Our Everyday Cup of Coffee: The Chemistry behind Its Magic

Article *in* Journal of Chemical Education · August 2005

DOI: 10.1021/ed082p1161

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abstract submitted for presentation to the
IV Symposium of the Latin-American Section of AOAC International
Montevideo (Uruguay), 18 - 22 November 2001

THE CHEMISTRY FOR COFFEE QUALITY

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Often referred to as the second most globally traded good after petroleum, coffee is mainly produced in emerging third world countries where it constitutes the gross portion of foreign income. Even if not a staple food commodity, it is one of the most important crops for the economy of many Latin-American countries. Moreover, its consumption is widespread all over the world, both in the producing countries and in the developed ones.

Coffee is not needed for nutritional purposes, but is much appreciated for its taste appeal along with its physiological effects on alertness and performance. The assessment of quality is of paramount importance to both of these aspects, in order to supply the customers with a pleasant and wholesome product. The high complexity of the raw seed matrix - and even more so when dealing with the roasted finished product - requires proximate analysis methods able to dig into the structure of families of compounds as ill-known as, for instance, melanoidins. Not secondarily, the sampling issue deserves some careful consideration.

Also the analyses relevant to the sensory sphere led to the development of increasingly sophisticated analytical approaches, where the parts per billion of volatile aromas are not the ultimate frontier of detection limits. No matter the progress of instrumental techniques, the good old cup-testing approach still remains the final assessment tool to obtain the green light for choosing the right plant and to conveying the product to the market. This is even more true when *espresso*, the rising star of coffee brewing methods, is considered.

Tempo totale per la lettura: 52 minuti

THE CHEMISTRY FOR COFFEE QUALITY

0. PREÂMBULO

Muito prezadas damas e cavalheiros, bom dia!
E' para mim uma honra grande este convite pelo comitê organizador, que agradeço, de apresentar os desenvolvimentos mais adiantados nas metodologias analíticas visadas à avaliação da qualidade duma das mercadorias mais presentes na nossa vida de cada dia, e mais importantes na economia dos países da América latina: o café.

Bueno, mis estimadas señoras y señores: come homenaje a la linda ciudad de Montevideo que nos está hospedando, ahorita me gustaría mucho seguir exponiendo esta mi charla en castellano, pero hay un problemito: es bastante peligroso para un Italiano alternar el español y el portugués. Hay el riesgo de acabar hablando una mezcla horrible, cuyo producto de reacción, desgraciadamente bien conocido, se llama 'el portuñol'. Entonces, voy a hablar ingles, que es el idioma oficial del mundo científico.

1. INTRODUCTION

1.1 Why do we drink coffee?

"Once upon a time"…this is the usual *incipit* of the very many legends about the discovery of coffee as a food. Sometimes they tell the story of shepherds consuming directly the seeds of some *Coffea* plant, by chewing either raw cherries or cooked, generally roasted, beans [Burton 1860]. For those of you who tried to munch a bean, it is hard to imagine that the success of coffee - today the most traded food commodity - could have ever occurred, if persisting in those primitive habits.

On the one hand, it seems logical that the appeal of coffee to those early "food science pioneers" derived essentially from their experiencing an arousal condition that proved to be beneficial to their activities [Ellis 1998]. In other words, the primordial reason for coffee consumption must have been the, by now well documented, physiological effects of caffeine in the human organism [Viani 1988].

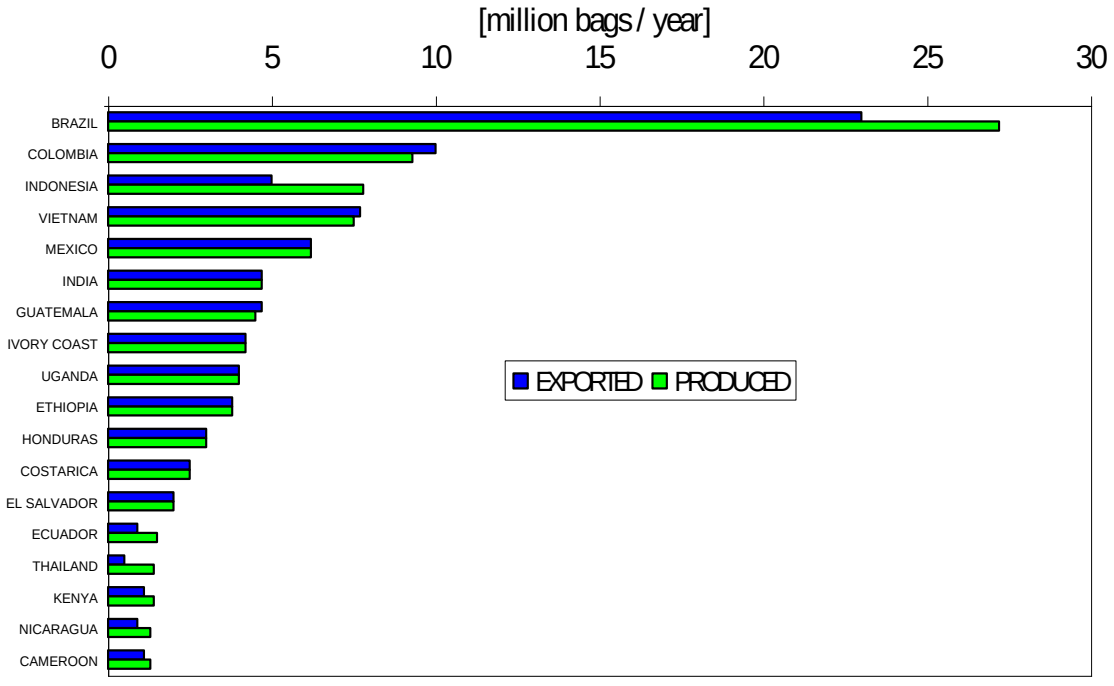
On the other hand, it is pretty obvious why coffee has become so popular, up to achieving the position of the second most largely consumed beverage after water: it is a matter of flavor, or better still of overall sensory impact. Whilst people like the flavor of coffee, they do not like the disturbing sensory feeling of chomping and swallowing hard particles deriving from a bean. This fact makes beverage preparation a key step for enjoying the benefits of this commodity, and sometimes for transforming it into a specialty.

