UNIVERSITY OF UDINE

FACULTY OF AGRICULTURE

Doctoral course in Food Science
Department of Food Science
(Course XXIV)

DOCTORAL DISSERTATION

Effects of Origin and Treatment of the Roasting Process on the Aromatic and Sensorial Composition of Coffee

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ACADEMIC YEAR 2011-2012

UNIVERSITY OF UDINE

Faculty of Agriculture

Doctorate in Food Science

XXIV cycle

This thesis is presented

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ACKNOWLEDGMENTS

Many thanks to Professor Roberto Zironi and Dott. Franco Battistuta, my major advisors. They have not only supported me with encouragement and challenges, but have also been exceptional mentors and friends.
I would also like to thank my colleagues who have helped me in many situations: dr. Piergiorgio Comuzzo, dr. Lara Tat, dr. Stefano Micolini, dr. Laura Brotto, dr. Sergiu, dr. Deborah and all the others, Paolo, Stefano, Lorena, Milena, Matteo, Lavinia and ACAUD association for my sensorial analysis.
I want to extend my gratitude to Dott. Alessandro Rettore, general manager Julius Meinl and quality manager Dott. Sonia Castracane who gave me opportunity to provide coffee samples and analysis support together with Ugo, Michele, Giovanna, Federico and Massimo.
I would also like to thank Luigi Pallotti manager at Pura Vida Cafè and Alberto Polojaz manager at Imperator coffee who gave me help and support in organizing sensory analysis and panel (judges) training for my project.
Finally thanks to my parents, Vito and Nicoletta my sister Michela my grandmother Margherita, who gave me a solid foundation not only for academics, but also for life and love.

ABSTRACT

The quality of espresso coffee generally arises from raw quality (genetic factors, typical of species and variety, soil, weather, agronomic processing, post harvesting processing) and roasting degree. From the ripe coffee cherry to roasted coffee roasting is the most important step in the processing chain. Physical and chemical properties of roasted coffee are highly influenced by process conditions during roasting, in particular by the time-temperature parameters (roast curve) within the coffee bean as a function of heat transfer. Chemical changes take place during the roasting process and reaction pathways lead to the formation of a wide range of volatile organic compounds that are quite abundant in coffee headspace. Numerous publications have appeared on the nature of reactions leading the roasted coffee flavour explaining that there are several hundred volatile compounds identified in coffee. Around 30 of these have been identified as aroma impact compounds. Scientific information on how new technologies affect the aroma quality of coffee roast and the brewed beverage is poor and sometimes contradictory. This scientific work was sampling of different single coffee raw and for every coffee was roasted with degree roast light, medium and dark. There were three replicates for treatment. The first step of the current work was the identification of the different provenance of 6 *Arabica* raw coffee samples and their "*quality markers*" analyzing thoroughly the respective peculiarity. This study had the merits of identifying the volatile compounds of single green coffee and so in this way, it was possible to conclude how the different provenance had present a strong influence on the chemical compounds affecting the final quality. Investigation of potent odorants from green coffees was performed using head space solid-phase microextraction in conjunction with gas chromatography-olfactometry. The second step of the current work was the identification of aroma compounds in roast coffee. All trials were run to an equal roast end point as defined by the lightness of coffee beans. The raw Arabica samples were roasted

to 3 different roast degree *(light, medium, dark).* The developing aroma compounds profiles were characterized by gas chromatography, mass spectrometry, and olfactometry. Another objective of this work was to verify how different roasting processes might affect sensory properties of coffees of different provenience. The third step was to identify the possible molecules called quality of "*markers"* able to correlate positively the singles roasted coffee based on the composition of the volatile fraction and the overall point of view of the sensory analysis. The main goal was to investigate, also the evolution of classes aroma compounds during roasting under with different time conditions and to compare experimental results with sensory analysis of espresso coffee. The results were evaluated by statistical analysis using PCA, Cluster Analysis, and Variance analysis.

It was observed that the aromatic profile of roasted coffee was totally different from raw coffee, and comparing different roasted coffee the effect origin green coffee results more important than effect treatment roasting. As a result of sensory analysis together with analytical valuation of the aroma compounds, there are several behavior of the coffees tested in relation to the roasting degree and the provenance. Moreover 19 note odours, possible *"markers"* were identified for all samples describing the coffee aroma in accordance with a few odours reported in literature. These possible "*markers"* provided sufficient differentiation for coffee samples of different origin and roasting treatment. The "*markers"* also present a good correlation with many flavour compounds and after-taste descriptors and it is possible to characterize the single coffee. A system is already in place to assess the intensity of the roasting process placing it alongside a sensorial analysis. Compounds like these are true examples of "cause and effect". They show that the variable degree of intensity of a process brings about a variation in the sensorial profile. With this in mind, a further series treatment "*markers"* has been established, although there is still some way to go before a definitive system can be set up due to the complexity of the "*markers"*.

CONTENTS

FIRST CHAPTER

"Introduction to green coffee"

1.1 Raw coffee Quality

It is well known that coffee cannot be considered a "food" consumed for its nutritional values, but it represents more the following a pleasure, a socio-cultural moment, an excuse to meet friends, a way to be ready and active for the daily jobs, and the quality must be the main requisite because it is directly related to positive moments in our lives. Coffee quality means a good raw material and flawless processes and techniques that go from the seed to final the beverage; each of them should be carried out correctly in order to respect the typicality of the coffee origin. The quality of coffee as a beverage is strictly related to the chemical constituents of the roasted beans, whose composition depends on the composition of green beans. Nowadays, the quality definition varies along the production consumer chain:

- at the farmer level: coffee quality is a combination of production level, price and easiness of cultivation;
- at the import-export level: coffee quality is linked to bean size, presence/absence of defects (fig 1), regularity of provisioning, tonnage available, physical characteristics and price. The ISO (2004) defined a standard for green coffee quality (ISO 9116 standard). It requires several pieces of information, like the geographical and botanic origins of the coffee, the harvest year, the moisture content the total defects, the proportion of insect-damaged beans and the bean size.

1.1.2 Main Defects in Coffee Beans


Black


Stinkers


Stones and Sticks


Floaters


Dried Cherries


Broken


Shells


Insect Damage


Malformed

G
Aromatic and Sensorial Composition of Coffee.

In the world of coffee world production it is more useful to talk about of a lack of quality meaning presence of defects instead of coffee quality; each coffee origin presents typical features related to the native place which should be valorised thanks to man's operations creating a product free of off-flavours but with the typicality of the origin state. Robusta coffees for instance have lower market value with respect to Arabica varieties, due to their organoleptic features, but it is important to underline that high quality coffees can come either from Robusta and Arabica, respecting their typicality. Robusta is an important resource for blends exactly for its peculiar sensory characteristics.

- at the roaster level: coffee quality depends on the botanical variety of coffee, processing, grinding, packaging and especially the roasting process, extraction method and moisture content;
- at the consumer level: coffee quality deals with price, taste and flavour, effects on health and alertness, geographical origin, environmental and sociological aspects. It is also fundamental to understand how the quality is understood by the customer: in terms of expected quality, perceived quality and effective quality related to the measurable coffee parameters (illy *et al*., 2005).

1.2 Raw Coffee (about Arabica and Robusta)

There are two main species commercially cultivated and economically important *Coffea canephora* (predominantly a form known as 'robusta') and *Coffea arabica*. The two species present considerable differences in their botanical, genetic, agronomical, chemical and morphological characteristics and are adapted to very different ecological environments. All coffee plants are classified in the large *Rubiaceae* family.

Arabica *(Coffea arabica)* (fig 2-3-4), the most highly regarded species, is native to the south western highlands of Ethiopia. The best known varieties are 'Typica' and 'Bourbon' but from these many different strains and cultivars have been developed, such as Caturra (Brazil, Colombia), Mundo Novo (Brazil), Tico (Central

America), the San Ramon and the Jamaican Blue Mountain. All these cultivars are diffused in subtropical zones at high altitude with low temperatures and season specific precipitations concentrated. The average *C arabica* plant is a large bush with dark-green oval leaves. It is genetically different from other coffee species, having four sets of chromosomes (2*n* = 44) rather than two. *Coffea* Arabica is characterizes by self-pollination, also known as autogamy (Lashermes *et al*., 1996). The fruits are oval and mature in 7 to 9 months. Since Arabica coffee is often susceptible to pest attacks and diseases, resistance is a major goal of plant breeding programmes. Arabica coffee is grown throughout Latin America, in Central and East Africa, India and to some extent in Indonesia Arabica coffee usually receives a premium for its superior flavour, aroma and lower bitterness. Arabica coffee costs twice as much as Robusta. It is more suited to higher cooler climates (600-2000 m altitude and 15-20 °C).

They are evergreen shrubs or small trees that may grow 5 m tall when unpruned. The leaves are dark green and glossy, usually 10–15 cm (4–6 in) long and 6 cm (2-4 in) wide. The flowers are axillary, and clusters of fragrant white flowers bloom simultaneously and are followed by oval berries of about 1,5 cm. Green when immature, they ripen to yellow, then crimson, before turning black on drying. Each berry usually contains two seeds, but 5–10% of the cherries have only one; these are called peaberries. Cherries ripen in seven to nine months.

Fig 2 - http://it.wikipedia.or

Fig 4 - http://it.wikipedia.org. Flowers – coffea Arabica

Robusta *(Coffea canephora)* (fig 5-6) is native to sub-sahariana West Africa (Uganda state). Robusta coffee is spread out in Brazil (a variety called Conillon), India and south-east Asia (Indonesia and Vietnam). In these latter countries the cultivation of the *C. canephora* was introduced in 1990 with intensive cultivation taking these country to high production levels. There are many different Robusta varieties. In general, they can thrive in hotter lowland areas (below 900 m altitude and above 20 °C of temperature). *Coffea canephora* (2*n* = 22) presents heterogamy where the pollen of a flower prevents the fertilization of the ovary of the flower from the same plant (Lashermes *et al*., 1996). The fruits are rounded and take up to 11 months to mature; the seeds are oval in shape and smaller than those of *C. arabica*.

Fig 5 - Fruit mature - Coffea canephora

Fig 6 - Flower – Coffea canephora

World coffee production (fig 7) is about 60% Arabica and 40% Robusta and it continues to show large annual fluctuations. Since 2009 Brazil has been the world leader in the production of green coffee. The Bahia State is considered among the biggest Brazilian coffee production regions, reaching 7% of the total product, just after the Paranà, Minas Gerais and San Paolo States followed by Vietnam, Indonesia and Colombia. Arabica coffee beans are cultivated in Latin America, eastern Africa, Arabia, or Asia. The mature coffee fruit is constituted of a red drupe (yellow for a few varieties) called cherry (fig 8).

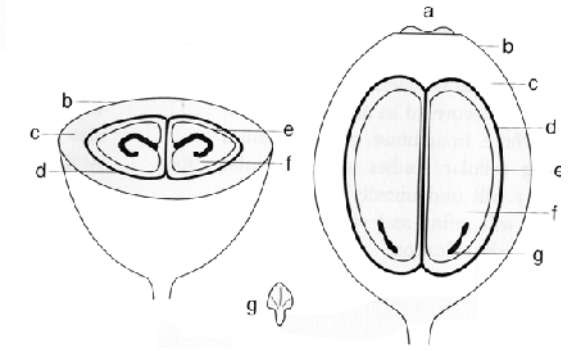


Fig 8. Anatomy of the fruit: a disk:

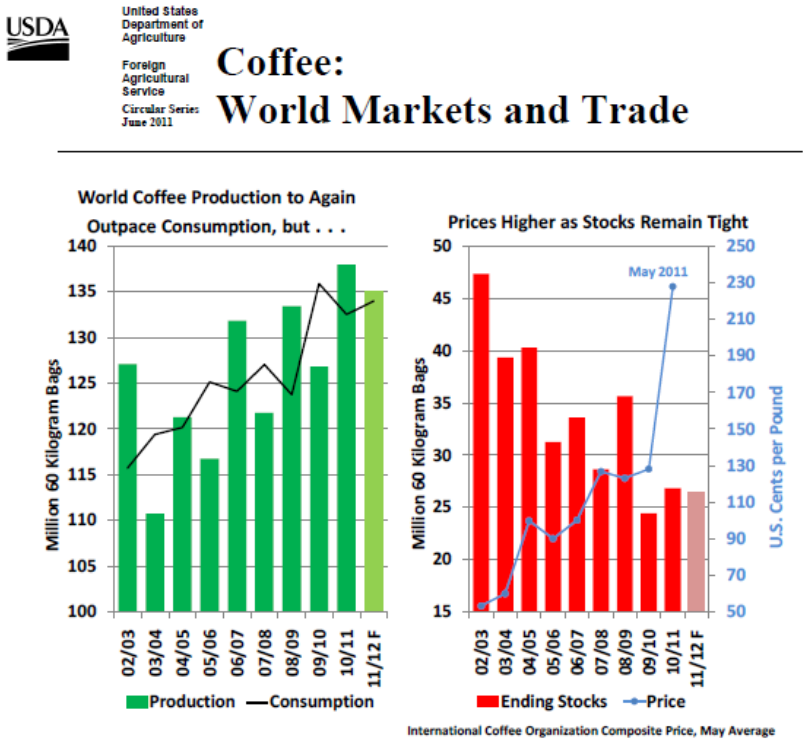
b esocarpo
c mesocarp
d endocarp (parchment)
e silvery pellicle "silver skin"
f endosperm
g embryo

(Wintgens, 2004).

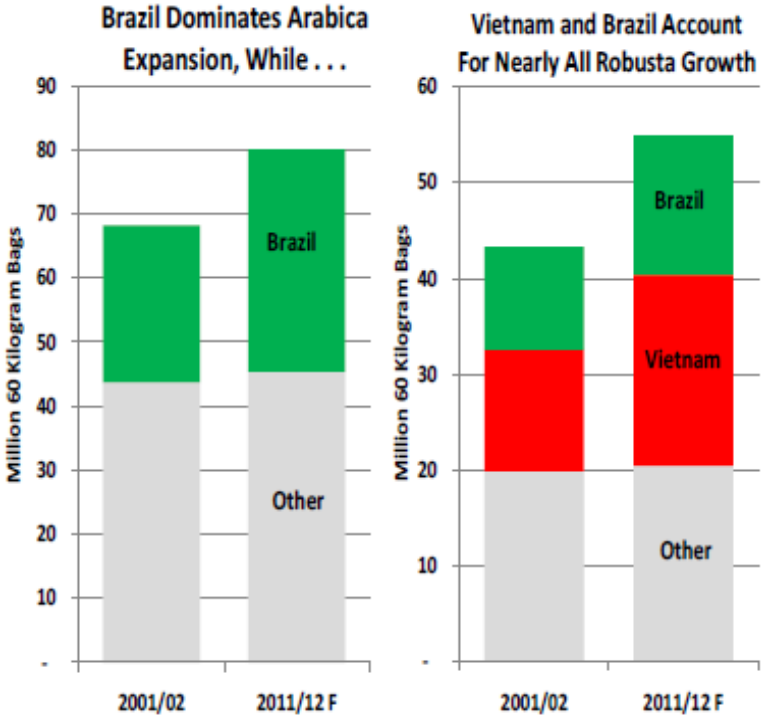
This fruit contains a thin layer of pulp, a hydrate layer called mucilage and two flat beans surrounded by a little cover called parchment. The chemical composition between two species is different. The principal differences are:

1) caffeine content: 1,2% in Arabica while 2,5% in Robusta coffee
2) lipid content: 16% in Arabica and 10% in Robusta
3) diterpenes cafestol and kahweol contents are typical in Arabica while only cafestol is present in Robusta coffee.

C. arabica and *C canephora* are not only different for their genetics, agronomics and chemicals characteristic, but for their overall beverage derived from beans. Arabica coffee is more acidic and sweet with aromatic notes (chocolate, caramel, floral, fruity). On the other hand Robusta produces a more bitter and astringent beverage with earthy, woody and medicinal odors. As shown below, official data graphs reported by USDA (United States Department of Agriculture) with regard to coffee production in 2010.

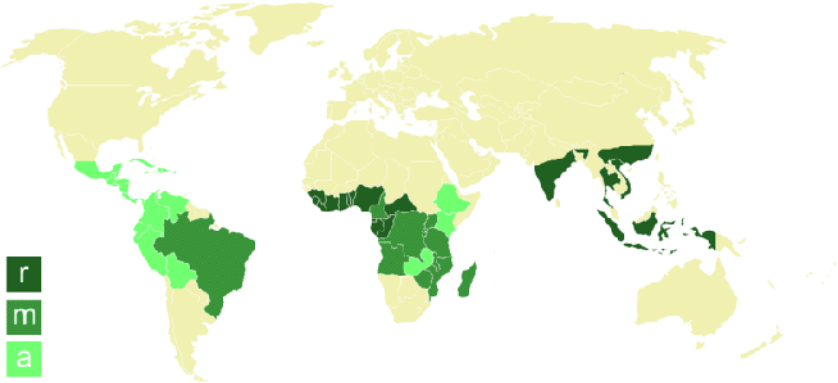


World coffee production in 2011/12 is forecast to outpace consumption for a second consecutive year, although ending stocks are expected to remain tight. Production is forecast to decline a moderate 2.9 million bags, whereas previous down years have declined as much as 16 million bags. However, stocks are forecast to remain at diminished levels, leaving little cushion should there be a supply problem. In response to the multi-year stocks drawdown, prices have spiked.



Arabica and Robusta productions have increased by nearly equal amounts in the last decade, expanding 11.9 million and 11.6 million bags, respectively. As a result, the ratio has not changed significantly from 60 percent Arabica to 40 percent Robusta. The vast majority of the Arabica growth has come from Brazil, with gains in Honduras, Peru and Nicaragua partially offset by losses in Colombia, India and Costa Rica. Nearly two-thirds of the Robusta gain has been from Vietnam and one-third from Brazil.

Map showing areas of coffee cultivation:



r= Robusta
a= Arabica
m= Arabica and Robusta

Fig 7- http://planetsave.com/

1.3 Harvesting

A fundamental pre-requisite for a good quality coffee is to harvest only ripe cherries which have to be processed immediately according to illy (1995 and 2005). Table 1 represents approximate different coffee harvest seasons.

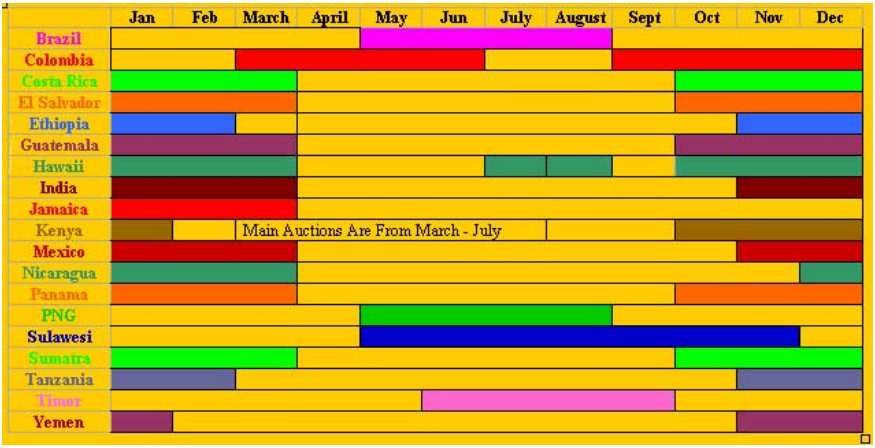
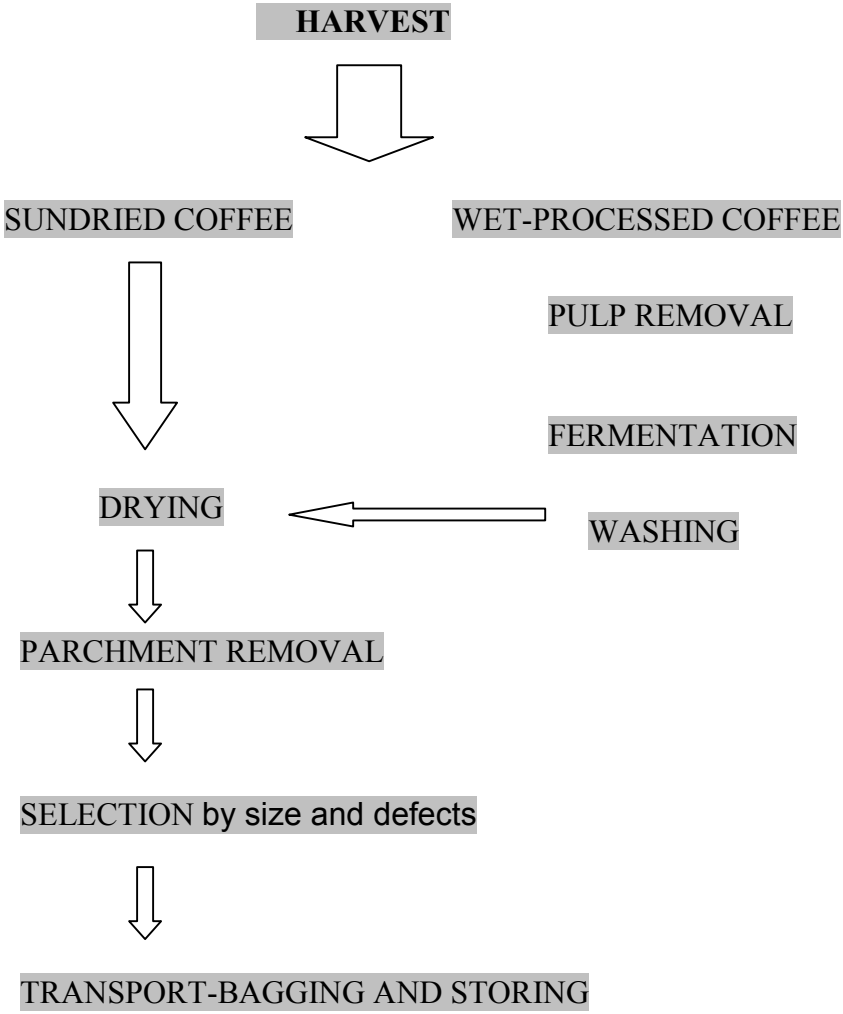


Table 1- www.lostdutchmancoffee.com

Harvesting can be done in two ways, either by stripping or by picking. In strip harvesting all the cherries are stripped together with leaves from the branches at the same time either by hand or mechanically. In the picking method, only ripe cherries are hand-picked, and collected in baskets or heavy pieces of cloth laid underneath the trees (illy 1998). The cherries can follow three different processing paths, depending on the kind of desired raw coffee: natural, wet and semi-wet processing.
In the following scheme explains the green coffee chain.

