Quantification of Compounds in Coffee Involved in Gastric Acid Regulation

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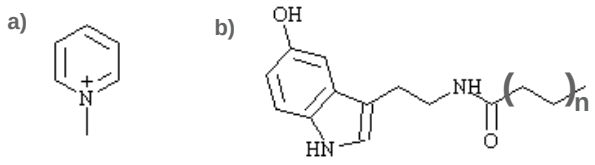
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SUMMARY

Some compounds present in coffee (e.g. $^{\beta}$*N*-Alkanoyl-5-hydroxytryptamides, caffeine, pyrogallol, and catechols) have been suggested to induce stomach irritation after coffee consumption. On the other hand, *N*-methylpyridinium has recently been shown exhibiting protective effect towards gastric acid secretion. In model brews and soluble-type coffees, *N*-methylpyridinium was found in the range of 13-115 mg/mL, its concentration being the highest in dark roasted brews. The $^{\beta}$*N*-alkanoyl-5-hydroxytryptamide concentrations range between 15-218 mg/L and are particularly enriched in green coffee brews. Based on this work, one could expect lower gastric acid secretion for coffee beverages prepared from dark roast.

INTRODUCTION

Coffee is one of the most popular beverages highly appreciated for its stimulating effect. Some consumers, however, experience symptoms of gastric irritation after coffee consumption, which might be caused by an increased acid secretion in the human stomach (Boekema et al., 1999; Nilsson et al., 2004). Complex mechanisms of gastric acid regulation have been described involving many cell receptors such as histamine H2, acetylcholine M3, cholcystokinin B or somatostatin receptors. The receptor diversity could explain the difficulty in identifying putative stomach irritating compounds in coffee beverage. In particular, $^{\beta}$*N*-alkanoyl-5-hydroxytryptamides (C-5HT), caffeine, pyrogallol, and catechols were incriminated for the gastric irritation[3]. In other studies, *N*-methylpyridinium (NMP), a trigonelline derivative, was shown to induce chemopreventive activities in-vivo and in-vitro, possibly also decreasing gastric acid secretion (Rubach et al., 2010; Somoza et al., 2003). The objective of this work was to investigate the levels of NMP and C-5HT derivatives (Figure 1) with the aim of mapping these stomach irritating / protective compounds in model coffees.



C18-5HT	N-steraoyl 5-hydroxytryptamide
C20-5HT	N-arachinoyl 5-hydroxytryptamide
C21-5HT	N-heneicosanoyl 5-hydroxytriytamide
C22-5HT	N-behenoyl 5-hydroxytryptamide
C23-5HT	N-tricosanoyl 5-hydroxytryptamide
C24-5HT	N-lignoceroyl 5-hydroxytryptamide

Figure 1. Chemical structures of compounds assessed in the coffee extracts: a) N-methylpyridinium, b) $^{\beta}$N-alkanoyl-5-hydroxy-tryptamides

MATERIAL AND METHODS

Sample preparation

A Vietnamese Robusta and a Colombian Arabica were roasted at three different levels (i.e. light, medium, dark) in a Probat RT3 roaster and finely ground. The roast and ground (R&G) coffees were extracted using a Dionex extractor in a single-step (i.e. 100 °C/10 min) or two-step (i.e. 100°C/10min + 180 °C/10 min) procedure to prepare brew-type and soluble-type coffees, respectively. The extracts were freeze-dried prior to analysis.

Quantification *N*-Methylpyridinium (NMP)

The chromatographic separation was achieved on a Kinetex 2.6 µm HILIC (100x2.1 mm) (Phenomenex) column coupled to a QToF 6520 (Agilent). A binary gradient of A/B (A: 2.5mM ammonium acetate with 0.1% acetic acid 90% ACN/10% water, B: 2.5 mM ammonium acetate with 0.1% acetic acid 10% ACN/90% water) was applied as follows: 0-2 min 100/0; 2-5.5 min 55/45; 5.5-6 min 0/100; 6-7 min 0/100. The flow rate was 700 µL/min. The column temperature was kept at 30 °C. The sample concentration was approximately 10 mg/mL in water. The injection volume was 2µL.

