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Variations in caffeine and chlorogenic acid contents of coffees: what are we drinking?

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The effect of roasting of coffee beans and the extraction of ground coffee with different volumes of hot pressurised water on the caffeine and the total caffeoylquinic acids (CQAs) content of the resultant beverages was investigated. While caffeine was stable higher roasting temperatures resulted in a loss of CQAs so that the caffeine/CQA ratio was a good marker of the degree of roasting. The caffeine and CQA content and volume was determined for 104 espresso coffees obtained from coffee shops in Scotland, Italy and Spain, limited numbers of cappuccino coffees from commercial outlets and several instant coffees. The caffeine content ranged from 48–317 mg per serving and CQAs from 6–188 mg. It is evident that the ingestion of 200 mg of caffeine per day can be readily and unwittingly exceeded by regular coffee drinkers. This is the upper limit of caffeine intake from all sources recommended by US and UK health agencies for pregnant women. In view of the variable volume of serving sizes, it is also clear that the term "one cup of coffee" is not a reproducible measurement for consumption, yet it is the prevailing unit used in epidemiology to assess coffee consumption and to link the potential effects of the beverage and its components on the outcome of diseases. More accurate measurement of the intake of coffee and its potentially bioactive components are required if epidemiological studies are to produce more reliable information.

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1. Introduction

Coffee beans are one of the most traded food products in the world[1] with around 500 billion of cups of coffee being consumed worldwide annually.[2] Consequently, the impact of the drink on human health is of great interest. *Coffea arabica*, and *Coffea canephora* var. robusta, are the most widely used and economically important species of coffee.[3] The pleasant aroma, taste, and rich colour of brewed coffee are a consequence of the roasting process that leads to profound changes in the chemical composition of coffee.[4] The roasted coffee beans are ground to a powder which is extracted with hot water, to produce what we know as "a cup of coffee".[1] In general, an arabica coffee brew is appreciated for its superior cup quality and aroma, whereas a robusta brew possesses a more aggressive harsher flavour and contains higher amounts of soluble solids, caffeine and phenolic compounds.[4] The main phenolic components in coffee are the chlorogenic acids (CGAs) among which the caffeoylquinic acids (CQAs), 5-CQA, 3-CQA and 4-CQA (Fig. 1) dominate along with lower amounts of feruloylquinic acids and dicaffeoylquinic acids. Although CGAs suffer losses during roasting, coffee beverage is a variable but rich, probably the richest, dietary source of CGAs. Regular coffee drinkers may have a daily intake of CGAs in excess of 1 g.[9]

Coffee is also a major dietary source of the purine alkaloid, caffeine (1,3,7-trimethylxanthine) (Fig. 1) with robustas containing about twice as much as arabicas.[4,10] Caffeine exhibits numerous and well-studied physiological effects[11] and exerts most of them through the antagonism of the A_1 and A_2 subtypes of the adenosine receptor, effectively potentiating the effects of sympathetic nervous system stimulation. The stimulatory effects of caffeine include enhanced perception, an increased capacity to remain awake for longer periods, and reduced fatigue.[12] Caffeine has also been shown to possess positive effects on long-term retention by enhancing memory consolidation[13] and may reduce symptoms associated with Parkinson's disease.[14,15] However, there are negative aspects of caffeine intake: in excess it can result in a state of excitement and anxiety including adverse reactions like tachycardia, headache, palpitations, insomnia, restlessness, nervousness, and tremor. Dose-response, and tolerance to regular consumption, varies between individuals, so even a single cup may cause sleeplessness with a

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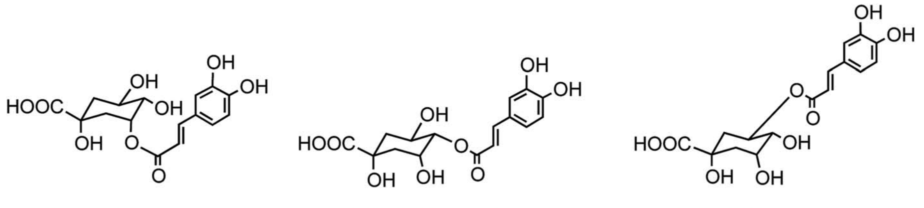

3-*O*-Caffeoylquinic acid (neochlorogenic acid) 4-*O*-Caffeoylquinic acid (cryptochlorogenic acid) 5-*O*-Caffeoylquinic acid (chlorogenic acid)