COFFEE CHERRY HARVESTING



1.3.1 The dry process called "Natural coffee or unwashed"

The dry process (fig 9) is the oldest and simplest method of processing coffee. The dry process is often used in countries where rainfall is scarce and long periods of sunshine are available to dry the coffee properly. The dry method is used for about 95% of Arabica coffee produced in Brazil, most coffee produced in Ethiopia, Indonesia and Paraguay, and some Arabica produced in India and Ecuador. Harvesting usually starts when ripe cherries make up 70-85% of the crop, depending on altitude, labour availability total crop output for the year, and the drying capacity at the farm. The whole crop must be harvested within 3-4 months to allow the tree to recover for the next season. Cherries brought from the field are first freed from impurities by compressed air or screens and by washing. The coffee goes through a rapid separation in order to remove impurities and to clean it from leaves, little stones, pieces of wood. It is usually done after a non-selective harvest, which means that cherries are usually harvested mechanically and present different stages of ripening. The coffee fruits are spread on the ground (earth, platforms, concrete or asphalt) in layers approximately 10 cm thick, heaped at night and respread each day (Schwan and Wheals *et al*., 2003). After that, the cherries are placed directly on drying patios until the moisture is around 12%. It is an economical method since it does not require particular machinery or water. The best patios should be in cement or brick, easily washable, and good heat conductors. It is quite common to place cherries on the ground exposing the coffee to the risk of absorbing strong off-flavours. It is also important to move the coffee often throughout the day in order to avoid mould formation responsible for off-flavours formation. The coffee is covered overnight and protected from the night cold and damp. This method usually goes from 15 to 20 days depending on the climatic conditions especially on the temperature and the relative humidity. Once dried, the parchment is removed with machinery, cleaned and bagged.

Fig 9 - Drying coffee (http://www.coffeeresearch.org/agriculture/harvesting.htm)

Over the course of 10-25 days of sun drying, natural microbial fermentation occurs which can influence the final quality of the product (Schwan and Wheals *et al*., 2003; Silva *et al*., 2000). If it is not performed correctly, undesired fermentations may occur in the sugary cherry mucilage: lactic, acetic, propionic and butirric fermentations; the latter two are responsible for important rancid off-flavours (illy, 2008). It is clear that acetic, lactic, butyric and propionic acids are products of microbial fermentation and their presence is correlated to the presence of microorganisms. The microbial succession is different (Gram-positive bacteria, yeast, fungi) and the number of individuals and species is influenced by the moisture and *aw* content, chemical composition of the coffee cherries, stage of ripeness, drying platform type, temperature, competition for substrates an the enzymatic capacity of the colonizing species and antimicrobial activity (Ferreira *et al*., 2008). During the dry method the exposure time varies because climatic conditions during fermentation can be different in each coffee-producing area. The presence of butyric acid generally gives an unpleasant flavour to the beverage and thus results in quality loss. Acetic acid present in the pulp and mucilage fractions is produced in an aerobic metabolic process that can be of bacterial origin or the product of the oxidation of yeast-produced ethanol (Silva *et al*., 2008). The bacteria present on the surface of coffee cherries produce this acid, which can then migrate to the pulp and mucilage, where it can interfere with the

organoleptic quality of the beans. On 8th day of fermentation, the pulp and mucilage can present the greatest quantified value of lactic acid; this is the last day when malic acid can be detected in a sample (Silva *et al*., 2008). Bacteria, yeast and filamentous fungi have already been reported during fermentation by the wet method (Masoud *et al*, 2006; Avallone *et al*., 2001), but only one comprehensive study of dry processing has been published (Silva *et al*., 2000). The microorganisms involved in dry processing are much more varied and complex than those found during wet fermentation.

1.3.2 The wet method called "washed"

After harvesting the cherries are cleaned from impurities like little stones, leaves, twigs, through sieves or nets. This separation is very important for the final quality in order to differentiate ripe cherries from immature ones. It is common to use water channel (fig 10) according to density differences: for example *boia* coffee (fig 11 unripe-not heavy) are separated from ripe beans. These *boia* cherries present low quality and Rio-off flavour.

Fig 10 - Coffee beans ready for separation step by water channel

Fig 11 - Coffee beans "*Boia*" (http://www.coffeeresearch.org/agriculture/flavor.htm)

After quality cherries are processed as "dispulpado" they are pulped. After that it is important to remove the sticky mucilage surrounding the bean by skinning with drum, skinning with disk or skinning a vertical drum (fig 12).

MUCILAGE COMPOSITION (%)

- Total pectines 33%
- Reducing sugars 30%
- No Reducing sugars 20%
- Cellulose, ash 17%
- Pectolitic enzyme
- Yeasts (*Saccaromyces* spp.)
- Microorganisms (*Erwinia* spp.)
- Lactic bacteria,
- Enzyme pectinolytic
- Moulds (*Apergillus* and *Penicillium* spp)

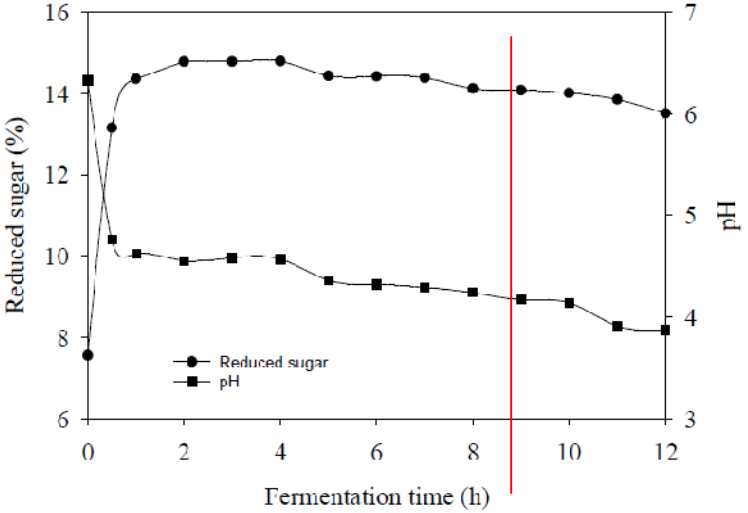
Fig 12 - Skinning a vertical drum

The "dispulpado" coffee still contains parts of pulp mucilage, insoluble in water, strongly hygroscopic and adherent to the parchment that must be eliminated by fermentation before processing to the drying. The main problem in this step is improper fermentations which must be avoided. The pulp left in the bean coffee contains about 17% of sugars, another 1% of pectin substances, and 13% protein. However, the conditions, they provide exceptionally useful nutrition for the wild microbial flora. The fermentation process starts after 24-48 hours digesting the residual pulp and the coffee beans acquire specific flavours and odours. The temperature of either of these processes is scarcely raised over 50 °C above ambient temperature reflecting the lack of oxygen diffusion to the heart of the mass.

1.3.2.1 Key concepts of the "wet" fermentation method

Field research has shown that coffee cherries contain a wide range of sugars and proteins depending on its species, weather, ripeness degree, management, soil, and sunlight. The green coffee bean affects the quality of brew coffee. It is very important to avoid irregular fermentation and undesired reactions. Determination of proper fermentation of coffee cherry is a key point. Well-fermented green coffee beans must be completely

cleared of mucilage. The fresh mucilage has pH original about 6.5 falling to a minimum of about 3.1 to 3.3 (Cheng-Chang Lin *et al*., 2010). Because improper fermentation, the badly odors coffee beans are required washing out with substantial amounts of water in tanks with machines. Coffee enzymes and bacteria break down the mucilage converting the structure from a hydrogel into a hydrosol (illy 2008). The first part of the reaction is caused by enzymes such as pectase and pectinase naturally present in the cherry. The mucilage which covers the pulped bean is a kind of pectin needed for a proper degradation to sugar which contributes a pleasant sweet and caramel flavour during roasting. Sugars and amino acids are precursors that drive the caramelization process called Maillard reaction. This reaction progresses during roasting to produce brownish, bitter-sweet glycosylamine and melanoidins donating a favorable coffee taste. Finally, the product taste is balanced sticky, thick, and mellow. In a research conducted (Cheng-Chang Lin *et al*., 2010) the fermentation was operated by mixture of *Aspergillus niger* and enzymes and shows good results (fig 1). As a result of the chemical reactions during fermentation, reduced sugar initially increases from 7,55% to the highest level of 14,78% at 2 hours maintaining the level up to 4 hours then gradually decreasing to 13,51% at 12 hours (graph 1).



Graph 1: Characteristics of green coffee beans fermenting at different time (Cheng-Chang Lin *et al*., 2010)

At the relative point, pH changes from the initial value of 6.33 sharply decreasing to 4.62 at 1 h, and down to 4.55 at 2 h, to 3.87 at 12 h. It is important to promote endogenous species in order to control pH values during fermentation; as these pH values decrease enzymatic activity slows down. The benefits of this fermentation were:

1. The fermentation time decreased to 1 h from the natural fermentation of 24 h. It was very economical for a small farmer to finish the treatment of coffee cherries in an day of harvest.
2. The product of green coffee bean produces higher reduced sugars (from 7,98 to 15,52%) and weaker acidity (pH from 3.07 to 4.64).
3. Coffee cupping resulting taste was more sweet, full bodies, pure and with caramel flavour.
4. The amount of waste water went from 10~18 kg to 1 kg for 1 kg of green coffee bean.

It is reasonable to say that the reduced sugar is a derivative compound of degrading mucilage (natural fermentation) or pectin by yeast, fungi, and acetic and lactic bacteria continually fermenting reduced sugar to produce alcohol, acetic acid, and lactic acid which lead to lower pH. (Moreover if the fermentation went on for 72 hours it could originate "*stinker"* with other defective formations). Instead if there are other (butirric, propionic) bacteria promoted in a short time at high temperatures and humidity they are responsible for rancid off-flavours. The optimal condition is to have reduced sugars at about 14% and pH 4.62 (4 hours). The strong acidity is a defect for the coffee taste and longer time fermentation causes weight loss that can reach 30-40% of volume reduction (mesocarp elimination) as well as higher costs. At the end of the fermentation (usually from 18 to 48 hours) there is a physical separation between the parchment and the containing sugars and pectin cells. The mucilage has to be removed through fermentation which takes place in tanks filled with large amounts of clean water with special washing machines. The coffee bean is releases part of component or nutrients during washing with high power water. Its clear that method wet and dry are two process different and fig 13 showed phisycal differences.

Fig 13- Differences between natural (right) and washed coffee (left) o the drying patio

1.3.3 Semi wet method

The method is collocated in the middle between the dry and wet methods. Cherries are cleaned from impurities and then separated by flotation and pulped with the same techniques adopted for the washed coffee. The mucilage is not fermented instead the cherries go directly to drying patios. During drying, the handling of the coffee layer is very important because the mucilage can ferment irregularly. At the end of the wet, dry and semi wet methods there is the drying. The correct management of this step is important for the quality of the coffee, because it presents several critical points that can affect the final results. There are two common methods for drying coffee: natural or mechanical (40 °C reaches a moisture not less than 13%). The best patios are in brick or cement with respect to natural patios on the ground (mould contamination), and the asphalt (polycyclic aromatic hydrocarbons contamination). During drying it is fundamental to maintain the bean humidity constant and to avoid undesired fermentation. It is important to cover and protect overnight or during the humid days with plastic covers.

There are several methods to control the critical points from harvest to bagging, storing and to improve the quality.

1) de-stoning: to eliminate heavy materials (stone) and metallic bodies
2) densimetric sorting (fig 14): it is a selection according to the weight and it eliminates impurities such as straws, light stones, broken beans
3) size selection: beans are selected by size and shape
4) colour selection: it eliminates black, green, brown beans which are known to be defective
5) moisture control must be < 12% (DM 20/05/76)
6) ochratoxins analysis must be < 8ppb for raw coffee (Reg CE nr. 123/2005 of the Commission of 26/01/05, Circ Min nr. 18 dd 16/11/2000)

Fig 14 - Densimetric sorting - www.comunicaffè.com

1.4 Bagging and storing

The preservation of the desirable sensory attributes of coffee essentially depends on the storage of the product. Storage is one of the stages following production that strongly influences the commercialization of coffee beans. Raw coffee beans are usually and traditionally stocked and transported in jute sacs of 60 Kg each.
During storing it is important to implement correct management to prevent mould formation and ochratoxins development and protect coffee from insects and other contaminants. A few step correct management are:

- hygienic conditions must be respected
- bag rotation in order to send the oldest ones first
- correct ventilation. Rapid deterioration in quality when the beans are stored without ambient air control. One of the challenges facing coffee bean exportation is maritime transport over long distances for prolonged periods and the quality of the product is degraded by the time the beans arrive at their destination (Harris and Miller, 2008). During shipping, prolonged storage under inadequate conditions in traditional jute sacks exposes beans to potentially harmful variations in ambient conditions, including fluctuations in temperature and relative humidity.

- sacks placed on pallets and not on the floor (at least 30 cm from walls and from other bags)

Jute is most frequently used because it is readily adaptable to small-scale commerce and because it is easily sampled for lot inspections but there are a few disavanteges (Ribeiro *et al*., 2011) that are:
- elevated operational costs that result from the need for manual handling
- rapid deterioration in quality when the beans are stored without ambient air control
- ease of mechanized handling, along with operational economies of scale
- jute is permeable to water vapour and to gases present in ambient air (Borém *et al*., 2008a)

1.5 Methods for evaluating the coffee quality (cupping)

The final coffee product is classified with respect to its aspect and its sensory properties before being marketed. Coffee experts analyze raw coffee only thanks to their experience in order to classify it by post harvesting methods and by the number of defects (European countries accept natural coffee with less than 20% of defects and washed with less then 10% of defects). Cupping (fig 15) is a method of evaluating different characteristics of a particular coffee of different origin before being roasted.

Fig 15 - Coffee Tasting Table (http://www.ineedcoffee.com/04/cupping)

The method consists of using the infusion type brewing. The raw coffee samples come roasted very light and grinded. Place the 10 g of grinded and added 100 mL of hot water 95 °C in a small bowl and to around 3-4 minutes. Take a deep spoon (a table spoon is a good substitute for the traditional cupping spoon) and fill it with infusion. To taste place the spoon in the mouth, and inhale drawing the coffee to the roof of the mouth to tickle the tongue and then fall into the back of the mouth. This creates a coffee vapour to stimulate that part of the sense of taste which is actually the sense of smell. The technique of valuation focuses on finding defects and differences on the inside of the raw coffee lot.

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SECOND CHAPTER

"Characterization of volatile compounds in raw Arabica coffee of different geographic origins"

2.1 Introduction

Different coffee varieties, growing conditions, and processing methods contribute to the distinctive aromatic compounds unique to each type/origin of green coffee. Coffee beans contains all the different necessary precursors and chemical compounds that affect the quality of final coffee products. The aroma of coffee, in particular, is one of its most important attributes, because coffee quality is assessed largely on the basis of its aroma flavour. There are more than 800 coffee volatiles of which 230 in raw coffee (Clarke, 2001) and vary in their aroma, quality, potency and concentration, changing the contribution of each one in the overall aroma. It is known that the volatile fraction of coffee beans develops primarily in the form of alcohols, acids, esters and aldehydes (Barel *et al.,* 1976; Full *et al*., 1999) during post-harvest processing. Those that result from the fermentation stage are alcohols, esters, aldehydes and acids. Therefore, during recent decades, much research has been devoted to elucidating the numerous factors that influence the chemical composition of raw coffee. Indeed, in different origin levels in aroma precursors may vary with genetic traits (Leroy *et al*., 2006), soil-climatic conditions (Bertrand *et al*., 2006), agricultural practices (Vaast *et al*., 2006), storage conditions and post-harvest techniques (Selmar *et al*., 2006) differences specific to the single place. For example about the post-harvest technique in Mexico, the mucilage removal occurs under dry conditions (Bailly *et al*., 1992a), while in Kenya, fermentation is often carried out in water to prevent over fermentation of the mucilage (Vincent, 1971; Mburu, 1999). Based on the type of post-harvest treatments and quality of raw coffee there are formation of specific compounds. In literature there is information about, possible fermentations during the drying step the generating the formation of several compounds and the final quality of the product (Schwan *et al*., 2003; Silva *et al*., 2000). For example two types of compounds are formed namely from thermal reactions during drying such as aldehydes, acids formed by the Maillard reaction between sugars and amino acids. The 2 and 3-methyl-butanal and butanoic acid

may also have a thermal origin by transamination and decarboxylation of amino acids, and through thermal degradation and oxidation of butanol (Spadone *et al*., 1990; Cantergiani et al., 2001). Pyridines are present in immature beans (Farah *et al*., 2008). Butyrolactone is present in immature or fermented beans with sweet notes (Toledo & Barbosa, 1998), acetic acid with sour notes formed from undesired spontaneous fermentation, 2 pentanol an alcohol is present in both healthy and stinking coffee at the same concentration level with harsh, chemical odour, reminiscent of fused oil, but not heavy (Flament 2002). 1-hexanol is an alcohol in healthy and stinking beans (coffee called in Brazil "*Rio*") with an phenolic unpleasant note. This off flavour is caused by the interaction of insects and bacteria (Bouyjou *et al*., 1999). The variegated coffee bug and other insects inflict wounds on unripe coffee cherries so that methoxypyrazine producing bacteria can penetrate them. The presence of 2,3 butanediol (*meso*) and 2,3 butanediol (*levo*), is identified in stinking green coffee. Its formation is due to undesired fermentations during the phase drying (Flament, 2002 and Gonzales-Rios *et al*., 2007). The propanoic acid, the 3-methyl butanal known to play a role in aroma (rancid flavour) development during fermentation (Gonzales-Rios *et al*., 2007; Flament 2002; Semmelroch & Grosch, 1996). Flavour compounds are significant contributors to the coffee aroma; they create a typical fingerprint characteristic for post-harvesting treatments beside the main families of chemical compounds responsible for the volatiles in roasted coffee. Based on published work, the most abundant compounds in the head space of green coffee are alcohols, in accordance with the most recent and comprehensive study on green Mexican and Hawaiian coffee (Kwang-Geun Lee *et al*., 2002). Although less abundant, aldehydes, hydrocarbons and organic acids are also in the more intense head space profile. Hydrogen sulphide, methanethiol and dimethyl sulphide have been proposed as indicators to distinguish coffees of different origin (Rhoades JW *et al*., 1960). Comparing the robusta coffee with arabica coffee in a work by Yeretzian (2001) the *C. arabica* yelds stronger methanol and ethanol peak presence. Also in this work the most abundant

class of compounds identified was aldehydes, 1% in Arabica and 3% in robusta. The third most abundant head space compounds were acids with 0,2% in arabica and 0,6% in robusta. This complex aroma is strongly influenced by numerous factors like the geographical origin of the plant, its botanical variety and the formalities of workmanship of the fruit and the seed after the harvest. All these factors contribute to giving to every type of coffee a unique aroma, distinguishing it from both the sensory and chemical point of view. The aim this chapter was to qualitatively investigate and characterize the volatile fraction of arabica coffee from different geographic origin (Africa, Central America and South America) by SPME technique. These origin-dependent coffee compounds could be correlated to the quality perceived by the final consumer in the cup of coffee. However, there are only a few reports on the identification and quantification of volatile components from raw green coffee beans in particular about different provenience geographic. In this paper we will try to determinate and establish potent odorants that contribute to the aroma in the coffee species of interest. An inquiry into potent odorants was performed using headspace solid-phase microextraction (HS-SPME) in conjunction with gas chromatography-mass spettrometry and gas chromatography-olfactometry.

