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Research Article

Influence of lipid content and stirring behaviour on furan and furan derivative exposure in filter coffee

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ABSTRACT

Coffee has been determined as the dominant source of furan within an adult's diet. This study investigates the influence of coffee condiment use and stirring on the retention of furan. Three condiment lipid compositions were investigated, 0%, 3.5% and 35%, and kept at either 4 °C, 20 °C or 70 °C before addition to a freshly brewed cup of filter coffee which was subsequently mechanically stirred at three intensities, not stirred and moderately or heavily stirred. While five furans were monitored, furan, 2-methylfuran, 3-methylfuran, 2,5-dimethylfuran and 2,3-dimethylfuran, only two were quantifiable: furan and 2-methylfuran. Increasing condiment lipid concentration significantly increased retention of furan and 2-methylfuran, whereas stirring the coffee significantly increased furan release. A condiment temperature of 70 °C was found to significantly increase furan release.

1. Introduction

Furan has been classified as a type 2B carcinogen, *possibly carcinogenic to humans,* by the International Agency for research on Cancer (IARC), due to its metabolic conversion into reactive metabolites upon consumption (IARC, 1995). These toxicologically relevant metabolic pathways do not discriminate between furan species (Becalski et al., 2010), giving any furan species potential to contribute to the product's toxicity. In this study, five furan species were investigated, including: furan, 2-methylfuran, 3-methylfuran, 2,3-dimethylfuran and 2,5-dimethylfuran.

Emerging as the most significant dietary source for adults (Fromberg, Mariotti, Pedreschi, Fagt, & Granby, 2014; Mariotti, Granby, Rozowski, & Pedreschi, 2013; Scholl, Scippo, De Pauw, Eppe, & Saegerman, 2012; Waizenegger et al., 2012), coffee's furan content is generated upon roasting (Becalski & Seaman, 2005; Limacher, Kerler, Davidek, Schmalzried, & Blank, 2008; Locas & Yaylayan, 2004; Van Lancker, Adams, Owczarek-Fendor, De Meulenaer, & De Kimpe, 2011; Yaylayan, 2006). While limited information is available regarding the content of other furans within coffee, 2-methylfuran has been consistently reported as being in far greater abundance than its parent furan counterpart (Becalski et al., 2010), potentially contributing significantly to consumer exposure.

Coffee is compositionally predisposed to form furan and furan derivatives upon roasting; nevertheless, consumer exposure is the net effect of an array of factors contributing to their formation as well as their loss before consumption, Fig. 1. Guenther and colleagues as well as Rahn and Yeretzian (Guenther, Hoenicke, Biesterveld, Gerhard-Rieben, & Lantz, 2010; Rahn & Yeretzian, 2019) both observed a net loss of 90% of furan from bean to cup. The continued reduction is dependent upon consumer behaviour, as furan is lost upon coffee cooling (Goldmann, Perisset, Scanlan, & Stadler, 2005; Guenther et al., 2010; Han, Kim, & Lee, 2017; Rahn & Yeretzian, 2019; Zoller, Sager, & Reinhard, 2007) that may be facilitated by stirring (Mesias & Morales, 2014). While Mesìas and Morales observed a 94% decrease in furan content from the coffee brew that was mechanically stirred, manual stirring was 10% less efficient than passive cooling, which seems counter-intuitive. However, the influence of stirring behaviour on coffee's furan content is a multivariant problem which depends upon the stirring intensity, mode of stirring as well as composition and temperature of the condiment added.