Fig. 1 Using the preferred IUPAC numbering[5] the structures of 5-CQA (chlorogenic acid) the related 3-CQA (neochlorogenic acid) and 4-CQA (cryptochlorogenic acid), all of which occur in coffee are illustrated. 5-CQA is readily available from commercial suppliers, some of whom including Sigma, continue to use pre-IUPAC nomenclature and sell chlorogenic acid as 3-CQA.[6] This continues to cause much confusion and two recent publications from well established groups have referred to their use of 3-CQA when according to the IUPAC nomenclature they were using 5-CQA.[7,8]

racing mind for some people, while for others drinking ten times this amount can still be pleasant and not interfere with sleep. The typical 4–6 h half-life of caffeine in adults can be extended up to 30 h among women taking an oral contraceptive, pregnant women, the developing fetus, young children, and those with liver disease.[16] These groups are, therefore, much more susceptible to the effects of caffeine toxicity. Typical caffeine levels in a cup of coffee are commonly cited as between 50 and 100 mg.[11] These figures probably relate to instant coffee as freshly brewed coffees can contain much more caffeine. Different coffee brews have been reported to vary widely with values as high as 323 mg per cup.[17,18]

The aim of this study was to evaluate the caffeine and CGA content of high street espresso and cappuccino coffees as well as the levels in commercial instant coffee brands.

2. Results

2.1 Impact of roasting on the caffeine and CQA content of arabica coffee beans

Grade A green Brazilian coffee beans (*Coffea arabica cv.* arabica, Finca lagoado morro) were treated under the conditions outlined in Table 1 to obtain light, medium and dark roasted beans. Subsequently the samples were ground, the moisture content determined and, after triplicate extraction with 50%

Table 1 Details of coffee beans, roasting procedures and their impact on caffeine and total CQA content[a]

Coffee beans	Roasting conditions: Temperature (°C)	Roasting conditions: Time (min)	Water (% FW)	Caffeine (mg g^{-1} FW)	Total CQAs (mg g^{-1} FW)	Caffeine/CQAs ratio
Green	—	—	8.4	12	41	0.3
Light roast	197	11.25	4.9	13	19	0.7
Medium roast	211	12.11	3.4	13	10	1.3
Dark roast	219	12.19	3.2	13	5	2.6

[a] Coffee beans 100% Finca lagoado morro Brazil (Grade A). Data expressed as mean values, standard error values <7% of the mean in all instances ($n = 3$). Total CQA content based on 3-CQA, 4-CQA and 5-CQA levels.

Table 2 Caffeine and chlorogenic acid content of espresso coffees made from light, medium and dark roasted beans with different barista procedures[a]

Coffees and Extraction	Coffee [g]	Serving size (mL)	Caffeine (mg/serving)	Total CQA (mg/serving)	Caffeine/CQA ratio
Light roast					
Regular extraction	18.6	23	165 *(68%)*	244 *(69%)*	0.7
Over extraction	18.6	55	203 *(84%)*	306 *(87%)*	0.7
Medium roast					
Regular	18.1	23	152 *(65%)*	119 *(66%)*	1.3
Over extraction	18.1	45	202 *(86%)*	160 *(88%)*	1.3
Dark roast					
Regular	20.4	22	174 *(66%)*	75 *(74%)*	2.3
Over extraction	20.4	43	232 *(88%)*	96 *(94%)*	2.4

[a] Caffeine and chlorogenic acid levels expressed as mean values with standard error values <7% of the mean in all instances ($n = 3$). Italicised figures in parentheses represent amounts extracted as a percentage of the levels in the ground beans. Total CQA content based on 3-CQA, 4-CQA and 5-CQA levels.

aqueous methanol, the caffeine and 3-, 4- and 5-CQA content of the beans was determined by reversed phase gradient HPLC with PDA and mass spectrometric detection.

The data obtained are presented in Table 1. It is evident that under the conditions used caffeine was stable, while with increased roasting time and temperature the CQAs declined. As a consequence there was a ~90% loss of CQAs in the dark roasted beans compared to the unroasted green beans while the light and medium roasted material contained, respectively, 46% and 24% of the CQAs of the green beans. These changes are reflected in the caffeine/CQA ratio which was 0.3 in the green beans and with increasing severity of roasting rose to 2.6 in the dark roasted beans (Table 1). This is in keeping with the data of Clifford[19] and supports the notion that the caffeine/CQA ratio is a good marker of the degree of roasting of coffee beans.[20] Our earlier study with roasted coffee beans showed that roasting can degrade caffeine as well as CQA[18] but this required much harsher roasting conditions, at temperatures of 270 °C and 350 °C.

