Aroma binding and stability in brewed coffee: a case study of 2-furfurylthiol

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Abstract: The aroma stability of fresh coffee brew was investigated during storage over 60 minutes, there was a substantial reduction in available 2-furfurylthiol (2-FFT) (84%), methanethiol (72%), 3-methyl-1H-pyrole (68%) and an increase of 2-pentylfuran (65%). It is proposed that 2-FFT was reduced through reversible chemical binding and irreversible losses. Bound 2-FFT was released after cysteine addition, thereby demonstrating that a reversible binding reaction was the dominant mechanism of 2-FFT loss in natural coffee brew. The reduction in available 2-FFT was investigated at different pH and temperatures. At high pH, the reversible binding of 2-FFT was shown to protect 2-FFT from irreversible losses, while irreversible losses led to the reduction of total 2-FFT at low pH. A model reaction system was developed and a potential conjugate, hydroxyhydroquinone, was reacted with 2-FFT. Hydroxyhydroquinone also showed 2-FFT was released after cysteine addition at high pH.

Key words: coffee brew aroma stability; 2-furfurylthiol (2-FFT); reversible and irreversible degradation; binding site stability

Chemical compounds studies in article: 2-furfurylthiol (PubChem CID 7363); L-cysteine (PubChem CID 5862); hydroxyhydroquinone (PubChem CID 10787); Sodium dihydrogen phosphate (PubChem CID: 23672064); disodium hydrogen phosphate (PubChem CID: 24203); hydrochloric acid (PubChem CID: 313); sodium hydroxide (PubChem CID: 14798); 3-heptanone (PubChem CID: 7802)

1. Introduction

After coffee roasting, furans, pyrazines, thiols, aldehydes and many other volatiles are present in the headspace (Yang et al., 2016) and together form the complex aroma profile of coffee brew (Semmelroch & Grosch, 1995). However, some of these coffee aroma compounds are unstable during storage of roasted coffee and coffee brew (Dulsat-Serra, Quintanilla-Casas, & Vichi, 2016). This aroma deterioration results from the interaction with the matrix (Fisk, Boyer, & Linforth, 2012; Yu et al., 2012) and aroma degradation, and is termed aroma staling (Hofmann & Schieberle, 2002, 2004; Müller & Hofmann, 2007).

2-Furfurylthiol (2-FFT), as a sulfur compound in coffee, has been established as one of the key aromas that contribute to the characteristic flavour of coffee based on sensory studies and model dilute experiments (Blank, Sen, & Grosch, 1992; Hofmann & Schieberle, 2002; Semmelroch & Grosch, 1995). However, 2-FFT rapidly reduces during coffee brew processing or storage due to these staling reactions. This loss could cause a significant reduction of sulfury-roasty aroma and is partially responsible for the inferior sensory quality of aged coffee brews (Hofmann, Czerny, & Schieberle, 2001; Mayer, Czerny, & Grosch, 2000; Semmelroch & Grosch, 1996).

The reduction of available 2-FFT during coffee staling can be divided into reversible and irreversible staling events (Charles-Bernard, Kraehenbuehl, Rytz, & Roberts, 2005; Guichard, 2002). The irreversible losses of 2-FFT are presumed to be due to physical diffusion/volatile loss and chemical degradation reactions, such as polymerization or oxidation (Blank et al., 2002; Charles-Bernard, Kraehenbuehl, et al.,

2005). This irreversible fraction is very hard to regenerate. Reversible losses are believed to be mainly through covalent bonding to non-volatile components in the coffee matrix, it is proposed that this 2-FFT lost due to reversible reactions could be subsequently released again by cysteine addition (Müller & Hofmann, 2007; Mestdagh, Davidek, Chaumonteuil, Folmer, & Blank, 2014; Sun et al., 2018).