2.2 Material and methods

2.2.1 Biological material

The arabica coffee samples (fig 1) used in this study were supplied by a roast company Julius Meinl S.P.A. The samples are reported in the following table.

COFFEE TYPOLOGY	MOISTURE (%)	QUANTITY (Kg)
KENYA	8,9	11
ETHIOPIA	8,3	11
HONDURAS SHG	9,3	12
GUATEMALA	8,9	11
NICARAGUA	9,1	11,5
BRAZIL	9,3	11

The sampling for single type of green coffee (10 Kg) was randomized for different bags within the same lot (for every sampling is been measure the moisture content was measured by "Microwave moisture measuring system MW 3150 of the TEWS Elektronik). Six green arabica coffee varieties were used to represent the major single origin coffee of different provenence and different post-harvest treatment conditions (wet method) processed. The coffee beans selected were Brazil (Santos bohne), Guatemala, Nicaragua, Honduras SHG (Strictly High Grown), Kenya and Ethiopia. The Brazil coffee, instead, was processed with the dry method and not wet.

Fig 1 - Photos of the six different types of raw coffee used in the experimental plan

ETHIOPIA

KENYA

NICARAGUA

HONDURAS
SHG

GUATEMALA

BRAZIL

2.2.2 Preparation of coffee samples to study volatile compounds

Green coffee samples (6,5 g) from each single origin were ground for 2 minutes. The grinding was carried out in a Perten grinder on a 500 µm setting. The ground coffee samples were placed in a hermetically sealed single flask (50 mL).

2.2.3 Extraction of volatile compounds from the ground coffee by SPME GC/MS technique

A divinylbenzene - carboxene - polydimethylsiloxane (DVB/CAR/PDMS) 2 cm-50/30 µm SPME fibre (Supelco Co., Bellefonte, PA, USA) was used to extract volatile fraction from the coffee headspace. Ground coffee samples (6,5 g) were placed for 30 min in a water bath oven thermostatically regulated at the temperature of 40 °C reaching sample headspace equilibrium. Then, volatile compounds were extracted by placing the fibre in contact with the headspace for 5 min at the equilibrium temperature. SPME was immediately followed by GC-MS injection. For desorption, the fibre was placed in the GC injector heated to 250 °C for 5 min. All the samples were taken in triplicate. In the following tables (1-2) the conditions used for analysis aroma compounds by GC-MS and GC-O (olfactometry) are reported.

Table 1. Instruments and working conditions (GC-MS) for aroma compounds identification in green coffee

Device	Description
Gas Chromatography	Model: GC-17A, Shimadzu coupled to a mass spectrometer QP-5000 Schimadzu
Column	Econo Cap, Carbowax Size: 3 m x 0,25 mm i.d Film thickness: 0,25
Detector	Mass Spectrometry, ionization current of 10 µA – ionization voltage 70 Ev
Acquisition system	Class 5000
Integration system	GCMS Solution, Version 2.0
Gas carrier	Helium Linear velocity: 35.0 cm/sec^{-1}
Chromatographic conditions	Initial isotherm: 40.0 °C for 5 min Rate: 4.0°C/min up to 240 °C Final holding time: 240.0 °C for 7 min Injector temperature: 250 °C Interface temperature: 240 °C Injection in splitless mode Sampling time: 30 sec
Injection system	Manual SPME Desorption time 5.0 min

Table 2. Instruments and working conditions (GC-0) for impact odorants identification in green coffee

Device	Description
Gas Chromatography	Model: GC-O, Carlo Erba Instruments HRGC MEGA 2 series
Column	Econo Cap, Carbowax Size: 30 m x 0,32 mm i.d Film thickness: 0,25
Detector	Was carried out independently by two judges
Acquisition system	Class 5000
Integration system	GCMS Solution, Version 2.0
Gas carrier	Helium Linear velocity:35.0 cm/sec^{-1}
Chromatographic conditions	Initial isotherm: 40.0 °C for 5 min Rate: 4.0°C/min up to 240 °C Final holding time: 240.0 °C for 7 min Injector temperature: 250 °C FID temperature: 240 °C Injection in splitless mode Sampling time: 30 sec
Injection system	Manual SPME Desorption time 5.0 min

The analysis of the aromatic fraction in green coffee made reference to a method used to evaluate the impact of ecological post-harvest processing on the volatile fraction of coffee beans: I. Green coffee (Gonzales-Rios *et al.*, 2007). Solid-phase microextraction (SPME) is well known as a simple, rapid, sensitive, and highly reproducible sampling method. This technique was used because the number of SPME applications in food analysis is vast, and recently SPME has been successfully utilized for the isolation of coffee aroma compounds. The method

used has undergone some modification. An additional make-up flow of nitrogen (21 ml min^{-1}) was supplied to improve the performance of the sniffing system, and an air flow (100 ml min^{-1}), humidified by bubbling in a distilled water reserve, was blown at the exit of the sniffing line, to cool and humidify the column flow. A three-phase fiber was used instead of a biphasic one. The DVB/CARBOXEN/PDMS fiber showed good sensitivity for coffee volatiles and besides the different chromatograms obtained it showed high similarity with the extract of steam distillation (Akiyama *et al*., 2007). On the other hand, the triple phase coating material proved to have better performance characteristics for sufficient isolation of analytes in green coffee having a wide range of physico-chemical properties (Risticevic *et al*., 2008). The amount of the sample was 6,5 g and not 1 g to reproduce the same conditions used in espresso coffee (see chapter 4) and to make possible comparisons. The same chromatographic conditions will be used to identify aroma compounds in roasted coffee samples (see chapter 5).

2.2.4 Identification of volatile compounds

The volatile fraction was identified by comparison of mass spectrum of the odorous peak with those reported in Wiley 5 mass spectra library, and by comparison of Kovats retention index, calculated from the retention times of n-alkanes, with those available in literature. The aromatic notes of the compounds perceived by olfactometry were also used as identification criteria by comparing them to references in the literature.

2.2.5 Statistical analysis

One way analysis of variance (ANOVA) was carried out on the absolute areas for all the aroma compounds. To verify the homogeneity of variance Levene's test was applied. The same analysis was applied for the compounds detected by SPME-GC-FID analysis of the headspace of green coffee samples. Means and standard deviations were calculated and represented with graphs box-plot, and significant differences were evaluated by the HSD Honest Significant Difference (HSD). Test variances were

homogeneous according to Levene's and results were considered significant at $p < 0,05$.

2.3 Results and Discussion

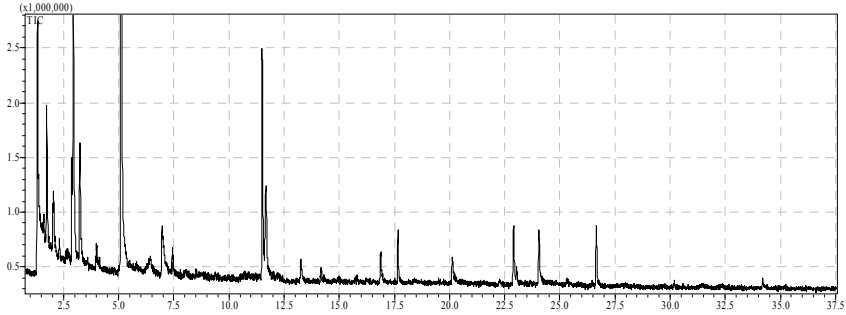
Table 3 gives a list of the 47 volatile compounds identified by SPME/GC-MS. This is quite a large number of compounds, given that some 230-300 compounds have been detected in green coffee in recent years when the HS-SPME extraction method is used (Holscher and Steinhart *et al*., 1995; Cantergiani *et al*., 2001; Clarke and Vitzthum *et al*., 2001; Grosch *et al*., 2001; Flament, 2002). The area of all compounds detected and the main odours notes identified by the sniffing technique were reported in the table. However, a dynamic analysis of the headspace of green coffee identified 47 compounds of interest composed of 5 aldehydes, 14 alcohols, 7 acids, 7 ketones, 1 terpene, 2 esters, 1 ether, 2 furans, 1 pyridines, 1 pyrazines and 1 ammine. The number of volatile compounds identified in green coffee was reduced if compared to those identified in roasted coffee (discussed in the chapter 5). The results obtained are similar with those reported in bibliography (Cantergiani *et al*., 2001; Lee *et al*., 2002; Gonzales-Rios *et al*., 2007, A Farah *et al*., 2008) and for these classes of compounds of major interest namely esters, ethers, furans, alcohols, aldehydes, acids, ketones and sulphur compounds. However, pyrazines were previously identified in green coffee (Czerny and Grosch *et al*., 2000; Cantergiani *et al*., 2001); their absence in this study was probably due to the use of the HS-SPME with the triphasic fiber chosen for its affinity for compounds in trace form or with low molecular weights (Roberts *et al*., 2000; Akiyama *et al*., 2003).

Table 3. The main compounds and odour/flavour descriptors identified in raw coffee samples as a result of the SPME/GC-MS and GC-O.

Compound Name	I	I Ref. [a]	Odour/Flavour detection by SPME-GC-O analysis	Odor description (literature) [a]	Group of compounds
Dimethylsulfphide	816	829 [b]			Sulphur compounds
2-Propanone**	822	814 [b]	sweet/pleasant	pervasive, sweet	ketones
2-Butanone	902	909 [b]			ketones
3-Methyl-butanal*	921	912 [b]	malt	malt/cocoa	Aldehydes
Ethanol	939	944 [b]			Alcohols
2,5-Dimethylfuran	958	n.d			Furans
Pentanal	976	978 [b]			Aldehydes
2-Butanol	1022	1027 [b]			Alcohols
Ethyl isovalerate	1060	1069 [b]			Esters
Hexanal**	1080	1087 [b]	fruity/green	fruity/green	Aldehydes
Isobutyl alcohol	1092	1105 [b]			Alcohols
3-Methoxy-1-butanol	1110	n.d			Alcohols
2-Pentanol	1129	1118 [f]			Alcohols
1-Butanol	1148	1151 [b]			Alcohols
3-Heptanone	1151	n.d			ketones
Pyridine	1179	1185 [b]			Pirydines
Limonene	1198	n.d			Terpenes
Isoamyl alcohol**	1205	1214 [b]	roasty coffee/nut/pungent	acrid/pungent	Alcohols
2-Pentyl furane*	1227	1229 [b]	pleasant	green/vegetale/butter [d]	Furans
1-Pentanol	1259	1240 [b]			Alcohols
3-Hydroxy-2-butanone**	1292	1282 [b]	mushroom/buttery	buttery	ketones
2-Hexanol, 3-methyl-	1329	n.d			Alcohols
1-Hexanol**	1362	1370 [c]	nut/pungent	resin, flower, green [d]	Alcohols
2-Pentanone, 4-hydroxy-4-methyl-	1357	n.d			ketones
1-Propene-1-thiol	1374	n.d			Sulphur compounds
2-Heptanamine, 5-methyl- 44(100); 55(20); 56(10);114(10);	1395	n.d			Ammines
1,3-Dichloro benzene	1435	1434 [b]			Benzene compounds
Acetic acid	1464	1449 [c]			Acids
Isopropyl vinyl ether	1453	n.d			Ethers
Furfural	1464	1457 [b]			Aldehydes
1-Ethyl-1-hexanol*	1483	1496 [c]	hay		Alcohols
2-Isobutyl-3-methoxypyrazine**	1515	n.d	earth, spice, green pepper	green pepper [d]	Pyrazines
2,3-Butanediol	1555	1546 [c]	buttery/herbaceou	Buttery [d]	Alcohols

(*levo*[e])**			s		
Propanoic acid	1541	1541 [b]			Acids
Butanal, 3-hydroxy-	1546	n.d			Aldehydes
Propanoic acid, 2-methyl-	1562	n.d			Acids
Dimethylsulphoxide**	1570	1571[b]	vegetable	vegetable	Sulphur compounds
2,3-Butanediol (*meso*[e])	1594	1580 [c]			Alcohols
2(3H)-Furanone, dihydro-5-methyl-	1597	n.d			Ketones
Butyrolactone	1632	1601 [c]			ketones
Isovaleric acid	1662	1672 [b]			Acids
Butanoic acid, 3-methyl-	1676	1689 [c]			Acids
Hexanoic acid	1828	1828 [f]	sweet	rancid/sweet [d]	Acids
2-Phenyl ethanol**	1894	1873 [f]	floral/rose	floral	Alcohols
2 Hexenoic acid*	1938	n.d	floral	must/fat [d]	Acids
Dimethyl phthalate	2279	n.d			Esters
Benzoic acid	2451	2430 [b]			Acids

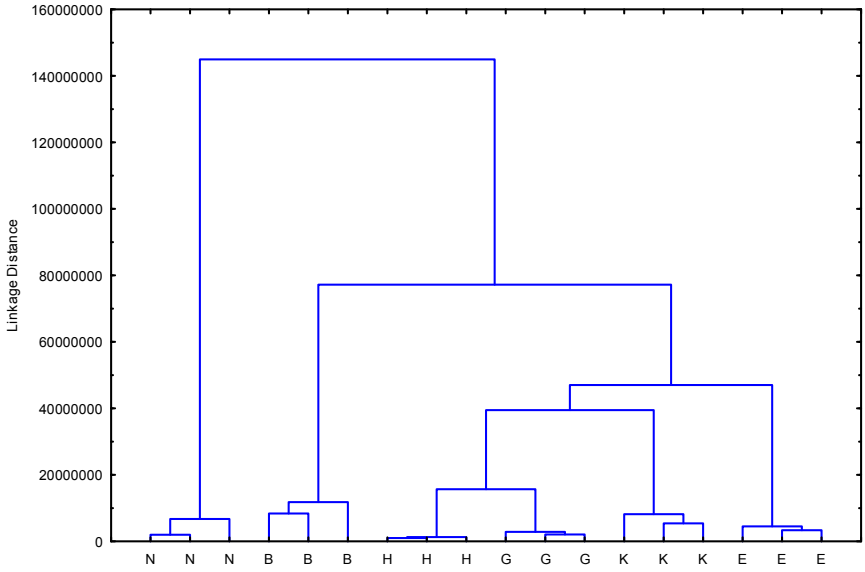
I: Kovats' retention index. [a] Reported by different authors: [b] Gonzales-Rios (2007); [c] A. T. Toci, A. Farah (2008); [d] reported in http://www.nysaes.cornell.edu/flavornet/chem.html. [e] Isomers according to Peinado, Moreno, Maestre and Mauricio (2007). [f] Holscher (1990), Cantergiani (2001), Sanz (2001); * Odour compound detected in the headspace by GC-O of a few green coffee samples; ** Odour compound detected in the headspace by GC-O of the green coffee in all the samples. N.d not detected.



Graph 1 - Chromatogram for the aromatic compounds in green coffee evaluation obtained by SPME/GC-MS

The cluster analysis is an explorative analysis which aims to create similarity groups. The case dendogram presented in graph

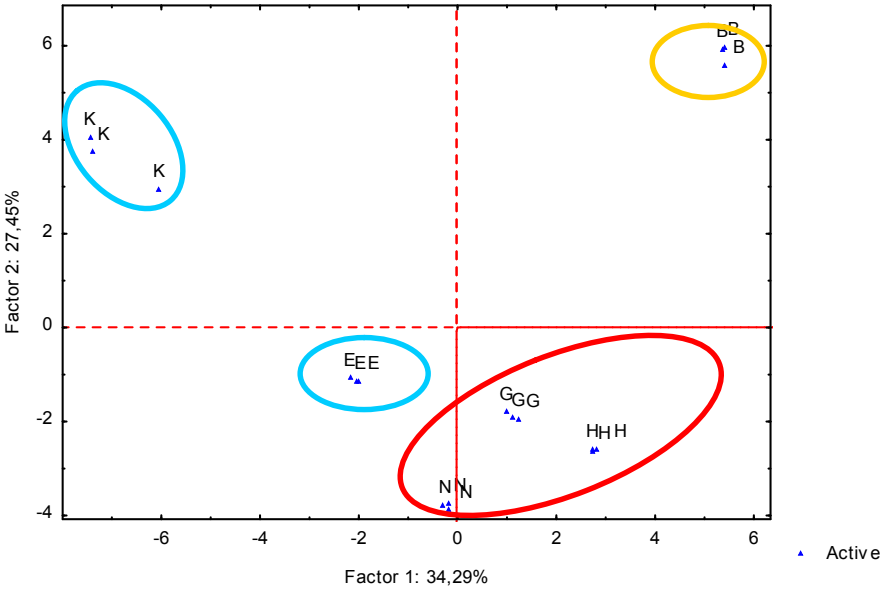
2 based on the Euclidean distances, shows that it is possible to group samples on the basis of the amount of aroma compounds into different groups with high difference from a qualitative and quantitative point view. Considering the graph 2 is important to underline the fact that the replicates of the different green coffee samples showed low variability then the goodness of the method used. It is interesting to show the aromatic fraction in Nicaragua coffee, presenting a marked difference with respect to the other groups of coffee in particular in the American Center class; information not evident and clear like in the PCA (graph 3). Nicaragua coffee showed a different composition with respect to the other group of coffees in terms of a high content of sulphur compounds and alcohols. Both these classes of compounds can take origin from spontaneous fermentations (not desired in the process) or incomplete mucilage removal during the post-harvest treatment. Instead the Brazil coffee even though it is processed with a different post-harvest manufacturing the drying method presented a greater similarity to coffees treated with the wet method. The Honduras and Guatemala coffees are similar, both constituting small groups. Not a very marked difference is explained between coffees originating from Africa. Observing again graph 3 the Kenya coffee does not establish a group with the Ethiopia coffee. In particular Kenya presented an aromatic fraction more similar to Honduras and Guatemala rather than Ethiopia.



Graph 2 - results of Cluster analysis carried out on the absolute areas in detected by SPME - GC-MS analysis of green coffee samples (E-Ethiopia, K-Kenya, G-Guatemala, H-Honduras, B-Brazil, N-Nicaragua)

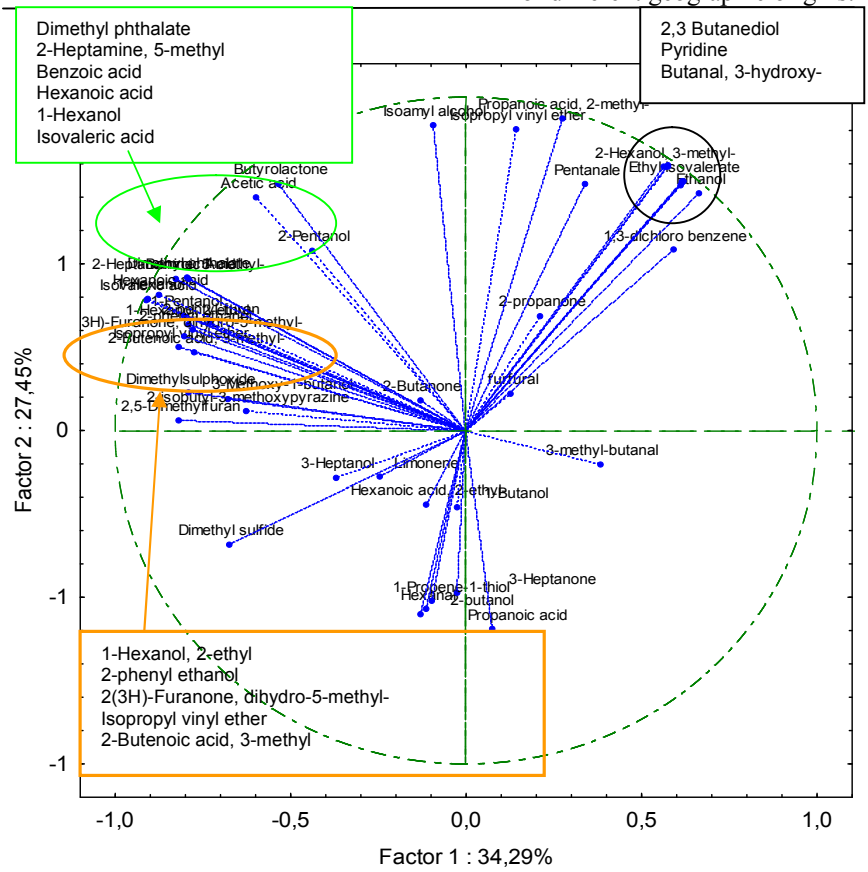
The following are biplot graphs. These graphs are often used to observe the structure present in a data matrix and to find grouping and correlation between variables or differences between the samples. In these plots, scores for both samples and variables are represented in the space of the two main components obtained in a PCA. Thus, a relationship between samples and variables can be seen on the same plot. The areas of these samples are showed by PCA (principal component analysis), considering the three replicates for every sample. As can be observed, the aroma fraction of the Arabica varieties seem to be giving good results in terms of grouping, according to their geographical origin. A poor grouping is given by the coffees of African origin. The PCA analysis led to the extraction of two main components having the initial eigenvalue >1, which contributed to (34,29 + 27,45) of the total variance of the data set.

