**3-methylfuran**	**2,5-dimethylfuran**	**2,3-dimethylfuran**	**Reference**

Current Study						
Fully Automatic[a]	74-99	188-264	10-13	7-8	<LOD	b
Filter[a]	47-53	138-128	7-6	4	<LOD	b
Instant [a]	<LOD	<LOD	<LOD	<LOD	<LOD	b
Capsule[c]	20-33	58-103	5	3	<LOD	b
EFSA						
Instant	0-10	-	-	-	-	f
Roasted bean	6-360	-	-	-	-	f
Roasted ground	0-228	-	-	-	-	f
Not specified	0-248	-	-	-	-	f
Whole beans	93-125	-	-	-	-	g
Ground coffee	41-158	-	-	-	-	g
Coffee	178	670	-	41	-	h
Coffee	93-129	-	-	-	-	i
Filter coffee[d]	8-66	-	-	-	-	j
Filter	20-78	-	-	-	-	k
Filter coffee – machine[e]	9-33	-	-	-	-	j
Filter coffee – manual[e]	17-24	-	-	-	-	j
French press[e]	33-66	-	-	-	-	j
Decaffeinated coffee	7-121	24-365	1-12	-	-	l
Medium roast	25-70	109-287	5-10	-	-	l
Espresso	35-352	135-1260	7-39	-	-	l
Espresso[d]	28-60	-	-	-	-	j
Espresso	43-146	-	-	-	-	k
Canned	25-36	-	-	-	-	i
Instant coffee	6-44	-	-	-	-	g

Instant	7-12	-	-	-	-	i
Pads	63-101	-	-	-	-	g
Capsule	68-244	-	-	-	-	k

[a] – Black coffee in both ceramic or ToGo cup at 75°C; [b] – current study; [c]- Black coffee in both ceramic or ToGo cup at 65°C; [d] – brewed from purchased coffee grounds; [e] brewed from freshly ground whole beans; [f]- (European Food Safety, 2011); [g]- (Test, 2004); [h]- (Shen, Liu, Jia, Jiang, Nie, Xie, et al., 2016); [i]- (Han, Kim, & Lee, 2017); [j]- (Kuballa, Stier, & Strichow, 2005); [k]- (Altaki, Santos, & Galceran, 2011); [l]- (Becalski, Halldorson, Hayward, & Roscoe, 2016); n.d. – not determined

Consistent with previous studies, 2-methylfuran concentrations exceeded those of the parent furan (Becalski, Halldorson, Hayward, & Roscoe, 2016). Concentrations of 2-methylfuran obtained within fully automatic and filter coffees (188-264 µg/L) and (138-128 µg/L) respectively, remained within the ranges previously reported by Becalski and colleagues (Becalski, Halldorson, Hayward, & Roscoe, 2016), **Table 2**. Levels of 2-methylfuran obtained in the current study for capsule systems (58-103 µg/L) are only comparable to levels reported by Becalski and associates (Becalski, Halldorson, Hayward, & Roscoe, 2016) for decaffeinated coffee. Absence of corroborative literature on 2-methylfuran is reflective of the emerging interest in methylfurans, resulting in this being one of the first investigations to report 2-methyl- and 3-methyl-furan content within capsule coffee. While 2-methylfuran levels obtained for capsule systems are lower than for other brewing methods, 3-methylfuran concentrations remain within the previously reported literature values independently of the brewing method employed, **Table 2**.

Presence of 2,5-dimethylfuran (41 µg/L) has periodically been reported in brewed coffee (Shen, et al., 2016). Concentrations found in the current study ranged from 3-8 µg/L depending upon the brewing method used, **Table 2**. No detectable levels of 2,3-dimethylfuran were found within any of the coffee samples in the current study (LOD ranging between 0.10-1.99 ng/mL depending upon the coffee matrix examined).

The following sections aim to examine the influence of different consumer habits on furan derivative exposure.

3.3. Impact of brewing method on furan derivative exposure

The initial consumer choice when drinking a coffee is the type of coffee to be prepared. Types of coffee distinguish themselves from one another by a multitude of different factors including the extraction equipment, brew ratio (ratio of coffee yield to grounds used), grind size of the coffee as well as water temperature and extraction duration. For the purposes of these experiments, four typical coffee preparations were chosen to determine consumer exposure to furans. Instant coffee examined in the current study did not contain any detectable levels of furans, resulting in the lowest coffee exposure levels possible. Instant coffee was the only coffee used in this study that originated from a different coffee source; all other coffees prepared came from a single batch of roasted coffee. Coffees were prepared using either a fully automatic, filter or capsule machine. While the capsules used contained the same coffee beans as those used for the fully automatic and filter coffee preparations, they were industrially packaged.