1.2 Production and consumption patterns

A little bit of botany first: the coffee tree belongs to the *Rubiaceae* family, where two species of the genus *Coffea* are economically important: *C. arabica* and *C. canephora*, the latter better known as Robusta coffee. Arabica accounts for three-quarters of the world production and is considered to give the best, mildest cup.

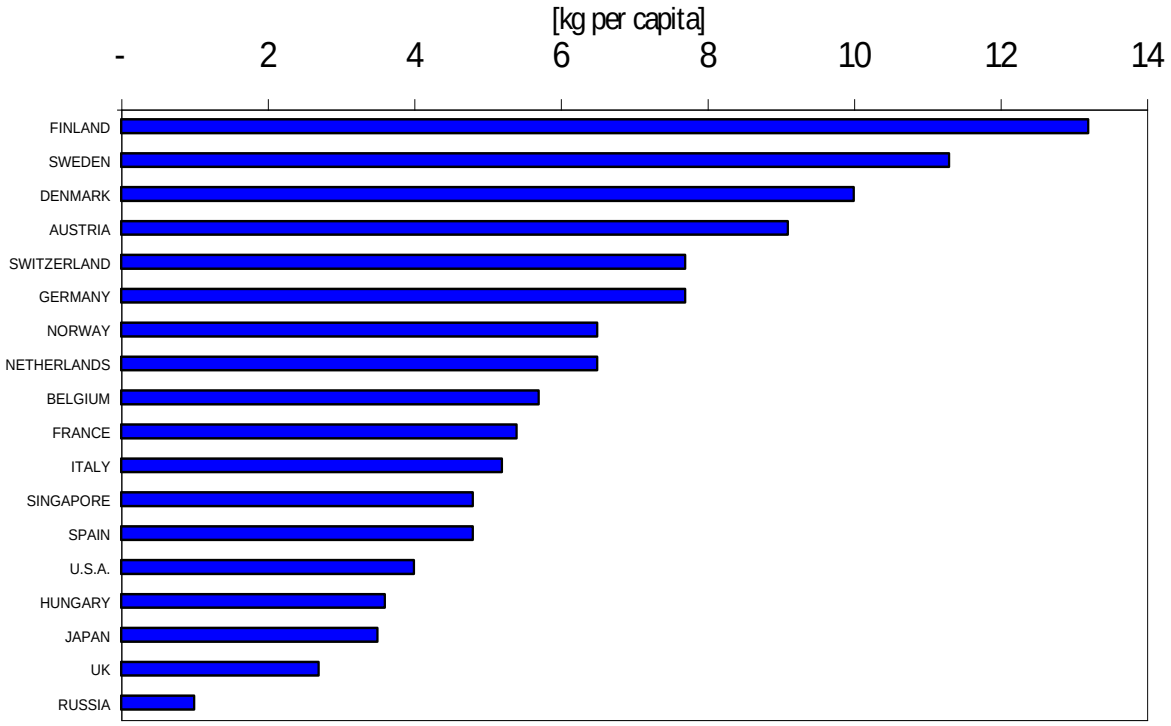
Coffee production is around 6 billion of kg/year [Illy and Viani (1995)] and ranks as the second commodity of international trading, after petroleum. Coffee cultivation mostly occurs in third-world countries, where it often represents the most important value income.

Table 1: Major coffee producing countries (data from ICO 1999 survey)



Coffee consumption is spread all the world, especially in Europe and in the USA, but the type of coffee beverages and the modality of consumption are strictly associated with social habits and culture of the single countries. Differences in the raw beans composition, in roasting conditions and in the extraction procedures used to prepare coffee brews result in a great diversity of the chemical composition in the final product: the cup. Also the size of a single serving is enormously variable, ranging from 15 ml of concentrated *espresso* to over 250 ml in the English-speaking countries, and it can derive from the brewing of a roast and ground coffee amount as little as 5 g, up to 15 g or more.

Table 2: Major coffee consuming countries (data from ICO 1999 survey)



A big difference between coffee and all the other beverages is the extraordinary variety of brewing techniques that have been developed and used traditionally in different countries: decoction methods (boiled coffee, Turkish coffee, percolator coffee and vacuum coffee), infusion methods (filter coffee and Napoletana), and the original Italian pressure methods (Moka and *espresso*) [Petracco 2001].

Espresso is a way to enjoy a cup of coffee that is gaining large popularity world-wide, especially in European countries of Latin origin and in recent years also in the USA and Japan. Its roots are to be searched for in the Italian culture of foods and beverages, which developed a typical lifestyle linked to coffee drinking [Illy and Illy 1990]. Its success lies in the greater satisfaction it gives to the consumers, when compared with coffee brews prepared by other methods: the rationale behind this statement will be made clear later in my speech.

2. PROXIMATE ANALYSIS

2.1 Raw coffee composition and changes on roasting

Let us have a look into what we know today on raw – also called green – coffee composition.
Green Arabica contains more than 50 % of carbohydrates, some 16 % lipids and 10 % of proteinaceous material, then 6.5 % chlorogenic acids and 1.2 % caffeine with traces of other purines.
The main compositional differences of Robusta coffee are higher caffeine (2.2 %), lower lipids content (10 %) and higher chlorogenic acids (10 %).