NMP was identified by combining retention time and exact mass (<3 ppm). Quantification was performed by external calibration. The spiked reference material showed excellent recovery (102%). The mass spectrometer QTOF 6520 (Agilent) was used in positive ESI-mode and was operated in MS mode to scan the 25-1600 *m/z* mass range. Nitrogen was used as drying gas (value of 10L/min) and the nebulizer was set at 35 psig. The source was operated at 300 C with Vcap at 5000 V, the fragmentor at 120V, the skimmer at 70V and the octopole at 300 V.

Quantification $^{\beta}$*N*-alkanoyl-5-hydroxytrypamides (C-5HT)

The chromatographic separation was performed using an Ultimate 3000 (Dionex) on a Luna 3µm C8 (150x3 mm) (Phenomenex) column. The isocratic condition 01% formic acif 93% MeOH/7% water was applied for 9 min. The flow rate was 450 µL/min. The column temperature was kept at 40°C. The sample concentration was approximately 6mg/mL in 33% THF/33% MeOH/33% water. The injection volume was 5 µL.

The mass spectrometer 4000QTrap (Applied Biosystems) was used in positive ESI-mode and operated in MRM mode to follow the $^{\beta}$*N*-alkanoyl-5-hydroxy-tryptamides derivatives ($[M+H]^+ \rightarrow$*m/z* 160 and $[M+H]^+ \rightarrow$ *m/z* 177). Nitrogen was used as the curtain (value of 22) and collision gas was set to high. The source was operated at 420 °C with ionspray at 5500 V; the nebulizer gas was set at 45 psi, the declustering potential at 100 V and the collision energy at 40e V. The C-5HT derivatives were identified and quantified by combining retention time and two distinct mass transitions. Quantification was performed by external calibration.

NMP and C-5HT levels in coffee beverages

The concentration in coffee beverage was further calculated for an average cup dosage, i.e. brew-type prepared at 50 g R&G/L, and soluble-type coffee at 15 g/L.

RESULTS AND DISCUSSION

N-Methylpyridinium (NMP) and $^{\beta}$*N*-alkanoyl-5-hydroxytrypamides (C-5HT) were quantified in coffee powders by advanced LC-MS techniques. The expected concentrations in the brew-type (11.2-13.7 g/L) and soluble-type coffee (15 g/L) were calculated as shown in Table 1. The roasting degree was by far the main factor affecting NMP and C-5HT concentrations in these coffee preparations. The effect of extraction type and coffee species was moderate.

NMP results from trigonelline degradation during roasting. NMP concentrations in coffee preparations significantly increase with roasting level from 13-25mg/L in light roast to 68-115mg/L in dark roast. NMP concentrations are lower in soluble-type preparations. Only small amounts of NMP (i.e. <15%) are additionally obtained at high temperature (i.e. 180 °C), the major part of NMP being already extracted at moderate temperature (e.g. 100 °C). At comparable roast level and extraction conditions, preparations from Arabica coffee are slightly richer in NMP.

C-5HT stands for a series of fatty acid amides of serotonin. C-5HT concentrations in coffee preparations drastically decrease with the roasting level from 149-218 µg/L in green coffee to 15-50 µg/L in dark roast. Major C-5HT components in green coffee brew-type are C_{20}-5HT (35%), C_{22}-5HT (55%) and C_{24}-5HT (5%). Increasing roasting level and/or extraction temperature (i.e. soluble-type) leads to a change in the C-5HT profile with increased level of shorter fatty acids, i.e. C_{18}-5HT (>10%), C_{20}-5HT (35-50%), C_{22}-5HT (20-50%), and C_{24}-5HT (<5%). At comparable roast level and extraction conditions, preparations from Robusta tend to be richer in C-5HT.