The addition of dairy condiments to coffee may counteract the

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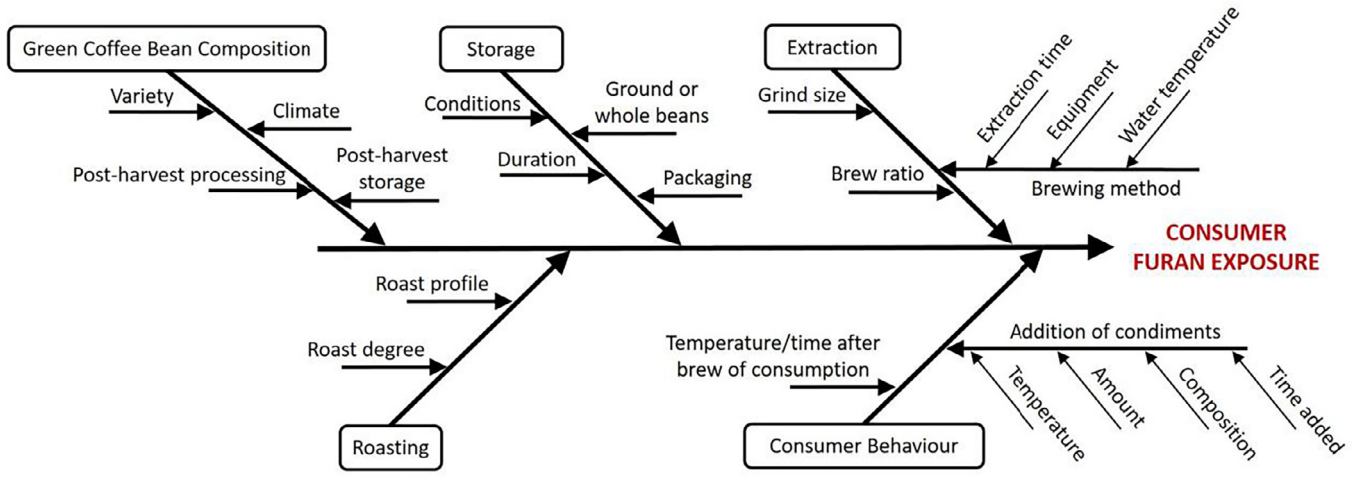

Fig. 1. Cause and effect diagram regarding parameters influencing furan exposure.

promotion of furan release caused by stirring, due to their lipid content. Furan literature suggests that the lipid content of the coffee is central to furan retention within the brew. In the present study, three intensities of mechanical stirring were investigated (none, moderate and fast), as well as the influence of condiment lipid composition at three different levels (water, 35% v/v lipid cream and 3.5% v/v lipid mock milk mixture), to explore their influence on the release of furans. As dairy products can vary in their protein and lipid composition, commercial cream was diluted to 3.5% v/v lipid content to simulate milk and increase compositional consistency between lipid condiments. Furthermore, these condiments were stored at three different temperatures (4, 20 and 70 °C) before being added to the coffee, in order to determine whether condiment temperature has an influence on furan loss.

2. Materials and methods

2.1. Chemicals

The following chemicals were used without further purification: furan (≥99%), d_4-furan (≥99%), 2-methyl 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 (Hamburg, Germany). 35% fat Qualité & Prix Coop full cream was purchased weekly (Zürich, Switzerland).

2.2. Standards preparation

Furan standards containing furan (40 μL), 2-methylfuran (120 μL), 3-methylfuran (10 μL), 2,3-dimethylfuran (10 μL) and 2,5-dimethylfuran (10 μL) in 20 mL of methanol were prepared fresh every two weeks in a 20 mL headspace vial as a methanol stock solution. 250 μL of the furan analogue stock solution was then added through the septum into a 20 mL headspace vial containing 20 mL of MilliQ 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.

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

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

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.

The 3.5% lipid content stock mock milk mixture was created by diluting a 6 mL aliquot of vortexed 35% lipid content cream tenfold with MilliQ water for a final volume of 60 mL, which was subsequently vortexed. The mock milk mixture along with the 35% cream and MilliQ water were then distributed, in triplicate samples, of 12 mL aliquots after vortexing into vials. Twelve millilitre vials were then incubated at either 4 °C for 24 h, 20 °C for 2 h or 70 °C for 15 min. All samples were vortexed again before addition to the coffee.

The coffee preparation process was designed to simulate typical consumer behaviour when drinking their coffee, while ensuring highest reproducibility along the way for the final samples for analysis.