Table 3: Typical composition of raw coffee for the two main botanical varieties, expressed as percentage of dry matter [after Illy and Viani 1995]

Components	Arabica	Robusta
Caffeine	1,2	2,2
Minerals of which Potassium	4,2 1,7	4,4 1,8
Lipids	16,0	11,0
Trigonelline	1,0	0,7
Proteins	10,0	10,0
Aliphatic Acids	1,7	1,6
Chlorogenic Acids	6,5	10,0
Sucrose	8,0	4,0
Polysaccharides	50,0	57,5

Raw coffee can hardly be defined as edible, and there are - to my knowledge - no claims about brewing green coffee bean material to produce a beverage, even if the active physiological component – caffeine - can be effectively extracted by hot water from triturated raw seeds. The grassy, astringent taste of such a brew is surely a deterrent against any commercial tentative in that direction. The roasting process is therefore a must to allow people to enjoy a coffee beverage, produced by various brewing techniques through a grinding step useful to increase the amount of solid surface exposed to water.

During roasting, pyrolytic reactions take place inside coffee cells, which – thanks to the thickness of their walls, unusual in other plants' seeds - can be compared to autoclaves resisting to an internal pressure of about 25 bar. The chemical composition of the beans is drastically modified with the release of large amounts of carbon dioxide and the formation of many hundreds of substances, which give roasted coffee its characteristic flavor and taste. In the meanwhile the beans change color to darker and darker brown. The highest final temperature applied to beans to be used for the preparation of *espresso* coffee may reach 220 °C [Illy and Viani 1995].

Most of the components of coffee beans undergo extensive transformation during roasting. The contents of free or protein-bound amino acids and sugars decrease substantially because of Maillard reactions, while about 60% chlorogenic acids are degraded. Only the overall content of caffeine and lipids remains practically unchanged. The consequences of all these reactions are the development of the typical coffee flavor and an intense browning due to polymeric materials, the melanoidins [Steinhart and Packert 1993].

Table 4: Typical composition of roasted coffee for the two main botanical varieties, expressed as percentage of dry matter [after Illy and Viani 1995]

Components	Arabica	Robusta
Caffeine	1,3	2,4
Minerals of which Potassium	4,5 1,8	4,7 1,9
Lipids	17,0	11,0
Trigonelline	1,0	0,7
Proteins	10,0	10,0
Aliphatic Acids	2,4	2,5
Chlorogenic Acids	2,7	3,1
Carbohydrates	38,0	41,5
Melanoidins (as difference)	23,0	23,0

Expounding a list of the methods of analysis used to determine those classes of compounds would take way longer than the time I am allowed to speak, so I shall concentrate only on the two following meaningful case histories.

2.2 Lipids: Arabica/Robusta discrimination (the ‰ range)

Lipids are, as anyone of you knows, a heterogeneous collection of components that share only one property: they are easily dissolved in organic solvents but can only hardly, or not at all, be dissolved in water. Coffee oil - a viscous liquid semi-solid at 25°C - is no exception to this respect: its amount is conventionally determined by Soxhlet extraction with non-polar solvents like petroleum ether, diethyl ether or chlorinated solvents [Folstar 1985]. Like many vegetal oils deriving from seed plasmalemma, coffee oil is mostly composed of triglycerides (or better triacylglycerols): the GC analysis evidences some 80 % triglycerides of linoleic and palmitic acids, with some oleic and stearic radicals, along with diterpene fatty acids esters (up to 20 %). There is also an unsaponifiable fraction, made of sterols (2-3 %) and non-negligible shares of waxes (hydroxytriptamide esters of longer chain fatty acids, up to 1 %) and of free diterpenes (8 ‰) [Speer 2001].
exclusive
Unlike protein and carbohydrate material, raw coffee lipids undergo little, if any, modifications on roasting. The most relevant one is the dehydrogenation of the diterpenes cafestol and kawheol, whose products increase in an approximately linear way with temperature. The cafestol/dehydrocafestol ratio could therefore be used as a roasting degree indicator [Kölling-Speer *et al.*1997].

But coffee diterpenes analysis delivers one more interesting application, namely the determination of the Arabica/Robusta ratio in a commercial blend of roasted coffee. This is a quite important issue in trade, where customers have learnt to appreciate the superior quality of 100 % Arabica blends, and do not accept unknown percentages of Robusta in it. Roasters have adapted to this request by appropriate labelling or color coding of their packages; nevertheless some fraudulent or cunning behaviour comes from time to time to the surface, requiring some evidence to be proofed. Caffeine analysis alone is not meaningful enough, and chemists used to turn to the tricky analysis of Δ5-avenasterol, which does come in higher amounts in Robusta but with varying natural content [Frega *et al.*1994].

The discovery of a key diterpene, 16-methoxycafestol, which is present in Robusta only [Speer and Mischnick 1989], has solved the issue, providing the ideal quality characteristic for a reliable detection of Robusta in coffee blends. Its determination by RP-HPLC with UV fluorescence detection at 220 nm, which has been validated and published in 1999 as DIN standard method 10779, allows the spotting of Robusta portions as low as 2 % in blends [Speer *et al.*1991].

2.3 Macromolecules: melanoidins (the % range)

The most dramatic chemical change underwent by coffee beans on roasting is caused by the Maillard reaction [Arnoldi 2001], which produces many hundreds of substances imparting roast coffee its characteristic flavor and taste. Another consequence is the development of an intense browning due to polymeric materials, the melanoidins. As already shown in table 1, melanoidins are largely present in roasted coffee and, being mostly water-soluble, are one of the major components of the coffee beverages where they are responsible for the dark color .

Along with aroma compounds, melanoidins are products of the final stages of Maillard reactions, which occur in all foods containing reducing sugars and amino acids when they undergo thermal treatment [Ledl and Schleicher 1990]. The exact course taken by the Maillard reaction in a food system depends on the temperature and time of heating, the reaction precursors and the pH and water activity of the system.

