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COFFEE TASTE ANALYSIS OF AN ESPRESSO COFFEE USING NUCLEAR MAGNETIC SPECROSCOPY

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ABSTRACT

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This thesis was done within a project dedicated to a better understanding of the chemistry occurring during the brewing process of espresso coffee.

This study aims to study the correlation of the 1H-NMR spectrum of an espresso coffee with the taste assessment of a trained coffee tasted in order to establish a relationship between the concentration of compounds present in coffee and the appreciation of the flavor of the espresso coffee. The study focuses on the concentration of some compounds: caffeine, acetate, formic acid, lipids and trigonelline.

The study has shown that the concentration of these compounds does not impact in a significant manner on the taste appreciation of the espresso coffees brewed.

Key words

acetate, caffeine, DMSO, espresso coffee, formic acid, lipids, 1H-NMR, taste assessment, trigonelline, WET water suppress technique.

INDEX OF ABBREVIATIONS

HMDS: hexamethylsiloxane
TCE: Tetrachloroethane
NMR: Nuclear Magnetic Resonance
ISO: International Organization for Standardization
ICO: International Coffee Organization
DMSO: dimethyl sulfoxide

ACKNOLWEDGEMENT

This work was performed at Koninklijke Philips Electronic N.V (Royal Philips Electronics), in the R&D department in Eindhoven Holland from July to December 2011.

I would like to thank my supervisor Dr. Freek Suijver, for the opportunity of being part of the coffee project, his tutoring and his availability. I would like to express my gratitude to all the team members from the coffee project for their knowledge, time and friendliness: MSc Nicole Haex, Dr Nico Willard, Dr Johan Marra, and MSc Mart Te Velde A special thanks to Dr. Jeroen Pikkemaat for assisting me with his expertise on the NMR. Finally I want to thank Richard Schukkink for the great coffees; I have learned to enjoy the subtleties of a good cup of coffee.

This work was supervised by MSc Jana Holm and Dr. Esko Johnson; I want to thank them both for their time and feedback.

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1 INTRODUCTION

For centuries coffee making was the operation of placing roasted or ground coffee beans in a pot and adding hot water to it and boiling it until the mixture smelled “right”. In the 70’s the first electric drip coffee makers made their apparition in households. From then on research to modify and ameliorate features such as heating elements, spray heads, filters, timers or clocks for automatic start, carafe design and build in coffee grinders have been completed. The industry of brewing coffee is growing all the time, aiming to provide customers with the experience of a good coffee at home. New developments are made to enhance the experience of a quality cup at home: grinder integrated to the machine, regulation of the strength of the coffee options, home roasters.

In the past decade a lot of studies on coffee have tried to determine the origin of the taste of coffee and to identify the compounds that contribute to a “good” coffee.

This study interest itself to the relation between the taste appreciation of a Barista and the composition of an espresso coffee cup. The study will focus on a set of taste related compounds and will aim to determine if those compounds are relevant to the taste in an espresso coffee. The taste of an espresso coffee will be assessed by a Barista, definition de Barista. The Barista will judge the coffee cups tasted, afterwards the concentration of the chosen taste compounds will be measured and correlated with the Barista’s appreciation in order to determine if the compounds chosen have an influence on the overall appreciation of the coffee cup. The determination of the concentration of those compounds will be done by Nuclear Magnetic Resonance spectroscopy. (NMR)

I performed this study during my internship at Koninklijke Philips Electronic N.V (Royal Philips Electronics) usually referred to as Philips. Philips is a Dutch company, founded in 1891 by Anton and Gerard Philips. The company started by producing carbon filament lamps, the company expanded quickly. Nowadays Philips is

organized in 3 main sectors: Philips Consumer Lifestyle dealing with consumer electronic products, Philips Lightning creating light applications for consumer and professional market, and Philips Healthcare that develops a wide range of solutions for healthcare management at home and in hospitals.

My internship took place at the Philips Research facility in the Consumer Lifestyle Sector. It is located in Eindhoven on the High Tech Campus. The High Tech Campus is an area, where R&D divisions of more than a hundred companies have regrouped for facilities and knowledge sharing.

I performed my internship within the project team on coffee research. The coffee research project had two main foci: fundamental research focusing on understanding the brewing process, its implications and an application focus aiming to improve coffee machines.

This particular analytical project goal was to determine if NMR spectroscopy could be used to evaluate the taste of an espresso coffee. My thesis subject is the bridging between the fundamental research focus and the practical focus since the taste of the final coffee brew is an important factor for coffee consumers. The study of the taste compounds correlated to the taste appreciation was an approach to being able to quantify taste. For my thesis I studied the correlation between the concentrations of certain compounds related to taste in coffee with the taste assessment of a professional coffee taster using NMR (nuclear magnetic resonance spectroscopy).

The first part of this report includes an introduction to the aspects of taste assessment of an espresso coffee using NMR spectroscopy. This section deals with the taste related compounds, their origin during the roasting process. In the course of which a set of reactions: Maillard reaction, Strecker degradation and caramelization, will result in an increase in the concentration of aromatic compounds. This section also includes theoretical background on the analytical technique used: NMR spectroscopy. The experimental standards are presented: Illy bean, automatic espresso machine. And the parameters that were chosen for investigation are described in brief.

The second part of the report describes the experimental procedure used for the treatment of each sample. The experimental section is divided into two sections: the preliminary investigation experiments and the experiments of espresso coffees concentration measurements and their correlation with the Barista's taste appreciation. Finally the results are presented and discussed.

The study is limited by the small amount of samples available for comparison, since the project was at its beginning stage. The choice of compounds that can be quantified or that are visible in the NMR spectra was another limiting factor in the study.

2 TASTE ASSESSMENT OF AN ESPRESSO COFFEE USING NMR SPECTROSCOPY

2.1 The coffee bean

Coffee is defined in the ISO (International Organization for Standardization) vocabulary by:

> The fruits and seeds of plants of the genus *"Coffea"* usually of the cultivated species, and the products from these fruits and seeds, in different stages of processing and use intended for human consumption.

There are two main varieties of coffee beans which are *Coffea Arabica*, commonly called Arabica and *Coffea Anaphora* commonly called Robusta. Arabica is predominant in South America and East Africa. It is usually cultivated at high altitudes. Robusta is predominant in West and Central Africa its cultivation is adapted to hot and humid region. (Oesteich-Janzen 2010.)

The biggest coffee producers in the world are: Vietnam, Colombia, Brazil, and Indonesia (International Coffee Organization). Many factors influence the quality and chemical composition of the raw bean such as the location, altitude, weather, and composition of the soil, cultivation, harvesting, the drying method used and the quality of the roasting process. (Viani, Andrea Illy&Rinantonio 2005.)

2.2 Taste in coffee

The main components present at various stage of coffee production (green bean, roasted bean, instant coffee) are: caffeine, carbohydrates, chlorogenic acids, fatty acids, other nitrogenous compounds, volatiles, and melanoidins. (Oestreich-Janzen, 2010) The concentration of the compounds present in the coffee bean varies during the process of treatment of the bean. (Table 1) The roasting of the coffee bean is the

key process to the formation of the aromatic compounds important to the taste of the final cup.

TABLE 1. Distribution of the concentrations of some of the different compounds presents in coffee in Arabica and Robusta coffees at different stages of production from the green bean to the roasted bean. (Adapted from Oestreich-Janzen 2010)

Chemical composition of coffee in mass percent in dry matter				
	Arabica green	Robusta green	Arabica roasted	Robusta roasted
Compounds	%DW	%DW	%DW	%DW
Caffeine	1,3	2,3	1,2	2,4
Trigonelline	0,8	0,7	0,3	0,3
Chlorogenic acids	8,1	9,9	2,5	3,8
Lipids	15,2	9,4	17,0	11,0
Melanoidins			25,4	25,9
Volatile aroma	Traces	Traces	0,1	0,1

The taste sensations experienced when drinking a coffee are the consequence of a specific balance between the concentration of the various aromatic and volatile compound present in coffee. An unbalance, or the excess of one compound over the other, will influence the taste of coffee especially in the case of acidic, sour and bitter compounds. The compounds listed are responsible for the sweet, salty, bitter, acidic and sour taste in coffee. (Coffee research.)

The ICO (International Coffee Organization) defines acidity in coffee as:

> A basic taste characterized by the solution of an organic acid. A desirable sharp and pleasing taste particularly strong with certain origins as opposed to an over-fermented sour taste.