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 D50 (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 coffee was weighed into a No. 4 Moccamaster filter (Amerongen, Netherlands) resting within the machine's brewing basket. The basket was then returned to the 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 replacing 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, after which 125 ± 2.25 g of coffee brew was poured into a porcelain cup (Cucina & Travola Prima, Migros, Switzerland) before samples were taken. A single pot of coffee was used to prepare three cups of coffee that were monitored using a testo 735 thermocouple (Germany) upon cooling. The 12 g of MilliQ water (0% lipid), "mock milk mixture" (3.5% lipid) or heavy cream (35% lipid) were added immediately after serving the coffee.

Three stirring levels were examined; fast, medium and no stirring (passive). The stirring levels requiring agitation were carried out mechanically using IKA RCT basic stirring mantels (settings 1–10, 50–1500 rpm capacity) (Staufen, Germany) and rods, at settings 1 and 9 for medium and fast stirring respectively. Stirring was carried out throughout the duration of cooling, to 35 °C. Manual stirring studies were conducted immediately after pouring for a duration of 15, 30 and 45 s stirring with a metal teaspoon. 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.

One 5 mL sample was taken from each cup of coffee prepared using a Supelco gastight syringe from each coffee preparation at drinking temperature 60 °C for the addition of lipid samples (0%, 3.5% and 35% lipid content) incubated at 20 °C or 70 °C before addition to the coffee. The 5 mL samples for lipid sample incubated at 4 °C were taken at 55 °C due to the immediate drop in the coffee's temperature.

Quantification was carried out according the FDA protocol for furan (USFDA, 2004). Standard addition curves were plotted using peak area ratios at characteristic retention times for furan (*m/z* 68/72) 2-methylfuran (82/72), 3-methylfuran (82/72) and 2,5-dimethylfuran (96/72) with R^2 value > 0.98, against their respective concentrations. Standard addition curves were subsequently corrected for background concentrations of furans, and then used as calibration curves to obtain the concentrations of the furans within samples taken at the drinking temperatures.

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 min with continual agitation occurring, 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 maintained for 12.5 min. Helium was used as the carrier gas with a constant flow rate of 1.7 mL/min. The capillary direct MS interface temperature was 225 °C; the ion source temperature was 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 µm 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, Two-way ANOVA in conjunction with a Tukey test to determine whether stirring rate, lipid content or lipid temperature had a significant influence on furan, 2-methylfuran and 2,5-dimethylfuran concentration for a confidence interval of 95%, where n = 3. Response surface analysis was conducted on the three factor Box-Behnken experimental design to obtain the contour plots found in Section 3.5 and Figs. S1a–h in the Supplementary material.

3. Results and discussion

Coffee has been found to be a significant dietary source of furan with potential to contribute considerably to the consumer's exposure to furan derivatives, such as 2-methylfuran (Chain et al., 2017). Nevertheless, consumer exposure to furan and its derivatives from coffee is strongly driven by post-roast processing, including consumer behaviour. In a previous study conducted by Rahn and Yeretzian (2019) it was seen that the brewing method as well as in-cup cooling had a significant impact on the consumer's exposure to furans. While brewing method and drinking temperature may be the predominant factors influencing exposure of drinkers of black coffee, many consumers add milk, water or another condiment to their coffee, requiring subsequent stirring.

The current study investigates the influence of lipid content and subsequent stirring separately in order to determine their respective influence on furan retention and release from the coffee brew. However, dairy products and their substitutes are also added to coffee brews at varying temperatures and quantities, for instance in a café one may add a room temperature creamer (10–12% lipid) whereas at home, milk (2–3.5% lipid) or cream (18–35% lipid) may be added out of the fridge. Thus, different cooling rates may result, potentially influencing evaporation of furans. To isolate the parameter responsible for furan retention, condiments were added at three temperatures 4, 20 and 70 °C. The quantity of condiments added to the coffee were kept constant at 12 g to ensure cooling was not further influenced by differences in volume, which would influence composition as well as cooling rate. Condiments were consistently added immediately after pouring.