The standard conditions of coffee roasting are well suited for Maillard reaction development: the presence of sugars and proteinaceous material, the high temperature and the low water activity lead to the formation of a roasted product containing melanoidins as the majority-constituent.

The molecular structure of melanoidins is largely unknown, so they are generically defined as macromolecular materials (MW >1000 Da) that are brown and contain nitrogen. From literature data it is clear that their composition strongly differs in different foods, so bread or beer melanoidins would be expected to be very different from those of coffee. In particular, for coffee melanoidins, it is not yet known whether the phenolic compounds present in the green beans (and missing in roasted) are reacted into the brown polymer, and to what extent. The lack of information about melanoidins has greatly hampered research on the physiological and nutritional properties of these compounds. This is particularly true for coffee, which is the dietary item that contains the relevant highest percentage.

While previous research on roasted coffee used to be mostly devoted to the comprehension of the parameters that could improve flavor properties, in the last decade much interest has been concentrated on melanoidins, because of their influence on flavor binding [Hofmann *et al.* 2001] and on foam stabilisation [Petracco *et al.* 1999]. Furthermore, it has been demonstrated that coffee melanoidins have some positive nutritional aspects, in particular an interesting antioxidant activity: on this aspect I shall elaborate right away in more detail.

Melanoidin analysis had so far mainly to resort to lengthy dialysis separation by molecular weight: newer techniques that have been proved useful in recent times are ultrafiltration on cut-off membranes, Gel Filtration Chromatography (GFC) or Size Exclusion Chromatography (SEC) [Anese and Nicoli 2000].

3. HUMAN PHYSIOLOGY

3.1 Coffee is more than caffeine (the ppm range)

Little is known with certainty about the physiological aspects of coffee, but a bewildering array of health and mood effects are attributed to it. For the reason mentioned above, any figure quoted in quantitative assessments of coffee consumption must be taken with due caution, notwithstanding the plethora of epidemiological studies on the effect of coffee that have been published in the last 20 years [Debry 1994] and whose conclusions did not always lead to clear-cut answers.

More than 90% of the research carried out on the physiological properties of coffee has been indeed devoted to caffeine, which is a pharmacologically active alkaloid that stimulates the central nervous system [Spiller 1998]. However, coffee is much more than caffeine: its complex composition and the presence of substances as yet unidentified, but with evident physiological effects, indicate that further research is needed to demonstrate both the wholesomeness of coffee consumption as well as the favourable effects this beverage can have in humans.

I shall not dwell here on confuting the long list of accusations moved in the past against coffee, impeaching it as an agent of very many aetiologies. Most of them have been disproved by later research, or re-ascribed to several confounding factors - like smoking - that are likely to occur in heavy coffee drinkers. I shall rather mention a few coffee compounds that may have beneficial effects on human health.

Green beans are rich of several phenolic compounds, among which chlorogenic, caffeic, p-coumaric and ferulic acids were identified. Most of those compounds display potent antioxidant activity *in vitro*, and are supposed to play an important protective role in several human pathologies acting as anti-inflammatory, anti-mutagenic and anti-carcinogenic agents. Their absorption, metabolic fate and availability for antioxidant protection in humans are not, however, fully understood.

Another non-minor compound present in green beans is trigonelline, which has received considerable attention as its thermal transformation products are important both from the sensory and nutritional points of view. Found in amounts ranging from 0.7 up to more than 1 %, trigonelline is readily degraded by 50 to 80 % into niacin or nicotinic acid, also known as vitamin B3 or PP (Pellagra Preventing). An average coffee consumer's niacin intake can represent up to 50 % of the recommended daily dose.
Traces of one more vitamin precursor – tocopherol - have been individuated in coffee. Being a lipophilic substance, also known as pro-vitamin E, its concentration is measured as coffee oil fraction, summing up to 600 ppm.

Furthermore, it has been reported that coffee decreases the level of gamma-glutamyltransferase (GGT), an enzyme which is a marker of liver damage induced by alcohol abuse. This is intriguing because epidemiological evidence have confirmed a negative association between coffee consumption and incidence of liver disease in alcoholics [Corrao 2001], but a mechanistic explanation and the knowledge of the active compound is still missing.

After highlighting the potent physiological effect of some minor roast coffee components, it is now time to deal with its majority-constituent, the ill-known melanoidin family, whose interest mainly resides in its antioxidant activity.

3.2 Antioxidant role of coffee

In recent years, nutritionists have dedicated much interest to the presence of antioxidant substances in vegetable foods. As an instance, antioxidants have been invoked to explain the so called "French paradox", *i.e.* the unexpected low association between high fat diet and risk of cardiovascular disease which has been observed in French and attributed to the high consumption of polyphenol-rich red wine [Maxwell 1997].

Various plant-derived foods and beverages rich in polyphenols have been reported to affect the total antioxidant capacity in plasma, with important beneficial effects on human health like the protection against the risk of all degenerative disease having at least in part a free radical component in their aetiology. In particular, red wine and green tea have a significant effect, which has been mainly attributed to their high content of flavonoids. Also some traditional Italian products, such as extra virgin olive oil and tomato, have received great promotion from a better comprehension of their antioxidant role in diet. Tomato is an especially interesting case because it has been demonstrated that, in contrast to what one might superficially expect, cooking enhances the bioavailability of licopene, the antioxidant component [Anese *et al.* 1999]. This fact has opened completely new scenarios on the possibilities offered by processed foods.

Recent research has given evidence that coffee too possesses an antioxidant activity [Daglia *et al.* 2000], which can be attributed to the development of Maillard reaction products. It increases with roasting up to the medium-dark roasted stage, then decreases with further roasting: an experimental observation explained with a partial decomposition of the freshly formed antioxidant compounds [Nicoli *et al.* 1997]. Particularly interesting is the finding that antioxidant activity is also present in human blood serum, as an acute result of the administration of 200 ml of coffee beverage prepared starting from 12 g of roast and ground coffee blend [Natella and Scaccini 2001].