Previous studies have shown that the thiol group of 2-FFT is a good nucleophile and could be involved in nucleophilic and radical reactions (Rowe, 2009). Through the binding reaction, 2-FFT could be reversibly bound to conjugates in coffee brew, such as 1, 4-bis (5-amino-5-carboxy-1-pentyl) pyrazinium radical cation (CROSSPY) that is found in coffee melanoidins (Hofmann & Schieberle, 2002; Tominaga, Blanchard, Darriet, & Dubourdieu, 2000). More recent studies have reported that hydroxyhydroquinone (HHQ), one of the chlorogenic acid degradation products, was the dominant conjugate to bind 2-FFT (Müller & Hofmann, 2007). In this binding reaction, 2-FFT is bound by HHQ through the reactive quinone converted from HHQ, leading to a rapid reduction of 2-FFT (Fig. 1) (Müller, Hemmersbach, van't Slo, & Hofmann, 2006; Müller & Hofmann, 2007). Hofmann and Schieberle showed that this covalent binding could be established within 15 min (Hofmann & Schieberle, 2002)

Cysteine has been shown previously to release 2-FFT that is bound by coffee matrix (Darriet, Tominaga, Lavigne, Boidron, & Dubourdieu, 1995; Mestdagh et al., 2014). After cysteine addition, the bound form of 2-FFT is competitively replaced by cysteine due to its mercapto structure and high reducing properties. High cysteine concentration could also prevent 2-FFT from forming dimers (Rowe, 2009). Cysteine

addition has also been shown to reversibly release bound 2-FFT in coffee brew. This has been used to determinate the total: bound 2-FFT in coffee brews. Hereinafter "total 2-FFT" refers to the whole available 2-FFT in the coffee brew at any time point, including both free 2-FFT and reversibly bound 2-FFT. "Free 2-FFT" means the 2-FFT fraction that exists in free form in a coffee brew (Sun et al., 2018).

pH is important for 2-FFT formation and loss in coffee brew. High pH conditions favor the formation of 2-FFT during coffee roasting and also increased 2-FFT loss during heating in model systems (Hofmann & Schieberle, 1998; Kumazawa & Masuda, 2003). However, the impact of pH on coffee aroma binding in coffee brews, and the relative understanding of free: bound 2-FFT in the coffee brew and their relative stabilities are not well understood and is therefore the focus of this study.

In this study, coffee aroma staling was evaluated and the mechanism behind 2-FFT loss investigated. The stability of free versus total 2-FFT was evaluated. Reversibly bound 2-FFT was released by the addition of cysteine enabling the calculation of the reversibly bound and irreversibly lost 2-FFT fractions with the further calculation of losses of 2FFT through volatilization. The effects of pH and temperature on 2-FFT binding capacity was evaluated for coffee brew. To elucidate the pH effect on potential conjugate, HHQ, a model system was developed to explain the interaction of HHQ and 2-FFT with or without cysteine addition. Aroma loss through binding reactions is a common costly problem for the soluble coffee industry, Robusta coffee is commonly used in this field, therefore Robusta coffee was selected as the target for this study.

2. Materials and methods

103 *2.1 Materials and reagents*

104 2-furfurylthiol (purity ≥ 98%), hydroxyhydroquinone (purity ≥ 99%),
105 homogenous series of *n*-alkanes (C6-C26) standard were purchase from Sigma-Aldrich
106 company (Poole, UK). Sodium dihydrogen phosphate (purity ≥ 99%), disodium
107 hydrogen phosphate (purity ≥ 99%), sodium hydroxide (purity ≥ 97%), methanol
108 (purity ≥ 99%), concentrated hydrochloric acid (purity ≥ 36.5%), L-cysteine (purity ≥
109 98%), 3-heptanone (purity ≥ 99%) were purchased from Acros Organic company (New
110 Jersey, USA). Robusta washed coffee beans from Vietnam were purchased from
111 Pennine Tea and Coffee Company (Halifax, UK).