As cooling affects coffee's furan content and the chosen parameters are liable to influence the rate of cooling, the following section will briefly discuss the sampling procedure used, prior to continuing the discussion on the influence of the parameters on furan exposure from coffee.

3.1. Influence of coffee temperature during sampling

Addition of an external liquid differing in temperature accompanied by agitation will influence coffee's cooling rate. Therefore, in order to compare the furan content between coffees, the cooling curves were monitored with a thermocouple and samples were taken at a typical drinking temperature, 55–60 °C, as well as once the coffee had cooled to 35 °C. When the lipid samples were pre-cooled to 4 °C, the coffee cooled rapidly to below 60 °C and therefore sampling took place at 55 °C. At 35 °C the cooling rate had slowed, facilitating sampling and reducing the standard deviation between data points. In addition to more consistent sampling, cooling to 35 °C emphasized the retention or release of furan by the coffee matrix. While the concentrations of furans collected at drinking temperature were consistently higher, the trends found within the datasets were identical, as illustrated in Fig. 2. The current study will focus on the parameters' influence on furan retention and release, and therefore all figures will contain data from coffee cooled to 35 °C in order to highlight the influence of the parameter on the furan content within coffee.

Furan levels (116.6 ng/mL) sampled at 60 °C upon the addition of 12 g of 20 °C water are in agreement with those of Han and associates (Han et al., 2017) of between 93 and 129 ng/mL and Shen et al. (2016) of 178 ng/mL for the unspecified category of coffee. Interestingly, unstirred coffee sampled at 60 °C contained similar furan and 2-methylfuran's concentrations, 116.6 ng/mL and 269.0 ng/mL respectively, as the same beans prepared using a fully automatic machine in our previous study, 74–99 ng/mL furan and 188–264 ng/mL (Rahn & Yeretzian, 2019). While the retention of furan within coffee brewed using a fully automatic machine was attributed to the extraction efficiency of the brewing method, the retention of furan within the brew due to the addition of 20 °C water is likely due to the difference in temperature, which will be discussed further in Section 3.4. Coffee's sampled at 35 °C (69.3 ng/mL) agreed well with filter coffee values reported by Kuballa, Stier, and Strichow (2005) of between 8 and 66 ng/mL, Altaki, Santos, and Galceran (2011) of 20–78 ng/mL as well as Rahn and Yeretzian (2019), 47–53 ng/mL.

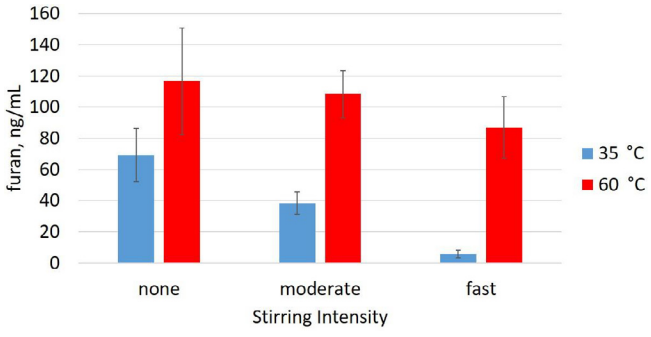

Fig. 2. Influence of stirring on furan concentration at two drinking temperatures, 35 °C and 60 °C, upon the addition of 12 g of water pre-heated to 20 °C.