This value is not high, but it showed effectiveness approximation in two dimensions in the sample space, attesting analysis reliability. It is noticeable that the Brazil green coffee is located at the positive side of PC1 and PC2. located at the positive end of PC1 and PC2. The Kenya green coffee is located at the negative end of PC1 and at the positive one of PC2. Ethiopia and Nicaragua green coffees are located at the negative end of PC1 and PC2. The coffee Guatemala and Honduras green coffee are located together at the positive end of PC1 and negative of PC2. These observations indicate that all the green coffee samples presented a different aromatic profile composition based type of the post-harvest process treatment, genotype, variety and environmental characteristics. However, if we observe the Euclidian distance represented in graph 2, we can understand how many are different.



Graph 3. Results of PCA analysis carried out on the absolute areas in detected by SPME - GC-MS analysis of green coffee samples

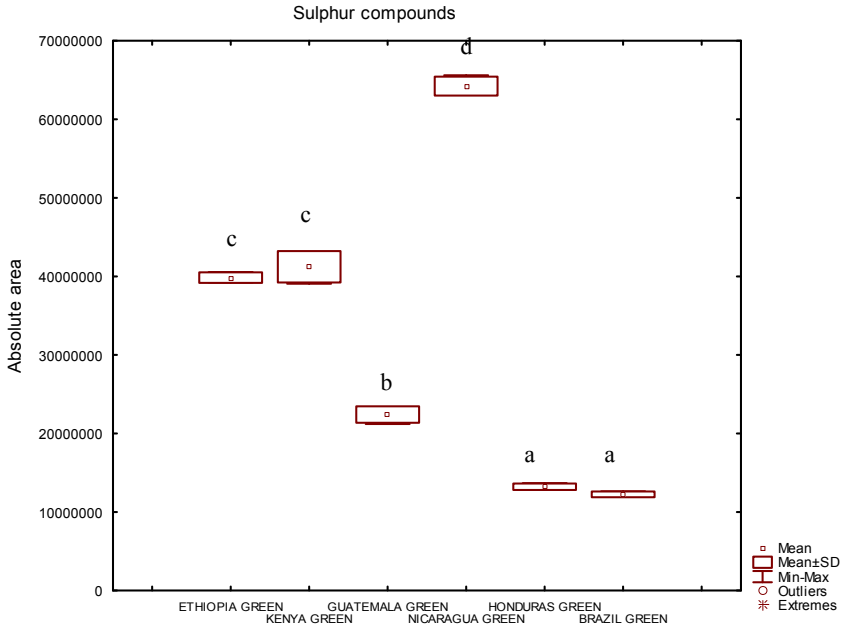
K=Kenya, E= Ethiopia, G=Guatemala, N=Nicaragua, H=Honduras, B=Brazil



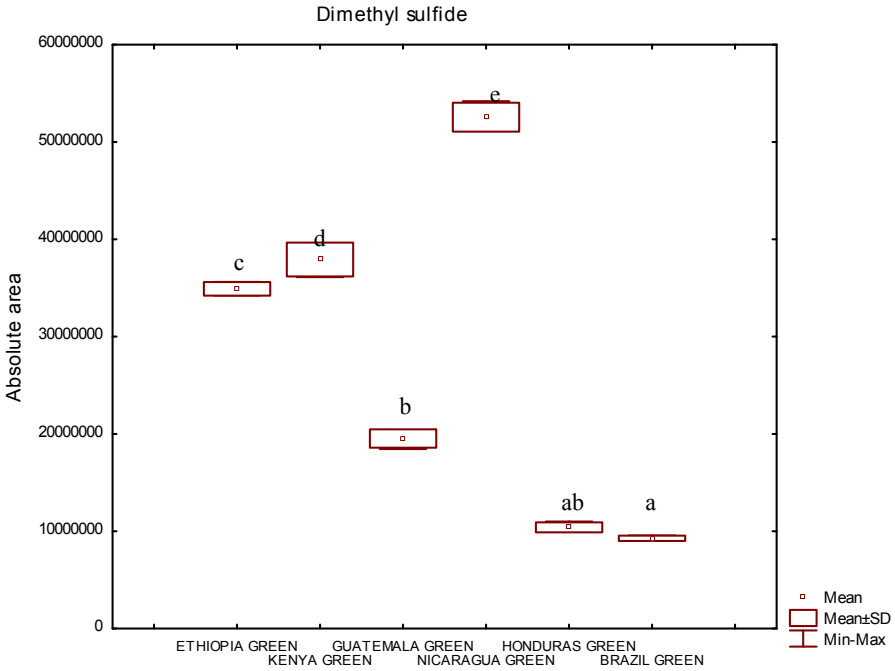
Graph 4. Results variables of PCA analysis carried out on the absolute areas in detected by SPME - GC-MS analysis of green coffee samples

In details the distribution of the variables represented in graph Biplot (graph 4), displays different projections of aroma compounds for a single type of raw coffee. Looking at graphs 3 and 4 it can be said that most of the content of aroma compounds is present in the Brazil and Kenya coffee areas. In the other coffees instead a low content in terms of quality and quantity of the aromatic fraction is present. For all the samples was executed Levene's test for to verify the homogeneity of variance and then

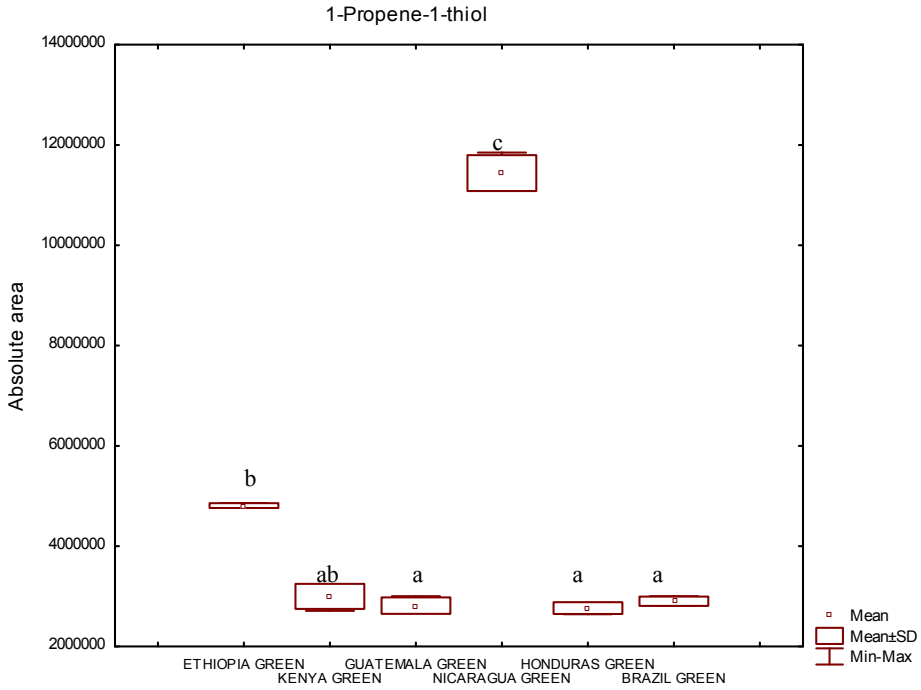
variance analysis for to understand which classes of compounds characterize the single coffees. Inside every group of compounds was investigate by the ANOVA test. As can be seen in the following graphs (5-9-11-12-13-14) the classes of compounds of major interest are represented by sulphur compounds, aldehydes, esters, phenols, furans and pyrazines. These compounds might be correlated with different origins. The sulphur compounds identified are mainly present in the Nicaragua coffee, following the Kenya and Ethiopia coffees and other varieties (graph 5). In the sulphur compounds class the dimethyl sulphide and 1 propene-1-thiol are the major compounds detected in the Nicaragua coffee identify by GC-MS (graph 6-7). Dimethyl sulfide present in green and roasted coffee is formed by oxidation of methanethiol (Flament, 2002). This compound is found naturally in green coffee at 0,1-0,3 ppm concentration (Rhoades *et al*., 1960). Guyot and Vincent (1990) found decreasing concentrations of methanethiol from "stinking" arabica coffee. Several authors found with high contents of dimethylsulfide showing a corresponding desirable bluish pigmentation in Colombia coffee, especially if stored under ventilated conditions (Gibson *et al*., 1974). The sulfphure compounds might produce defects if they present an olfactory threshold at concentrations higher than the recognition threshold. On the contrary a good odour is generated if the threshold of concentrations is low (Lorenzi *et al*., 2008). Besides the concentration they also depend on the geographical origin and climatic conditions. The odour of dimethyl sulphide is extremely diffusive, repulsive, reminiscent of wild radish, sharp, green, cabbage like (Flament, 2002) and only in very high dilution it becomes bearable and almost acceptable, pleasant, vegetable-like (Arctander *et al*., 1967).



Graph 5. Box Plot representation and variance analysis (HSD Tukey Test) of total areas of Sulphur compounds identified by GC-MS.



Graph 6. Box Plot representation and variance analysis (HSD Tukey Test) of the Dimethyl sulfide compounds identified by GC-MS (Table 3).

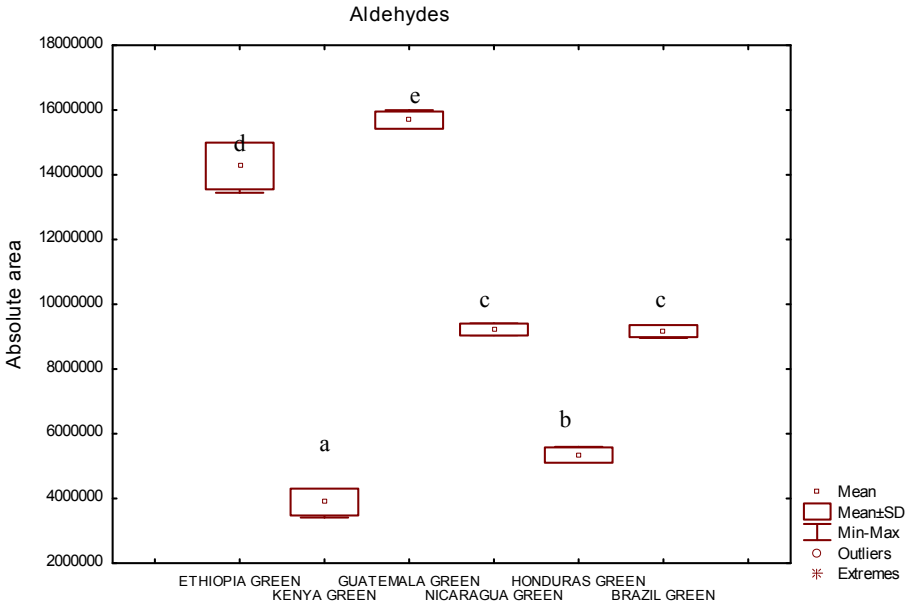


Graph 7. Box Plot representation and variance analysis (HSD Tukey Test) of total areas of 1-Propene-1-thiol identified by GC-MS (Table 3).

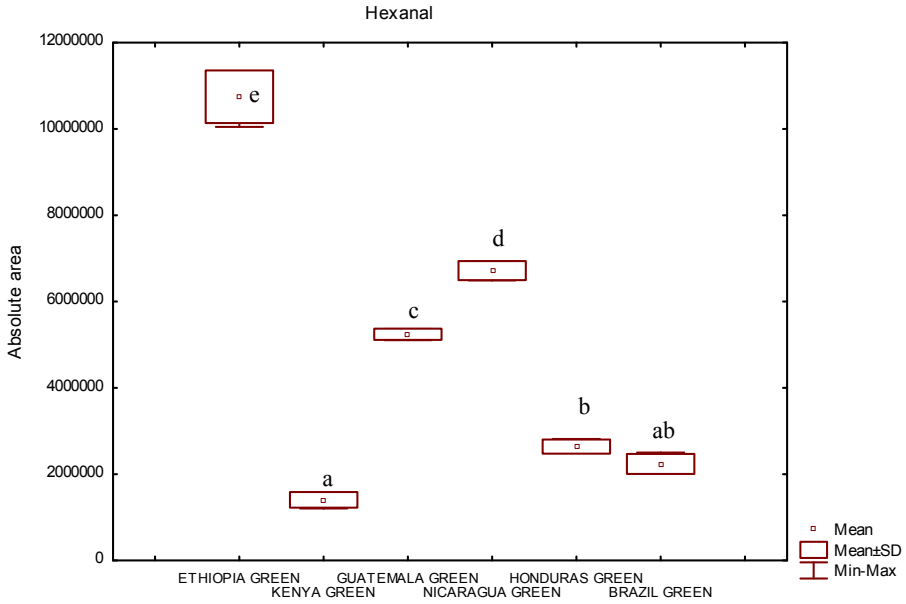
In general most of these volatile substances are produced during the alcoholic fermentation by yeasts, therefore, incorrect management of fermentation parameters can notably shape the development of an off-flavour. For example it is known that during fermentation a low value of nitrogen, high pH and the handling temperature could influence the production of sulphur compounds.

Another compound group identified by GC-MS that presented a significant difference by variance analysis are the aldehydes (graph 8). A high level of this class of compounds is detected for all the coffees at different concentrations in particular in the

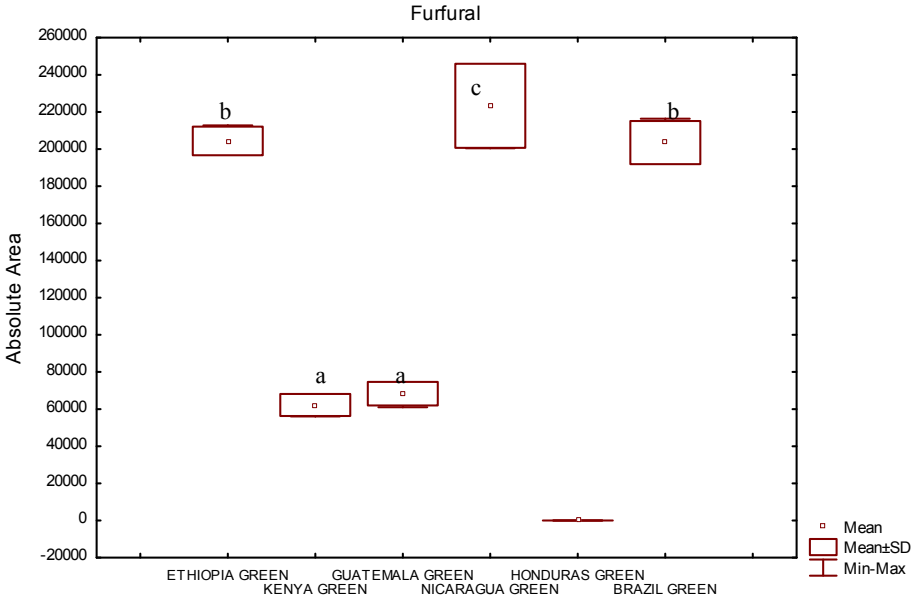
Ethiopia and Guatemala coffees. A middle value was found for the Nicaragua and Brazil coffees, while a low one for Honduras and Kenya coffee. The formation of various aldehydes by autoxidation of unsaturated fatty acids thanks breakdown of hydroperoxide intermediates is well established in the literature contributing particularly to the typical green - coffee odour. Thus their presence is not surprising considering that green coffee beans contain 13% of lipids half of which is linoleic acid ($C_{18:2}$). It is probable that the value of aldehydes for the Ethiopia and Guatemala coffee is high, since there is a high level of lipids. In fact in a study conduced on fatty acid profiles as discriminant parameters for coffee variety differentiation the presence of linoleic acid was very different in several coffee varieties (Gonzales *et al*., 2000).
The main aldehydes identified are hexanal and furfural. Both green coffee Ethiopia and Kenya showed a different behaviour.



Graph 8. Box Plot representation and variance analysis (HSD Tukey Test) of the Aldehydes compounds identified by GC-MS.



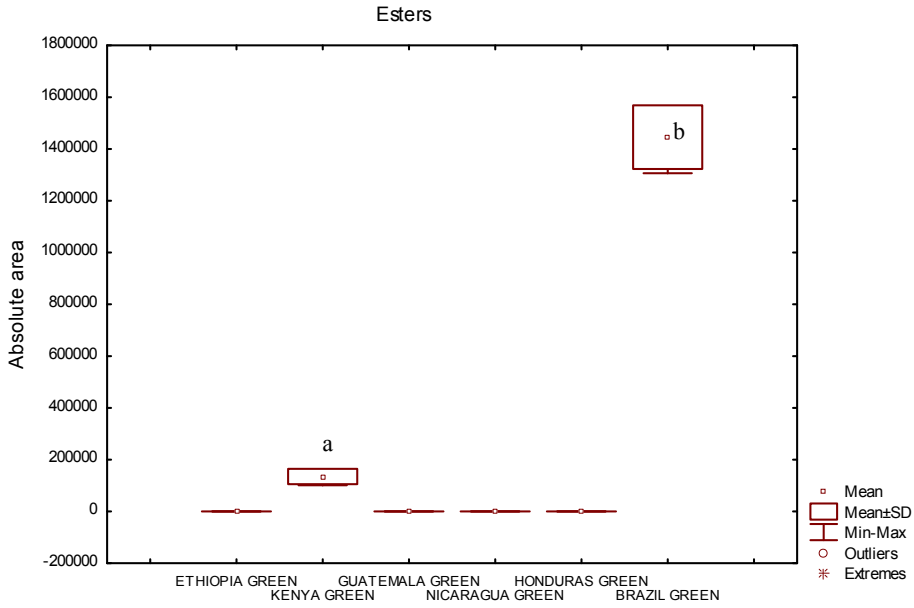
Graph 9. Box Plot representation and variance analysis (HSD Tukey Test) of the Hexanal compound identified by GC-MS (Table 3).



Graph 10. Box Plot representation and variance analysis (HSD Tukey Test) of the Furfural compound identified by GC-MS (Table 3).