Bitterness is defined as:

> A primary taste characterized by the solution of caffeine, quinine and certain alkaloids. This taste is considered desirable up to a certain level and is affected by the degree of roast brewing procedures. (ICO)

Sourness is defined as:

> An excessively sharp, biting and unpleasant flavor (such as vinegar or acetic acid). It is sometimes associated with the aroma of fermented coffee. (ICO)

The importance of certain taste related compounds in the overall taste of a cup of coffee also depends on the taste threshold of these compounds. The taste threshold is the minimum concentration at which the taste sensitivity is reached. If a compound is below its taste threshold then it most likely will not affect the taste perceived by the coffee drinker. (Viani et al. 2005) The relative impact on taste of compounds in coffee will be highly dependent on their concentration and their tasting threshold. (Table 2)

TABLE 2 Examples of some human thresholds (Adapted from Hypertexts for Biomedical Sciences)

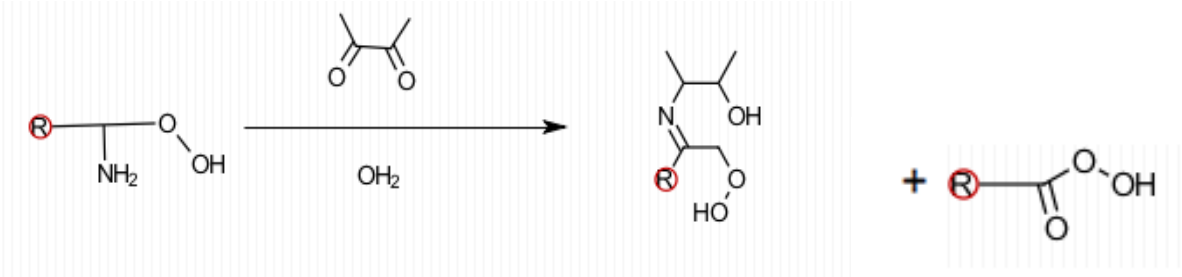
Taste	Threshold for tasting
Salty	0,01 M
Sour	0,0009 M
Sweet	0,01 M
Bitter	0,000008M

The taste of coffee takes its origins in the roasting process. During the roasting process the green bean is treated in order to be grinded for coffee brewing; it is treated to become a roasted bean or the ground coffee that is used for brewing coffee. Temperature, roasting time and roasting techniques are the most important variables when it comes to treating the raw coffee bean. The aromatic and flavor

characteristics of coffee result from the roasting process. Roasting begins with a simple heating stage during which the bean absorbs the heat and gives away the excess water in the form of steam as it slowly dries, taking a yellow color and a toasted smell begins to develop. The bean swells, doubling in size and becomes a light brown color. The roasting temperature increases, the color deepens and the bean loses in weight and density as it becomes brittle. CO_2 is released during this phase and will continue to be released many days after the roasting process has ended. The high pressure and temperature inside the bean during the roasting process will trigger the formation or alteration of volatile aromatic compounds and other flavor related compounds. The final balance of the different compounds in coffee will depend on the pressure and temperature during roasting. A temperature that is too high or a roasting time that is too long will result in the destruction of aromatic and flavor compounds rather than their creation. (Viani et al. 2005)

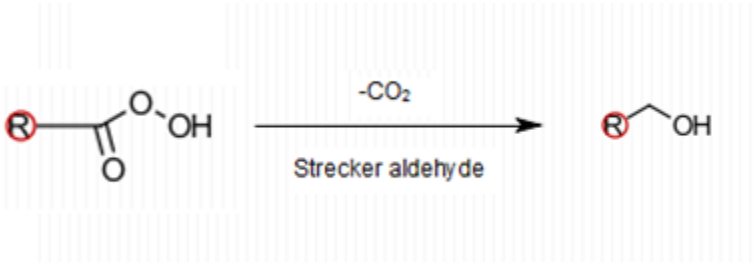
The chemical reactions occurring during roasting are responsible for the flavor and aromatic compounds present in coffee. These reactions are: the Maillard reaction, the Strecker degradation and the caramelization. The process occurs in 3 phases and is characterized by a heat transfer process which releases aromatic compounds.

The Maillard reaction is a reaction between a reducing sugar and an amino acid that will lead to the formation of melanoidins which are brown colored molecules important to the crema which is the foam layer that can be observed on top of the liquid phase of the espresso when it is brewed correctly.



GRAPH 1. The Maillard reaction

The products of the Maillard reaction will react with carbonyl groups during the Strecker reaction leading to the formation of a Strecker aldehyde and an amino ketone, compounds responsible for the brown pigment observed in coffee and volatile aromatic compounds.



GRAPH 2. The Strecker reaction leading to the formation of a Strecker aldehyde

Caramelization will require high temperatures and will begin with the loss of water from the sugar molecule. The sugar will be converted into a furfuryl. The same compound is produced during Maillard reactions but it is during prolonged exposure to high temperature that various types of aromas are formed. The chemical reactions occurring during the roasting products are not always predictable because different sugars reacting with different amino acids will produce different aromatic compounds, resulting in slightly different proportions in final compounds. (Ohio University presentation; Fayle, Gerrard and Belton, 2002). The reaction is dependent on the: amino acids available, the pH, the amount of water and the period of time held at a temperature. The Maillard reaction, the Strecker degradation and caramelization all occur during the roasting of the coffee bean, making roasting the key step in the formation of a good coffee cup. These reactions result in the formation of over 800 aromatic compounds. The coffee aroma is dependent on the concentrations and the odor and taste thresholds of these compounds. (Viani et al.2005.) (Appendix 1)

3 ANALYTICAL METHOD USED IN THIS STUDY

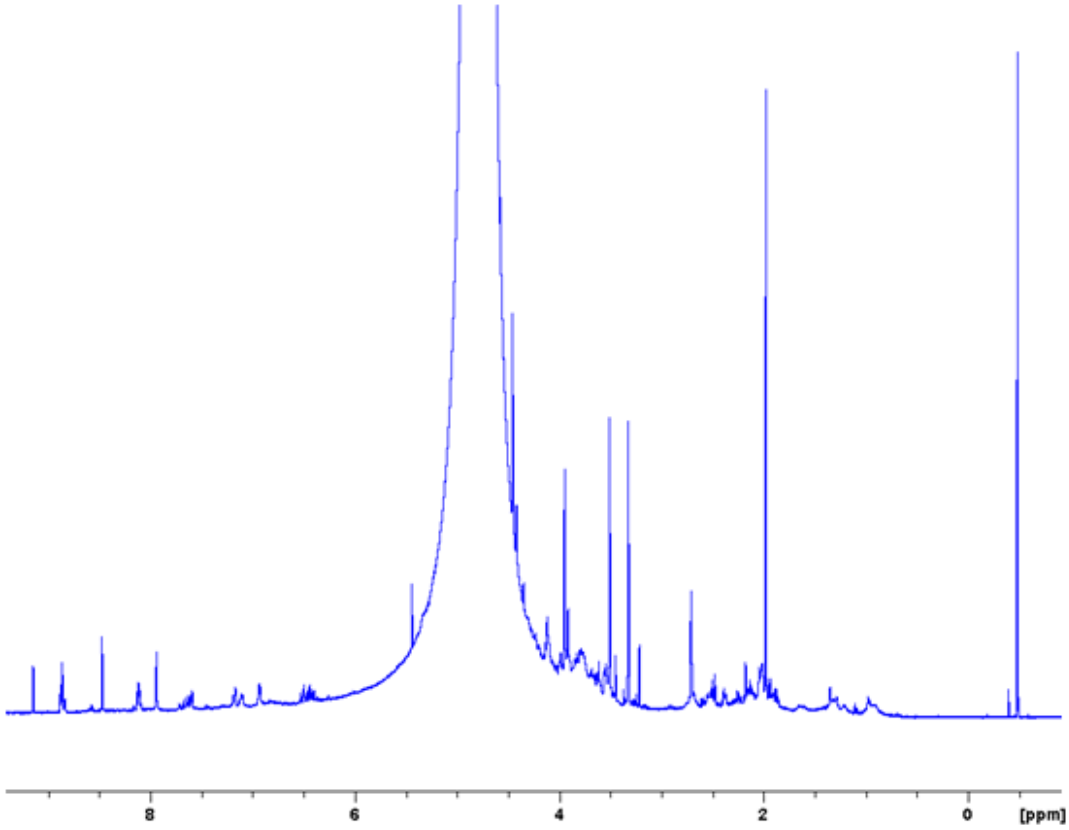
The analytical method used in this study to quantify the concentrations of various taste-related compounds was ^{1}H nuclear magnetic resonance spectroscopy (^{1}H-NMR). NMR uses the magnetic properties of atomic nuclei to obtain information on the nuclei such as: molecular structure and concentrations. (Joseph P. Hornak 1997-2011)

Most atomic nuclei possess a physical property called spin, a rotation of the nucleus around its own axis. As a result of its electrical charge, the spinning nucleus will possess a magnetic moment allowing it to act as a tiny bar magnet with its axis along the axis of rotation. The orientation of nuclear spin is quantified. A typical NMR experiment will consist in bringing a sample into a magnetic field. Radio frequency waves, when transmitted to the sample will induce transition between energy levels. This process is called excitation. In NMR techniques, nuclei are excited with short radio frequency pulses containing a continuum of frequencies. When the spins return to equilibrium distribution, they emit a RF signal at the resonance frequency which is detected by the NMR spectrometer. (Hornak 1997-2011).