2.2 Effect of barista procedures on the caffeine and CQA content of arabica espresso coffee

Data on the effect of preparing espresso arabica coffees from light, medium and dark roasted beans using a regular or over-extraction with, respectively, ~23 mL and ~55 mL of water, are presented in Table 2. The amount of ground beans extracted ranged from 18.1 to 20.4 g, which is typical for a "double shot" espresso coffee. The amounts of caffeine and total CQA in the final beverages were similar when expressed as the percentage recovered from the ground beans. Regular extraction resulted in ~67% recoveries, which increased to over 85% with over-extraction. In keeping with the data presented in the previous section, roasting had little impact on the caffeine content of the coffee, at 152–174 mg per cup with a regular extraction, increasing only to ~200 mg per serving with over-extraction. In contrast, roasting reduced total CQAs. Regular extractions yielded 244 mg per serving from lightly roasted beans, 119 mg with a medium roast and 74 mg in coffee produced from dark roasted beans.

2.3 Caffeine and total CQA in commercial espresso coffees

Espresso coffees were purchased from city-centre coffee shops in Scotland, Italy and Spain. Four coffees were obtained from each outlet over a three-week period, and at different times of the day.

2.3.1 Scottish coffees. Data on espresso coffees purchased in the west end of Glasgow near the University of Glasgow are presented in Table 3. There were substantial variations in the volumes of the espresso coffees served by the different outlets, with the smallest serving sizes coming from the local boutique coffee shops Little Italy and Artisan Roast. Larger sized servings were provided by the chain outlets, Costa Coffee, Caffè Nero and Starbucks. The largest servings were obtained from University Café and Patisserie Francoise with substantial variation in the cup size from the latter outlet (Table 3). The caffeine content per serving, as a mean of four samples from each outlet, ranged

Table 3 Volumes and quantities of caffeine and CQA per serving of Scottish espresso coffees purchased on four separate occasions[a]

Outlet (coffee type and weight)	Volume (mL)					Caffeine (mg/serving)					Caffeine (mg mL^{-1})	CQAs (mg/serving)					Caffeine/CQA ratio				
	1	2	3	4	Mean	1	2	3	4	Mean	Mean	1	2	3	4	Mean	1	2	3	4	Mean
University Café (blend, 18 g)	49	53	45	64	**53**	276	191	180	201	**212**	**4.0**	157	112	93	101	**116**	1.8	1.7	1.9	2.0	**1.9**
Little Italy (blend, 9 g)	21	17	16	22	**19**	130	112	101	140	**121**	**6.4**	88	75	68	89	**80**	1.5	1.5	1.5	1.6	**1.5**
Patisserie Francoise (blend, 9 g)	38	47	90	25	**50**	111	124	126	110	**118**	**2.4**	45	51	60	49	**51**	2.5	2.4	2.1	2.2	**2.3**
Artisan roast (arabica, 18 g)	17	16	15	13	**15**	99	104	101	116	**105**	**7.0**	122	138	118	137	**129**	0.8	0.8	0.9	0.9	**0.9**
Costa coffee (arabica, 9 g)	36	34	30	34	**34**	101	86	89	94	**93**	**2.7**	60	47	46	55	**52**	1.7	1.4	1.8	1.7	**1.7**
The Tinder Box (arabica, 9 g)	23	27	25	25	**25**	77	83	90	86	**84**	**3.4**	17	26	31	28	**26**	4.5	3.2	2.9	3.1	**3.3**
Caffè Nero (blend, 9 g)	32	33	34	31	**33**	72	87	82	89	**83**	**2.5**	48	61	57	60	**57**	1.5	1.4	1.4	1.5	**1.5**
Starbucks (arabica, 9 g)	33	31	27	27	**30**	79	76	66	67	**72**	**2.4**	8	7	6	7	**7**	9.9	10.9	11.0	9.6	**10.4**

[a] Data expressed as mean value, standard deviation <7% of the mean in all instances (n = 3). Total CQA content based on 3-CQA, 4-CQA and 5-CQA levels.

from 212 mg from University Café to 72 mg from Starbucks. The high concentration of caffeine was found in the low volume espressos from Little Italy and Artisan Roast. There were different reasons for this. Little Italy used an 9 g shot of a robusta/arabica blend while Artisan Roast used 18 g of arabica coffee per serving, The mean caffeine content per serving of an espresso coffee from University Café was 212 mg, due the use of 18 g of blended coffee per cup coupled with the larger, 50 mL, mean extraction volume.