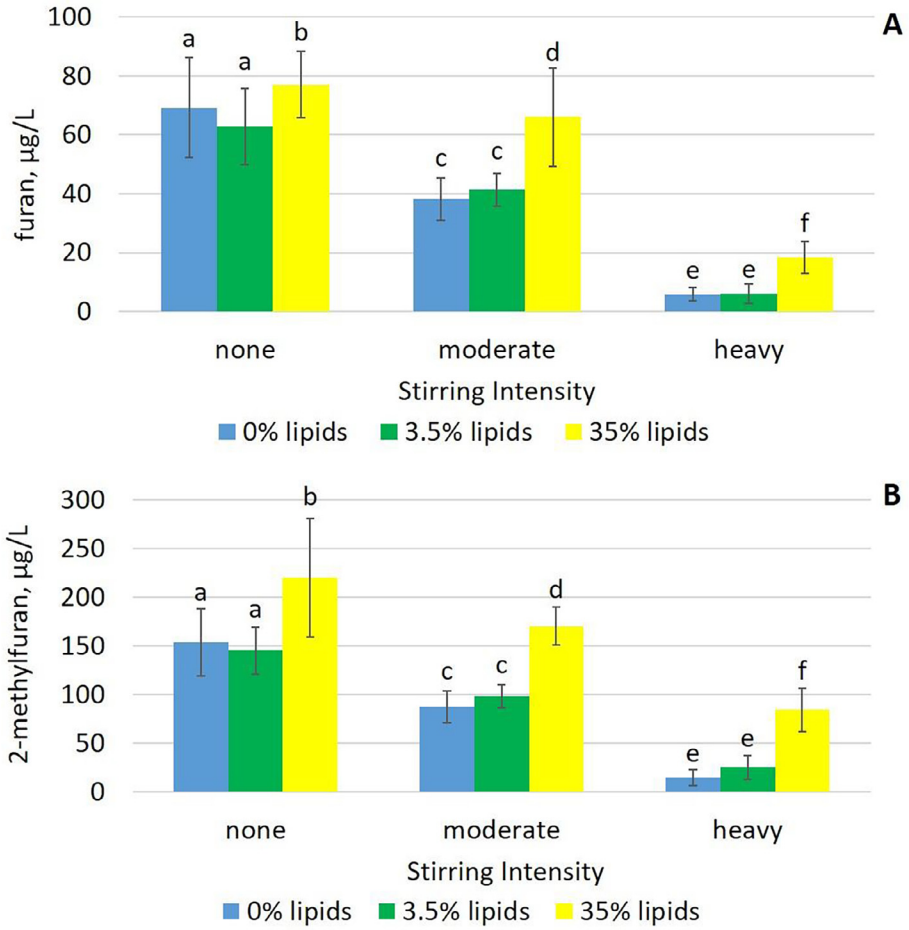

Fig. 3. Influence of adding 12 g of 20 °C water, 3.5% milk or 35% cream to 125 g of filter coffee on furan (A) and 2-methylfuran (B) loss when not stirred or continuously stirred at a medium and fast rate. Lower case levels reflect outcome of Tukey test of condiment lipid content versus stirring intensity.

While other furan derivatives were monitored in the current study, including 2,5-dimethylfuran, 2,3-dimethylfuran and 3-methylfuran, only 2,5-dimethylfuran was detected within aqueous condiment systems. The inability to detect 2,5-dimethylfuran after the addition of lipid containing condiments is a reflection of its low native concentration in addition to its lipophilic nature, consequently eliminating it from the headspace and concealing it from analysis. Due to the absence or incomplete dataset Box Behnken analysis could not be carried out on 2,5-dimethylfuran, 2,3-dimethylfuran or 3-methyfuran.

3.2. Influence of stirring on furan retention

The influence of stirring on coffee's furan release was evaluated by continuous mechanical stirring after the addition of the lipid component and until the final temperature of 35 °C was reached, which was within approximately 20–30 min depending on the conditions. Stirring levels chosen represented the two extremes, not stirring and stirring vigorously (creating an eddy within the cup), as well as an intermediate level of moderate stirring, which was a gentle agitation.

Mechanical stirring significantly decreased furan exposure, Fig. 2. Stirring at the highest intensity was found to reduce furan exposure by 90% relative to the coffee that was not stirred at all, indicating that passive cooling retains more furan than when the coffee is agitated and that increasing the stirring intensity facilitated furan's release from the coffee matrix.