The two most common nuclei used to detect NMR signals are ^{1}H and ^{13}C, in this case ^{1}H-NMR was used to investigate the content of an espresso coffee

^{1}H-NMR can provide information on:

- The number and different types of hydrogen present in the molecule
- The number of hydrogen in the neighborhood
- The electronic environment of the nucleus

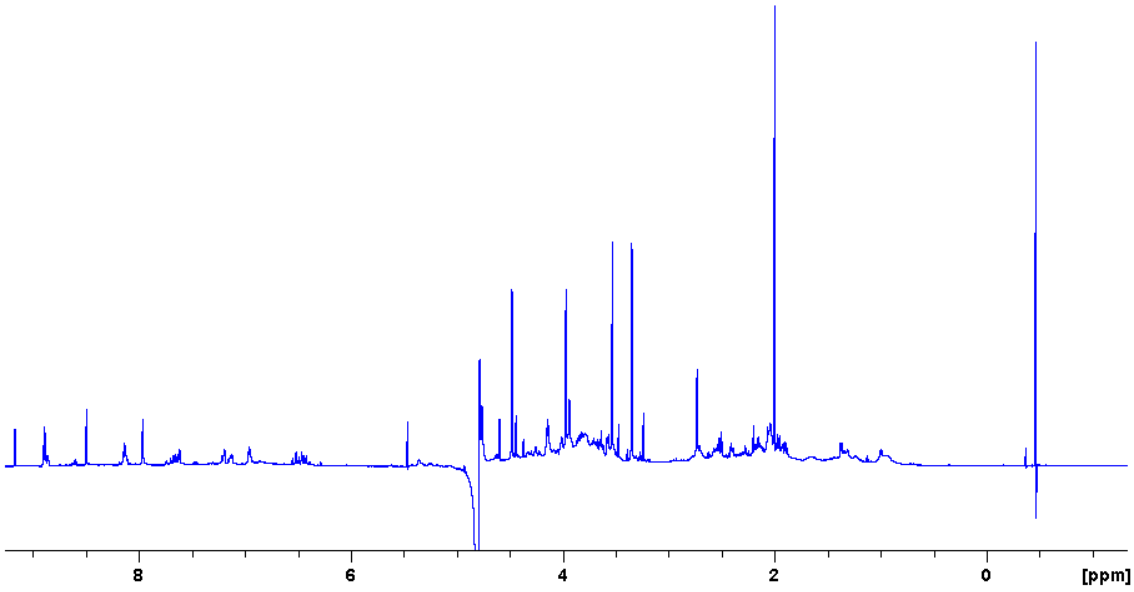


GRAPH 3. Typical NMR spectrum of an espresso coffee, no suppression of the water peak

The basic NMR spectrum is a plot of intensity against the frequency of the radio frequency signal detected by the spectrometer. The measurement by NMR gives us a spectrum that has to be further more analyzed. (Graph.2) First the peaks corresponding to the compounds that need to be quantified are identified. The position of the peaks corresponding of a compound can be found in literature and are referred to as the chemical shift (Spectral database for organic compounds).The intensity of a NMR signal is proportional to the concentration of the corresponding atom in the sample. Signal intensities are used to determine relative concentrations or the number or equivalent atoms. (Introduction to spectroscopy 2011).

The difficulty with analysing a NMR spectrum of coffee resides in the broadness of the water signal that can overlap other signals and the numerous signals that overlap each other rendering integration difficult. Therefore a so-called “WET water

suppression" technique (McKay, 2009.) may be used to improve the accuracy for the integration of the chosen peaks and to prevent the noise introduced by the digitizer. (GRAPH 3)



GRAPH 4. Spectra of an espresso coffee after using the "WET water suppression" technique.

In the following experiments the taste appreciation of an espresso was correlated to the NMR spectra of the same espresso coffee sample. The compounds investigated were chosen with respect to their visibility in NMR spectra of an espresso coffee and their relative importance to taste in the final cup of coffee. The compounds targeted were: acetate, lipid, trigonelline, formic acid and caffeine. (d'Amelio, Fontanive, Uggeri, Suggi-Liverani, Navarini 2009)

4 CULTURAL AND SOCIAL ASPECTS OF COFFEE CONSUMPTIONS

Coffee was first used as a physiologic-stimulant for physical activities. Nowadays coffee is considered not only a stimulant but also a flavored beverage to be enjoyed. The rites of preparation and consumption of coffee differ from country to country. (Viani et al. 2005.)

Finland is one of the biggest coffee-consuming countries in the world. With 12kg per year per person it is twice as much as many of the Europeans countries. Comparatively French only consume 5 kg per year. Coffee has become part of Finnish culture; Finland is one of the only countries in the world where coffee breaks are statutory. Coffee is also part of many celebrations such as name's day, weddings, and funerals. It is common when receiving guests in Finland to offer them a cup of coffee. Finns have a preference for drip filter coffee and the particularity of the coffee consumed in Finland is that it is light roasted. (Ojaniemi 2010.)

In Italy and France the aroma and taste of coffee are importance. Consumption of coffee is part of the culture and in France the name "café" stands for the drink as well as the place where it can be served. Espresso is a preferred beverage in Italy and France because of the strong aroma of the coffee. (Viani et al. 2005)

5 PROCESS PARAMETERS

5.1 Experimental standards

A certain number of standards were chosen for the purpose of this study:

- A standardized coffee bean: Illy roasted coffee beans. Illy coffee beans are a blend of 9 different Arabica coffee beans. Illy beans are distinct because of their particular flavor, “always identical in any espresso cup wherever it is consumed in the world”. (Illy, 2011)
- The coffee machine used was the Syntia from Philips-Saeco. (Fig.5)
- The coffees were all tasted by a recognized and professional coffee taster and Barista in order to establish a quality benchmark.

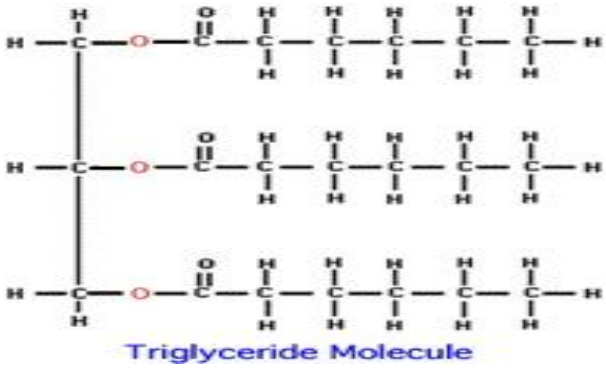
GRAPH 5. Illy logo and the Syntia: a fully automatic espresso machine by Saeco.

5.2 Parameters chosen for the research of the taste compounds

The parameters that were investigated were pH, Brix and the concentration of caffeine, acetate, formic acid, trigonelline and lipids. The pH and Brix were indicative measurements, done prior to the analysis by NMR spectroscopy. The compounds chosen for investigation are compounds that are known for having a bitter or acidic taste and are present in sufficient concentrations to be visible in the NMR spectra of a coffee sample.

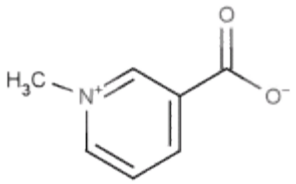
Compounds investigated

Lipids are fatty acid containing compounds present in coffee, mostly triglycerides. Lipids are present in the coffee liquid phase but also in the foam layer on top of the coffee. Lipids are known to have a taste and will trap volatile compounds within the foam layer. (Farah, Monteiro, Calado, Franca, Trugo 2006)



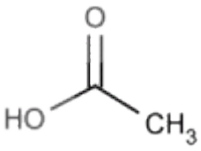
Triglyceride molecule

Trigonelline (1-methylpyridium-3-carboxylate) is a compound that contributes indirectly to the formation of flavor compounds responsible of the bitter taste of coffee. The concentration of trigonelline is proportional to the degree of roast (Farah et al. 2006).