CONCLUSION

NMP concentrations in model brews and soluble-type coffees were in the range of 13-115 mg/mL. NMP concentration is highest in dark roasted brews. At this level, NMP in coffee matrix was shown to reduce the gastric secretion[3].

C-5HT concentrations in model brews and soluble-type coffees range between 15-218 mg/L. C-5HT concentrations are highest in green coffee brews. Such C-5HT concentrations were reported to already induce gastric acid secretion (Lang et al., 2010). Upon roasting and/or at high temperature extraction, the proportion of C-5HT derivatives with shorter fatty acid chains increase. This could reduce the impact of coffee beverage on the gastric acid secretion, as shown by Lang et al. (2010) when comparing activity on gastric secretion of C_{20}-5HT vs. C_{18}-5HT[5].

Based on this work, one could expect lower gastric acid secretion for coffee beverages prepared from dark roast. However, the mechanisms of the physiological response and the coffee molecules involved in receptor activation are far from being well understood.

Table 1. Concentration of N-methylpyridinium and βN-alkanoyl-5-hydroxytrypamides in Arabica and Robusta coffees roasted at different levels (i.e. light to dark) and extracted under different preparation conditions (i.e. brew-type and soluble-type).

Coffee	Roasting	Yield	Type	Cup Dosage	N-methyl pyridinium	C18-5HT	C20-5HT	C21-5HT	C22-5HT	C23-5HT	C24-5HT	Total C-5HT
		(g/100g)		(g/L)	(mg/L)	(ug/L)	(ug/L)	(ug/L)	(ug/L)	(ug/L)	(ug/L)	(ug/L)
Arabica	Green	23.0	Brew	11.5	0.0	3.0	74.9	1.3	124.0	1.7	12.7	217.6
Arabica	Light	22.4	Brew	11.2	16.9	1.6	34.8	0.8	68.4	1.0	7.8	114.4
Arabica	Medium	23.1	Brew	11.6	39.4	0.8	15.7	0.4	19.1	0.3	2.5	38.8
Arabica	Dark	24.1	Brew	12.0	114.5	0.4	6.6	0.3	7.0	0.1	0.8	15.2
Robusta	Green	20.2	Brew	10.1	0.0	14.3	85.1	0.6	45.5	0.2	3.3	149.0
Robusta	Light	24.8	Brew	12.4	25.3	14.8	12.9	0.6	9.0	0.1	0.8	38.3
Robusta	Medium	26.6	Brew	13.3	51.8	17.0	12.9	0.0	6.9	0.1	0.5	37.5
Robusta	Dark	27.4	Brew	13.7	114.3	3.6	13.5	0.4	18.8	0.3	3.2	39.7
Arabica	Green	42.6	Soluble	15.0	0	3.5	71.0	1.2	105.2	1.4	10.6	192.9
Arabica	Light	47.6	Soluble	15.0	12.5	1.7	29.4	0.7	51.8	0.7	5.7	90.2
Arabica	Medium	49.0	Soluble	15.0	28.1	1.2	18.6	0.5	15.9	0.2	2.0	38.3
Arabica	Dark	49.6	Soluble	15.0	77.9	0.7	10.2	0.4	7.9	0.1	0.8	20.0
Robusta	Green	38.8	Soluble	15.0	0.0	21.7	94.4	0.9	45.5	0.3	3.2	165.8
Robusta	Light	53.8	Soluble	15.0	17.4	20.0	26.6	0.8	12.3	0.1	0.9	60.8
Robusta	Medium	55.7	Soluble	15.0	35.1	19.1	19.7	0.6	7.6	0.1	0.5	47.6
Robusta	Dark	57.3	Soluble	15.0	67.6	4.3	19.4	0.4	22.0	0.4	3.5	50.0

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