112 *2.2 Coffee sample preparation*

113 Green Robusta washed coffee beans (250 g) were roasted by a convection oven
114 (Mono Equipment, Swansea, UK). Before roasting, the oven was pre-heated for 5 min
115 to reach 225 °C. The coffee beans were roasted for 15 min at 225 ± 2 °C and, after
116 cooling to 20 °C by convective cold air (around 10 °C cooling for 5 min), were ground
117 using a coffee grinder for 11 seconds (KG 49, Delonghi, Australia). The ground coffee
118 was screened by a metal sieve (700 µm, Endecotts, Essex, UK) to control particle size.
119 The color of roasted ground coffee was analyzed by an UltraScan Pro (Hunterlab, US)
120 in a 1 cm Petri plate. The result was: L^* (lightness) = 41 ± 0.3, a^* (red, green) = 3.74 ±
121 0.2, b^* (yellow, blue) = 3.81 ± 0.3. The L^*, a^* and b^* values were in accordance with
122 the optimal roasting degree of medium roasting (Mendes, de Menezes, Aparecida, &
123 Da Silva, 2001). All ground samples were stored at -80 °C under nitrogen gas. Before
124 brewing, ground samples were defrosted at 15 °C for 15 min.

The ground coffee (9 g) was brewed using a French press (3 Cup Black Cafetière, Argos, UK). Before brewing, the French press was rinsed by deionized water (around 92 °C) to preheated it for 10 seconds. Then the ground coffee (9 g) was mixed by deionized water (180 mL, 92 °C) in the French press coffee maker. The plunger was pressed down until the filter touched the coffee liquid surface and stood for 4 min before depressing the plunger. The resulting fresh coffee liquid was shaken gently in French press for 5 seconds to make coffee brew uniform.

In order to minimize the volume of headspace and exclude the aroma physical diffusion, the coffee brew was poured from French press to fill full an amber vial of 40 mL which was sealed by screw top with PTFE-lined silicone septa (Supelco, Sigma Aldrich, UK). The sealed amber vials with fresh coffee were cooled to 40 °C by water bath and following experiments were started from these "sealed amber-vial samples" respectively.

2.3 Coffee aroma stability

The sealed amber-vial samples were stored in a water bath (FB60307, Fisher Brand, UK) up to 60 min (0, 15, 30, 45, 60 min) at 40 °C prior to solid-phase microextraction-gas chromatography mass spectrometry (SPME-GC-MS).

2.4 Effect of storage time on free and total 2-furfurylthiol concentration under open-vial condition

Five milliliter of fresh coffee brew was transferred from the sealed amber-vial samples (prepared from item 2.2) to GC headspace vials (20 mL, 22.5 mm × 75.5 mm, Sigma-Aldrich, UK) by pipette (Argos, UK). The GC headspace vials were left open

147 (open-vial condition) to allow aroma volatilization so that the physical diffusion loss
148 was evaluated. The headspace vials were stored for up to 60 min at 40 °C in a water
149 bath before sealing for free and total 2-FFT SPME-GC-MS analysis.

150 *2.5 Effect of pH and storage temperature on free and total 2-FFT in coffee brew*

151 The sealed amber-vial samples were adjusted to pH 3 to 9 with 1 M hydrochloric
152 acid solution and 1 M sodium hydroxide solution. Samples were stored at 20, 55 or
153 90 °C in a water bath for 1 h respectively. After cooling down to 40 °C by water bath,
154 the free 2-FFT concentration of samples was analyzed by SPME-GC-MS. Another
155 aliquot of 40 mL amber-vial sample was subjected to the same pH incubation (without
156 different temperature storage). Then the total 2-FFT concentration was analyzed by
157 SPME-GC-MS.

158 To further study the pH effect on 2-FFT binding and release, additional
159 experiments were also performed:

160 **Cysteine addition after pH incubation:** The sealed amber-vial samples (40 mL) were
161 adjusted to pH 3 or 9, and stored for 1 h in water bath at 55 °C. After that, the pH of
162 samples was adjusted back to 6 prior to total 2-FFT analysis by SPME-GC-MS.

163 **Cysteine addition before pH incubation:** Cysteine of 0.64 g was added into the sealed
164 amber-vial samples (40 mL) after cooling down to 40 °C by water bath (to prevent
165 cysteine from undergoing Maillard reaction at high temperature) (Sun et al., 2018) and
166 stirred at 1000 rpm for 5 min on a magnetic stirrer (IKA RET control-visc, UK). The
167 pH of samples was adjusted to 3 and 9. After 1 h storage in a water bath at 55 °C, the
168 total 2-FFT was analyzed via SPME-GC-MS.