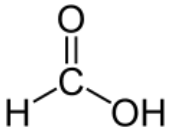


Trigonelline molecule

During roasting carbohydrates chains will breakdown leading to the formation of aliphatic acids like acetic and formic acid.

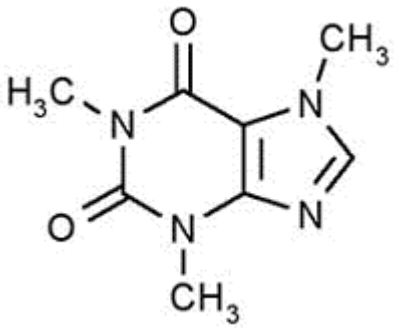


Acetic acid



Formic acid

Caffeine is one of the most consumed psychoactive substances, mostly in beverages ranging from coffee to energy drinks. It is found naturally in more than 60 plant species and is present in many products derived from these plants. Caffeine is a bitter tasting white crystalline powder that belongs to the family of alkaloid compounds. (Illy, The science of Coffee.)



Caffeine molecule

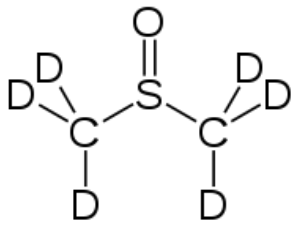
TABLE 3. Estimated chemical shifts of the studied compounds in coffee

Compounds	Estimated chemical shift (ppm)
Lipids	0.9
Acetate	2.0
Caffeine	3.4 - 3.6 - 3.8 - 7.8
Formic acid	8.5
Trigonelline	9.0

Chemical shifts are the relative difference in resonant frequency compared to a standard signal. For proton NMR the standard is tetramethylsilane (TMS). The chemical shifts of different compounds can be found in tables. (Richards & Hollerton 2011)

6 EXPERIMENTAL

A part of the caffeine contained in coffee is transiently bound to large compounds. This fraction of caffeine is not visible in the 1H-NMR spectrum as a result of the large line width associated with these large molecules. It was found in literature that the addition of dimethyl sulfoxide (DMSO) to the coffee sample prevented the binding of caffeine with larger molecules, thus allowing an accurate NMR quantification (Nicolas d'Amelio et al. 2009.). To prevent the presence of an additional solvent signal in the 1H-NMR spectrum deuterated DMSO (DMSO-d6) was used.



DMSO-6

Not only does DMSO-6 prevent the binding of caffeine to chlorogenic acid but it also allows an excellent LOCK signal for frequency locking and is optimal for the dissolution of less hydrophilic molecules.

6.1 Standard procedure for the treatment of samples

The experimental samples were processed according to a standard procedure:

- A sample of 300 µL was taken from the coffee, weighted, the weight was recorded.
- Then 200 µL d DMSO-d6 was added thoroughly mixed and the weight of the total mixture was recorded.
- The sample is then added to an NMR tube (wilmad labglass, 5mm thin wall 7'')

- A coaxial capillary insert containing a mixture of hexamethyldisiloxane in deuterated tetrachloroethane (HMDS) was used as a reference for the ^{1}H - NMR chemical shifts and concentrations. (Richards & Hollerton 2011).
- ^{1}H-NMR spectra were recorded on a Bruker 600MHz NMR spectrometer using a standard pulse-acquire (Ryan T.McKay 2009) experiment preceded with a WET sequence (Ryan T.McKay 2009) for the suppression of the water signal.
- Following the integration of the obtained spectrum, the concentrations of the studied compounds was obtained by applying the following formula:

$$[x] = \frac{d_{H2O}}{MW_{H2O}} \times \frac{I^{ref}_{HMDS}}{I^{ref}_{H2O}} \times \frac{n_{H2O}}{n_x} \times \frac{I_x}{I_{HMDS}}$$

$[x]$ is the concentration of the compounds measured.

d_{H2O} and MW_{H2O} are the density (998 g/L at 25°C) and molecular weight (18.02 g/mol) of deionised water.

I^{ref}_{H2O} and I^{ref}_{HMDS} are the intensities of the water and the hexamethyldisiloxane CH_3 signals in the ^{1}H-NMR concentration reference measurement

n_{H2O} and n_x are the number of hydrogen atoms contributing to the respective ^{1}H-NMR signals

I_x and I_{HMDS} are the intensities of the water and the hexamethyldisiloxane CH_3 signals in the actual ^{1}H-NMR concentration measurement.

Due to the addition of DMSO a correction factor had to be applied to the concentration values obtained from the previous calculation. (See Appendix 7 for the detail of the experiment).

$$C' = \alpha \left(\frac{Wt}{Wc}\right) C$$

C': exact concentration

C: measured concentration

α: correction factor = *0.9181*

Wt: Total weight of the sample

W_c: Weight of the compound in solution

The pH and the Brix of each sample were also measured and recorded.

6.2 Preliminary study of the effects of DMSO on the compounds studied

It was found that a part of the caffeine in a cup of coffee will have a tendency to bind with larger compounds. (D'amelio et al. 2009.) When measuring caffeine with NMR in solution of known amount of caffeine, it was observed that part of the caffeine was "missing". This observation was verified when coffees of known amount of caffeine were measured by NMR. (Appendix 5) Therefore it is necessary to prevent this binding for more accuracy in concentration determination. The prevention of binding was done by adding a known amount of DMSO to each sample before measuring the concentrations of the chosen compounds. (D'Amelio et al. 2009)

The aim of the following experiment was to study if the addition of DMSO to coffee samples affected the concentration of the other compounds studied with NMR analysis.

6.2.1 Materials and methods

The analyses were realized with a Bruker 600 MHz NMR spectrometer. The solutions analyzed were prepared by diluting the chosen chemicals in distilled water. The DMSO used was pure (40 (v/v) % in coffee). The chemicals investigated were caffeine, formic acid, acetate and trigonelline.
Each compound was diluted with distilled water. Two spectra were measured: one prior to the addition of DMSO and one after the addition of DMSO.
The exact experimental procedure can be found in the appendixes. (APPENDIX 4, APPENDIX 5)

The concentration of the compounds measured before addition of a known amount of DMSO and after the addition of DMSO were recorded and compared to the values obtained by calculation with the correction factor.

Table 4 shows the measured concentrations of the compounds before the addition of DMSO, after the addition of DMSO and the third column called “Calculated” shows the calculated value of the concentration.

TABLE 4 Concentration of compounds measured before DMSO addition, after DMSO addition.

Concentration	Before DMSO	After DMSO	Calculated
Caffeine (M)	0.0033	0.0021	0.0034
Formic acid (M)	0.025	0.016	0.025
Acetate (M)	0.44	0.21	0.43
Trigonelline (M)	0.0010	0.00063	0.0010

6.2.2 Discussion

From the results listed in Table 4, it can be observed that the values of the concentration of the compounds before the addition of DMSO-d6 and those obtained by calculation are almost equal. It can be conclude that, except in the case of dilution, the addition of DMSO-d6 did not affect the concentration of the compounds studied by NMR.

It can therefore be concluded that the use of DMSO-d6 to prevent the complexion of caffeine with other larger compounds does not affect the concentration of the other compounds studied. DMSO can be used effectively to target specifically prevention of the binding of caffeine.

6.3 Taste assessment experiment

For the experiment on the correlation of taste appreciation against the concentration of compounds, coffee brewed by the Syntia was rated against itself by the Barista.

6.3.1 Materials and methods

In this experiment 24 cups of coffee were brewed, the Barista tried every fifth cup and gave his appreciation of each cup comparing them to each other.

The coffees tasted by the Barista were rated according to his appreciation of the taste:

- 3, bad tasting coffee
- 6, average tasting coffee
- 9, pleasant tasting coffee

The parameters measured and/or recorded were the pH, the Brix, in-cup temperature of the coffee after brewing, the extraction time and the concentration of the chosen compounds: caffeine, acetate, formic acid, trigonelline and the lipids.

These parameters were later correlated with the taste impressions of the Barista.