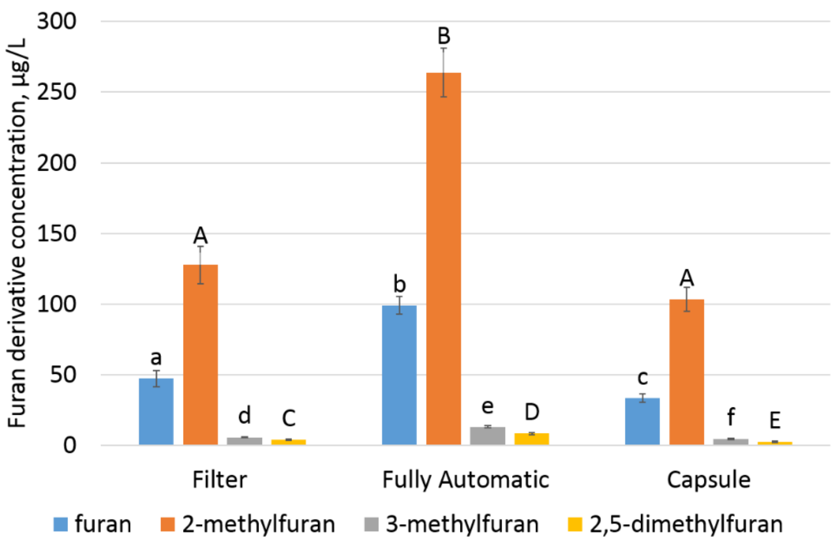

Figure 1: Dependence of coffee furan composition on brewing method

(refer to **Table S4** for statistical summary)

Consumer brew preferences have a significant influence on their furan exposure. EFSA findings report that adults are chronically exposed to on average 0.17 - 0.54 µg/kg b.w./day (Knutsen, et al., 2017) of the parent furan, which translates to a daily exposure of between 11.9 – 37.8 µg furan/day for a 70 kg adult. Freshly brewed coffee, in this study, was found to result in a furan content of between 4.2 and 12.4 µg per cup (**Table S4**). One cup of coffee freshly brewed with a fully automatic machine (12.4 µg furan/125mL cup, at 75°C), has the capacity to exceed the average lower exposure threshold of 11.9 µg furan/day (Knutsen, et al., 2017), whereas one would have to consume two cups of filter coffee (5.9 µg furan/125mL cup, at 75°C) or three of capsule coffee (4.1 µg furan/125mL cup, at 65°C) to exceed the lower threshold reported by EFSA. This demonstrates the significant influence, viewed statistically, that the brewing method has over the consumer's daily exposure.

Coffee furan concentration has been associated in the literature with its lipid content (Arisseto, Vicente, Ueno, Tfouni, & Toledo, 2011; Van Lancker, Adams, Owczarek, De Meulenaer, & De Kimpe, 2009). In 2009, Van Lancker and associates (Van Lancker, Adams, Owczarek, De Meulenaer, & De Kimpe, 2009) demonstrated the ability of lipids to retain furan by measuring d_4-furan retention within various lipid systems. Moreover, these authors compared deuterated furan's retention within normal and defatted coffee matrices demonstrating furan loss when the coffee was de-fatted. In addition, Pavesi Arisseto and colleagues (Arisseto, Vicente, Ueno, Tfouni, & Toledo, 2011) suggest that Arabica's greater lipid content is responsible for its greater furan retention within the beverage, in contrast to Robusta varieties that are more susceptible to generate furan during roasting yet are less inclined to transfer furans into the brew. Interestingly, Glöss and colleagues (Glöss, Schönbächler, Klopprogge, D'Amrosio, Chatelain, Bongartz, et al., 2013) as well as Ratnayake and associates (Ratnayake, Hollywood, Ogrady, & Stavric, 1993) compared the lipid content of coffee between a variety of brewing methods, demonstrating that filter coffee has the lowest lipid content when compared to any other preparation method. Ratnayake *et al.* (Ratnayake, Hollywood, Ogrady, & Stavric, 1993) demonstrated that the lipids are mainly (90%) retained in the grounds, whereas the retention in the filter paper itself only accounts for just over nine percent, and only approximately 0.4% of the fat will be found within the brew. Ratnayake and colleagues (Ratnayake, Hollywood, Ogrady, & Stavric, 1993) also determined that the lipid composition remained the same, independently of where the lipids were retained. If these authors (Glöss, et al., 2013; Ratnayake, Hollywood, Ogrady, & Stavric, 1993) are correct, then the reduced furan content, and possibly furan release, observed within filter coffee may be attributed to the partial removal of coffee lipids during brewing. This suggests that the brewing method influences the furan content of the coffee through its ability to extract lipids. Furthermore, lipid content of the coffee, potentially enhanced by the addition of cream, may significantly influence a consumer's exposure to coffee furans.