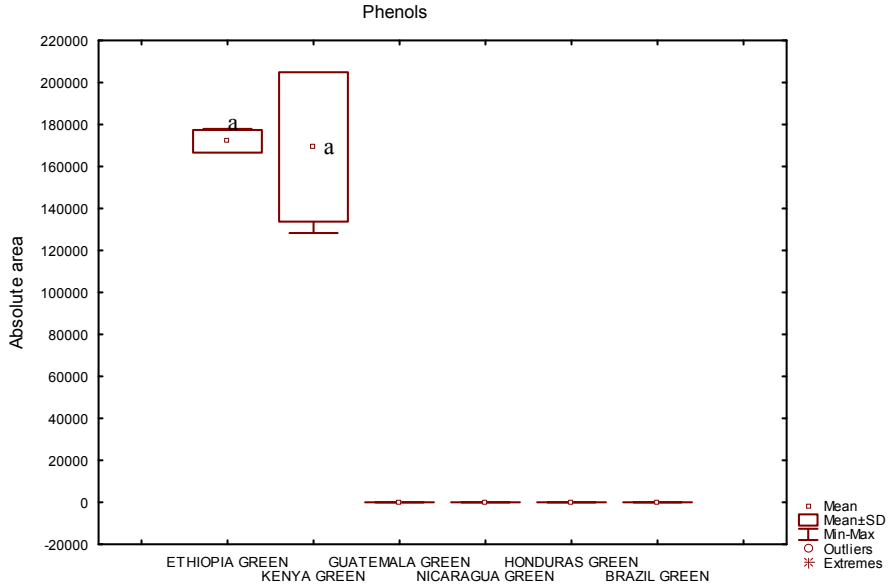
If we observe graphs 9 and 10 we can see the Ethiopia coffee has a higher content for both the aldehydes than the Kenya coffee. Also the Central America coffee presents a variable content of aldehydes depending on the variety. In particular in the Honduras coffee the furfural content is absent. Hexanal is a saturated aldehyde identified in healthy and in stinking green beans (Guyot *et al*., 1983; Cantergiani *et al*., 2001). Hexanal is involved in the staling of coffee in the presence of oxygen and in the overall oxidation of lipids, index of oxiding environment. A significant increase in hexanal content has been found in green coffee with "*Rio*" defects, with damages of cell membranes (lipids are more susceptible to oxidation) after massive invasion by mould species (Spadone *et al*., 1990). The odour is very powerful, penetrating, fatty-green, grassy. In extreme dilution it

becomes more reminiscent of freshly cut grass and unripe fruit (apple). According to Guyot (1983) the fruity character is more perceptible in stinking than in healthy beans. The flavour is described as leafy, green, fatty, fruity (Chemisis *et al*., 1994). Often the defects mentioned (example stinker, Rio) in the bibliography and common language not have a interpretation unique and are marked with different compounds without a knowledge real cause-effect and without recognizing the defects during cupping tasting. The presence of furfural and its level in green coffee was observed in different degrees of maturity cherries, collected from green to red (Guyot *et al*., 1982). It is probable that the low content of furfural detected in the Kenya and Guatemala coffee is an indication of poor maturity these coffees during harvest. Furfural has been described as pungent, but sweet, bread-like and caramellic (Kroes *et al*., 1983).



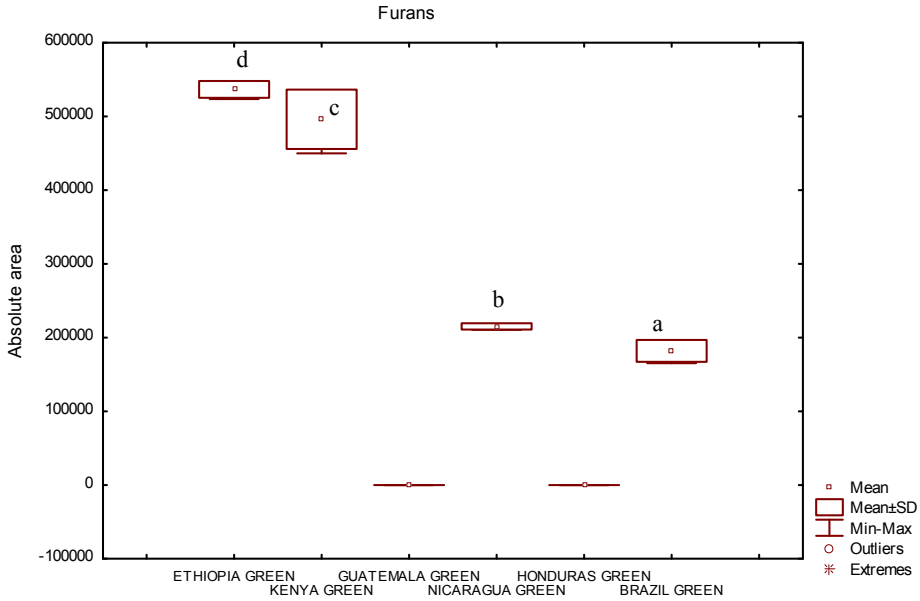
Graph 11. Box Plot representation and variance analysis (HSD Tukey Test) of Esters compounds identified by GC-MS.

A greater content of esters has been found in coffee Brazil (graph 11), and low content in the Kenya coffee, instead it was absent in others coffee. According to several authors (Barel *et al*., 1976; Full *et al*., 1999) the esters compounds originate from the fermentation (fermentation compounds) stage during post-harvest processing. A high content of esters in the Brazil coffee probably derived from possible fermentation during the long drying stage. 2,3-butanediol is a major confirmation (possible marker) of this presence in the Brazil coffee (see graph 4), and this alcoholic compound plays a role in aroma development during fermentation (Spadone *et al*., 1990; Cantergiani *et al*., 2001). In wine this compounds derived from malo-lactic fermentation. The latter authors observed that more esters are present in green beans than in roasted ones and they are products also in connection with the metabolic processes in the fruit.



Graph 12. Box Plot representation and variance analysis (HSD Tukey Test) of Phenols compounds identified by GC-MS.

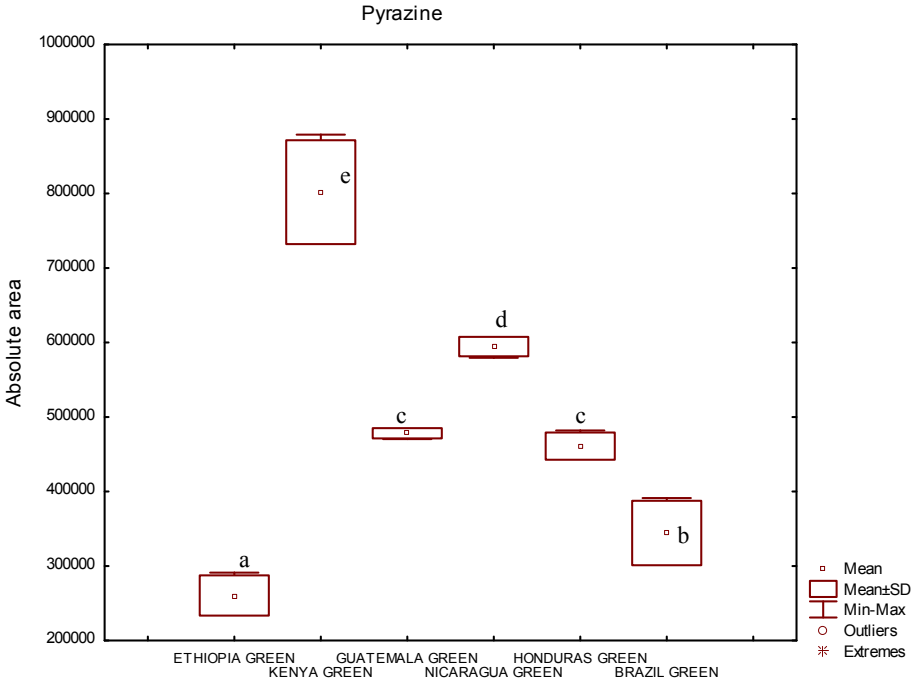
Observing graph 12 the high content of phenols is evident in the coffee originating from Africa. Phenols are present in nature and are essential contributors to the typical quality of coffee flavour. The quantities and types of phenols depend on the varieties of coffee varieties. Phenols at low concentrations are described as having sweeter, warm, floral, balsamic with pleasant vanilla, note odours (Flament, 2002). In this case there is a good correspondence between the Kenya and Ethiopia coffees in terms of the phenol content because they are known thanks to the present delicates, floral, sweet note.



Graph 13. Box Plot representation and variance analysis (HSD Tukey Test) of Furans compounds identified by GC-MS.

The Africa coffee presented a high value of furans, while they were absent in the Guatemala and Honduras coffees. Sometimes furans result from terpenic precursors already present in green

beans (Gautschi *et al*., 1967). In a study the furans concentration was measured to be be 4,2 ng/g in headspace in different green coffee by the SPME technique (Hamide *et al*., 2007).

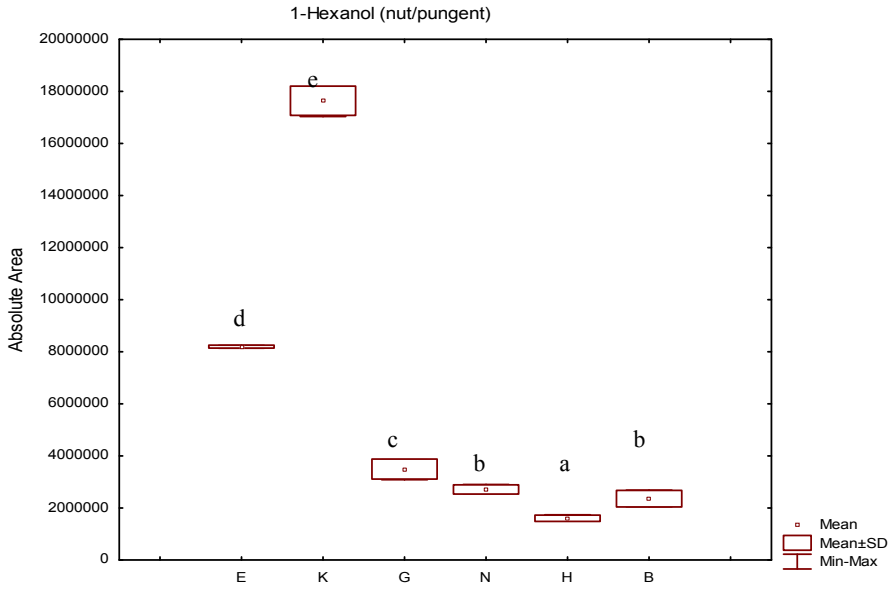


Graph 14. Box Plot representation and variance analysis (HSD Tukey Test) of Pyrazines compounds identified by GC-MS.

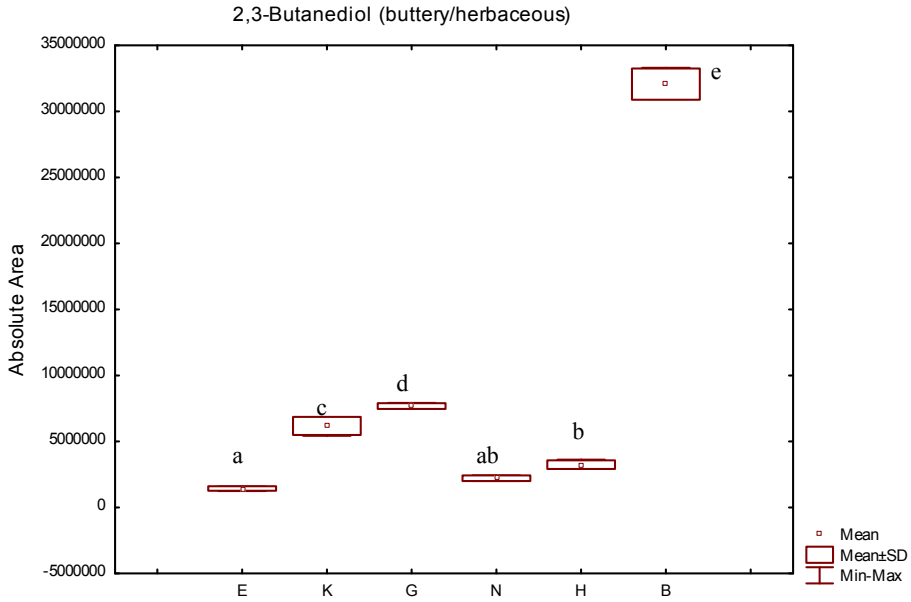
Pyrazine was detected in a greater concentration in the Kenya coffee followed by Nicaragua, Guatemala, Honduras and Brazil. In particular 3-isobuthyl-3-methoxypyrazines were identified which are not formed by Maillard reaction but are of biogenetic origin (pre-fermentative aroma). Their formation in plant tissues occurs by condensation of amides of α–amino acids with α–

dicarbonyl derivates (Murray *et al*., 1970). The content of methoxypyrazines is influenced also by the cherry harvesting season which must bot be too late (high presence of vegetable with strong odours notes), and the plant breeding type. Methoxypyrazine identified is responsible for negative notes in coffee (Bortoli *et al*., 2001; Cantergiani *et al*., 1999, Spadone *et al*., 1990). This off flavour is so strong that is most likely caused by the interaction of insects and bacteria (Bouyjou *et al*., 1999). The variegated coffee bug and other insects inflict wounds on unripe coffee cherries so that methoxypyrazine producing bacteria can penetrate them. These compounds have proved to have a predominant good pea-like odorant in raw coffee at a concentration of 97 µg/Kg (Holcher *et al*., 1995).
To confirm the previous results and validate the effect on the aroma quality of green coffees, an olfactory analysis was carried out on samples of different origins. The results of SPME-GC-O analyses of the green coffee are reported in table 3 with a comparison of the experimental notes and reference notes for the characterized compounds. The most important olfactory impact note of 9 compounds was perceived. The olfactory notes were varied mixing pleasant (sweet/pleasant, fruity/green, roast coffee/nut/pungent, mushroom/earthy, nut, green pepper, and unpleasant odours (sour, acrid). Different studies have been published on the aroma characterization of green coffee by GC-O, and only a few descriptors have been cited in these studies as buttery, vegetable, pungent, fruity and floral (Spadone *et al*., 1990; Holscher and Steinhart *et al*., 1995; Czerny and Grosch *et al*., 2000, Sarrazin *et al*., 2000; Cantergiani *et al*., 2001, Gonzales *et al*., 2007), instead the descriptors of green pepper, nut and mushroom never been cited. Nevertheless, there are a few differences in table 3 between the odour descriptions reported in literature and those detected by olfactometric analysis. It is that evident some of these compounds may confer pleasant or unpleasant notes depending on their concentrations. For instance the behaviour of the dimethylsuphoxide and 2,3 butanediol are characterized to give off-flavours. The SPME method used could not identify all potent odorants because of its dependence on

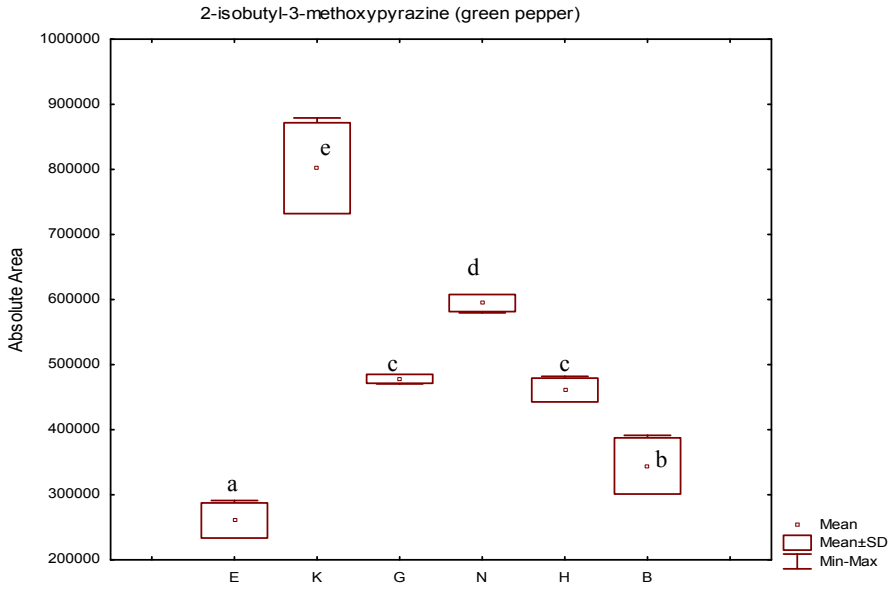
different factors such as the influence of the sample preparation process, GC-O method applied and solute extraction procedures. The peak area of a volatile compound is not necessarily connected with its contribution to the overall flavour, because the threshold value of the compound plays a major role. The identification and integration of area peaks of compounds allowed to differentiate coffee from samples. In particular only four pleasant volatile compounds (1-hexanol, 2,3-butanediol, 2-isobutyl-3-methoxypyrazine, hexanal) resulted marked significant differences by variance analysis, the ANOVA test (graphs 15, 16, 17, 18 where E=Ethiopia, K= Kenya, G=Guatemala, N=Nicaragua, H=Honduras, B=Brazil).



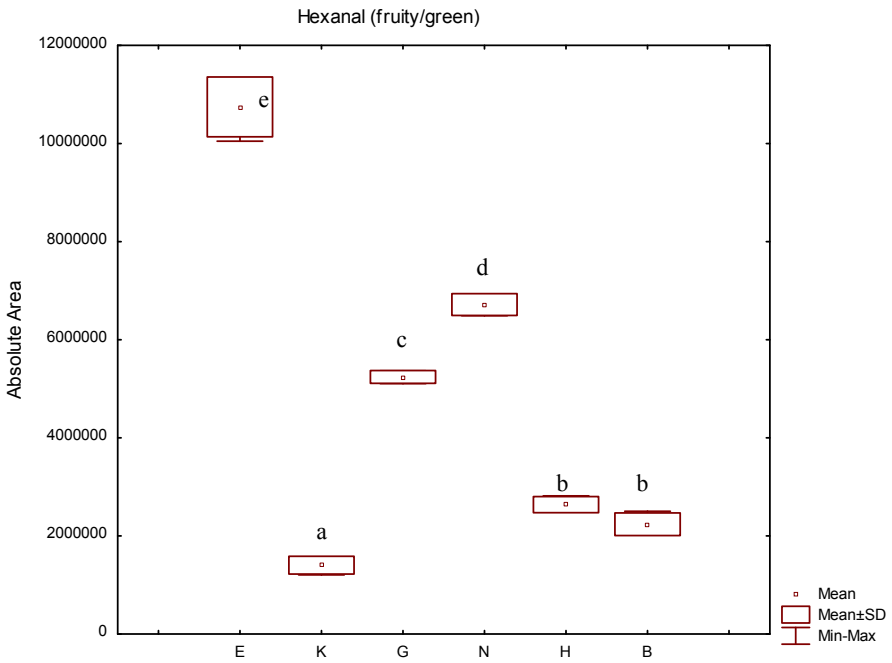
Graph 15. Box Plot representation and variance analysis of 1-Hexanol (HSD Tukey Test) compound identified by GC-O (Table 3).



Graph 16. Box Plot representation and variance analysis of 2,3-Butanediol (HSD Tukey Test) compound identified by GC-O (Table 3).



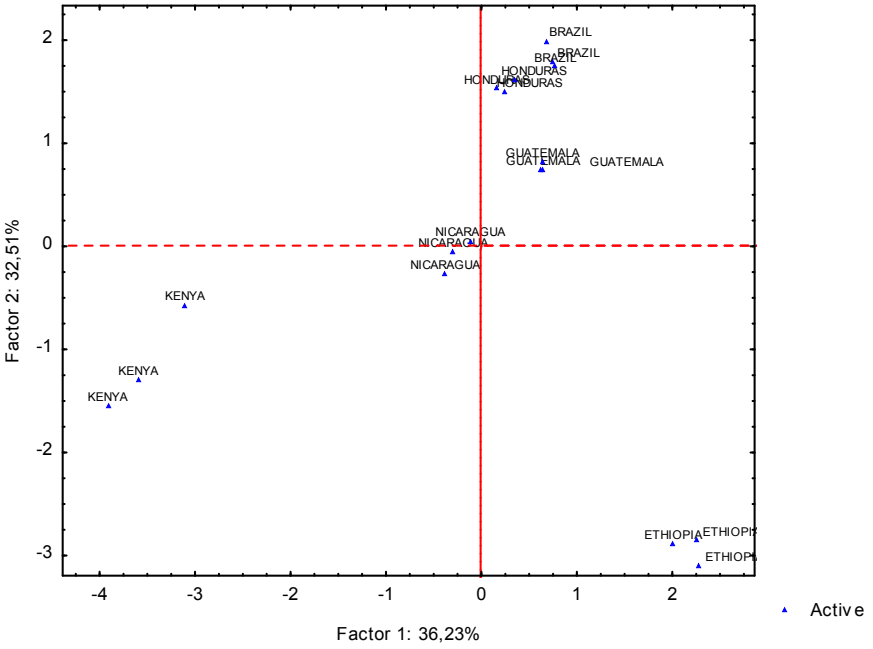
Graph 17. Box Plot representation and variance analysis (HSD Tukey Test) of 2-isobutyl-3-methoxypyrazine compound identified by GC-O (Table 3).