The CQA contents of coffees from the Scottish outlets varied enormously, from 129 mg (Artisan Roast) to 7 mg (Starbucks) per serving. This was reflected in the caffeine/CQA ratio of these coffees at, respectively, 0.9 and 10.4. These data clearly demonstrated the more extensive roasting of beans used by Starbucks. In an earlier study with coffees purchased in 2008, 24 mg of CQAs was detected in a serving of Starbucks espresso coffee.[18] As the volumes of the coffees were similar to those in the current study, this suggests that Starbucks are now using beans that are roasted at a much higher temperature which enhances the decline in CQA.

2.3.2 Italian coffees. Information on Italian espresso coffees purchased from boutique coffee shops in Parma is presented in Table 4. In Parma they appear to be more precise with the way they make espresso with smaller and more consistent cup volumes than Glasgow coffees. There were also more consistent mean caffeine contents, ranging from 73 to 135 mg per serving, compared to 72 to 212 mg in the Glasgow espressos. The CQA content and caffeine/CQA ratios suggest that in general Italians roast their coffee more than Scots but very much less than the Glaswegian Starbucks espresso.

2.3.3 Spanish coffees. Figures on the volumes, caffeine and total CQA contents of espresso coffee from small Spanish boutique outlets in Pamplona are given in Table 5. It is evident that the Spanish produce much larger volume espressos than either the Italian and Scottish outlets. The caffeine content per cup appeared more uniform while the concentration of caffeine was lower than in the Parma and Glasgow samples, because of the higher Spanish cup volumes. The Pamplona espressos were also characterised by a consistently high CQA content and a very low caffeine/CQA ratio, indicating a preference for lightly roasted beans.

2.4 Caffeine and total CQA in other coffees

2.4.1 Cappuccinos. Cappuccino and latte coffees are made using espresso methods. Table 6 provides data on five cappuccino coffees purchased from some of the outlets that had supplied espresso coffees in Glasgow. The quantities of caffeine and CQA in the cappuccinos from University Café and Artisan roast were similar to the amounts found in their espresso coffee (Table 2) while the quantities in cappuccinos from the other three coffee shops were about double the amounts in the comparable espressos. Caffeine contents of the cappuccinos from Patisserie Francoise and Costa Coffee were in excess of 200 mg per cup. Once again, the caffeine/CQA ratios indicated the use of mild roasting conditions by Artisan Roast and harsher roasting by Starbucks.

Table 4 Volumes and quantities of caffeine and CQA per serving of Italian espresso coffees purchased on four occasions[a]

Outlet (coffee type and weight)	Volume (mL)					Caffeine (mg/serving)					Caffeine (mg mL^{-1})	CQAs (mg/serving)					Caffeine/CQA ratio				
	1	2	3	4	Mean	1	2	3	4	Mean	Mean	1	2	3	4	Mean	1	2	3	4	Mean
Caffetteria Pellico (blend, 7.5 g)	20	21	22	22	**21**	121	129	140	150	**135**	**6.4**	67	65	79	81	**73**	1.8	2.0	1.8	1.9	**1.9**
Camst (blend, 8 g)	28	23	24	29	**26**	132	114	121	108	**119**	**4.6**	50	43	46	42	**45**	2.6	2.7	2.6	2.6	**2.6**
Castagnoli (blend, unknown)	24	19	19	20	**21**	108	118	105	126	**114**	**5.5**	57	63	58	73	**63**	1.9	1.9	1.8	1.7	**1.8**
Marcheselli (blend, 7 g)	19	18	19	18	**19**	100	102	115	111	**107**	**5.6**	21	22	26	25	**24**	4.8	4.5	4.5	4.4	**4.5**
Kikko (blend 7 g)	27	27	23	31	**27**	106	98	94	105	**101**	**3.7**	35	34	33	36	**35**	3.0	2.9	2.9	2.9	**2.9**
Regina (arabica 8 g)	14	18	15	13	**15**	95	101	108	96	**100**	**6.6**	21	22	21	20	**21**	4.5	4.5	5.3	4.8	**4.8**
San Marco (blend 8 g)	16	18	21	16	**18**	100	85	113	102	**100**	**5.5**	48	41	46	44	**45**	2.1	2.1	2.4	2.3	**2.2**
Lo Sfizio (blend 7 g)	21	16	15	24	**19**	99	101	85	104	**97**	**6.1**	55	64	43	57	**55**	1.8	1.6	2.0	1.8	**1.8**
Bar Topino (blend 5 g)	17	16	13	19	**16**	93	89	82	109	**93**	**5.8**	47	44	47	58	**49**	2.0	2.0	1.8	1.9	**1.9**
Alfa Cafè (arabica 8 g)	23	16	23	20	**21**	75	54	84	80	**73**	**4.3**	57	33	52	50	**48**	1.3	1.6	1.6	1.6	**1.5**