169 *2.6 Effect of pH on free and total 2-FFT in model system*

170 The buffer solution was prepared using disodium phosphate solution (0.2 M),
171 sodium dihydrogen phosphate solution (0.2 M) and sodium hydroxide solution (0.1 M)
172 and hydrochloric acid solution (0.1 M) to adjust the pH to 3 or 9. An aqueous solution
173 of hydroxyhydroquinone (HHQ) (0.004 g HHQ diluted in 30 mL Milipore water) was
174 added (300 µL) into phosphate buffer solution (40 mL) and placed in amber vials (40
175 mL), the samples was then stored for 1 h in a water bath at 40 °C to allow incubation
176 (Müller & Hofmann, 2007). 2-FFT (36 µg in 31.7 µL of methanol) was added into HHQ
177 buffer solution. 2-FFT was reacted with HHQ for 1 h at 40 °C prior, to evaluate free
178 and total 2-FFT by SPME-GC-MS.

179 *2.7 Quantification of volatile compounds*

180 The quantification of 2-FFT was carried out using the internal standard
181 quantification method (Sun et al., 2018). 3-Heptanone (4.1 µg in 5 µL of methanol) was
182 added into calibration solution as an internal standard (IS) to accommodate for
183 instrument drift. For coffee brew, the calibration curve was established by adding 2-
184 FFT (0, 1.8, 3.6, 7.2, 14.4 µg in 31.7 µL of methanol) into a prefabricated coffee model
185 which had a similar matrix to coffee brew. To prepare this coffee model, cysteine (1.6
186 g) was presented into fresh coffee brew (200 mL) at 40 °C. The coffee brew was dried
187 by rotary vacuum evaporation at 40 °C. The dried sample was then dissolved in 200
188 mL of ultra-pure water. This part of experiment (from adding cysteine to samples
189 dissolved into water) was repeated once more to release maximum 2-FFT from the
190 coffee brew (Sun, et al., 2018). For the model experiment, the calibration curve was

191 established by adding 2-FFT (0, 9, 18, 36, 44 μg in 31.7 μL of methanol) into the
192 phosphate buffer solution. The concentration of 2-furfurylthiol could be calculated by
193 the calibration curve made by relative peak area (peak area of standard 2-FFT to that of
194 internal standard) to the concentration of standard 2-FFT (y = 0.0055 x + 0.0381, R^2 =
195 0.998) .

196 Approximate quantification of all volatiles for the aroma stability study (Item 2.3)
197 were calculated by comparing the GC peak areas of volatiles with the peak area of 3-
198 heptanone internal standard, using a response factor of 1 to give relative concentrations
199 (Liu, Yang, Linforth, Fisk, & Yang, 2019). The percentage of volatile was calculated by
200 the comparison of their relative concentration after storage to their initial relative
201 concentration in fresh coffee. All samples were analyzed in one run in randomized order.

202 *2.8 SPME-GC-MS*

203 Five milliliters of samples were placed into GC headspace vials (20 mL, 22.5 mm
204 × 75.5 mm, Sigma-Aldrich, UK) from the amber vials by pipette. 3-heptanone (4.09 μg
205 in 5 μL of methanol) (Caporaso, Whitworth, Cui, & Fisk, 2018; Liu et al., 2019) was
206 added into the samples as the internal standard before vials were sealed by screw top
207 with PTFE-lined silicone septa. For total 2-FFT analysis, cysteine powder was added
208 to each vial before closure at 16 g/L (Sun et al., 2018) so that the bound form of 2-FFT
209 was released to the free form. With cysteine, the free 2-FFT currently detected by
210 SPME-GC-MS was the amount of total 2-FFT. Aroma analysis for free 2-FFT in coffee
211 brew had no cysteine addition.