Numerous brew parameters are varied in the generation of a specific type of coffee that may influence furan derivative content. One of the main parameters likely to influence the concentration of furans within the final cup is the brew ratio. The brew ratio is an indirect expression of the extract concentration by comparing the brew yield, in grams, to the mass of grounds used during preparation. In the current experiment, brew ratios typical of the brewing method were used: 16.5 for filter, 13.9 fully automatic and 20.8 for capsules. Concentrations of furans observed in **Figure 1** reflect the same trend anticipated from dilution, reflected by the brew ratio. High brew ratios reflect a higher dilution, whereas low brew ratios reflect a higher concentration. Nevertheless, brew ratio is not directly proportional to furan derivative content and therefore can only give a relative indication of a consumer's furan derivative exposure. The indicative nature of the brew ratio with respect to furan derivative exposure suggests that other brewing parameters influence the final cup concentration.

In the current investigation, meticulous attention was paid to each step within the brewing procedure due to their potential impact on the coffee's furan derivative concentration. The highest furan derivative concentrations were found in fully automatic machines that employ an enclosed grinding and extraction module. Furthermore, the fully automatic machine used the finest mean particle size, 366 µm of all brewing procedures employed in this study, which should have promoted furan release (Guenther, Hoenicke, Biesterveld, Gerhard-Rieben, & Lantz, 2010). However, coffee was brewed within 10.6 ± 0.3 s after grinding, minimizing furan loss promoted by grinding. Filter coffee with a coarser mean particle size 766 µm took significantly longer to brew: 3 - 4 minutes, which is likely to have contributed to the lower furan derivative content measured. Probing into cup furan concentration's dependence on particle size (760, 540 and 327 µm), in filter coffee, a weak correlation was found (results not shown), suggesting that furan's concentration has a stronger dependence with respect to other brewing parameters, such as the time interval between grinding and extraction. Most coffee prepared is ground well in advance of extraction, as mentioned by EFSA in their report, where over 90% of the products reported were analyzed as purchased (European Food Safety, 2011). Considering the high volatility of furan, this will potentially reduce the furan content significantly during storage (Guenther, Hoenicke, Biesterveld, Gerhard-Rieben, & Lantz, 2010) affecting the consumers' final exposure.

In addition to monitoring the time between grinding and extraction, the duration of extraction was also recorded. Similar to their grinding interval, filter machines had a significantly longer extraction time 4.0 ± 0.2 minutes when compared to their fully automatic counterparts, 18.9 ± 0.3 s. Nevertheless, associating extraction time with a higher or lower furan content is complex, due to the brewing setup. La Pera and colleagues (La Pera, Liberatore, Avellone, Fanara, Dugo, & Agozzino, 2009) found that over 60% of furan is extracted within the first minute of brewing when water is close to boiling, as in the current set of experiments (92 - 96 °C). Therefore, if the initial coffee extract was richest in furan, then letting it incubate on the filter machine hotplate for the entire extraction period, 4 minutes, would reduce the consumer's exposure by driving the furan out of the brew. In addition to the potential loss of furan during the carafe's incubation, various authors (Goldmann, Perisset, Scanlan, & Stadler, 2005; Guenther, Hoenicke, Biesterveld, Gerhard-Rieben, &

Lantz, 2010) have observed a 10% decrease in furan content when coffee was poured, which was only done with filter coffee.