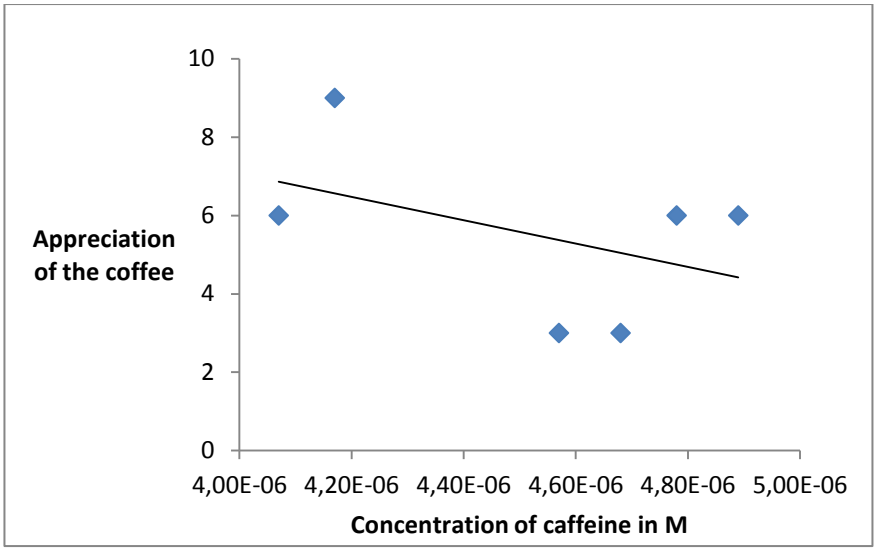
6 samples were chosen to be analyzed and correlated with the barista's appreciation. The samples chosen for detailed analysis were the samples that drew the most extreme comments ranging from "the best coffee of the day" to "very foul tasting coffee". The samples were chosen with the assumption that if there were to be differences in content to be noticed by NMR analysis, they would most probably be the most flagrant in samples that show the most taste appreciation contrast.

The NMR measurements were realized with a Bruker 600 MHz NMR spectrometer. The compounds measured were caffeine, acetate, formic acid, lipids and trigonelline. DMSO was added to each sample according to the analytical procedure followed when using DMSO.

6.3.2 Results

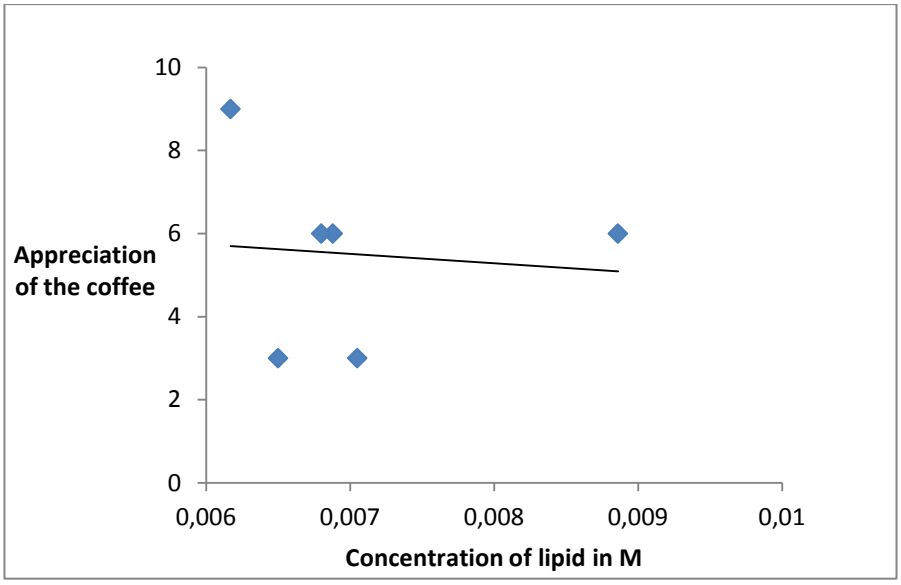
The following graphs present the appreciation of the coffee by the Barista as a function of the concentration of the studied compounds. Indicative trend lines are displayed in black to draw attention to the absence or presence of correlation between the appreciation and measured values. The concentrations of the compounds are in Molar and the appreciation grades of the coffees are from a scale of 0 to 10.

GRAPH 12 presents the concentration of caffeine as a function of the appreciation of the espresso coffee cup. Caffeine is a key compound in the coffee brew.



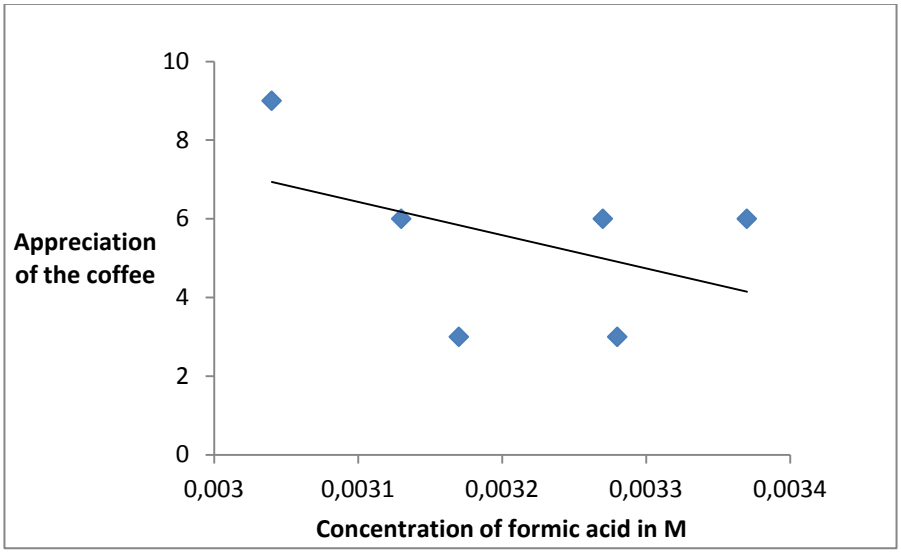
GRAPH 12. Appreciation as a function of caffeine concentration in molar (M).

GRAPH 13 is the plot of the concentration of lipids against the appreciation grade attributed by the Barista. The lipids in this case represent the fatty compounds mostly present in the crema layer on top of the espresso.



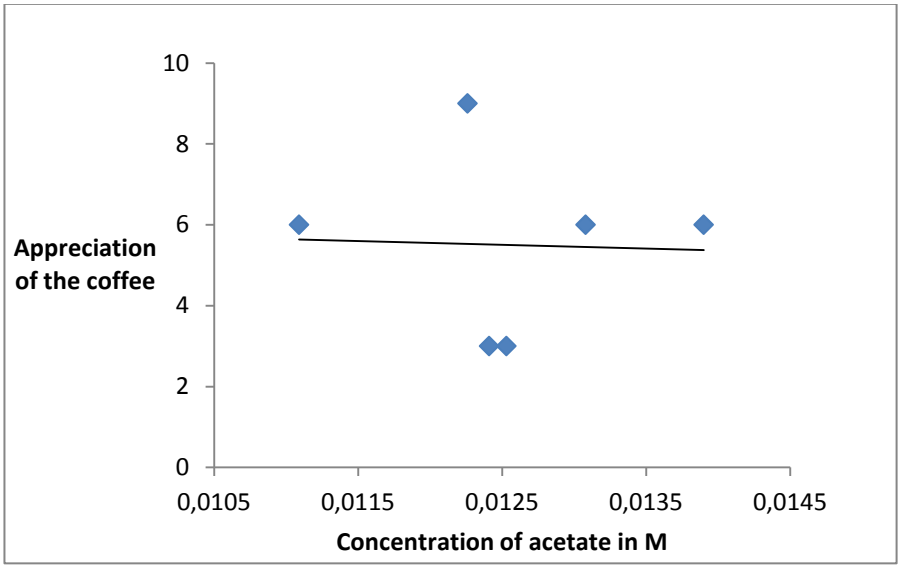
GRAPH 13. Appreciation as a function of lipid concentration in molar (M)

GRAPH 14 is the plot of the concentration in formic acid against the appreciation.