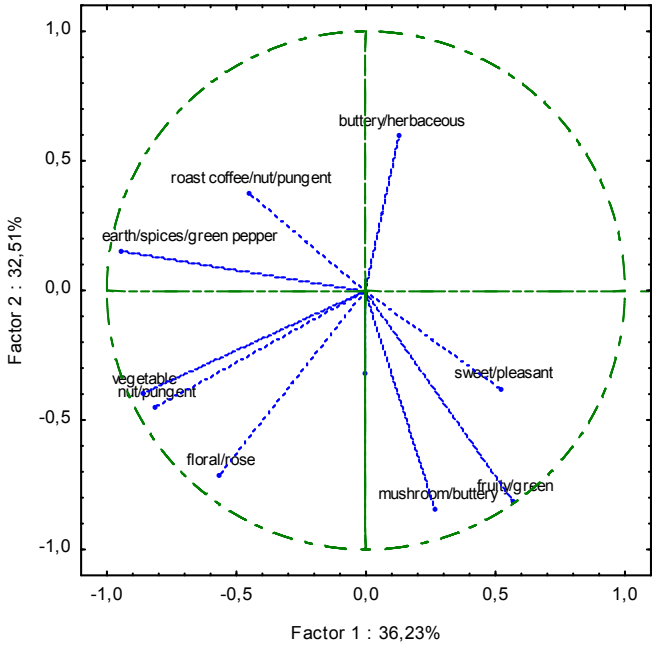
Graph 18. Box Plot representation and variance analysis of Hexanal (HSD Tukey Test) compound identified by GC-O (Table 3).

Regarding these compounds this behaviour could easily be connected to different fermentations that can distinguish different coffees for example 2,3 butanediol. These compounds can represent marker potential for the characterization of different coffees from the point of view of the geographic origin. In particular the 1-hexanol in general for African coffee 2,3-butanediol for Brazilian coffee, 2-isobutyl-3-methoxypyrazine for Kenya coffee and hexanal for Ethiopia coffee, but these observations should be further investigated to confirm the present results and identify new possible *markers*. If we consider all volatile compounds instead we can see in the PCA (graph 19) the distinction between different green coffees with a combination of

main components PC1 and PC2 accounts for 68,74% total of information. The Ethiopia coffee (graph 20) gave sweet/pleasant notes, mushroom/buttery and fruity/green odours. In particular these last two notes presented two vectors with a long distance meaning a high effect for to characterized Ethiopia coffees. The Nicaragua coffee characterized by mixed notes between Kenya and Ethiopia coffees. The Kenya coffee showed vegetable/nut, pungent, floral/rose mixed notes, instead the other coffees (Brazil, Honduras, Guatemala) distinguished themselves prevalently with buttery/herbaceous note.



Graph 19. Results of PCA analysis of the odour identified by GC-O for the six green coffee samples coffee samples.



Graph 20. PCA projection of all variables on the factor-plane relative to the pleasant flavours detected by GC-O.

2.4 Conclusions

Our results show that this method in SPME with a few modifications helps give a clear discrimination between different samples. In the present work, a total of 47 different volatile compounds were observed in raw coffee they were 5 aldehydes, 14 alcohols, 7 acids, 7 ketones, 1 terpene, 2 esters, 1 ether, 2 furans, 1 pyridines, 1 pyrazines and 1 ammine. They permitted to make several observations. The acquired data set was subjected to main component analysis and the corresponding geographical origin discriminations of coffees samples originating from South - Central America and Africa were successfully established. The results from PCA showed that the data were sufficiently separated (61,29% explained variance) into different areas on the

graph, one containing coffees from Africa, coffees from Central America and from Brazil. It was possible to identify the prevalent compounds characterizing every single origin thanks to analysis variance. The present work confirms how the effects of numerous factors may affect a single raw coffee origin and its aromatic fraction. The different soil composition, agricultural practices, genetics and climate condition can influence the volatile fraction together with harvest and post-harvest treatments in particular. Also the presence of specific microorganisms naturally selected in a particular environment or during fermentation might influence the volatile profile in coffee both positively and negatively. The presence of specific compounds is in good correlation with spontaneous fermentation. This concept also underlines once again the importance of applying good handling techniques during harvest and post harvest to obtain high quality coffees. There are important differences also between green coffees cultivated in near states. For example the Nicaragua coffee (Central America) presented important difference with respect to the Guatemala and Honduras coffees (Central America), and there are specific compounds of biogenetic origin. The same supposition is valid for coffees from Ethiopia and Kenya. Also the results obtained from the analysis in GC-O of volatile compounds, provided valuable information. Of all the aroma compounds determined only 9 were detected with the sniffing technique and allowed to distinguish and characterize the green coffees of different origins.

In particular four pleasant compounds were identified (1-hexanol, 2,3-butanediol, 2-isobutyl-3-methoxypyrazine, hexanal) where marked significant differences between coffee samples were found. Considering the fact the flavour of a green coffee is influenced by many variables and analyzed a only sample for state origin, is not possible, call quality of "*marker*" the flavour compounds detected by GC-O technique, but possible "*marker*". Is necessary to analyze more samples of the same typology of green coffee harvested in different places of the same original state. Also the method SPME used in this work might to be used

create and build a database that takes into account the origin and the variables involved.

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THIRD CHAPTER

"Characterization of the Aromatic Precursors in Arabica Raw coffee from Different Geographic Origins"

3.1 Introduction

The presence of non-volatile aroma compounds in coffee beans plays an important role in the beverage's final quality. In base their typology the content and ratio produce important differences in terms of taste and flavours in coffee beverage. The major storage compounds of mature *Coffee Arabica* beans are cell-wall polysaccharides, mainly galacto-mannans and arabinogalactan-proteins, lipids, proteins, sucrose, and chlorogenic acids (De Castro & Marraccini, 2006). Each of these major storage compounds plays several crucial roles in the complex roasting chemistry (Flament, 2002). The precursors are synthetized throught the metabolism of the plant in reply at climate and mode of coffee processing. Recently, it has been shown that during the post-harvesting stage various metabolic activities are present in green coffee beans (Selmar *et al*., 2002; Breitenstein *et al*., 2006). Beans that had been exposed to a longer period of drying were affected by a decreased in the contents of fructose and glucose within the first day of drying (Kleinwächter *et al*., 2009). The chemical composition of wet and dry processed raw coffee may differ significantly, as observed for free amino acids, organic acids, and non-structural carbohydrates (Bytof *et al*., 2005; Selmar *et al*., 2006). According to some research studies climate conditions are empirically known to have beneficial effects on coffee quality, these effects occurs through the climate changes of shade and altitude. These two factors are empirically known to have beneficial effects on the coffee quality. In addition other environmental factors such as air temperature have proven to have a considerable influence on the coffee beans. Cooler temperatures conditions have a positive attributed on the effects of the quality of coffee. Cooler temperatures conditions have a positive attributed on the effects of the quality of coffee. The effects of low temperatures have suggested to slow down the ripening process, which in turns leads to higher accumulation of aroma precursors (Guyot *et al*., 2006). It is generally accepted that high altitude improves coffee quality (Avelino *et al*., 2005; Decazy *et al*., 2003; Guyot *et al*., 1996). A study performed by

Thierry Joët (2009) explains the relationship between altitude and temperature and the final presence of CGA (chlorogenic acid) plays an important factor on sugars, lipid and caffeine thus appear to be highly dependent on the climate changes, even with a common trend towards 5-CQA (caffeoyl quinic acid). The levels in aroma precursors may vary with genetic traits (Leroy *et al*., 2006), soil-climatic conditions (Bertrand *et al*., 2006) agricultural practices (Vaast *et al*., 2006) and post-harvest techniques (Breitenstein *et al*., 2006). The aromatic precursors are classes of different compounds, which are known as sugars, in particular the sucrose including glucose and fructose, the proteins, caffeine, trigonelline, chlorogenic acids and carboxylic acids. These compounds take place in Maillard reaction and Strecker degradation during roasting process. The purpose of this paragraph is to quantify the precursor aromatic contents by different analytical techniques with the possibility to characterize the six variety of green coffee and their various origins. These important researches that were conducted through the characterization of precursors will allow researchers to improve and obtain better results and quality in roasted coffee.

3.1.1 Sucrose Glucose and Fructose

The sucrose is the principal low molecular weight carbohydrate or sugar in green coffee and the others mono-saccharides contents are relatively low (fig 1). Different publication showed several variations among bean types, although, in general, arabican varieties tend to contain about twice as much sucrose as robusta coffee. Sucrose is the major free sugar present in green coffee, it is found in specific amounts which are dependent upon the species and sources of the coffee (Trugo *et al*., 1985). Sucrose content has a relevant cherry ripeness marker, which is mainly influenced by post-harvest processing (Meenakhsi *et al*., 2007). The immature beans present a lower sucrose content than mature ones according to Mazzafera (1999). The sucrose is also the major sugar representing a long-term storage compound, that unlike exoses which covers the short term energy demand, is not catabolised before the reserves are required for seeding

development. The stored sucrose should be catabolised during storage to maintain the energy supply (Kleinwachter *et al*., 2010). Coffees that have been processed by wet or dry traditional methods showed a relationship between the kind of post harvesting and the fructose and glucose contents. It has been seen that wet treatment leads to low levels of these sugars and the dry method leads to higher amounts. A comparison with the fresh controls indicates that the low levels of both sugars could be the consequence of a decrease of glucose and fructose concentration during the dry. Sucrose, the major low molecular sugar in green coffee beans, is not significantly affected by coffee processing (Knopp *et al*., 2005). Whereas all other coffees lost viability within the first 6 months of storage, coffee beans stored within the parchment remained viable for >1 year. Glucose and fructose decreased slightly in the course of storage and glutamine content declined significantly (Selmar *et al*., 2007). Most literature values for sucrose are in the range of approximately 5% to 8,5% for arabicas (Clifford *et al*., 1985). Through specific factors a researcher can determine the profile of green coffees beans of different countries by the content of sucrose and by analyzing the low molecular weight carbohydrate (fructose, glucose, mannose, arabinose, rhamnose). Concentrations of glucose, fructose and sucrose evolve in a very similar way during maturation in Arabica grains. In particular glucose and fructose are usually the major free sugars of young coffee grains, glucose being consistently twice the concentration of fructose. By the end of the grain development, concentrations of glucose and fructose decrease for both species to 0,03 and 0,04% dry weight and a increase of sucrose in endosperm place (Rogers *et al*., 1999). When ripening or processing occurs it leads to the presence of monosaccharides, which derives from the polysaccharides and from small yields of arabinose. These elements are also found in all coffees, and in addition mannose is found in most coffees as well. The content of fructose found was 0,02% - 0,55%, instead the glucose was range < 0,01 – 0,50% (Silwar and Lüllman *et al*., 1989), instead of 0-2,2% and glucose of 0-1,9% fructose (Murcovic *et al*., 2006). Sucrose has an

important role in the coffee aroma: during the roasting process it creates different reaction by producing different flavour compounds (Oosterveld *et al*., 2003). The roasting process generates several classes of aromatic compounds such as furans, aldehydes and carboxylic acids (Perrone *et al*., 2008; Mazzafera *et al*., 1999). Trugo and Macrae (1985) showed that sucrose loss for a light roast was about 87% and for a dark roast about 99%.

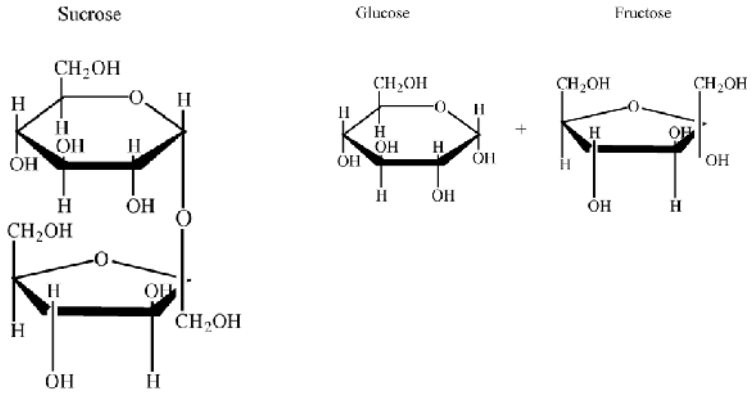


Fig 1 – Sucrose, glucose and fructose molecular structure

3.1.2 Caffeine

Caffeine called 1,3,7 trimethyl-xanthine is found in various kinds of foods and drinks that we consume daily (Singh & Sahu, 2006). It is a purine alkaloid and a secondary metabolite of the coffee plant: the biosynthesis starts from xanthosinemonophosphate (ribonucleoside monophosphate).

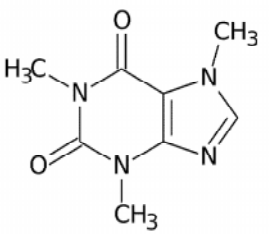


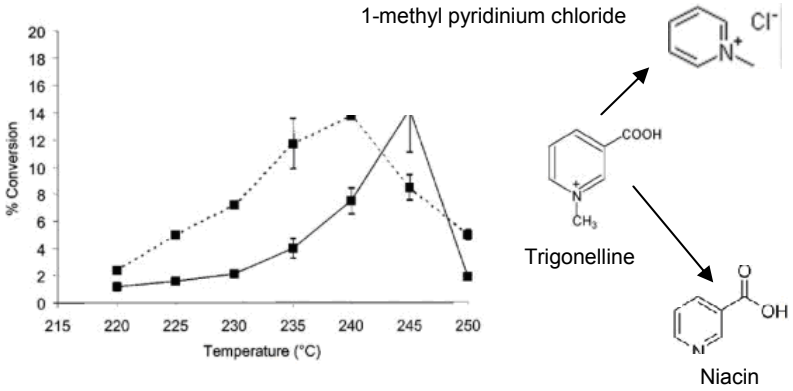
Fig 2 - Caffeine molecular structure

Research studies have proven that a higher caffeine contents is associated with highest quality samples compared to other Arabic samples (Oliveira *et al*., 2005). Therefore, establishing a rapid and cheap analytical method for the determination of caffeine in coffee beans, is considered a very important factor because it leads to the discovery of a wide range of physiological effects on the human body and quality controls. The coffee beans contain between 0,8 and 2,8% caffeine dry matter, depending species and origin, besides contributes to 10 to 30% of bitterness perceived in coffee beverage and this action depending on the concentration (Keast & Roper, 2007). It may also make a small contribution to its strength and body. The caffeine is thermally stable and showed a slight decrease after 10 min of roasting and it would be expected that caffeine sublimation might have occurred to a higher extent when its sublimation temperatures was reached at 185 °C (Casal *et al*., 2000). This observation should be related with porosity and the internal pressure created within the beans that may cause some difficulties for caffeine sublimation (Macrae *et al*., 1989).

3.1.3 Trigonelline

Trigonelline, the N-methylpyridinium-3-carboxylate, is, after caffeine, the second most important alkaloid of coffee, with about 1% of the green bean. In particular it is a pyridine derivative (Perrone, 2008) and known to contribute indirectly to the formation of desirable and undesirable aroma compounds during roasting (Macrae, 1985) and (Moreira *et al*., 2000). The direct precursors are nicotinic acid and nicotine amide, deriving from the pyridine nucleotide cycle (Ashihira *et al*., 2004). Trigonelline is

rapidly degraded during roasting in Niacin and 1-methyl pyridinium chloride (graph 1).



Graph 1 - Formation Niacin (dashed line) and 1-methyl pyridinium chloride (solid line) from trigonelline hydrogen chloride as a function of temperature, expressed as percent conversion on a molar basis. Pyrolysis time = 15 min.

Trigonelline products have an impact on the overall aromatic perception of roast coffee and beverage and has been correlated to good cup quality (Trugo *et al*., 2006). Niacin (nicotinic acid), the degradation product of trigonelline only about 1,5% and its formation depends on the roasting temperature and not on the process duration (Clarke Macrae *et al*., 1985). Instead in according with Casal (2000) the niacin content increased after 8 mn of heating and continued to 15 min. For an exposure time greater than 15 min a gradual decrease of the nicotinic acid content was observed. It is needed for vitamin supply in human nutrition; it is an accepted vitamin in European legislation (directive 90/496/EEC).

3.1.4 Chlorogenic acids (CGA)

The CGA are the main phenolic compounds in coffee and are widely distributed secondary metabolites in plants, and these are also present in the coffee beans in relatively large quantities. The

parent structure is a conjugate of tetrahydroxy-cyclohexane carboxylic acid (quinic acid) and caffeic acid. A whole family of chlorogenic acids is formed due to isomers and epimers in the cyclohexane part and substitutions at the aromatic ring. Chlorogenic acids are biosynthesized in the perisperm and accumulated in the beans endosperm. CGA content varies mostly according to genetic factors and the degree of maturation. The less important factors are climate, soil, agricultural practices (Clifford *et al.*, 1985; Farah *et al.*, 2006). The chlorogenic acid composition of coffee products is extremely complex with at least five major groups of compounds present: caffeoylquinic acids (CQA), dicaffeoyl-quinic acids (diCQA), ferloylquinic acids (FQA), *p*-coumaroylquinic acids (CoQA) and caffeoylferuloylquinic acids (CFQA). In fig 4 is shown the general structure of CGA

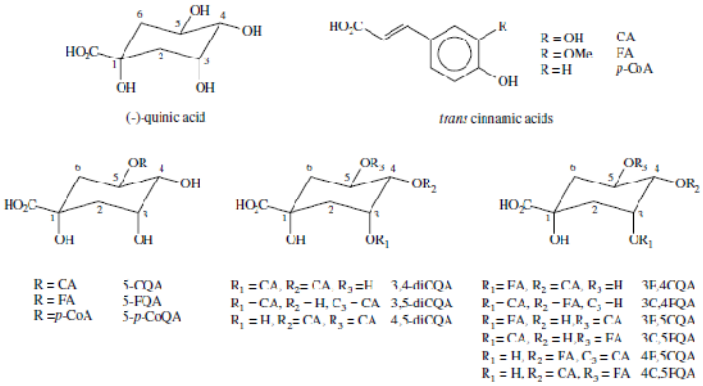


Fig. 3. Structure of chlorogenic acids precursors – quinic acid, caffeic acid (CA), ferulic acid (FA), p-coumaric acid (p-CoA) – followed by CGA main subclasses: caffeoylquinic acids (CQA), feruloylquinic acids (FQA), p-coumaroylquinic acids (p-CoQA) (example of 5-isomers for CGA monoesters), dicaffeoylquinic acids (diCQA) and caffeoylferuloylquinic acids (CFQA). We adopted the IUPAC numbering system (IUPAC, 1976) for chlorogenic acids.

Although CGA (primarily as 5-caffeoylquinic acid) are widely distributed in plant materials, their content in green coffee is

among the highest found in plants, ranging from 4 to 14% (Farah & Donangelo, 2006; Trugo & Macrae, 1984), with 45 different CGA compounds already identified (Clifford, Johnston, Knight, & Kuhnert, 2003; Clifford *et al*., 2006a; Clifford *et al*., 2006b). CGA play an important role in the formation of roasted coffee these compounds are important factors in determining a distinct flavour characteristic in a cup of coffee (Farah, Monteiro, Calado, Franca, & Trugo, 2006). Moreover, several beneficial health effects have been attributed to CGA and may be largely explained by their potent antioxidant activities (Moreira, Monteiro, Ribeiro-Alves, Donangelo, & Trugo, 2005; Natella, Nardini, Gianetti, Dattilo, & Scaccini, 2002; Pereira, Pereira, Trugo, & Neto, 2003). During coffee roasting, CGA are partially degraded as a result of pyrolysis, generating phenolic lactones and other derivatives. Cinnamoyl-1,5-c-quinolactones (CGL) are the main CGA lactones in roasted coffee, being produced through the loss of a water molecule and formation of an intramolecular ester bond between positions 1 and 5 of QA (Farah, De Paulis, Trugo, & Martin, 2005). Phenolic volatiles along with CGA, CGL are major contributors to coffee flavour, (despite their low concentrations, their impact on the final cup quality may be significant (Ginz & Engelhardt, 2001). They contribute to the final acidity (Macrae *et al*., 1985) and confer astringency (Clifford *et al*., 1985) and bitterness to the beverages. As a result of Maillar and Strecker's reactions, bitterness increases during roasting due to release of caffeic acid and formation of lactones (De Maria *et al*., 1995). However, according to Silva (1999), the total CGA levels present an inverse association with the quality of coffee, with higher CGA content has been observed in lower quality samples. Considering that CQA accounts for at least 60% of CGA content in roasted coffee, higher levels of CQA would be more likely to be associated with low quality in a cup of a brewed coffee. The changes in these chlorogenic acid lactones and quinic acid lactones are involved with the generations of a bitter, sour taste in a coffee brew which is also determined by another important factor whether it has stood for some hours on a hot plate. The CGA are attributed potential biological properties that

are: antioxidant, antibacterial, anti-mutagenic, anti-obesity, antiviral, immuno-stimulating, hypoglycaemic (Farah *et al*., 2008).