Interestingly, concentrations of all furan derivatives shared a similar ratio between brewing methods. Coffee brewed with the fully automatic machine consistently contained approximately twice the concentration of all furan derivatives when compared to filter coffee, whereas filter coffee contained approximately 1.5 times the content found in capsule coffee in the current study. The consistent ratios found between brewing methods is also a reflection of a constant ratio within a brewing method between furan derivatives, **Table S5**. 2-methylfuran was consistently found at more than three times the furan concentration, which has also been observed in previous studies (Becalski & Seaman, 2005), with the exception of Fromberg and colleagues (Fromberg, Mariotti, Pedreschi, Fagt, & Granby, 2014). In contrast, concentrations found for 3-methylfuran and 2,5-dimethylfuran were only 12 - 14% and 8 - 9% of the furan concentration, respectively. Percent 3-methylfuran levels found within this study are consistent with those presented in the EFSA (Knutsen, et al., 2017). Given a constant ratio between individual furans, the brewing method primarily influences the extent of exposure to furans, where capsules present the least exposure followed by coffees prepared using a filter and fully automatic machine.

3.4. Influence of cooling temperature on furan derivative loss in ceramic cups

Coffees brewed for this study were initially between 65 – 75 °C, classifying them as very hot beverages and therefore also a type 2B, *possibly carcinogenic,* product according to IARC (IARC, 2016). Those who drink their coffee at these high temperatures are therefore more susceptible to developing esophageal cancer (IARC, 2016), than those that wait for their coffee to cool. Furan derivative exposure was also found to be greatest immediately after brewing, decreasing significantly upon cooling, **Table 3**, independently of brewing method and furan derivatives. Continuous furan derivative loss upon cooling in a ceramic cup can partially be attributed to the evaporation of the beverage, **Figure S2**, where approximately 3.2 g of coffee is lost over 20 minutes.