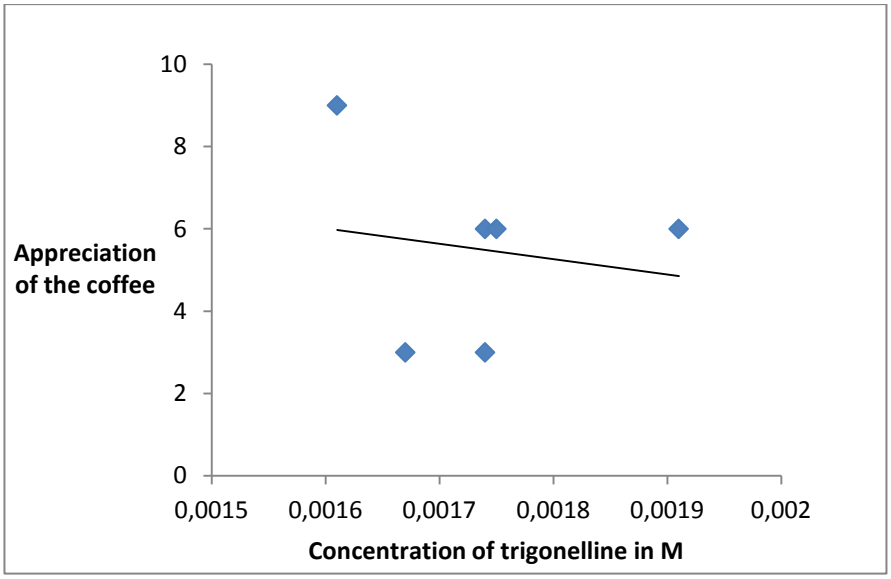
GRAPH 14. Appreciation as a function of formic acid concentration in molar (M)

GRAPH 15 is the plot of the concentration of acetate against the appreciation of the coffee.



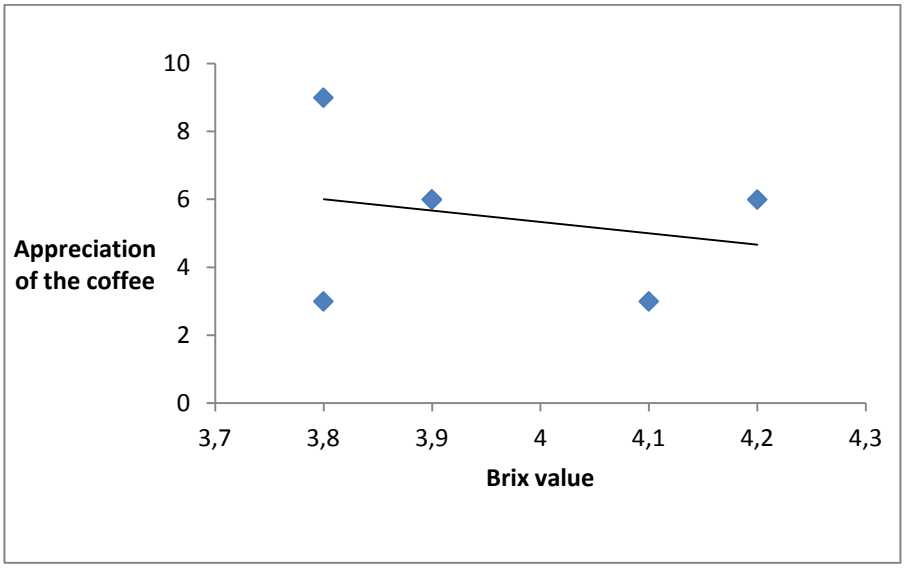
GRAPH 15. Appreciation as a function of acetate concentration in molar (M)

GRAPH 16 is the plot of the concentration of trigonelline against the appreciation of the coffee.



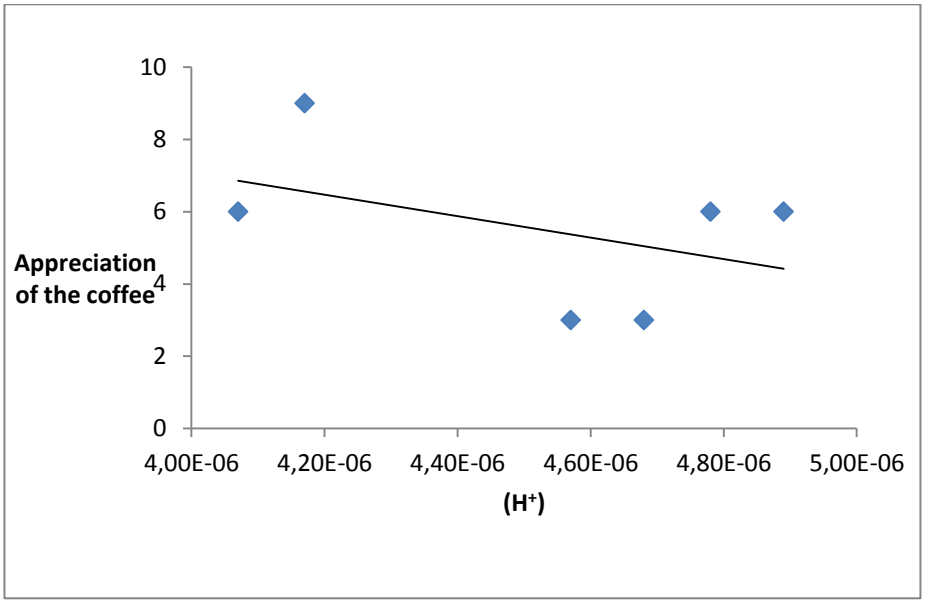
GRAPH 16. Appreciation as a function of trigonelline concentration in molar (M)

GRAPH 17 is the plot of the concentration of the Brix value against the appreciation of the coffee. The Brix measurements were indicative of an over or an under extraction. Brix measurements were performed for each sample before measuring the concentration with the NMR.



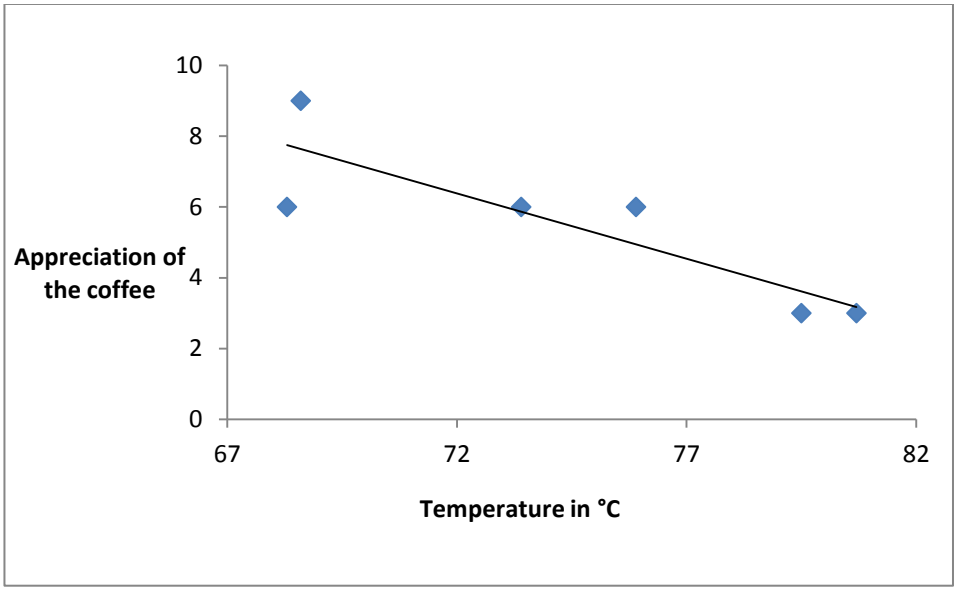
GRAPH 17. Appreciation as a function of Brix

GRAPH 18 is the plot of the concentration of (H+) ion against the appreciation of the coffee. The concentration of (H+) ions corresponds to the pH measured in each espresso coffee cups.