212 The analysis was carried out using a gas chromatography coupled with the Single-

213 Quadrupole Mass Spectrometer (Thermo Fisher Scientific, Hemel Hemptead, UK). A
214 50/30 µm DVB/CAR/PDMS SPME fiber (Supelco, Sigma Aldrich, UK) was used to
215 extract volatile aroma compounds from the samples headspace. Samples were
216 incubated at 40 °C for 2 min. The SPME extraction procedure was presented at 40 °C
217 for 10 min. The fiber desorption was at 250 °C for 3 min in the injector and then
218 analyzed by GC-MS.

219 A ZB-WAX column (30 m × 0.25 mm I.D., 1 µm film thickness; Phenomenex Inc,
220 Macclesfield, UK) was used to separate constituents. Analytical GC conditions in GC-
221 MS were as follows: Helium was used as carrier gas (1 mL/min). Injector temperature
222 was 250 °C. Splitless injector mode was used. The oven temperature program was held
223 at 40 °C for 5 min, then raised at a rate of 3 °C /min to 160 °C. After that, oven
224 temperature was raised at 20 °C /min to 240 °C and held for 2 min; Energy voltage was
225 70 eV. Single ion monitoring (SIM) and Scan mode was used (30 – 300 m/z). Samples
226 were run in triplicate randomized order.

227 2-FFT was identified by comparing the detected 2-FFT mass spectra and linear
228 retention indices (LRI) with 2-FFT standard mass spectra and LRI value from reference
229 data libraries (NIST 11 and WILEY 07 databases). 114 m/z was used as the
230 characteristic ion for 2-FFT quantification (Mestdagh et al., 2014; Tominaga &
231 Dubourdieu, 2006). The identification of other volatiles was identified by comparing
232 the detected volatiles mass spectra and LRI value with the mass spectra and LRI value
233 from data libraries (NIST 11 and WILEY 07 databases). The linear retention indices
234 values were calculated with homogenous series of n-alkanes (C6-C26) standard in the

same GC conditions.

2.9 Statistical Analysis

Data were presented as mean values ± standard deviation. Statistical analysis was conducted by SPSS 19.0 (SPSS Inc., Chicago, USA). Duncan's multiple range tests was used, and $p < 0.05$ was considered as significant. All samples were measured in triplicate.

3. Results and discussion

3.1 Coffee aroma stability

To identify which coffee volatiles are directly impacted by chemical changes over storage, samples were stored in fully filled vials. This allowed the investigation of storage time on aroma, while excluding the effects of physical diffusion and losses into the headspace. Eighty-nine volatile aroma compounds were detected in the fresh coffee brew and after 1 h storage at 40 °C, there were substantial changes over storage time in the aroma concentration of four aroma compounds, shown in Fig. 2 A to D. 85 of the volatile aroma compounds did not change their relative headspace concentration by more than 50%. The headspace relative concentration of 2-furfurylthiol, methanethiol and 3-methyl-1H-pyrrole decreased by 84%, 72%, 68% respectively; 2-pentylfuran increased to 165% compared to its relative concentration in coffee brew before storage. Of all the compounds evaluated, 2-FFT showed the fastest decrease, with a 68% reduction in free 2-FFT within 15 min. It should also be noted that of the four compounds that changed, 2-FFT has the highest odour activity value (OAV) and highest flavor dilution factor (FD factor) by aroma extract dilution analysis (Semmelroch &