Coffee melanoidins represent a peculiar case of antioxidant Maillard compounds, because it has been demonstrated that they contain, besides approximately 30 % carbohydrates and 9 % proteins, a good 33-42 % of polyphenols [Nunes and Coimbra 2001]. This suggests the possibility of the presence of a non-colored carbohydrate skeleton, bearing a variety of chromophoric substructures coming in part from the Maillard reaction and in part from chlorogenic acids decomposition. The polyphenol residues are likely to be found out in the future to play a certainly important role for the antioxidant activity.

These facts indicate very clearly that a better knowledge of the coffee melanoidin structure, that until now is still rather elusive [Rizzi 1997], should be very desirable.

Since the issue is nowadays a very popular one among scientists, very many methods are proposed for the analysis of antioxidant capacity (AC), which can be assessed in a variety of ways based on different properties of the food under investigation. I shall briefly review just three approaches:

- direct determination of the content of known antioxidants in the specimen (e.g. chlorogenic acid)
- measure of the oxidation rate of lipids when protected by the specimen (e.g. rancimat method)
- evaluation of the radical scavenging effect of the specimen (e.g. crocin bleaching method).

Direct analysis of specific compounds, albeit sometimes useful, is prone to misestimation of AC due to the presence of other active substances or, on the contrary, of additional oxidants within the food matrix, or even to an interfering effect of the matrix itself.

Indirect methods, based for instance on peroxide value or conjugated dienes hydroperoxides measurements, or on chemiluminescence and fluorescence tests, are useful inasmuch as they give qualitative data on the attitude of a product to delay the oxidative degradation, while no information on the all-important underlying mechanism of action can be obtained.

I shall put forward two final remarks:

- regardless to the method put at work, it is often useful to compare the AC data with those obtained by standard antioxidants. The most used of these is Trolox, the water-soluble equivalent of vitamin E
- as AC in foods evolves depending on compositional and technological factors, more reliable data can be obtained by the contextual use of different methodologies.

3.3 Mycotoxin prevention (and sampling issues) (the ppb range)

Food contamination, both by natural or artificial agents, is an important field where chemical analysis is a vital tool. In coffee industry, the prevailing and most actual issue there is mycotoxin control and prevention [Petracco 2000].

Mould spoilage of grain foods is a historically well known, regrettable phenomenon [Stoloff 1976]. Along with the obvious reduction in sensory food appeal caused by an evident infection, the hidden threat of a damage to human health has drawn much attention of the scientific world. While the toxicity of metabolites of some fungi - for example *Claviceps* - has indeed been studied for centuries, only in recent years has the refinement of analytical techniques allowed for detection of tiny amounts of mycotoxins, even in commodities where they were not thought to be present.

Coffee, if compared to other basic foods like cereals and nuts, has begun late to be considered an issue inasmuch as it was seen exclusively as a problem of heavily decayed mouldy lots deriving from damage. Of the three aspects of quality loss of traded coffee beans, the two evident ones of mouldy external appearance and of tainted sensory character were well known and under control. The third aspect, healthiness, used not to be considered for the lack of scientific evidence.

The first attempts to screen for mycotoxins large populations of commercial raw coffee date from the late seventies. Ochratoxin A (OTA), a fungal metabolite originally named after *Aspergillus ochraceus*, happened to be the first toxin reported in coffee. The earliest reference that comes to memory is an American survey [Levi *et al.* 1974], who found OTA levels up to 360 μg/kg (ppb) in mouldy bags, but just traces of the mycotoxin in commercial lots. Later on, [dePalo *et al.* 1977] some 500 raw coffee samples were examined in Italy without detecting any OTA-positive one.

It must be pointed that those researchers were using Thin Layer Chromatography analyses (TLC), whose detection limits hardly reached the sub-ppm (mg/kg) range. By the way, still in the 1995 edition of the AOAC Manual of Official Methods of Analysis, the procedure recommended for green coffee [Scott 1995] is the TLC one described by Levi twenty years earlier, where a detection limit around 20 ppb can be inferred.

OTA toxicity has been actively monitored: it has been pointed out as a possible cause for Balkan nephropaty, an endemic disease in some rural areas and, since it induces renal tumors in experiment animals, the International Agency for Research on Cancer (IARC) classified it as possibly carcinogenic to humans [Plestina 1996]. On the other hand, the genotoxicity of OTA is still under debate: recent studies have observed that OTA is unlikely to form reactive intermediates capable of binding to human DNA because, unlike in animals, it is not metabolised by cytochrome P450. A genotoxic carcinogenic effect is therefore implausible [Zepnik *et al.* 2000].

For all these reasons, it is understandable that industry is tackling the issue of OTA prevention in coffee very actively: to this purpose both analytical aspects and correct sampling insight are of great importance.

Before speaking about its chemical analysis, I always use to stress that OTA determination begins with the sampling stage [Petracco 1996]. The latter is a notably delicate one for raw coffee, as ever is the case when dealing with a material constituted by pellets (grains, seeds, or “beans” in the case of coffee) [Park and Pohland 1989]. While for most determinations of raw coffee trade characters (colour, sensory, moisture, caffeine) simple sampling plans - as the one coded by ISO method 4072/1982 - are adequate, mycotoxin analyses of seeds are a different question. There is in fact little doubt that sampling contributes the major portion of the variability normally encountered in mycotoxin determination when the lot sample is large. In cases of extreme inhomogeneity, such as with aflatoxin in peanuts, the sampling error is so large that the analytical errors are inconsequential [Whitaker *et al.*1995]. To overcome this, large amounts of several aliquots of seeds taken at different sites in the lot are requested to avoid lack of repeatability, and must be analysed after complete grinding.