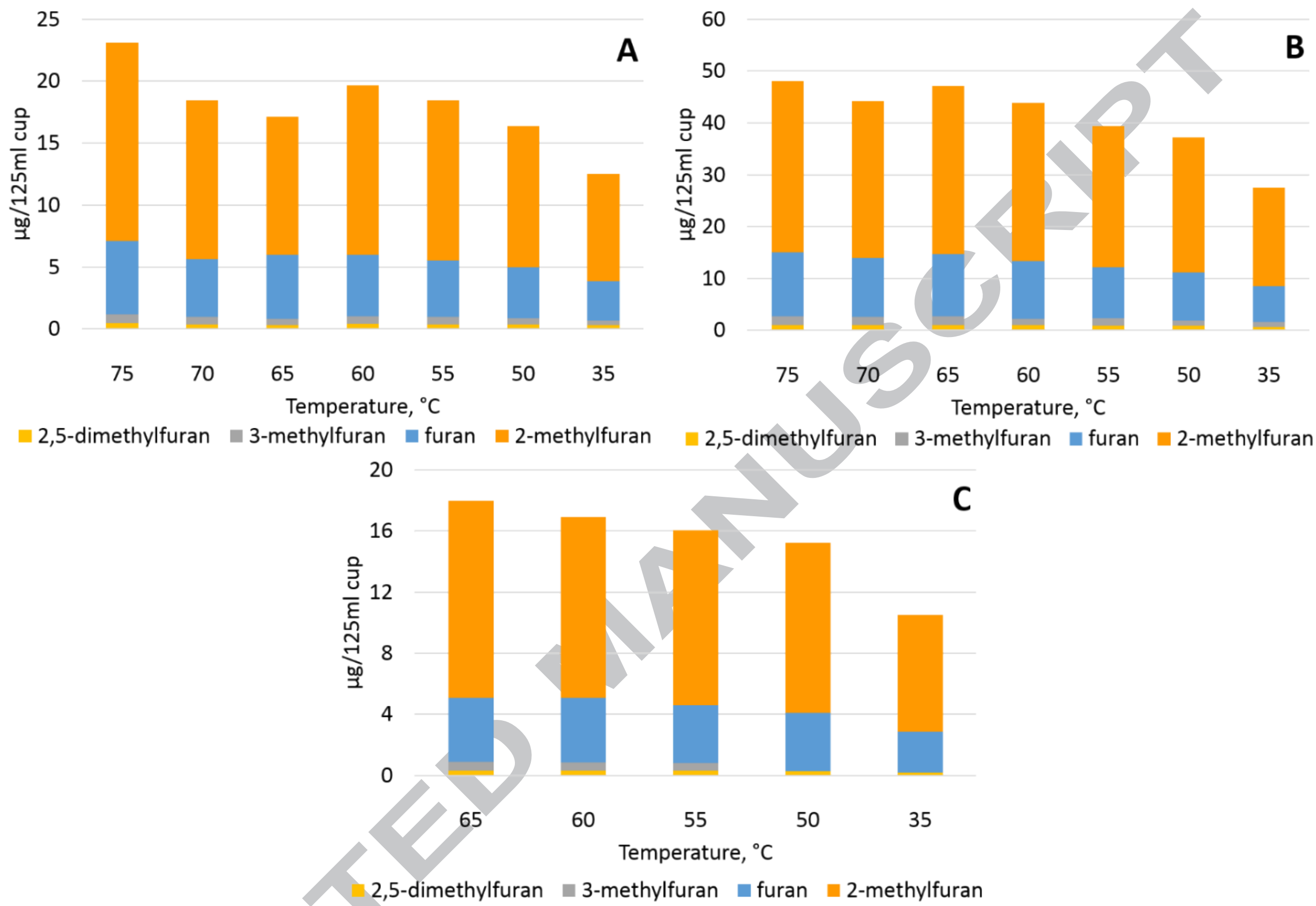

Figure 2: Concentrations of (blue) furan, (orange) 2-methylfuran, (grey) 3-methylfuran and (yellow) 2,5-dimethylfuran in coffee brewed from a (A) filter, (B) fully automatic or (C) capsule machine served in a ceramic cup (refer to **Table 3** statistical summary)

Furan and 2-methylfuran account for 95% of a consumer's exposure to furan, **Figure 2**. While coffee is often consumed at 60 °C independently of its strength (Lee & O'Mahony, 2002), this temperature is reached at different rates depending on the coffee brewed. In the current experiments, capsule coffee, cooled in a ceramic cup, reached 55-60 °C in approximately 2.9 minutes, whereas filter coffee required an additional minute and a half, while coffee prepared with a fully automatic machine needed six additional minutes. As anticipated, rapid cooling, experienced by capsule coffee, resulted in the smallest furan loss, on average 8.0%, before consumer consumption. Lengthier cooling periods for fully automatic and filter coffees resulted in a greater average loss of 14.3 and 17.2%, at 55-60 °C respectively. Han and colleagues (Han, Kim, & Lee, 2017) report a 20% reduction in furan from an open vessel of filter coffee after five minutes, which aligns well with our findings where a 22.4% reduction was found within six minutes, corresponding to a temperature of 55 °C. 2-methylfuran, 3-methylfuran and 2,3-dimethylfuran were all found to decrease in a proportional manner, for example all by between 20 - 22% at 55 °C, for filter and capsule coffees. Rate of loss for furan derivatives in fully automatic machines seem to be dependent on the furan derivatives; however, this has to be corroborated by additional studies.

The significant retention of furans, 85%, by coffee brewed with a fully automatic machine, despite the longer cooling period of nine minutes, suggests the presence of a barrier to the release of furans. This barrier, as previously discussed, may be due to the greater lipid extraction efficiency anticipated by brewing with a fully automatic machine, or may also be due to the formation of a visible crema layer upon brewing, which is the formation of a foam layer on the surface of the coffee upon brewing and which may present a physical barrier to furan loss.

Table 3: Statistical results for Dunn pairwise comparison of furan derivative concentrations of over cooling temperatures

	Temperature, °C						
	75	**70**	**65**	**60**	**55**	**50**	**35**
furan	A	ab	ab	bc	cd	cd	D
2-methylfuran	A	AB	BC	BCD	CDE	DE	E
3-methylfuran	E	ef	fg	gh	gh	h	h
2,5-dimethylfuran	F	F	FG	GHI	HI	HI	I

The parent furan content of a cup, 125 mL, of coffee cooled to 55-60 °C does not exceed the daily exposure threshold reported by EFSA, 11.9-37.8 μg/day (Knutsen, et al., 2017) for a 70 kg adult, independent of brew method, **Table S6**; nevertheless, cumulative furan exposure does, **Figure 2**. All coffees served in a ceramic cup and cooled to 55-60 °C, irrespective of brewing method, exceed the lower chronic exposure threshold established by EFSA (Knutsen, et al., 2017), with coffee brewed using a fully automatic machine exceeding the upper threshold.