Mechanical stirring was selected to ensure the conditions could be consistently reproduced under all conditions; however, mechanical stirring is seldom used by consumers. Therefore, to compare the mechanical stirring to a more realistic manual stirring scenario, coffees were manually stirred with a teaspoon for three durations, 15, 30 and 45 s with an effort being made to maintain the same level of vigour in each case. In all samples, 12 g of water at 20 °C was added to reproduce the cooling and dilution effect of added condiments. The furan loss between the three durations of manual stirring were found to be insignificant. Nevertheless, it was remarkable to see that manually stirring for as little as 15 s was more efficient than mechanically stirring at a moderate intensity for over ten minutes, Fig. S1. These results suggest that furan release provoked by stirring occurs quickly after agitation begins. Additional studies would be needed to confirm these observations, taking into consideration that manual stirring was consistently conducted on fresh coffee brew, where the high temperatures may have assisted in furan release. Furan release due to stirring may be less efficient if conducted on cooled coffee.

3.3. Influence of lipid content on furan retention

Furans are hydrophobic molecules, leading several authors to suggest that coffee's lipid content is responsible for the variances in furan concentration found between different coffee preparations (Van Lancker, Adams, Owczarek, De Meulenaer, & De Kimpe, 2009). That said, furan concentrations between different brewing methods are difficult to compare as they vary according to coffee variety, extraction procedure as well as brew ratio ($\frac{\text{brewyield, g}}{\text{coffeegrounds, g}}$). Moreover, brewing methods resulting in higher furan exposure levels are also efficient at extracting lipids as well as other components, including hydrocolloids. Therefore, in order to determine whether lipids have a significant effect