Back to chemistry, it must be acknowledged that OTA molecule is a quite active one, thanks to its several functional groups. It is soluble in polar and apolar solvents, water being a good extractant both in acidic and alkaline environment. It is intensely fluorescent under UV light at 335 nm, but in green coffee this fluorescence is hindered by the overwhelming presence of scopoletin, a relative of the cumaric moiety of OTA. Furthermore, the molecule is thermally stable, partially explaining its endurance after heating at temperatures as high as 270°C. Data obtained on roasting contaminated coffee samples at laboratory conditions are often conflicting, while at a larger scale has been shown that in an industrial process only 16 % of the original OTA was found to remain in roast coffee [Blanc *et al.* 1998].

The analysis itself comprises three steps: extraction, isolation and measurement. OTA extraction, by the commonly used solvents like acidified chloroform or alkalised methanol, is only seemingly easy: several other substances are co-extracted (to be noticed that green coffee contains some 500 chemical compounds, and roast coffee way more: up to 2000), hence the need for purification steps. The most reported clean-up columns are made of bicarbonate-celite or alkaline diatomaceous earth ones, and more recently immunoaffinity columns specific for OTA have become available on the market [Zimmerli et al. 1996] [Sharman 1992]. HPLC on C18 is nowadays the most used technique to separate OTA peaks, evidenced then by fluorimetric detectors.

The sensitivity of chemical analytical methods for OTA determination has this way improved to detection thresholds down to fractions of ppb. Some open problems are anyhow actual:

- numerous interfering substances are present, some of them co-eluting at ordinary HPLC conditions
- the reported recovery rates of pure OTA added as a spike are quite dispersed, putting a question mark on quantitation capability

- no report of inter-laboratory proficiency test exists up-to-day for OTA in roasted coffee. For what concerns green coffee, a round robin exercise organised by the Food Analysis Performance Assessment Scheme showed that only 88 % of the participating laboratories scored satisfactorily, i.e. within ± 84 % of the target value [FAPAS 1999].

To conclude this issue on a positive note, the recent application of mass spectrometer as a detector of an HPLC eluate appears to be a promising technique, which will hopefully help both to reject interferents - making OTA analysis less prone to overestimation - and to minimise the need of clean-up stages, preventing that way OTA loss and poor recovery [Gianelli 1998]. It must also be mentioned that AOAC is currently active in sponsoring a collaborative study for the validation of a method for green coffee, which is ongoing under the lead of a Brazilian team.

4. SENSORY IMPACT

4.1 Five senses are involved

When considering that coffee is the second most largely consumed beverage after water, we must acknowledge that its popularity has been achieved for its flavor or, better still, for its overall sensory impact. In this context, *espresso* is the brewing method that offers the consumer the most powerful experience, even if a high quality cup it is not easy to obtain: *espresso's* very strength - the ability to concentrate aromas - is also its weak point because, while enhancing qualities, it shows up in the same time all the defects of the raw material [Petracco 2001].

The main features of *espresso* coffee derive from its preparing rules:

- extemporaneous preparation, on express order
- brewing by a specific method (percolation), using high water pressure
- rapid extraction, admitting into the cup just the best material.

Percolation is a process in which a small amount of hot water under pressure is squeezed through a tightly packed layer of ground roasted coffee, the so-called coffee cake. This very efficiently produces a concentrated brew containing not only soluble solids, but also lipophilic substances lacking in all other brews.
The resulting beverage is very peculiar from a physical and chemical perspective, inasmuch as the foam on the top and the opaque brew are unique to *espresso*. This is due to the presence of a dispersed phase formed by very small oil droplets in emulsion, which are perceived in the mouth as a special creamy sensation - the body. Furthermore, the oil droplets preserve many aromatic components, which would otherwise either escape into the atmosphere or be destroyed by contact with water as in the other brewing techniques, so that the rich coffee taste lingers in the mouth for several minutes [Petracco 1989].

However, *espresso*'s main characters are of sensory nature. All human senses, with the exception of hearing, are involved in appreciation of an *espresso* cup:

- vision evaluates foam's aspect, examining its color and its consistency and persistence
- touch assesses the beverage mouthfeel, or "body", a property linked with density and viscosity
- taste judges the bitter/acidic balance and the presence of a sweet caramelic after-taste
- olfaction appreciates both fragrance, by direct inhaling of the vapours arising from the cup, and flavor, or nasal perception of the volatile substances evolving in the mouth.

4.2 Cup-testing protocols

In the coffee industry routine, some form of objective evaluation is needed to ascertain product overall quality along with the constancy of that quality on time and on varying process conditions [Amerine *et al.* 1965]. The "tool" commonly put to use is a panel of assessors, who may be either coffee experts (professional cup-testers) or naïve consumers after a very basic training. The reason for employing more than one people is obvious: by averaging responses, the risk of incorrect judgement due to a possible bad shape or minor illness of one person is minimised. Another panel potential is the synergy that can be gained by debating coffee characteristics among the assessors during open sessions: this procedure may extract more information, since individual sensitivities and perception thresholds may be different. Sensory tests may be grouped in three basic types, listed by increasing difficulty for the panel members:

- trio tests, used to simply determine if any perceivable difference exists between two samples. In this configuration each beverage is split in two cups, but one cup is discarded and only three of them are presented to the panel. The assessors are requested to tell what the "foreign" single cup is, opposed to the pair of "sisters" cups. A variation called duo-trio presents the panel with five cups, where two “sister foreigners” are shuffled with three “sister controls”: this approach has the advantage to make “hits by chance” much less likely (1/10 vs.1/3)

- duo tests, where two or more single cups are presented to the panel, asking to rank them in relationship to one sensory variable. When more than one variable is to be determined, a pre-filled card proves to be useful to summarise the evaluations

- absolute tests, in which some complex variable, like aroma or overall merit, is to be determined by comparison with a mental paradigm present in each assessor's memory by previous experience. Coffee aroma profiling [I.C.O. 1991] as well can be included in this type, since it is based on assessors' recalling of variegated flavor knowledge present in their experience.