3.1.5 Raw proteins

The protein content of green coffee is about 10-13% (Macrae *et al*., 1985; Franca *et al*., 2005). The protein profile of coffee changes during roasting; the proteins are both fragmented and polymerized, and integrated into melanoidins. Their concentration in the brew is at the end about 6-7% (Cliffors *et al*., 2006). The proteins in green coffee consist of water soluble (15% albumin and 85% globulin) and in water insoluble fraction, present almost in similar proportion and are stored in endosperms (Rogers *et al*., 1997). Both of the fractions are high temperatures-sensible. The content in proteins and free amino acids did not vary appreciably with species or with maturity, and had only a very slight tendency to a higher content in mature beans. According to a study conducted by Mazzafera (1999) found a higher protein content in mature beans than in the immature beans, but a lower content of free amino acids. The dry methods had no effect on % crude protein (Tetteh Lower *et al*., 2007). The proteins, peptides and free amino acids play an important role in the coffee flavour formation being involved in the development of colour, aroma and flavour compounds in Maillard reaction (Clarke *et al*., 1985; Murkovich *et al*., 2006). Protein degradation is proportional to the degree of roasting and varies from 20 - 40% in medium degree roasts up to 50% in darker roasts. The content and degradation depends on the initial composition, and, therefore on species and varieties. Besides the content their might change based on the polyphenol oxidase activity, in this case oxidation products of CGA can bind to proteins producing a defective bean discoloration. A great deal of free amino acids (the major are glutamine, glycine, asparagine, aspartic acid) are involved in a series of reactions giving rise to many of the volatile substances in the aroma and colour in roasted coffee (illy *et al*., 1985).

3.1.6 pH and total acidity

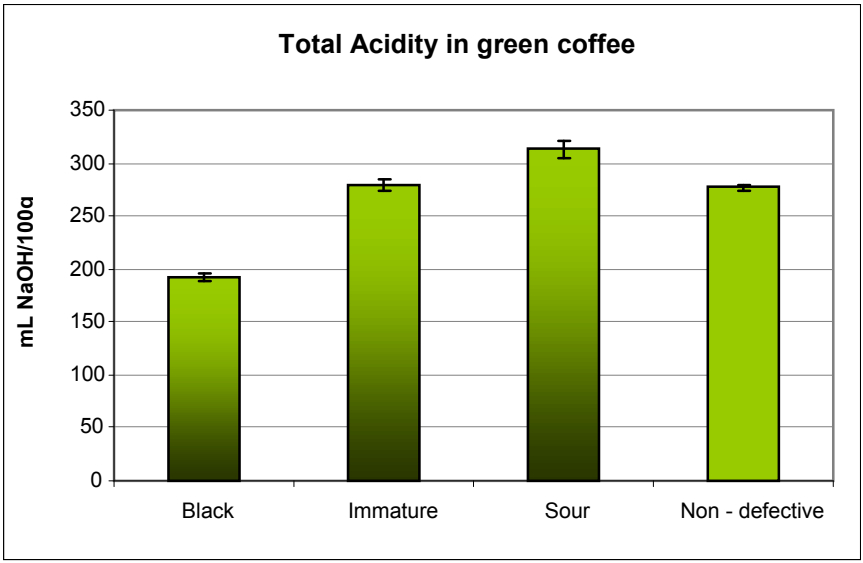
The pH is the hydrogen ion concentration of an aqueous solution. It is related to the acidity concept, in particular at the ionization or dissociation of acids present in an aqueous solution. The content is a very important element of concentration of H^+ ions about perceived acidity. Certainly there are a few important factors that influence the acidity which are:

- Botany: there are several varieties of coffee beans. Usually Arabica coffee beverages pH 5.3-5.5 (Franca *et al*., 2004).
- Altitude: coffee shrubs and beans grow more quickly in lower, warmer, and moister climates than in higher, cooler mountain areas. Higher grown coffee beans make more acid-tasting beverages, which are recognized for their good flavour and aroma and are therefore more expensive (Colombian Arabica beans grown at up to 1800 meters above sea level). Central American Arabicas (Costa Rica, Nicaragua, Honduras, Salvador, Guatemala) grown between 750-1500 meters. Brazilian Arabicas are grown between 400-600 meters under a natural cloud cover near the Tropic of Capricorn.

Kenyan coffee beans are well known for their well developed acidic character which is often described as "fine acidity" (Vitzthum *et al*., 1976).

- Wet and dry processing: except for Brazil, almost all Arabica coffee countries use the wet method. And their beans make the most-acid tasting coffee. Dry processing gives a higher pH than wet processing (Tetteh Lower *et al*., 2007).
- Age of Beans: coffee beans yield the most acid tasting beverages just after harvesting and processing (new–crop beans). As the years pass, these same beans produce beverages that are progressively less acid, the rate of change depends on temperature and moisture conditions while in storage (Sivetz, 1963). Many authors subsequently reported the same effect–an increase of acidity during storage which could have been detected by a decrease in the pH and a corresponding increase in titratable acidity (Cros *et al*., 1980; Walkowsky *et al*., 1981).

- Degree of roasting: illustrated in the chapter on sensory analysis the acidity of coffee depends upon the degree of roast (Balzer *et al*., 2001).
- Presence of defectives: the presence of defective beans is usually a consequence during harvesting and pre-processing operations saw in the first chapter. Acidity values for green coffee were the highest for sour beans, which could be associated to bean fermentation. Acidity levels were the same for both immature and non defective beans which could be due to the loss of acids during soil contact (graph 2). According to previous studies, acidity should increase as coffee quality decrease (Franca *et al*., 2005; Mazzafera *et al*., 1999).



Graph 2 - Average value ± standard deviation of total acidity (mL NaOH/100g) of the samples Arabica green obtained from Minais Gerais, Brazil (Vasconcelos *et al*., 2007)

The commercially available defective coffee beans also showed higher titratable acidity like the other category of defective coffee

beans (Ramalakshmi *et al*., 2007). The acidity and sourness of coffee brews have always been recognized as important attributes of their sensory quality (Ginz *et al*., 2000). Acids are responsible for sourness, which together with aroma and bitterness are a key contributor to the total sensory impact of a coffee beverage. Green coffee contains present citric, malic that are major acids, oxalic, tartaric, pyruvic, quinic, CGA and acetic acids (Maier *et al*., 1982). Their contents are affected by factors such as age, processing and fermentation. Analysis of the Arabica coffees gave an average of 5,6 g/Kg for malic and 12,3 g/Kg for citric acid (Clarke & Vitzthum *et al*., 2001). In general the acids present in coffee are responsible for about 11% of the green beans weight and for 6% of roasted coffee beans weight (Maier *et al*., 1987). In table 1 are reported the principal acids in different coffees (Kampmann *et al*., 1982; Scholze *et al*., 1984).

Provenance	Quinic acid	Malic acid	Citric acid	Phosphoric acid
Santos Aabica	5.6	6.15	13.81	1.07
Burundi Arabica	5.7	5.12	13	1.11
Kenya Arabica	4.7	6.62	11.65	1.37
Colombia Arabica	5.5	-	-	-
Mocca Arabica	-	4.60	10.55	1.47
Burundi Robusta	3.5	3.78	10.01	1.42
Angola Robusta	-	2.78	9.17	2.16
Guinea Robusta	3.9	-	-	-

Table1. Contents (g/Kg) of quinic, malic, citric and phosphoric acids of different green coffees. (Data from Kampmann & Maier; 1982; Scholze & Maier, 1983, 1984)

3.2 Material and Methods

3.2.1 Sucrose/Glucose/Fructose

Sugars determination was carried out on the raw coffee diluited extract (Sucrose/D-Glucose/D-Fructose enzymatic analysis, R-Biopharm. Roche). Raw coffee (10 g) was dried under vacuum condition (Vuomatic 50.Bicasa) overnight at T=40 °C. Dried raw coffee was ground for 120 seconds in a coffee mill and 0,5 g of ground coffee was added to 30 mL of Milli Q water. The sample was sonicated in a water bath (Ultrasonic Cleaner VWR) at T=80 °C for 10 minutes. The samples was cooling in a ice bath for 5 minutes. The samples was centrifuged (Centrifuge Beckmen. Ireland. Model TJ-6) for 5 minutes at 2500 rpm. After 20 mL of this first extraction were collected in a falcon and added to the second one that was the result of the same operations. The relative concentrations were determined before and after the enzymatic hydrolysis, by 340 nm spectrometric measure (Spectrophotometer Jasco V-530 UV-VIS) following the instruction proposed by R-Biopharm enzymatic Kit. The measures were carried out in triple.

3.2.2 Proteins

The nitrogen (method 920.87) contents of the green coffee samples were determined according to official AOAC procedures (AOAC, 1995). Nitrogen content (N_2) was determined with a Kjeldahl struments. A 1g sample of green coffee beans was grinded and carried out in the digestion system for 240 minutes using 20 mL of sulphuric acid 0,1N and potassium sulfate as catalysts (method 979.09). Termined the digestion was title with HCl 0,1N. Protein levels for green coffee were also in the range reported in the literature. It is noteworthy to mention that this range is based on the determination of crude nitrogen and multiplication by the factor 6,25. After corrections for caffeine-nitrogen and trigonelline nitrogen, protein values should be in the range of 9–13 g/100 g. The measure were carried out in triple.

3.2.3 Alkaloids (caffeine, trigonelline, chlorogenic acids)

The analysis about alkaloids are performed by HPGF (Hight Performance gel Filtration) which is a technique and a method used that allows to determine simultaneously the presence of caffeine, trigonelline and CGA (De Maria *et al.*, 1995). Raw coffee (10 g) was dried under vacuum condition (Vuomatic 50 Bicasa) overnight at T=40 °C. Dried raw coffee was ground for 120 seconds in a coffee mill and 0,5 g of ground coffee was added to 30 mL of Milli Q water. The sample was sonicated in a water bath (Ultrasonic Cleaner VWR) at T=80 °C for 10 minutes. The samples was cooling in a ice bath for 5 minutes. The samples was centrifuged (Centrifuge Beckmen, Ireland, Model TJ-6) for 5 minutes at 2500 rpm. After 20 mL of this first extraction were collected in a falcon and added to the second one that was the result of the same operations. The samples were diluted 20 times the measure were carried out in triple. The analytical conditions are summarized in Table 2.

Device	Conditions
HPLC Jasco UV-VIS 875 UV/880 02/ 880PV	
Column	TSK-GEL G2000SW (Tosoh Corporated) Size: 7,8mm x 30 cm
Guard Column	TSK Guard column SW (Tosoh Corporated) 6mm x 4 cm
Detector	UV detector at 272 nm of wavelength
Integration System	Chrom card for Windows version 1,19 (1997)
Mobile phase	Water MilliQ Flow rate: 1 mL/min
Chromatographic conditions	Isocratic conditions Flow rate: 1 mL/min Analysis time: 24 min
Injection system	Manual injection Valve 7725i (Reodyne) Injection Volume: 20 µL

Table 2: HPGF Conditions for determination of alkaloids of raw coffee (De Maria 1995 method)

The compounds are analyzed and the areas of relatives chromatogram were prepared then the standards of caffeine, trigonelline and CGA are measured in order to build calibration curves and obtained the linearity equation for quantitative analysis how reported in table 3.

Standard Compound	Calibration Equation	R^2
Caffeine	y=0,000000026x	R^2=0,988
Trigonelline	y=0,0000000619x	R^2=0,997
Chlorogenic Acids	y=0,000000618x	R^2=0,999

Table 3 Linearity equation obtained by injection standard caffeine, trigonelline and CGA

3.2.4 Determination of pH

The pH of green beans measurements was used the automatic titration by struments called CRISON (AOAC Official method 1995). The samples of green coffee were grinded for 2 minutes. 0,5 g sample grinded was placed in a test tube and added with 50 mL deionized water. The pH analysis was made using a simple pH meter. The electrode used was HAMILTON Flushtrode/P at 25 C°. The measures were carried out in triple.

3.2.5 Determination total acidity

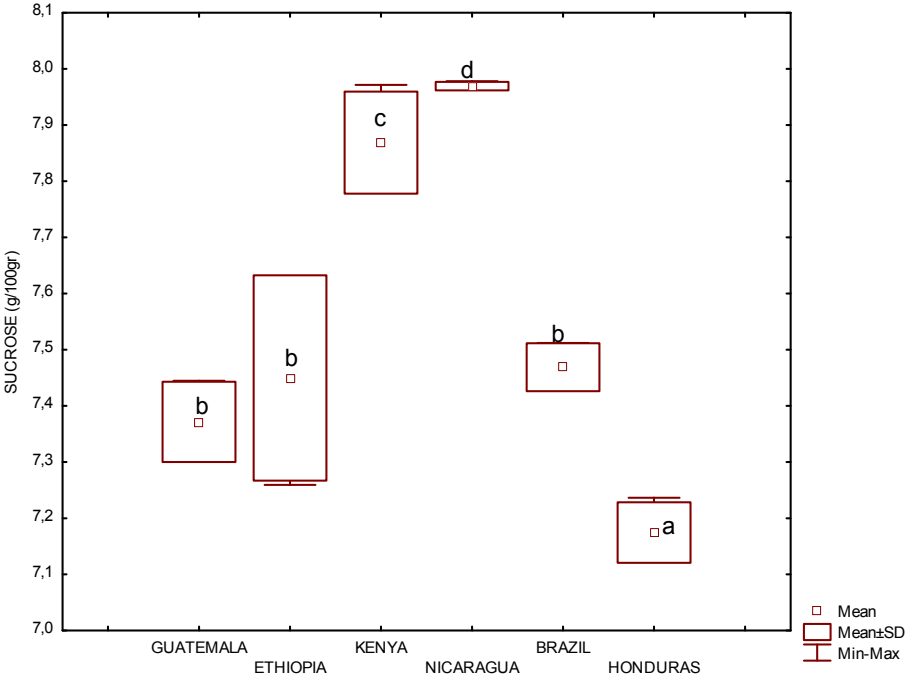
The total acidity of green beans was measured by automatic titration, with instruments called CRISON (AOAC Official method 1995). The samples of green coffee were grinded for 2 minutes. 10 g prepared grinded sample was placed in test tube Erlenmeyer and added 75 mL 80% alcohol, stopper, and let stand 16 h shaking occasionally. Filter, transfer aliquot of filtrate (25 mL for green coffee) to beaker, dilute to ca 100 mL with deionised water and titrate with 0,1N of NaOH, using phenolphthalein. Express results as ml 0,1N of NaOH required to neutralize acidity of 100 g sample. The end value is taken at pH 8. For this analysis was took 50 mL of sample. The measures were carried out in triple.

3.2.6 Statistical analysis

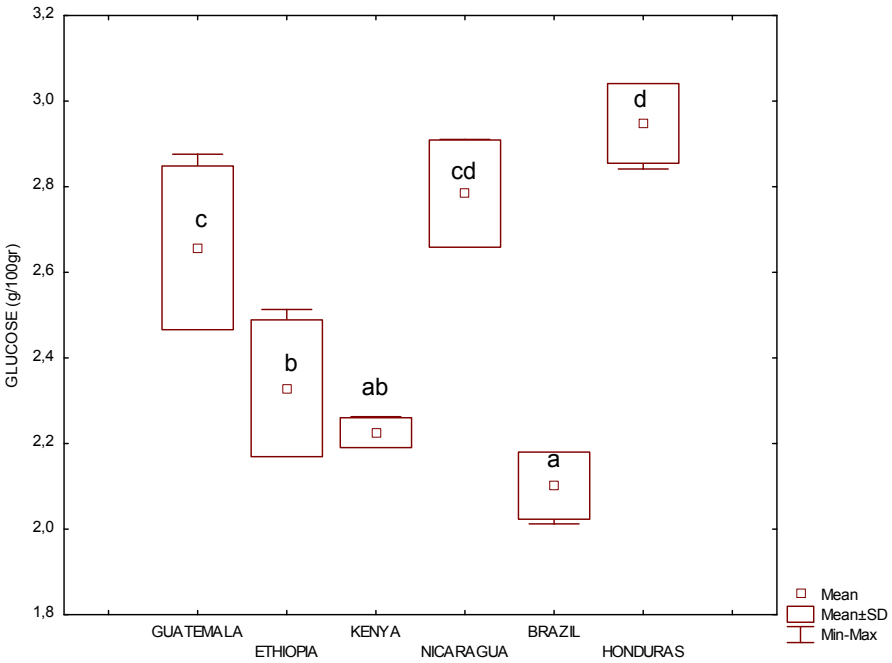
A one way analysis of variance (ANOVA) was carried out for all the result obtained. To verify the homogeneity of variance it was applied the Levene's test. Means and standard deviations were calculated and represented by box-plot, and significant differences marked with letters were evaluated by the LSD Fischer Test. Leven's test variances were homogeneous and results were considered significant at $p < 0,05$.

3.3 Results and discussion

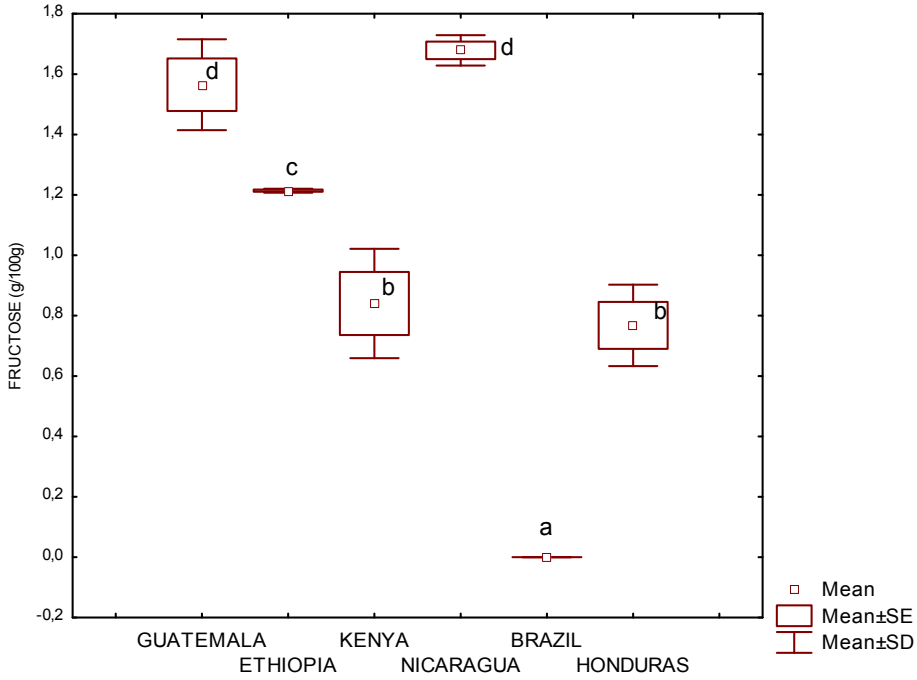
Observing the following graph it is possible to make some observations about the content of the presence of aromatic precursors in different quantities for every single origin. The experimental trials about sugars (sucrose, glucose, fructose) explained in graph 3 - 4 - 5 showed that sugars are related to origin and different post-harvesting techniques.