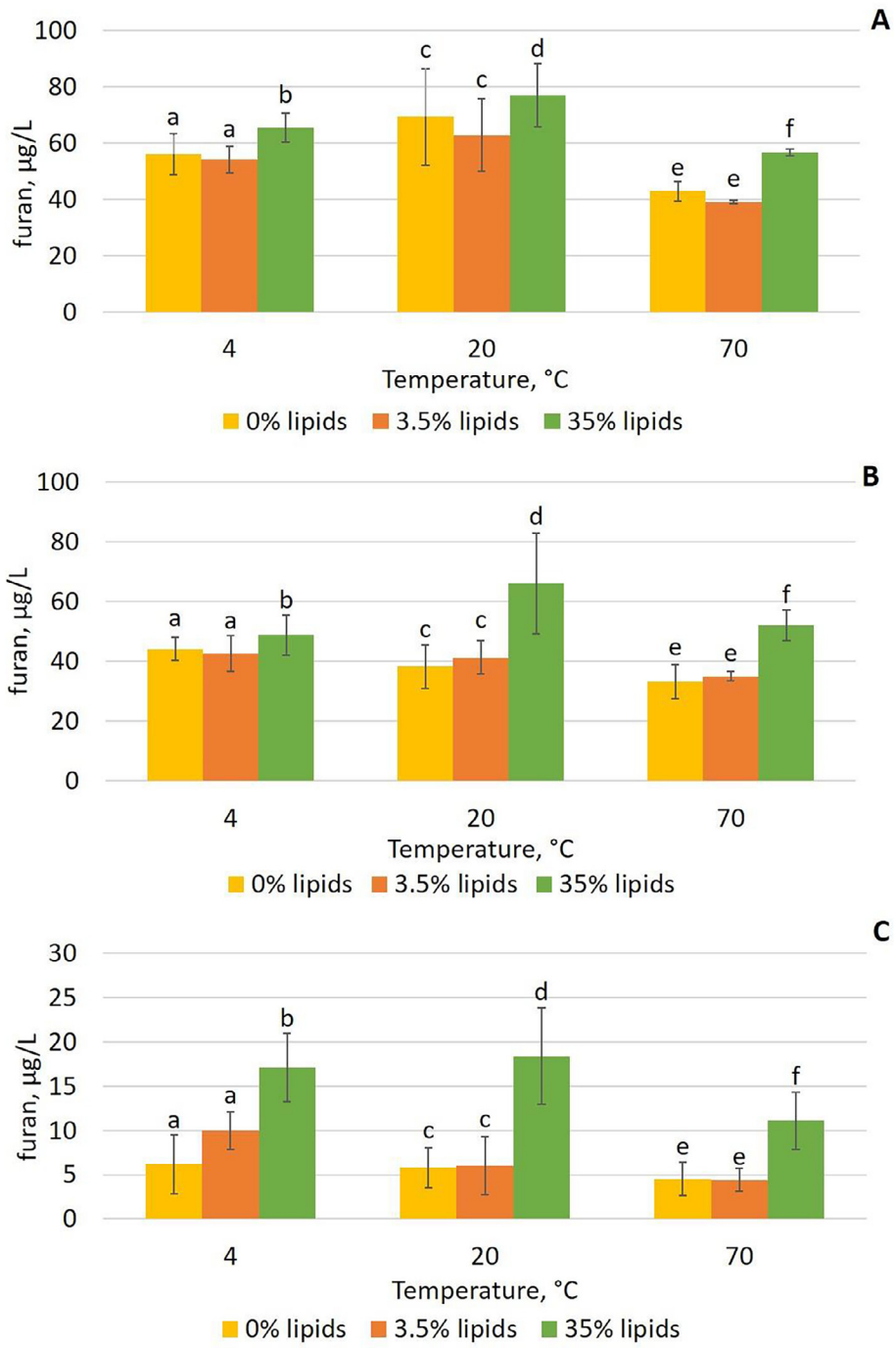

Fig. 4. Influence of adding 12 g of water, 3.5% milk or 35% cream kept at either 4, 20 or 70 °C to 125 g of filter coffee on furan retention when not stirred (A) or continuously stirred at a moderate (B) and vigorously (C) intensity. Lower case levels reflect outcome of Tukey test of condiment lipid content versus temperature.

on coffee furan retention, filter coffee was chosen as the brewing method, as only 0.4% of the lipids in the grounds have been reported as being transferred into the brew (Ratnayake, Hollywood, Ogrady, & Stavric, 1993). Three levels of lipids were subsequently added to the freshly brewed and poured coffee, simulating the addition of water, 3.5% milk or 35% cream by a consumer. To minimize compositional discrepancies between dairy products, the heavy cream purchased was diluted to a lipid content of 3.5%, resulting in a mock milk mixture. Once the coffees had been prepared, they were mechanically stirred as described in Section 3.2.

Stirring, as seen in the previous section, was found to have a significant effect on furan release from the coffee mixtures. As illustrated in Fig. 3, the addition of the mock milk mixture (3.5% lipids) slightly impeded furan and 2-methylfuran release from the coffee brew when compared to the addition of water, however this difference was not significant. Conversely, the addition of heavy cream (35% lipids) significantly retained the furan species within the brew, reducing stirring efficiency in releasing furans. While coffees with added water or mock milk lost as much as 90% of their furan content when vigorously stirred, relative to their non-stirred equivalent, coffees containing heavy cream did not lose more than 40% upon vigorous stirring. Unstirred coffee containing heavy cream retained an extra 11% furan upon cooling to 35 °C than coffees with added water or 3.5% lipid solution.

The data obtained in the current study suggests that a critical coffee lipid content is required within the coffee beverage before significant retention of furans can be observed. Theoretical lipid content of our

filter coffee is 0.0021%, assuming 0.4% of the lipids used for the filter coffee made it to the brew (Ratnayake et al., 1993), adding 12 g 3.5% (v/v) lipids would only increase the coffee's lipid concentration to 0.42%, which may account for the insignificant difference between water and the mock milk added. Addition of heavy cream increased the coffee's lipid concentration to 4.20%, suggesting that the critical lipid content for the retention of furans within coffee lies between 0.42% and 4.20%. The influence of dairy proteins on furan retention was not investigated.