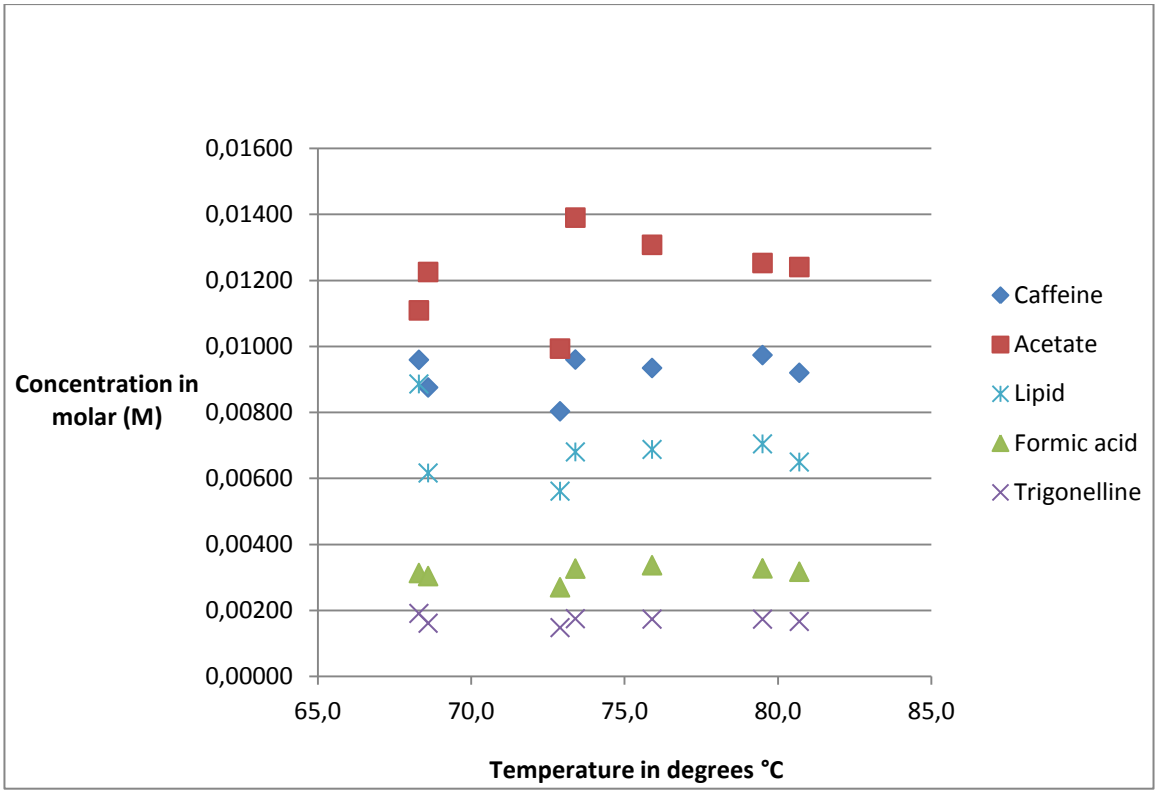
GRAPH 18. Appreciation as a function of the concentration in (H^+) ions

GRAPH 19 is the plot of the in-cup temperature of the coffee cups just before being tasted against the appreciation of the coffee cup.



GRAPH 19. Appreciation of coffee as a function of temperature

GRAPH 20 is the plot of the concentration of the compounds in Molar against the in-cup temperature of the coffee before being tasted.



GRAPH 20. Concentration of analytes in coffee as a function of temperature

7 DISCUSSION

The study presented aimed at correlating the levels in caffeine, acetate, trigonelline, acetate, formic acid with the appreciations of a professional coffee taste. The analysis of the taste compounds was done by 1H-NMR spectroscopy, the concentrations of the compounds was assessed and plotted against the grades accorded to each coffees by the Barista.

The parameters chosen were the concentrations of taste related compounds: caffeine, acetate, trigonelline, formic acid. Other parameters such as the pH, the Brix and the temperature were recorded as well as indicative measurements. Prior to the study of these compounds preliminary experiments concerning the accuracy of the concentrations measured by NMR spectroscopy were done and proved to be conclusive. The choice of the studied compounds was motivated by the facts that not only are they taste related compounds but also because they are present in such a concentration that their quantification by NMR is feasible.

The preliminary experiment aimed to verify that the concentrations of the studied compounds were not altered by the addition of DMSO to the coffee samples. From literature it was found that part of the caffeine remained bound to larger molecules, which is why DMSO was needed to prevent the complex formation, in order to accurately quantify caffeine. It was necessary to verify whether or not the addition of DMSO would affect the concentrations of the other compounds as well. It was successfully established that the addition of DMSO did not alter the concentration of the studied compounds.

Following the preliminary experiments the concentrations of the taste compounds were assessed. In the case of the concentrations of caffeine, acetate, trigonelline, formic acid and the lipids no correlation could be found between the concentrations and the appreciation of the taste. The concentration in (H+) ions and the Brix couldn’t either be related to the taste appreciation. The extraction rate of the compounds studied did not appear to be affected by the temperature of the water either, but the

Barista seemed to be more appreciative of the coffees that had a lower in-cup temperature (lower water temperature during extraction).

At this point no obvious correlation could be found between the appreciation of the taste of a coffee cup and the concentration of the compounds measured with NMR.

The absence of correlation between the concentrations of the bitter compounds and the appreciation could be explained by the fact that the wrong compounds were targeted to be studied. The absence of conclusive correlation could also result from the lack of sufficient data points and approximation in the translation of the Barista's comments into a numerical form to allow statistical analysis.

The project being at its preliminary stage when the study was performed, the conclusion can't be deemed definitive, the provision of extra data points could confirm or infirm the absence of correlation.

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Table 1. Important aromatic compounds in coffee as summarized by Grosch. Click on compound name for more information.

Volatile[1]	Conc. (mg/L)[1]	OAV[1]	Coffee Aroma Description[2]
(E)-ß-Damascenone	1.95×10^{-1}	2.60×10^{5}	honey-like, fruity
2-Furfurylthiol	1.08	1.10×10^{5}	roasty (coffee)
3-Mercapto- 3-methylbutylformate	1.30×10^{-1}	3.70×10^{4}	catty, roasty
3-Methyl-2-buten-1-thiol	8.20×10^{-3}	2.70×10^{4}	amine-like
2-Isobutyl-3-methoxypyrazine	8.30×10^{-2}	1.70×10^{4}	earthy
5-Ethyl-4-hydroxy-2-methyl-3(2H)-furanone	1.73×10^{1}	1.50×10^{4}	
Guaiacol	4.20	1.10×10^{4}	phenolic, spicy
2,3-Butanedione (diacetyl)	5.08×10^{1}	3.40×10^{3}	buttery
4-Vinylguaiacol	6.48×10^{1}	3.20×10^{3}	spicy
2,3-Pentanedione	3.96×10^{1}	1.30×10^{3}	buttery
Methional	2.40×10^{-1}	1.20×10^{3}	potato-like, sweet
2-Isopropyl-3-methoxypyrazine	3.30×10^{-3}	8.30×10^{2}	earthy, roasty
Vanillin	4.80	1.90×10^{2}	vanilla
4-Hydroxy-2,5-dimethyl- 3(2H)-furanone (Furaneol)	1.09×10^{2}	1.70×10^{3}	caramel-like
2-Ethyl-3,5-dimethylpyrazine	3.30×10^{-1}	1.70×10^{2}	earthy, roasty

2,3-Diethyl-5-methylpyrazine	$9.50x10^{-2}$	$1.00x10^{2}$	earthy, roasty
3-Hydroxy-4,5-dimethyl- 2(5H)-furanone (Sotolon)	1.47	$7.50x10^{1}$	seasoning-like
4-Ethylguaiacol	1.63	$3.00x10^{1}$	spicy
5-Ethyl-3-hydroxy-4-methyl- 2(5H)-furanone (Abhexon)	$1.60x10^{-1}$	$2.00x10^{1}$	seasoning-like

Table References

1) Grosch, 151.
2) Blank et al., 124.

From Research, Coffee. [Online] http://www.coffeeresearch.org/.

APPENDIX 2 Compounds contributing to bitterness found in coffee. (1/2)

Compound	Concentration in Roasted Coffee (mg/L)	Taste Threshold (mg/mL)
Quinic	3200-8700	10
5-hydroxymethylfurfural	10-35	200
2-Methyl Furan	0.05	
Furfuryl Alcohol	300	19, 24, 40
Trigonelline	3,000-10,000	
Chlorogenic Acid	20-100	20,26,27
Caffeic Acid		10-90
Citric Acid	1,800-8,700	96-590
Malic Acid	1,900-3,900	107-350

Lactic Acid	0-3,200	144-400
Pyruvic Acid	400-1,700	
Acetic Acid	900-4,000	22-70
Pyrazine	17-40	1
Caffeine	10,000-20,000	78-155

APPENDIX 3 Coffee Acidity Chart: Acids Present in Coffee. (1/2)

Acids Present in Coffee [3]	Notes [3]	Comments
Formic	a	pK_a = 3.75, 130-159 µmole/100 mL.[1] 0.05-0.1% dry matter at med roast. Max at light roast.[2]
Acetic	a	pK_a = 4.75, 74-226 µmole/100 mL.[1] 0.12-0.4% dry matter, max concentration at light roast.[2] Derived from carbohydrate degredation.[2]
Lactic	b	pK_a = 3.08, 22 µmole/100 mL.[1] 0.11% dry matter. Concentration independent of roast.[2]
Pyruvic	b	0.06% dry matter. Concentration independent of roast.[2]
Malic	b	pK_a = 3.40 / 5.11, 58-76 µmole/100 mL.[1] 0.17-0.5% dry matter at med roast. Max at light roast.[2]
citric	b	pK_a = 3.14 / 4.77 / 6.39, 75-189 µmole/100 mL.[1] 0.37-0.5% dry matter, max at light roast.[2]
3-monocaffeoylquinic acid	d	pK_a = 3.40, 96-291 µmole/100 mL.[1]