In regular day-to-day *espresso* cup-testing sessions for support to the purchasing activity, the panel should be presented with a maximum of a dozen samples, each served in three different cups according to three fundamental preparation techniques:

- infusion: it is a brewing method that is widely used in northern Europe and in the U.S.A., in which boiling water is poured on coarsely ground coffee powder and allowed to rest for a given period before filtering away the spent grounds from the remaining clear beverage. The concentration of the beverage is low (below 20 g/l) and only the soluble substances may pass into the cup, giving an aromatic pattern typical of filtered coffee beverage

- *espresso*: the classic espresso cup is prepared under standardised and thoroughly controlled conditions, allowing the panel to evaluate its foam and body along with the taste and flavor characteristics. Its concentration may exceed 60 g/l

- diluted *espresso*: a little aliquot of the above mentioned espresso beverage is taken, and then diluted with hot water up to reach the infusion's concentration. This way the high concentration of regular espresso does not hinder any longer the evaluation of some weaker aroma's nuances, and the difference between the solution's aromatic pattern and the emulsion's one can be determined.

Espresso cup-testing sessions cannot be too long nor frequent during the day, because some fatigue develops after the first dozen or so of espresso cups. This is due to the lingering after-taste deriving from the sticking of coffee oil droplets on the tongue and mouth membranes. Rinsing the mouth with water, albeit necessary between each sampling, is not effective to remove the taste completely. On the contrary, cool whole milk seems to act better to this purpose, perhaps because, being itself an oil-in-water emulsion, it can displace coffee oil droplets from the tongue by dilution.

The libraries of sensory data collected can be capitalised for the calibration of instrumental screening methodologies like, for instance, Near Infrared Reflectance (NIR). This analysis, based on spectroscopy in the range of the near-infrared wavelengths (1100 - 2500 nm), is a technique that measures the absorption of monochromatic light by the material to be examined, whose energy is dissipated in rotational and vibrational movements of the molecular bonds, and ultimately transformed into heat [Murray and Williams 1987]. Energy absorption pattern provides information about the molecular configuration of tested material.

NIR applications take advantage from the fact that it is a rapid, non-destructive fingerprinting technique apt to supply simultaneous forecasts of many chemical characteristics of the sample examined, provided that a good calibration has been previously obtained by statistical correlation with traditional, time-consuming analytical methods. This secondary method is suitable also for modeling sensory data, bearing in mind that since several pre-processing steps are needed to obtain the actual coffee beverage as it is tested, regression coefficients not better than 60 % are to be expected.

4.3 Chromatography of volatiles (the ppt range)

Since it is clear that coffee flavor determines most of the quality of a roast coffee beverage, a considerable amount of money - mainly in the major companies - is therefore spent for coffee aroma research.
To make the distinction between aroma and flavor clear, I may explain that the human sense of olfaction perceives the presence of odorous volatile molecules - the ensemble of the ones given off by coffee is, in this context, called aroma - by means of thousands of receptors located in the inner *mucosae* of the nose. Olfaction appreciates both odor, by direct inhaling of the molecules arising from the roast and ground coffee or from the cup, and flavor, the nasal perception of the volatile substances evolving in the mouth and reaching the nose cavity by the pharyngeal pathway [Petracco 2001].

Green coffee beans, as coming from the tree via traditional agricultural practices, do not yet show either roasted beans' color or aroma. Both are formed during the roasting process, where the latter develops mainly as a consequence of Maillard reactions and concentrates mostly in the coffee oil, an effective aroma carrier. Albeit made up of almost one thousand of volatile compounds, aroma constitutes just the 0.1 % in weight of roast and ground coffee [Vitzthum 1998].

The identification of some early aroma compounds - like guaiacols and furfurilmercaptans – was done by food chemistry pioneers already in the thirties [Prescott *et al.* 1937]. From then on, their number has grown greatly thanks to gas-chromatography techniques. Anyhow, as an authentic key coffee aroma compound was never found, it appeared making little sense to increase them just as an outcome of lower and lower detection thresholds. A newer concept, GC-olfactometry, made the search of sensory active compounds possible, spotting them via sniffing ports at the end of a chromatographic column [Schmid and Grosch 1986].

Today, the aroma analysis of a coffee sample includes several steps:

- first, volatile substances have to be isolated by steam distillation and extracted therefrom by lipophilic solvents like diethylether or pentane. The relevant standard method is the Simultaneous Distillation Extraction (SDE), also known as Likens-Nickerson technique; another suitable approach is the High Vacuum Distillation according to Grosch. The resulting concentrate must be stored in a freezer because of its instability: it turns rapidly from colorless to dark red due to degradation reactions. To keep away from this, a solvent-free sampling technique based on adsorption begins to gain popularity, also for the little sample amount needed: Solid Phase Microextraction (SPME)

- second, fractionation can be performed by a variety of chromatographic configurations, where either packed or, more frequently, capillary columns are widely employed. Column oven and injection temperature programming are also often implemented. Flame Ionization Detectors (FID) are still used for peak sensing, whereas Pulsed Flame Photometric Detectors are indispensable when looking for sulphured compounds. Of course, for sensory correlation gas-chromatographs must be equipped with sniffing ports, where trained or naive assessors can record their qualitative and quantitative impressions

- third, identification of peak compounds is nowadays facilitated by the universal use of inexpensive analysers based on Mass Spectrometry (MS). For deeper investigation and better recognition of more complex compounds, also multiple fragmentation instruments are at hand: the so-called MS/MS system in the triple quadrupole configuration or even in the MS^n configuration, made possible by the use of an ion trap

- fourth, final confirmation of structure would ideally require a Nuclear Magnetic Resonance (NMR) analysis or an IR analysis, best if assisted by Fourier Transform algorithms (FT-IR), if just the sample quantity could allowed it. For this purpose, some attention is currently being paid to the exploitation of preparative GC.