3.4. Influence of lipid content temperature on furan retention

The motivation behind a consumer adding cream, milk or water to coffee can be manifold, including sensory preference, related to specific coffee preparation (such as a cappuccino or Americano) or even to rapidly lower the coffee to drinking temperature. Irrespective of the rationale, these condiments do not only vary in composition, but also in temperature, potentially influencing furan release by influencing the coffee's immediate temperature upon addition and the subsequent cooling rate. To assess the influence of condiment temperature on furan release from coffee, the lipid mixtures discussed in Section 3.3 were incubated at 4, 20 and 70 °C before being added to the freshly brewed coffee.

Unlike stirring intensity or lipid content, condiment temperature did not demonstrate a dominant trend. Not stirring showed a significant difference between all condiment temperatures, but no clear trend. Furan retention increased from 4 °C to 20 °C and decreased significantly when the lipid mixtures were incubated at 70 °C, Fig. 4. This increase and then decrease trend in furan retention over incubation temperature was no longer seen under moderate stirring conditions, however the significant decrease in furan retention upon incubation at 70 °C was once again seen when coffees were stirred vigorously. Similar trends in furan content were observed for the three lipid compositions between 4 °C and 20 °C, either increasing insignificantly with temperature or remaining constant, while a decrease was consistently observed between 20 °C and 70 °C. The significant decrease in furan content observed at 70 °C for a number of conditions may reflect the initial retention of heat by the brew upon addition of the condiment, facilitating furan loss and reducing consumer furan exposure from coffee.

3.5. Integrating consumer preferences

Results obtained from the lipid content, temperature and stirring analyses were integrated in a second order function to consolidate the findings. Fig. 5 presents a slice of the virtual environment created by the algorithm illustrating the two parameters that principally influence furan retention, condiment lipid content and stirring intensity.

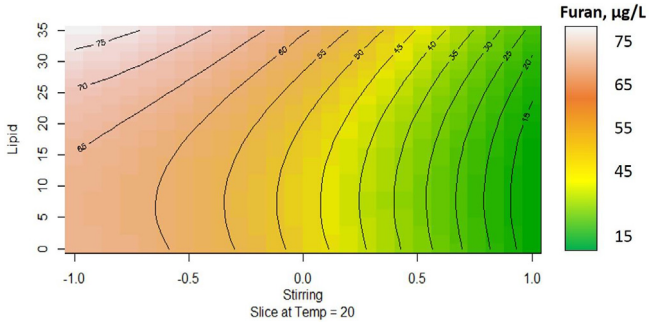

Fig. 5. Contour plot depicting furan retention, in µg/L, cooled to 35 °C as a factor of added lipid content, at 20 °C, and stirring intensity (−1.0 = no stirring, 0.0 = moderate stirring and 1.0 = heavy stirring). Colour contouring is an expression of furan concentration as elucidated by the accompanying legend. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Additional contour plots are available in the Supplementary material, Fig. S2a–h.

Stirring, as previously mentioned in Section 3.2, has a positive effect on furan release, increasing furan loss with increasing stirring intensity, irrespective of lipid content added. Nonetheless, the efficiency of furan release caused by stirring is reduced by the promotion of furan retention upon adding a condiment containing lipids, increasing the cup lipid concentration. Therefore, if increasing the cup lipid composition increases furan retention, then brewing methods that enhance lipid extraction will increase furan exposure. Similar trends in furan retention and release are observed at all temperatures.

Temperature of the lipid fraction added to the coffee had a modest influence on the virtual space. While a statistically insignificant maximum furan content is seen at 25 °C, a significant decrease occurs when the condiment is added at 70 °C. Therefore, coffee consumers can significantly reduce their furan exposure by adding hot water, milk or cream to their coffee and subsequently stirring their coffee while hot.

4. Conclusion

The current study extends our understanding of coffee's contribution to furan exposure, extending beyond black coffee into the use of coffee condiments, such as milk or cream. Condiment lipid content was found to have a significant effect on furan retention, demonstrating that creams high in lipids would increase the consumer's furan exposure. Nevertheless, the retention of furan by the coffee can be significantly decreased by stirring, mechanically or manually. Temperature of the condiment was found to have a modest effect, revealing that adding a heated condiment can significantly reduce the consumer's furan exposure.

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Declarations of interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2019.01.207.

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