[a] Data expressed as mean value, standard deviation <7% of the mean in all instances ($n = 3$). Total CQA content based on 3-CQA, 4-CQA and 5-CQA levels.

Table 5 Volumes and quantities of caffeine and CQA per serving of Spanish espresso coffees purchased on four separate occasions[a]

Outlet (coffee type and weight)	Volume (mL)					Caffeine (mg/serving)					Caffeine (mg mL^{-1})	CQAs (mg/serving)					Caffeine/CQA ratio				
	1	2	3	4	Mean	1	2	3	4	Mean	Mean	1	2	3	4	Mean	1	2	3	4	Mean
Tahona (arabica 9 g)	59	68	82	38	**62**	117	134	139	117	**127**	**2.0**	152	163	165	142	**156**	0.8	0.8	0.8	0.8	**0.8**
Taberna (arabica 9 g)	67	64	63	64	**65**	123	119	128	119	**122**	**1.9**	145	145	161	150	**150**	0.8	0.8	0.8	0.8	**0.8**
Faustino (blend 9 g)	70	67	67	65	**67**	112	118	116	129	**119**	**1.8**	131	138	138	147	**139**	0.9	0.9	0.8	0.9	**0.9**
Café de Iruña (arabica 9 g)	71	104	63	92	**83**	125	128	82	132	**117**	**1.4**	166	176	112	188	**161**	0.8	0.7	0.7	0.7	**0.7**
Café y Te (arabica 9 g)	40	50	68	42	**50**	91	107	127	111	**109**	**2.2**	92	108	129	108	**109**	1.0	1.0	1.0	1.0	**1.0**
Manterola (arabica 9 g)	90	43	103	55	**73**	112	92	121	111	**109**	**1.5**	110	104	152	136	**126**	1.0	0.9	0.8	0.8	**0.9**
Rumbos (blend 9 g)	70	34	84	62	**63**	116	101	128	113	**115**	**1.8**	158	122	167	134	**145**	0.7	0.8	0.8	0.8	**0.8**
Café de Pio (arabica 9 g)	53	72	56	72	**63**	101	96	99	92	**97**	**1.5**	140	136	130	133	**135**	0.7	0.7	0.8	0.7	**0.7**

[a] Data expressed as mean value, standard deviation <7% of the mean in all instances ($n = 3$). Total CQA content based on 3-CQA, 4-CQA and 5-CQA levels.

Table 6 Volumes and quantities of caffeine and CQA per serving of Scottish cappuccino coffees purchased on four occasions[a]

Coffee Shop	Volume (mL)					Caffeine (mg/serving)					Caffeine (mg mL^{-1})	CQAs (mg/serving)					Caffeine/CQA ratio				
	1	2	3	4	Mean	1	2	3	4	Mean	Mean	1	2	3	4	Mean	1	2	3	4	Mean
University Café	195	260	295	265	**254**	237	143	252	145	**195**	**0.8**	142	86	151	92	**118**	1.7	1.7	1.7	1.6	**1.7**
Patisserie Francoise	310	300	255	255	**280**	168	311	304	317	**275**	**1.0**	55	103	103	104	**91**	3.0	3.0	3.0	3.0	**3.0**
Artisan Roast	178	230	115	165	**172**	89	138	85	92	**101**	**0.6**	113	187	114	126	**135**	0.8	0.7	0.7	0.7	**0.7**
Costa	260	220	250	190	**230**	241	237	211	201	**222**	**1.0**	123	131	90	88	**108**	2.0	1.8	2.3	2.2	**2.1**
Starbucks	285	265	235	305	**273**	170	129	166	157	**156**	**0.6**	24	25	23	19	**23**	7.1	5.3	7.1	8.3	**7.0**

[a] Data expressed as mean value, standard deviation <7% of the mean in all instances ($n = 3$). Total CQA content based on 3-CQA, 4-CQA and 5-CQA levels.