257 Grosch, 1995; Vermeulen, Gijs, & Collin, 2005), and a sulfury-roasty aroma, which is
258 highly characteristic of coffee aroma (Akiyama et al., 2008; Dulsat-Serra et al., 2016).
259 Therefore, 2-FFT was selected as the key instable aroma compound for further studies.
260 *3.2 Effect of storage time on reversible and irreversible binding of 2-FFT under open-*
261 *vial condition*
262 Total available 2-FFT can be lost by irreversible losses and reversible binding
263 reactions (Charles-Bernard, Kraehenbuehl, et al., 2005; Guichard, 2002). These two
264 losses affect the total and potential for free 2-FFT in coffee. As the total 2-FFT includes
265 free 2-FFT and reversibly bound 2-FFT, the difference between the total 2-FFT amount
266 and free 2-FFT amount is defined as "reversibly bound 2-FFT"; On the other hand,
267 irreversible losses occurred and increased during coffee storage. So, the difference of
268 total 2-FFT between original and stored coffee is defined as "irreversibly lost 2-FFT".
269 Thus, the total and free 2-FFT of the stored coffee were determined to investigate the
270 extent of reversibly bound and irreversibly lost 2-FFT during storage. Over 1h storage,
271 the concentration of both total and free 2-FFT decreased significantly (Table 1). The
272 biggest change happened within the first 30 minutes and then plateaued. Total 2-FFT
273 decreased from 153 to 138 µg/L (10% loss) ($p<0.05$), and free 2-FFT decreased from
274 3.4 to 0.4 µg/L (88% loss) over 1 h storage ($p<0.05$). The total 2-FFT concentration was
275 much higher than free 2-FFT concentration, indicating that a large amount of 2-FFT
276 was reversibly bound to coffee components within the fresh coffee brew.
277 Using the free and total 2-FFT amounts, the reversibly bound and irreversibly lost
278 2-FFT amount could be calculated using the equation below. After a defined period of

storage (X min).

$$Reversibly\ \ bound\ \ 2\text{-}FFT = Total\ \ X - Free\ \ X$$

$$Irreversibly\ \ lost\ \ 2\text{-}FFT = Total\ \ O - Total\ \ X$$

X: the concentration of aroma in coffee brew stored at time X min;

O: the concentration of aroma in original fresh coffee brew at time 0 min.

Reversibly lost 2-FFT and irreversibly lost 2-FFT are presented in Fig. 3. Over the 1 h storage period, the proportion of reversibly bound 2-FFT reduced from 149.6 to 137.1 µg/L). The irreversibly lost 2-FFT increased from 0 to 15.5 µg/L. These low (~10%) losses suggest that the volatilization and irreversible chemical degradation have a limited effect on 2-FFT loss compared to the predominant reversible binding in natural coffee brew (~pH 6.2). Due to this, in natural coffee (~pH 6.2), reversible loss is proposed to be the main reason for the loss of free 2-FFT, which is expected to play a significant role in the loss of the characteristic aroma of coffee during staling (Hofmann & Schieberle, 2002; Mayer & Grosch, 2001).

3.3 The effect of pH and storage temperature on free 2-FFT concentration in coffee brew and the model system

Coffee brew samples were stored at different pH and temperatures to investigate the impact on free 2-FFT stability. Both pH and temperature (Fig. 4 A) had a significant impact on free 2-FFT concentration. After 1 h incubation at elevated temperatures, free 2-FFT concentration decreased significantly (2.7 to 0.4 µg/L at 20 °C; 1.9 to 0.5 µg/L at 55 °C; 1.6 to 0.5 µg/L at 90 °C), the loss of free 2-FFT was greatest at highest pH values at all temperature (Fig. 4 A). This resulted in almost all free 2-FFT being

301 reversibly bound by the coffee matrix at pH 6-9. The same trend was also found in the
302 model system (Fig. 4 B) where the concentration of free 2-FFT decreased from 383 to
303 0.02 µg/L when the pH of buffer solution was increased from 3 to 9.

304 This pH sensitivity can be explained by the impact on quinone. As discussed
305 previously, during the reversible binding reaction between 2-FFT and HHQ, the
306 reactive quinone is formed from HHQ first, then it reacts with 2-FFT (Fig. 1) (Müller
307 & Hofmann, 2007). The highly reactive quinone is unstable at low pH due to its
308 carbonyl property (Li & Chen, 2005). So, under low pH conditions, the reversible
309 binding reactions are inhibited resulting in a greater concentration of free 2-FFT at low
310 pH as shown in Fig. 4 A and B. The inverse of this means that at high pH the conversion
311 of quinone is not be inhibited and more free 2-FFT is reversibly bound.