3.5. Impact of coffee container on furan derivative loss: ceramic versus disposable cups

Coffee is commonly consumed from either a ceramic or disposable cup. Han and associates (Han, Kim, & Lee, 2017) investigated the impact of open and closed systems, using paper cups, on furan reduction upon coffee cooling demonstrating that closed vessels lose less furan over five minutes than their open system counterparts. While Han *et al.*'s (Han, Kim, & Lee, 2017) findings are corroborated by the results presented in the current study, their results are strictly time-dependent, neglecting a potential temperature-dependent consumer drinking bias. Lee and O'Mahony's (Lee & O'Mahony, 2002) established that consumers preferentially drink their coffee at around 60 °C, however filter coffee cooled in an open ceramic cup for five minutes will have cooled to almost 55 °C, while coffee in a lidded disposable cup will have barely cooled to 65 °C. The rate of cooling therefore is likely to have a significant influence on a consumer's drinking behavior and consequently their furan exposure. Coffee consumed from a ceramic cup will reach a drinking temperature, 60 °C, more rapidly after brewing, approx. 3 minutes in the case of filter coffee, than a disposable cup, approx. 9 minutes, possibly resulting in more immediate consumption. If the coffee is consumed at 60 °C, instead of after five minutes of cooling, disposable cups contribute a statistically equivalent furan exposure when compared to their ceramic counterparts for all brewing methods, according to Dunn's *post hoc* test, **Figure 3** and **Table S4**. Therefore, time-dependent differences in exposure found in the literature for open and closed systems may be a reflection of different temperatures, which is a function of the cup dynamics.

Coffee served in a disposable cup maintains a desirable drinking temperature for a longer period of time, cooling at a rate of approx. 1 °C/min for filter coffee ($R^2 = 0.9954$), possibly leading to a more gradual consumption of the beverage. Interestingly, while the rate of cooling in disposable cups revealed a nearly linear relationship between temperature and time, ceramic cups demonstrate a greater rate of initial cooling. Preliminary experiments with regard to heat dissipation, **Table S7**, demonstrates that pre-heating a cup has minimal influence on the first five minutes of cooling, whereas selection of cup geometry may have a more significant effect. In addition to the cup materials influencing the rate of cooling, coffees cooled in an open system were found to lose over 2.5% of their weight over 20 minutes or approx. 30 °C of cooling, whereas closed systems lost less than 0.4% over the same time period or approx. 30 °C of cooling (**Figure S2**).

The potential influence of cup choice on cooling rate and thereby consumer drinking behavior emphasizes the importance of examining the dependence of furan derivative exposure on temperature.