4-monocaffeoylquinic acid	d	The chlorogenic acids have an astringent taste due to its ability to precipitate salivary proteins onto the mucous membranes. Therefore it may also be responsible for heightened body.[2]
5-monocaffeoylquinic acid	d	At dark roasts, 80% of the CGA's may be lost resulting in a residual CGA content of 2.2-2.4%.[2]
Quinic	e	pK_a = 3.40, 123-242 µmole/100 mL.[1] 0.6-0.8% dry matter at med. roast. Concentration increases inversely with chlorogenic acid.[2]
Phosphoric	f	pK_a = 2.12 / 7.21 / 12.67, 65-108 µmole/100 mL.1 0.54% of dry matter.[2]

Notes

a. Volatile Aliphatic Carboxylic
b. Non-Volatile Aliphatic Carboxylic
c. Heterocyclic furanoid carboxylic
d. Chlorogenic
e. Alicyclic/phenolic
e. Inorganic

Sources

1. Clifford, M. *Tea and Coffee Trade J*. 159: 8. 1987. 35-39.
2. Illy, A. and Viani, R. Espresso Coffee: The Chemistry of Quality. 107-110.
3. Clarke, R.J. *The Flavour of Coffee*. In Dev. Food Science. 3B: 1-47. 1986. 1-47.

APPENDIX 4 Experimental procedures for the study of the effects of DMSO on the compounds studied by NMR. (1/3)

Caffeine

Solution 1: 13 mg pure caffeine was diluted to 20 mL of distilled water and mixed thoroughly to form a homogenous solution.

Sample 1 (S1): A sample of 500 µL was taken from the solution 1 and put into a NMR tube (Wilmad Labglass 5 mm thin wall 7”). A coaxial insert containing hexamethyldisiloxane (HMDS) in deuterated chloroform was added into the sample tube. The sample was placed through a spinner and into the NMR spectrometer. The spectra was obtained using a standard pulse acquire experience. The phase and baseline of the spectra were manually corrected prior to the integration of the HMDS and caffeine peaks.

Sample 2 (S2): A sample of 300 µL was taken from solution 1 and weighted. Then 200 µl of DMSO was added to the 300 µL of solution 1, the mixture was shaken and weighted. Afterwards the mixture was put into a tube, the coaxial insert containing HMDS in deuterated chloroform was added to the sample and the sample was placed in the NMR spectrometer. The spectra was obtained using a standard pulse acquire experience. The phase and baseline of the spectra were manually corrected prior to the integration of the HMDS and caffeine peaks.

Formic acid

Solution 2: 80µL of formic acid was diluted to 20 mL of distilled water and mixed thoroughly to form a homogenous solution.

Sample 3 (S3): A sample of 500 µL was taken from the solution 1 and put into a tube. A coaxial insert containing hexamethyldisiloxane (HMDS) in deuterated chloroform was added into the sample tube. The sample was placed through a spinner and into the NMR spectrometer. The spectra was obtained using a standard pulse acquire

experience. The phase and baseline of the spectra were manually corrected prior to the integration of the HMDS and formic acid peak.

Sample 4 (S4): A sample of 300 µL was taken from solution 2 and weighted. Then 200 µl of 40% DMSO was added to the 300 µL of solution 2, the mixture was shaken and weighted. Afterwards the mixture was put into a tube, the coaxial insert containing HMDS in deuterated chloroform was added to the sample and the sample was placed in the NMR spectrometer. The spectra was obtained using a standard pulse acquire experience. The phase and baseline of the spectra were manually corrected prior to the integration of the HMDS and formic acid peak.

Acetate

Solution 3: 2mL of ethylacetate was diluted to 50 mL of distilled water and mixed thoroughly to form a homogenous solution.

Sample 5 (S5): A sample of approximately 500 µL was taken from the solution 3 and put into a NMR sample tube. A coaxial insert containing HMDS in deuterated chloroform was added into the sample tube. The sample was placed through a spinner and into the NMR spectrometer. The spectra was obtained using a standard pulse acquire experience. The phase and baseline of the spectra were manually corrected prior to the integration of the HMDS and formic acid peak.

Sample 6 (S6): A sample of 300 µL was taken from solution 3 and weighted. Then 200 µl of 40% DMSO was added to the 300 µL of solution 3, the mixture was shaken and weighted. Afterwards the mixture was put into a tube, the coaxial insert containing HMDS in deuterated chloroform was added to the sample and the sample was placed in the NMR spectrometer. The spectra was obtained using a standard pulse acquire experience. The phase and baseline of the spectra were manually corrected prior to the integration of the HMDS and formic acid peak.

Trigonelline

Solution 4: 10mg of trigonelline was diluted to 20 mL of distilled water and mixed thoroughly to form a homogenous solution.

Sample 7 (S7): A sample of approximately 500 µL was taken from the solution 4 and put into a tube. A coaxial insert containing hexamethyldisiloxane (HMDS) in deuterated chloroform was added into the sample tube. The sample was placed through a spinner and into the NMR spectrometer. The spectra was obtained using a standard pulse acquire experience. The phase and baseline of the spectra were manually corrected prior to the integration of the HMDS and formic acid peak.

Sample 8 (S8): A sample of 300 µL was taken from solution 4 and weighted. Then 200 µl of 40% DMSO was added to the 300 µL of solution 4, the mixture was shaken and weighted. Afterwards the mixture was put into a tube, the coaxial insert containing HMDS in deuterated chloroform was added to the sample and the sample was placed in the NMR spectrometer. The spectra was obtained using a standard pulse acquire experience. The phase and baseline of the spectra were manually corrected prior to the integration of the HMDS and formic acid peak.

APPENDIX 5 (1/2)

The effect of DMSO on the concentration of caffeine in the coffee was investigated. It could be noticed that for coffee solution of known concentration the values measured without DMSO were always far-off from the expected theoretical values. The table below present the concentrations of caffeine measured before and after the addition of DMSO-d6 in standard coffee solutions of 2g/l caffeine.

The standard solutions were prepared by mixing 3 bag of Nescafe Decaf (weight: 1.8g) in 90mL of boiled tap water and adding to it a known amount of caffeine 180 mg. In order to obtain a 2g/l caffeine concentration.

Concentration of caffeine before the addition of DMSO (g/L)	Concentration of caffeine after the addition of DMSO (g/L)
1.13	1.80
1.09	1.77

APPENDIX 6

Sample reference	Temperature	Comments
Rinse water	63.4	
B2.1	68.3	1st cup of Syntia, stale, smells woody, old coffee, tastes ashy (old beans), lacks acidity. Temperature tak
B2.2	72.9	2nd Syntia
B2.3	72.7	3rd Syntia
B2.4		4th Syntia
B2.5	77.3	5th Syntia, lack of viscosity:More bitter and less acid then B2.1
B2.6	75.6	
B2.7	77.7	
B2.8	78.9	
B2.9	77	
B2.10	79.5	Big difference in smell, almost no aroma. Big variation versus B2.1 and B2.5. No nice smell.
B2.11	79.2	Very little difference with B2.5 slightly more bitter. Would not recognize it as Illy coffee. Lots of bitter ar
B2.12	79.4	
B2.13	78.2	
B2.14	78.5	
B2.15	80.7	Even more bitter than than B2.10. Much less enjoyable than B2.5 and B2.1
B2.16	80.5	
B2.17	78.7	
B2.18	79.4	
B2.19	78.7	
B2.20	79	A little less bitter. Same as B2.15
Brewing unit cleaned and cooled under flowing water.		
B2.21	68.6	Huge difference. Smells like coffee again. Best so far! Pretty.
B2.22	75.9	Again more bitter
B2.23	71.2	Brewing unit cleaned again and cooled down. Slightly less bitter than B2.22 but worse than B2.21
B2.24	73.4	Slightly less bitter than B2.21. Less astrigent than B2.21 Still way too bitter

APPENDIX 7

Correction factor

The correction factor was calculated experimentally in this manner:

- 300 µL of D2O was weighted
- 300 µL of DMSO was weighted
- The DMSO-d6 and the D2O were mixed and a sample of 300 µL was taken from the mixture and weighted.
- The value for the correction factor was found by dividing the weight of the 300 µL water by the weight of the mixture of D2O and DMSO-d6.