Table 7 Quantities of caffeine and CQAs per serving of instant coffee made with 2 g of coffee and 125 mL of water[a]

Instant coffee	Caffeine (mg/serving)	Total CQAs (mg/serving)	Caffeine/CQA ratio
Fortaleza Natural	88	58	1.5
Marcilla Crème	78	35	2.2
Nescafé Classic	70	37	1.9
Nescafé Green Blend	60	152	0.4
Nescafé Gold Blend	55	70	0.8
Nescafé Espresso	48	73	0.7
Nescafé Colombia	48	112	0.4
Nescafé Alta Rica	48	42	1.1

[a] Data expressed as mean value, standard deviation <7% of the mean in all instances ($n = 3$). Total CQA content based on 3-CQA, 4-CQA and 5-CQA levels.

2.4.2 Instant coffees. Details of the caffeine and CQA contents of eight instant coffees are presented in Table 7. Drinks were prepared from 2 g of instant coffee, equivalent to one teaspoonful, dissolved in 125 mL of boiling water. The amount of caffeine per serving ranged from 48 to 88 mg, which is generally lower than found in commercial espresso coffees. In most instances, the caffeine/CQA ratio indicates that the beans used to prepare the instant coffees were subjected to relatively mild roasting conditions. Nestlé Green Blend had the highest CQA content at 152 mg per serving, in excess of almost all espresso coffees from Scotland and Italy (Tables 3 and 4). This, presumably, is a consequence of the coffee being prepared from both roasted and green beans. Interestingly, although this is not longer the case, Nestlé Green Blend was originally marketed as a high antioxidant coffee containing 9% polyphenol antioxidants.[21] In strict chemical terms this is not correct, as the main antioxidants in coffee are the 3-,4-,and 5-CQAs[22] which are not polyphenols but hydroxycinnamates which have a single phenol ring.[23]

3. Discussion

There is widespread public interest in caffeine and its health associations, with specific concerns about the safety of caffeine in pregnancy, which have resulted in government health agencies recommend an upper limit of 200 mg per day.[24,25] Coffee is the major natural dietary source of caffeine, but guidance to consumers on the caffeine contents of coffee is very limited. Coffee preferences in different countries vary, and to some extent the colossal market has been modified by commercial pressures to maximise profit, seeking cheaper beans and ways to minimise the amount of beans used per cup. The palates of consumers change over time to accept altered taste, thus for example, American palates have generally accepted much weaker coffees than are usual and expected in Europe, the Middle East or Australia, and they have accepted the harsher taste of robusta beans which are less acceptable in other countries. In Europe, the influx of American-style chain outlets, such as Starbucks, generally drew coffee-drinkers away from the previously more frequent instant coffees, but a wide range of other coffee shops have also become popular. It is clear from previous publications,[17,18] and the data in the present paper that the coffees sold commercially vary widely. While not necessarily representative of the different countries studied here, it appears that the variations in Glasgow are rather greater than in Parma and Pamplona, but a two-fold range in the caffeine content of espressos is common. Individual outlets sell espresso coffees which contain over 200 mg, and the popular cappuccino, and presumably latté coffees, often contain over 200 mg in a single cup. There were also striking variations in the CQA content and volumes of espresso coffees summarized in Table 8, which allow some conclusions to be drawn about difference in the coffee making practices, drawing on the data we have gathered on the effects of roasting conditions on CQA and caffeine/CQA ratio as an index of roasting severity.

The Parma Italian coffees had consistent small sized servings while in Pamplona the Spanish were served a large cup size, which ranged from 34–104 mL. The median caffeine content of a cup was not greatly different at just over 100 mg but the 66–276 mg range in Scotland indicated much greater outlet-to-outlet variability than in Italy or Spain. Coffees prepared in Spain were very lightly roasted compared to the beans used to make Italian espressos. As with volume, there was much more variability in Scotland where the median caffeine/CQA was 1.8 and the range 0.8–11.0. The major contributor to this wide range were the espressos purchased from Starbucks which had an extremely low CQA content (Table 3) and the resultant very high caffeine/CQA ratio indicated that the beans had been subjected to intensive roasting. Assuming that globally Starbucks use a standard roast procedure, this is likely to be a feature of Starbucks coffee worldwide, rather than a uniquely Scottish phenomenon.