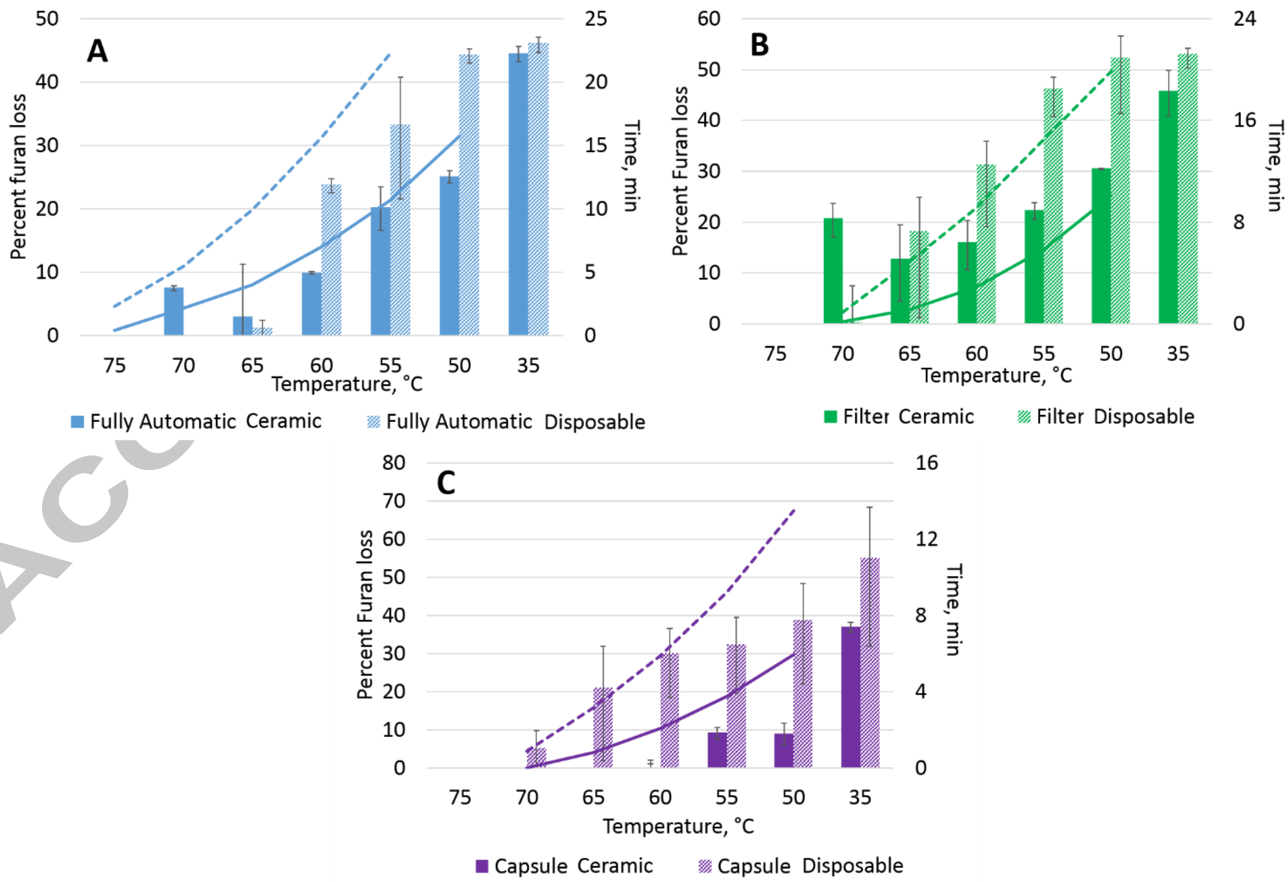

Figure 3: Furan loss in coffee upon cooling comparison between ceramic and disposable cups prepared from either a fully automatic (A), filter (B) or capsule (C) machine (refer to **Table S8** and **S9** for statistical summary)

Similar results were obtained for all furan derivatives and are included in the supplementary materials (**Figures S3-S5**).

Conclusions

Furan and 2-methylfuran account for 95% of the furan derivatives found within coffee. While whole beans contain the highest furan levels, less than 21% of these furan derivatives will be extracted into the cup. The extraction efficiency of furan derivatives is dependent upon prior processing steps and the brewing method employed. Filter coffee was found to result in the lowest level of furan derivative extraction, approximately 10% of furans were found within the brew in contrast to coffee brewed with a fully automatic machine that was twice as efficient. Capsule coffees demonstrated the highest extraction efficiency, at 32.6%. However, the furan content within the brew is significantly influenced by the loss during capsule manufacturing, of 34.3%. The compounded loss during capsule manufacturing and brewing resulted in the consumer being exposed to approximately 11% of the furan theoretically transferable into the brew, based on the brew ratio. The initial concentration of furans within a brew are therefore dependent on the brewing method as well as the amount of coffee being extracted and the brew yield. This initial concentration of furans within the brew decreases significantly upon cooling, with longer times being associated with a greater loss. Cooling rate is dependent upon brewing method where capsule coffees cooled most rapidly, followed by filter and finally coffee brewed with a fully automatic machine. Coffee brewed with a fully automatic machine did not lose as much furan (14.3%) as its filter (17.2%) machine counterpart, despite requiring twice as long to cool. The limited furan derivative loss undergone by coffee brewed with a fully automatic machine was attributed to the higher lipid content of these coffees as well as the formation of a crema layer that could serve as a physical barrier to the loss of furans. Finally, while serving vessels appeared to have a significant influence on furan loss, this was found to not be statistically significant. Serving vessels were found to influence the cooling rate but not the furan exposure at a given temperature.

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Declarations of interest: none.

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Highlights

- Brewing method has a significant influence on furan and furan derivative exposure
- Drinking temperature influences furan and furan derivative exposure
- Cup choice affects cooling dynamics but not temperature dependent furan exposure