312 The 2-FFT in coffee brew stored at 20 °C had the highest free 2-FFT concentration
313 (Fig. 3 A), while at 90 °C there was less free 2-FFT compared to samples stored at 20
314 and 55 °C. This result suggests that the high temperature may increase the efficiency of
315 the reversible binding reaction leading to more efficient 2-FFT binding.

316 *3.4. The effect of pH on total 2-FFT concentration in coffee brew and model system*

317 pH also impacted the total 2-FFT level. After 1 h storage, total 2-FFT showed a
318 significant increase when the incubation pH was increased (Fig. 4 C), increasing from
319 2.6 to 159 µg/L. In the model system, the similar increase was found, but to a lesser
320 extent (351 to 524 µg/L) (Fig. 4 D).

321 To study the reason for the low level of total 2-FFT at low pH and high level at
322 high pH value, 2-FFT releasing ability of cysteine was further studied as cysteine might

be sensitive to pH. Before cysteine addition, samples were incubated at different pH and were adjusted back to pH 6, as cysteine has previously been shown to effectively release reversibly bound 2-FFT at pH 6 (Mestdagh et al., 2014; Sun et al., 2018). After control of the pH, the result still showed a low total 2-FFT level when stored at low pH and high total 2-FFT level when stored at high pH (Fig. 5: Pre pH incubation). The total 2-FFT at pH 9 (105 µg/L) was over twice as the amount retained when the coffee brew was stored at pH 3 (50 µg/L), suggesting that the 2-FFT releasing ability by cysteine was not susceptible to pH.

With the addition of cysteine, total 2-FFT at pH 3 was much lower than pH 9 as shown in Fig. 4 C. This low amount of total 2-FFT might be a result of irreversible losses as the reversible binding is inhibited at low pH (Discussed in Item 3.3). To further verify this irreversible loss effect on total 2-FFT, cysteine was added into coffee brew before pH incubation (Fig. 5.). After pH incubation, total 2-FFT under both pH 3 and 9 incubation presented a much lower level (56.8 µg/L and 49.9 µg/L). This result indicated that when the bound 2-FFT was released out of the "protection" of conjugates (such as HHQ) prior to incubation, the total 2-FFT was equally lost by the irreversible losses occurring during incubation. Suggesting that 2-FFT is protected by reversible binding during incubation.

It should also be noted that the total 2-FFT concentration in the model system at pH 3 (Fig. 4 D) was not degraded as much as the total 2-FFT in the coffee brew (Fig. 4 C). Suggesting other effects caused by the matrix difference between the coffee and the model system. In coffee brew, the matrix is much more complex and would increase

irreversible losses through other mechanisms such as radical delivery from Fenton reaction (Charles-Bernard, Roberts, & Kraehenbuehl, 2005). This suggests that additional coffee non-volatiles do contribute to 2-FFT irreversible losses (Charles-Bernard, Kraehenbuehl, et al., 2005; Charles-Bernard, Roberts, et al., 2005).

4. Conclusion

This study identified four unstable aroma compounds in coffee brew, the availability of which changed significantly over a 1h holding period, these were 2-furfurylthiol, methanethiol, 3-methyl-1-pyrole and 2-pentylfuran. 2-furfurylthiol suffered the greatest losses and was selected for further investigation. It was shown that reversible binding with HHQ was the dominant reason for 2-furfurylthiol staling in natural coffee. To further explain this reversible binding reaction, 2-FFT binding was studied and showed that at low pH, the 2-FFT binding reaction to hydroxyhydroquinone (HHQ) is inhibited and the availability of free 2-FFT level increased compared to free 2-FFT at higher pH values. This work also showed that without the protection of conjugates (reversibly bound to HHQ) in coffee brew, free 2-furfurylthiol could be irreversibly lost.

The key finding that high levels of reversible binding of 2-FFT occurs during storage at an optimal pH and this stabilizes key aroma compounds from irreversible loss could be applied to the production of instant coffee. Furthermore, flavour houses that extract and stabilize coffee aromas could store aroma isolates and brew concentrates at high pH to preserve key aromas, and ready-to-drink coffees could apply these findings directly to their products to reduce the staling phenomenon.

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