Table 8 Summary of the volumes, caffeine and total CQA contents of espresso coffees purchased in Scotland, Italy and Spain[a]

	Volume (mL)		Caffeine (mg/serving)		Total CQA (mg/serving)		Caffeine/CQA ratio	
Source	Median	Range	Median	Range	Median	Range	Median	Range
Italy espresso	20	13–31	102	54–150	46	20–81	2.1	1.3–5.3
Spain espresso	66	34–104	116	82–139	142	92–188	0.8	0.7–1.0
Scotland espresso	31	13–90	100	66–276	59	6–157	1.8	0.8–11.0
Scotland cappuccino	255	115–310	180	85–311	103	19–187	2.3	0.7–8.3
Instant coffees	125	125	58	48–88	64	35–152	1.0	0.4–2.2

[a] Total CQA content based on 3-CQA, 4-CQA and 5-CQA levels.

The caffeine levels detected in espresso coffee in Glasgow were not as high as those in an earlier study where four out of 20 coffees purchased in 2008 contained over 200 mg per serving.[18] The change may reflect the considerable local publicity over extreme caffeine contents after this publication.[26–28] Nonetheless it is evident that drinking 2–3 cups of espresso coffee on a daily basis, in Scotland, Spain or Italy, can result in a caffeine intake well above the 200 mg daily safe limit advised for pregnant women.[24,25] This safe limit can also be exceeded by a double-shot cappuccino or even by 2 to 3 cups of coffees made from more than one teaspoonful of instant coffee. The use of robusta beans from *Coffea canephora* is becoming more popular and they now comprise 40% of the world coffee market. The increasing blending of arabica with robusta beans, which have double the caffeine content of arabica,[4,10] will result in a greater caffeine contents of commercial coffees. A clear declaration of the type of bean used by outlets would help guide consumers.

Although ingestion of even small amounts of caffeine gives unpleasant symptoms to some people, and there is a growing market for caffeine-free beverages, most adults enjoy coffee and suffer no ill-effects. Most regular coffee-drinker develop tolerance to the multiple pharmacological effects of caffeine, and indeed may suffer symptoms such as headache and fatigue from caffeine withdrawal. Unexpectedly large consumption of caffeine does still commonly generate symptoms, including anxiety, sleeplessness and tachycardia, even for regular coffee drinkers. For children, the elderly, and especially for pregnant women, there is an additional hazard, leading to more chronic symptoms of 'caffeinism' and behavioural problems in children, because the normal hepatic capacity for metabolism of caffeine is greatly reduced. As a consequence, caffeine accumulates in the body, such that the half-life extends from the normal 4–6 hours to as long as 30 hours. Unwitting consumption of high-caffeine coffees aggravates this problem. High caffeine consumption in pregnancy has been associated with impaired fetal growth and with miscarriage.[25] The evidence is weak, and observational, but for obvious reasons a controlled trial could not be considered on ethical grounds. Following the precautionary principle, agencies such as the UK Food Standards Agency and the American College of Obstetrics and Gynaecology have set a safe upper limit of 200 mg per day for caffeine consumption in pregnancy.[24,25] and as a result many women are beginning to avoid coffee completely during pregnancy. Guidance would also appear to be required for people with liver disease as their tolerance to caffeine can be limited.[16]

The high variations in CQA content found in this study, with amounts ranging from 6 mg to 188 mg per serving, reveal the substantial impact of roasting and coffee making procedures on the level of the hydroxycinnamates in a serving of the beverage. CQAs have been linked to potential health benefits of coffee consumption as a consequence of their *in vitro* and *in vivo* chemopreventive, anticarcinogenic, antithrombotic, anti-inflammatory and hypoglycaemic effects.[29–33] Epidemiological studies indicate lower risk of type 2 diabetes, cardiovascular diseases, and endometrial and hepatocellular cancer in habitual coffee consumers which might be linked to the presence of CQAs in coffee.[34] Arguably, this raises the possibility that regular consumption of low CQA coffees may have less beneficial effects than those with high CQA levels.

It should be stressed that epidemiological studies have not focussed on the composition of coffee brew and coffee intake is assessed by the number of cups consumed. From the considerable variability in the composition of the coffee beverage coupled with significant differences in cup size, demonstrated in the current study, it is evident that the term "one cup of coffee" is not a reproducible measurement for consumption, yet it is the prevailing method to determine the amount of coffee consumed and to link the potential effects of coffee and its components on the outcome of diseases. Other ways should be sought for a more accurate measurement of the intake of coffee and its potentially bioactive components in order to assess the effects of the beverage on human health in a more realistic manner.

4. Experimental

4.1 Chemicals

5-CQA and caffeine were purchased from Sigma-Aldrich (Poole, Dorset, UK). HPLC-grade methanol and acetonitrile were obtained from Fisher Scientific (Loughborough UK).