Using these techniques, several hundreds of compounds belonging to the following chemical families have been demonstrated:

Table 5: Roasted coffee aroma compounds so far identified, grouped by chemical families [after Grosch 1996]

Family	Number of compounds
Aliphatic hydrocarbons	45
Aromatic hydrocarbons	35
Alcohols	25
Aldehydes	37
Ketones	85
Acids	28
Esters	33
Amines	13
Pyrrols	72
Pyridines	20
Pyrazines	89
Quinoxalines	11
Furanones	128
Oxazols	35
Thiols	7
Sulphides	13
Disulphides	10
Thiophenes	28
Thiazoles	27
Phenols	49
others	45

Among them, some have been highlighted as key aroma compound using the concept of Flavor Dilution Factor, namely the order of a consecutive halving of the amount of concentrate injected into the column, and still perceivable from the sniffing port at the relevant elution time. Some important compounds are:

Table 6: Main key aroma compounds identified in roasted coffee [after Grosch 1996]

Compound	Dilution factor
3-mercapto-3-methylbutylformiate	2048
2-ethyl-3,5-dimethylpyrazine	2048
(E)-ß-damascenone	2048
4-vinylguaiacol	512
2-isobutyl-3-methoxypyrazine	512
2,3-diethyl-5-methylpyrazine	512
3-hydroxy-4,5-dimethyl-2[5H]-furanone	512
5-ethyl-3-hydroxy-4-methyl-2[5H]-furanone	512

In parallel with the search for volatile compounds advantageously contributing to coffee aroma, the same, or even more, attention has been paid to spotting the agents responsible for off-flavors, namely the obnoxious sensations caused by rotten or defective beans. Unfortunately, those "bad guys" have proved to be present in very tiny amounts even if well noticeable by our sense of olfaction. Among them there are some infamous coffee taints, like the "old crop" note, the "stinker" defect, the "jute bag" flavor and the peasy flavor: each of those flaws is responsible for substantial value reduction of the affected lots.

The most important achievement in this field were the identification of the compounds responsible for two frequent negative traits: the so-called Rio-taste and the flavor typical of Robusta species. Both have been ascribed to metabolic pathways of infecting micro-organisms.

The latter was singled out and recognised as 2-methylisoborneol (MIB) by a Kraft Jakobs Suchard reseach team [Vitzthum *et al.* 1990] using a 2-dimensional GC-MS technique equipped with a sniffing port. Further confirmation was obtained by HR-tandem-GCMS with a deuterated internal standard. The amount of this compound in roasted coffee is extremely low - less than 300 ng/kg (ppt) – but it is easily recognisable due to its low threshold sensory level.

The former compound was elucidated in a collaborative research led by Nestlé scientists [Spadone and Liardon 1987], who used temperature-programmed GC equipped with a capillary column and coupled to a MS operating in the Single-Ion Monitoring mode. They identified the active compound in 2, 4, 6 trichloroanisol (TCA), possibly the most potent odorant discovered so far: its sensory detection concentration threshold has been reported [Maarse *et al.* 1985] to be as low as 0.03 ng/kg, corresponding to a few thousandths of ppt !

5. CONCLUSIONS

In my direct experience, any research strategy aimed to developing a fruitful knowledge basis, worthy of being called "coffee science", must allow scientists with different backgrounds (food technology, food chemistry and biochemistry, nutrition, consumer science) to work closely with coffee companies to investigate both aspects that are crucial to product acceptance:

- the health properties of coffee and the potential of industrial processing to optimise them. This aspect is critical for coffee beverages, which are currently regarded by consumers largely as a useful booster of mental performance, but also as a potential source of health problems

- the chemistry and psychology of the pleasure associated with the daily habit of sipping a cup of coffee. Of course, the complexity of an approach collating analyses in the parts-per-trillion range with a consumer acceptance based mostly on hedonistic criteria cannot be under-emphasised.

To these regards, both quality control/optimisation and clinical research (along with *in-vivo, in-vitro* and cell culture experiments) crucially depend on analytical support and ask hence for increasingly sophisticated methods to be made available. Nevertheless, no matter how great the progress of instrumental techniques may get, the good old cup-testing approach still remains the final assessment tool to obtain the green light for choosing the right plant and to conveying its fruit to the market.

A conclusione di questa bellissima giornata, dopo aver sentito tanti esperti parlare in dettaglio di scienza del caffè, credo non sia più necessario far ulteriormente notare come questa scienza possa e debba essere messa al servizio della qualità. In un prodotto complesso come il caffè ciò può essere realizzato efficacemente solo affrontandone lo studio con un approccio multidisciplinare, che coinvolga scienze avanzate come la genetica molecolare, la chimica analitica più raffinata, la teoria della complessità, la statistica aiutata dall'intelligenza artificiale.

Ma tutto ciò non servirebbe poi a molto se non implicasse anche e soprattutto l'utilizzo della qualità al servizio del consumatore: dunque la scienza alleata ed ispiratrice dell'industria quando questa si sforza di produrre qualità costante. Se questo è vero, diviene comprensibile come l'Italia abbia un così grande successo nell'esportare un prodotto alimentare che non deriva dalla sua terra, in quanto viene dai paesi tropicali, ed è ancora più strano che lo esporti addirittura negli stessi paesi di produzione, dopo averlo nobilitato. Infatti, non riesportiamo semplicemente il contenuto di caffeina di un chicco di caffè importato ad esempio dalla Colombia, ma gli aggiungiamo quel background di conoscenza e quella tecnologia particolare che esalta i potenziali di questo chicco, e lo fa diventare attuale in una preparazione complicatissima e magistrale.

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