4.2 Coffees

Green coffee beans (*Coffea arabica*, *cv.* arabica Finca lagoado morro; Brazil; Grade A) were purchased from Artisan Roast (15-17 Gibson Street, Glasgow G12 8NU, UK) who subjected the beans to three different roasting conditions to yield light, medium and dark roasted coffee. Artisan Roast also prepared espresso coffee brews from the three types of roasts with regular and over extraction volumes of water (Table 2) using a commercial coffee maker (La Marzocco, Florence, Italy). Water temperature and pressure during the extraction were 92 °C and 9 bars, respectively.

Other espresso coffees were purchased on four separate occasions from 26 different outlets in Scotland, Italy and Spain. The volume of each single shot espresso coffee was measured after which aliquots were diluted 20-fold with MeOH : H_2O (1 : 1, v/v). During the aliquoting, the coffee was stirred to homogenize the sample. The diluted aliquots were stored at −20 °C prior to analysis of caffeine and CQA levels.

In addition to the espresso coffees, cappuccino coffees were obtained from five coffee shops in Glasgow. Eight brands of commercial instant coffee were purchased from a Pamplona supermarket and brews prepared by adding 125 mL boiling water to 2 g instant coffee. Prior to analysis aliquots were diluted 20-fold with MeOH : H_2O (1 : 1, v/v).

4.3 Analytical procedures

For HPLC analysis of the coffee brews, the samples were centrifuged at 16 200*g* for 5 min. Quantitative analysis of CQAs and caffeine in the coffees used a Surveyor HPLC with a PDA detector scanning from 200–600 nm, an autosampler cooled at 6 °C and a LCQ Duo ion trap mass spectrometer fitted with an electrospray interface (ESI) (Thermo Fisher Scientific, San José, USA) using conditions described previously.[22] The injection volume was 100 μL. Chromatographic separation was performed at 40 °C using a Synergi 4 μm Polar-RP 250 × 4.6 mm reversed phase column (Phenomenex, Macclesfield, UK) and a linear 35 min gradient of 5–8% acetonitrile in HPLC grade water containing 1% formic acid. The flow rate was 1 mL min^{-1} 3-CQA, 4-CQA and 5-CQA were quantified by PDA at 325 nm, by reference to a 20–1000 ng 5-CQA standard calibration curve (R^2 = 0.9999) whereas caffeine was quantified at 280 nm, by reference to a 20–1000 ng caffeine standard calibration curve (R^2 = 0.9999).

5. Conclusions

Caffeine is much more stable than CQAs during the roasting of coffee beans and, as a consequence, an increasing caffeine/CQA ratio is a good marker of the extent to which coffee beans have been roasted. The caffeine and CQA content of espresso coffees purchased in Scotland, Spain and Italy varied with the caffeine/CQA ratio ranging from 0.7 to 11. In Spain espresso coffees were prepared from lightly roasted beans, in Italy more extensive roasting procedures were used while the ratio along with serving size varied more extensively in Scotland with coffees from Starbucks, with a mean caffeine/CQA ratio of 10.4, being prepared from the most heavily roast beans. Caffeine levels, like serving size, varied most in Scotland, with mean amounts ranging from 72 to 212 mg per serving. This compared to a range of 73–135 mg per serving for Italian espressos and 97–127 mg in Spain. The mean caffeine content of cappuccino coffees purchased in Scotland varied from 101 mg to 275 mg per cup, presumably depending upon whether it was one or a two-shot serving. Coffees prepared from 2 g of instant coffee, equivalent to a one tea spoon serving, contained from 48 to 88 mg of caffeine. The levels of CQAs, which have potential beneficial effects on health, were also variable with the mean amounts per serving ranging from 7 to 156 mg.

In order not to impair fetal growth and risk miscarriage USA and UK health agencies advice a maximum intake of caffeine, from all sources, of 200 mg per day for pregnant women. As they too have a lowered capacity to remove caffeine from the circulatory system, a similar, if not lower restriction would appear to be an appropriate precaution for people with liver disease. It is evident that regular coffee drinkers, whether they favour an espresso, cappuccino or an instant beverage, can, without realising it, have a caffeine intake well in excess of 200 mg per day.

In these circumstances, it is evident that providing consumers with readily available information on bean variety and caffeine levels, including labelling of instant coffees, and providing guidelines for coffee-making, would benefit consumers and coffee-shops alike.

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