Graph 3 - Box Plot representation and the variance analysis (LSD Fischer Test) of sucrose content (%) identified by enzymatic Kit



Graph 4 - Box Plot representation and the variance analysis (LSD Fischer Test) of glucose content (%) identified by enzymatic Kit

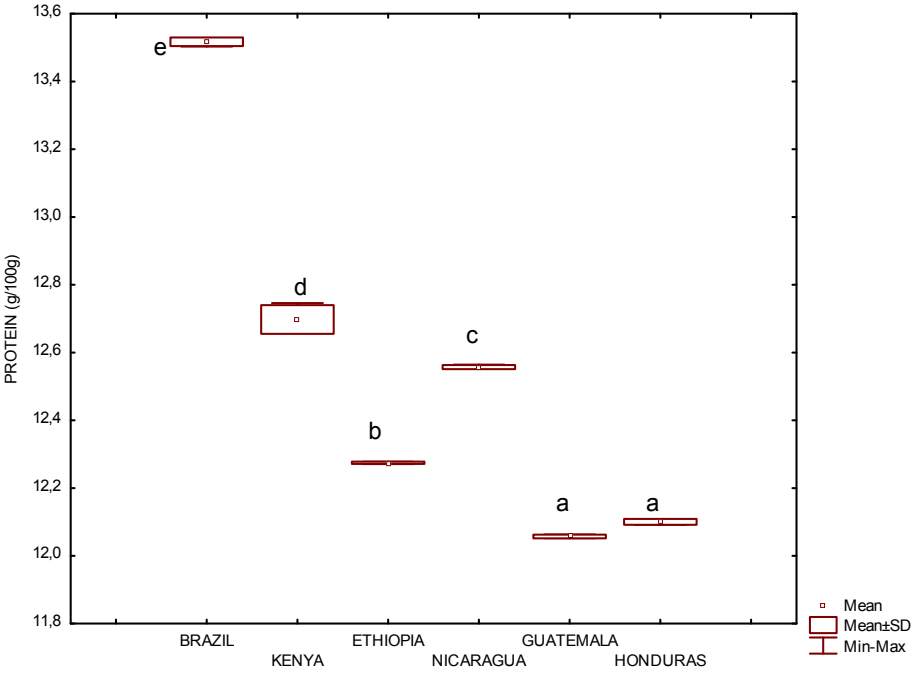


Graph 5 - Box Plot representation and the variance analysis (LSD Fischer Test) of fructose content (%) identified by enzymatic Kit

The sucrose content data are in accordance with different authors who declared that the content differed significantly between species (Chabrillange *et al*., 2000). The values obtained of the content of sucrose and glucose fructose are in agreement with the literature and data (Farah *et al*., 2006). Referring to the graph 3 it wasn't evident a difference between Brazil coffee post-harvest dry method and others coffee with post-harvest wet method. The factors post-harvest have a major influence on the coffee's quality and therefore the content aromatic precursors. The contained lower part of sugars in the Brazilian coffee samples can be justified from the fact that the coffee has been mechanically harvested. It is well known that in Brazil the coffee often comes

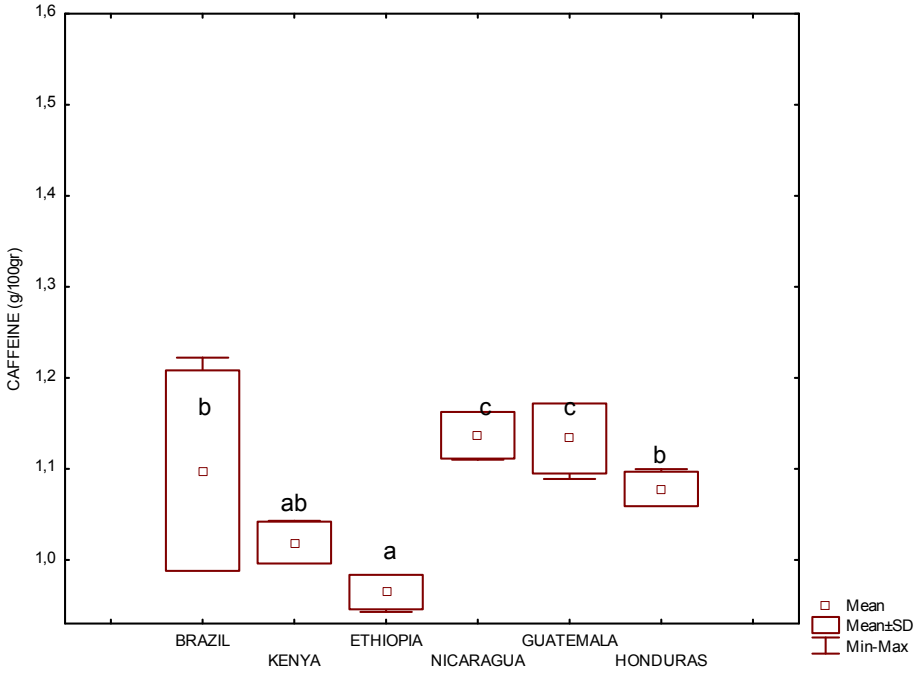
mechanically harvested in a lot of zones of the Bahia State and the different crops both good and bad are often blended and used for export. The mechanically harvesting depreciates the quality of the coffee beans because the immature beans amount is very relevant and often high. These immature beans present lower sugar content than the mature ones according to Mazzafera (1999). It is also necessary to point out the other variable, that the sample coffee from Brazil was imported directly from the origin country and it's been sampled from numerous stock locations. The highest sucrose level in green beans was found in the Kenya and Nicaragua coffee, the medium level in Guatemala, Ethiopia, Brazil and the lowest level in the Honduras coffee also as a consequence of environmental characteristic. The other varieties of coffee surely introduce a taller value of reducing sugars in particular the coffee Nicaragua and Guatemala. These coffees are been treated by post harvesting method washed and moreover it is well known (Illy and Viani, 1995, 2005; Clarke Vizthum, 2001) that washed coffee usually comes from a manual harvesting where only ripe cherries are picked. As a result of this the content of sugars varies with the maturation degree and the ripening speed (Geromel, 2008), and the higher of sugars, in particular sucrose, in washed coffee respect to natural or semi-wet one is explained (Kleinwachter *et al*., 2010). The manual harvest allows to select the good and healthy beans you deprive of defects where they present a lower content of sugars (Mazzafera *et al*., 1999; Vitzhum *et al*., 2010).

The total crude proteins content of coffee beans were presented in graph 6. The crude proteins level were in the range reported in literature. The earlier studies had found small differences in total protein between different green variety (Underwood and Deatherage 1952; Thaler and Gaigi 1962) and in graph 6 the variance analysis revealed that significant differences between green samples. In particular a high value of proteins was been obtained in Brazil coffee, probably this result was affected also by the post harvesting treatment method dry.



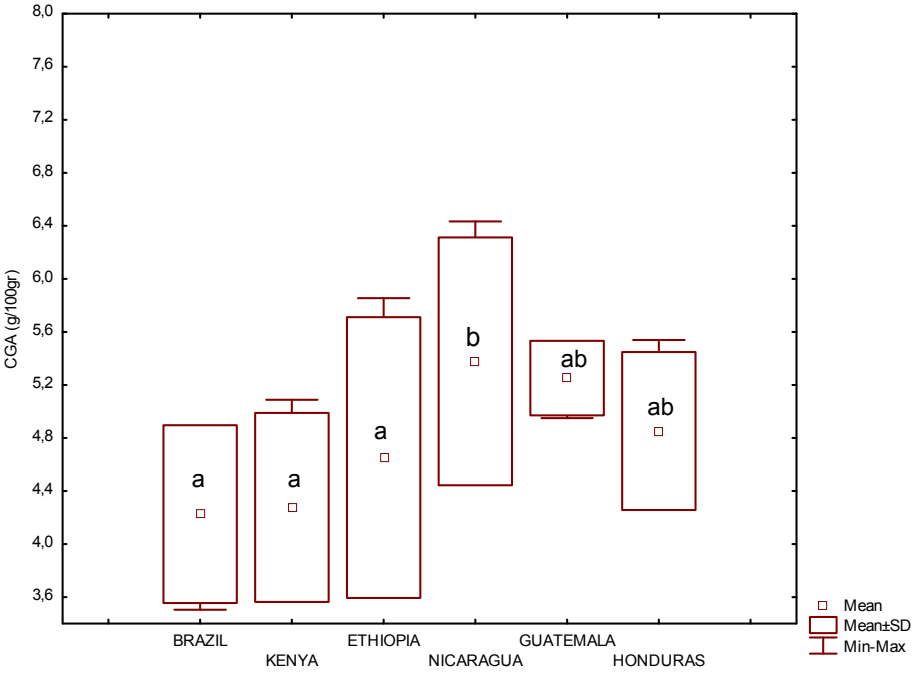
Graph 6 - Box Plot representation and the variance analysis (LSD Fischer Test) of protein content (%) identified by Kjeldahl (method 920.87)

The caffeine contents is illustrated in graph 7 and showed a small but a few significant difference. The caffeine is related to genetic factors, in particular to the variety and the species: Nicaragua, Guatemala, Honduras showed average contents of 1-1,22 g/100 g respect to light low values of Kenya and Ethiopia coffee. How is been explained in literature the caffeine is not affected in base post-harvest processing in agreement with general coffee data (illy 1985; Clarke 1985) and the value higher caffeine are related highest quality sample (Franca *et al*., 2005).



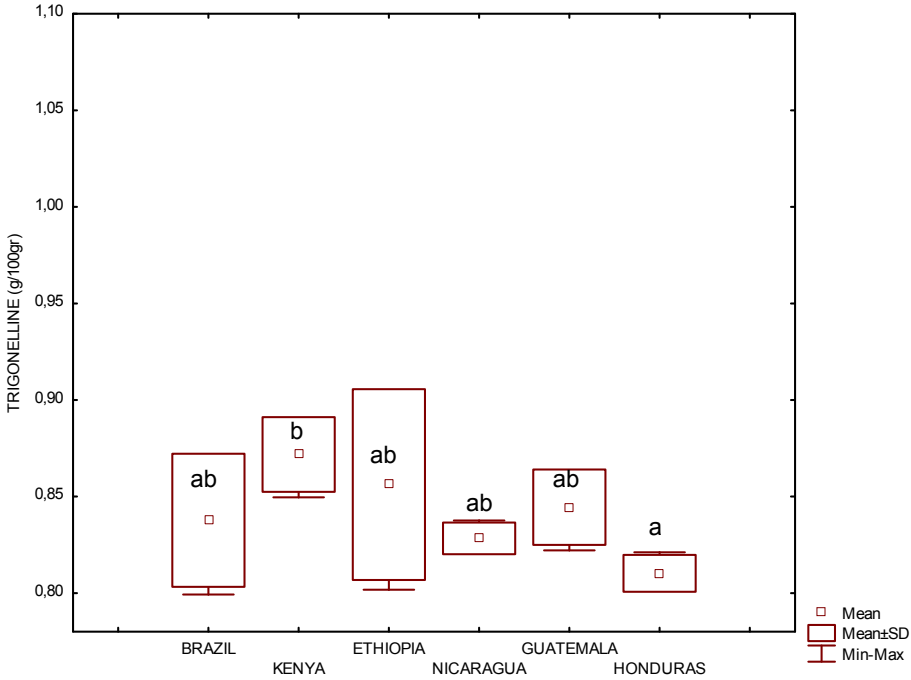
Graph 7 - Box Plot representation and the variance analysis (LSD Fischer Test) of caffeine content (%) identified by HPGF technique

Despite the content of CGA is influenced by different factors but the analysis of the variance not underlined significant differences in CGA content between single green coffees. The factors that might influence the content of CGA are the treatment post-harvest, genetics, climate, ground and presence of defects, in particular in immature bean the content their increase.



Graph 8 - Box Plot representation and the variance analysis (LSD Fischer Test) of CGA content (%) identified by HPGF technique

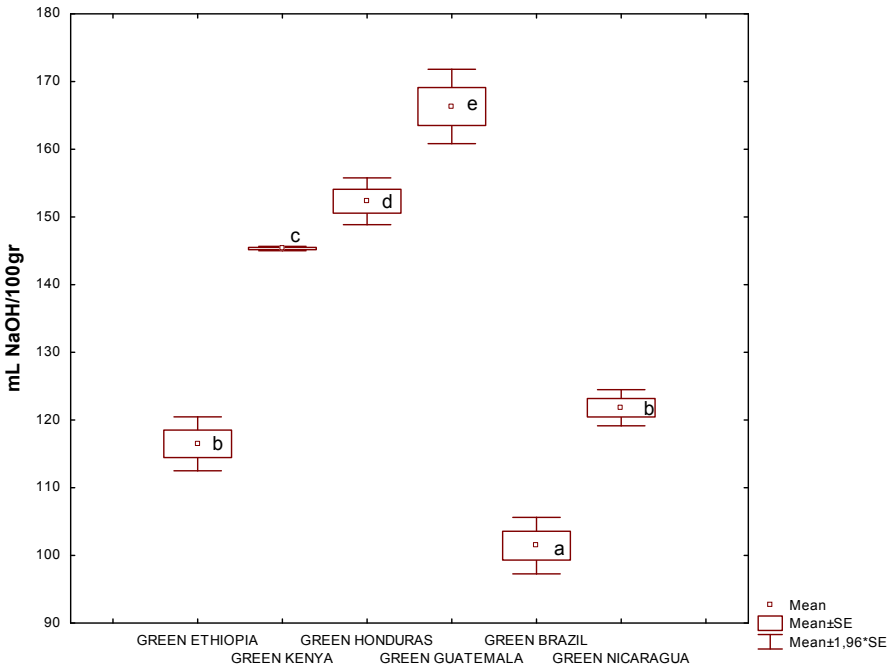
The trigonelline contents are presented in graph 9 that showed differences between origin. Trigonelline content seems to be highly dependent on the variety, but also on the extraction and dosage methods implemented (Campa *et al*., 2004). The content ranged from 0,80 to 0,90 g/100 g in agreement with data reported in literature; and only Kenya coffee showed higher value respect to Honduras with value more loss.



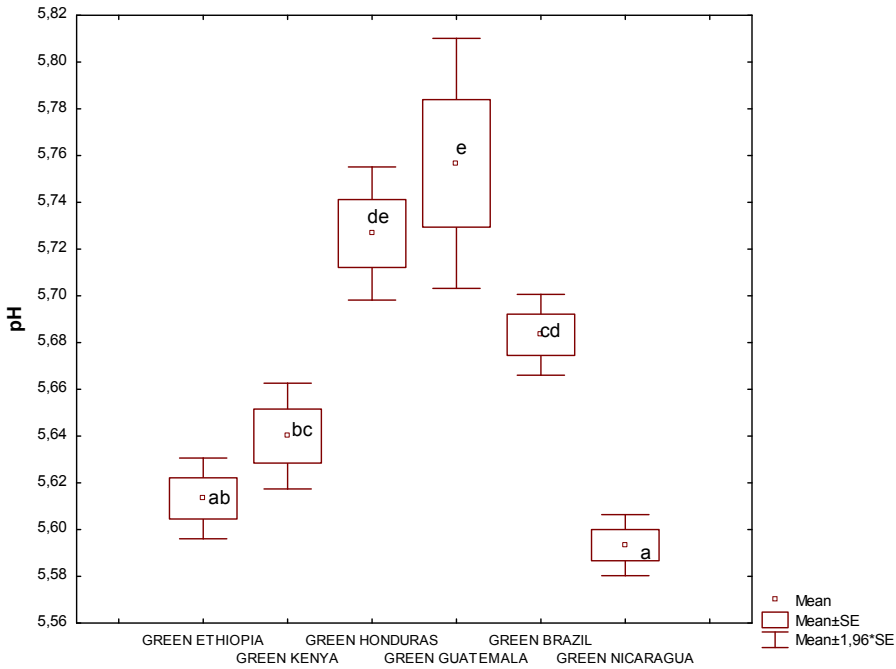
Graph 9 - Box Plot representation and the variance analysis (LSD Fischer Test) of trigonelline content (%) identified by HPGF technique

The acidity of coffee is an important attribute of coffee brews influenced by several factors as discussed in the introduction in particular country of origin and post harvesting method. The graphs 10-11 showed the trend of acidity and pH of the single coffees from different origins. Looking at the results showed in the graphs is easy to see that there is a significant difference between the different coffees. Each coffee has an acidity value different, except for the coffee Ethiopia and Nicaragua that have the same value. High levels of acidity is been found in coffee Guatemala and Honduras. In general, the Central American coffees are characterized to have a high acidity. There is also an

indication that acidity should increase and pH should decrease as cup quality diminishes. This could be associated to the effect of sour beans on cup quality. According to Mazzafera (1999), low quality coffee is associated also with high acidity contents, mainly due to bean fermentation.
Brazil coffee instead presented a low acidity. In fact except for Brazil, almost all Arabica coffee growing countries use the wet method, and their beans make the most-acid tasting coffee. When wet or natural fermentation occurs under adverse conditions, the beans may become sour and acid tasting. Similarly, dried beans stored under humid conditions will absorb moisture and also ferment. The method dry-processed gives a less acid taste. Besides the dry processing gives a higher pH than wet processing. The graph 11 showed higher level of pH in coffee Honduras and Guatemala despite a high value of acidity. This phenomenon is given by the different mechanism of dissociation of the carboxylic acids present as a function of pk_a It is also probably that over the presence of principle acids (citric, malic, quinic) are present minor acids that might influence the different dissociation mechanism responsible of acidity perceived. Would be necessary to make a major investigate about content of carboxylic acids in green coffee samples.



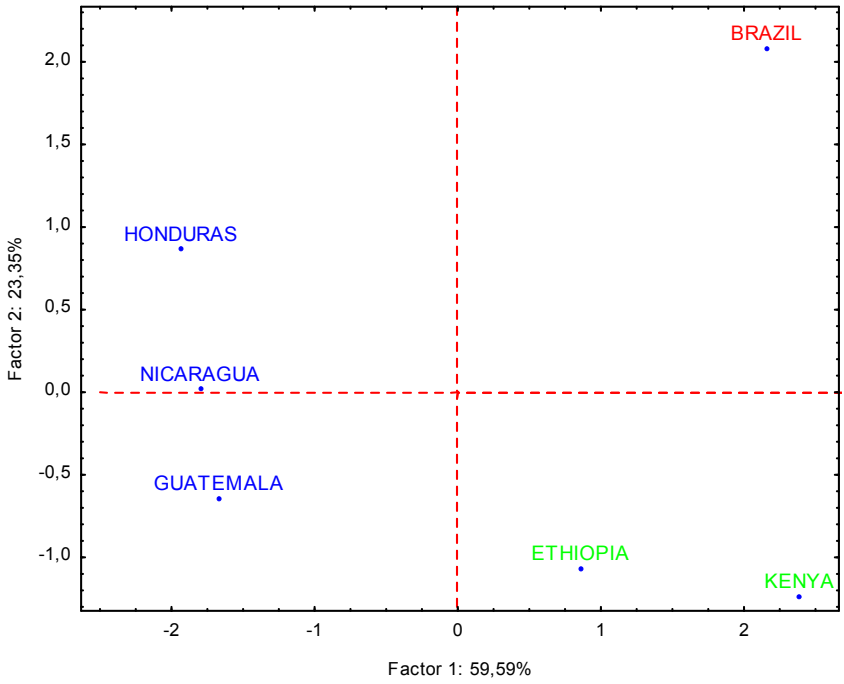
Graph 10 - Box Plot representation and the variance analysis (LSD Fischer Test) of total acidity (ml NaOH/100 g) measure by Crison strument



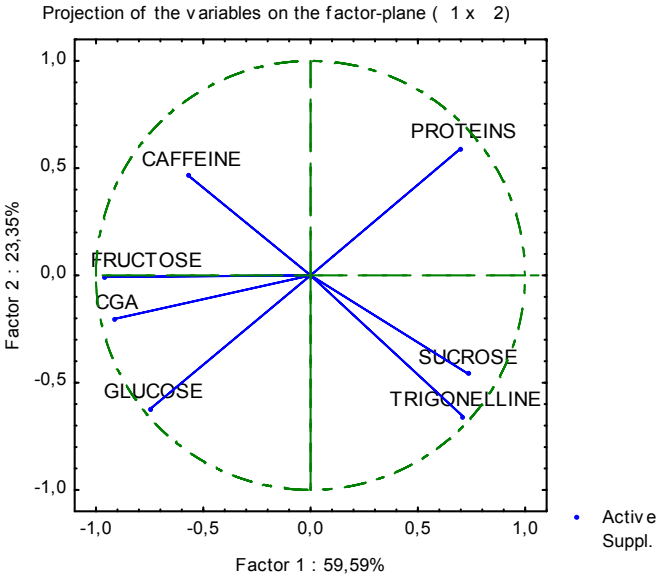
Graph 11 - Box Plot representation and the variance analysis (LSD Fischer Test) of pH measure by instrument Crison

In order to visualize the data structure and the discriminating efficiency of the selected features, scatter plots of samples using the first two principal components (Factor 1 and Factor 2) obtained from PCA. Graph 12-13 shows the biplot of the studied samples and variables representing content of precursor analyzed in green coffee samples. The scores plot explains 82,94% of the total variance, so that Factor 1 accounts for 59,59% and Factor 2 for 23,35% of the total information. At a cursory glance, good separation of the samples can be observed. for graphs 12-13 show a clear separation of the origin of green coffee samples analyzed. Explained the variability in these graphs is of 82,94%. African coffee samples are located at positive scores of Factor 1, well separated from American center

samples which are located at negative scores of Factor 1. These results are in relative agreement with the fact that samples are different based on the amount of aromatic precursors and not only aromatic fraction as stated in chapter 2. The detail in figure 13 shows that the variables that characterize the coffees. It is easy to see how the American coffee center are characterized to have a higher content of caffeine, glucose, fructose and CGA, the African samples have the highest values of sucrose and trigonelline and the Brazilian coffee is rich in proteins.



Graph 12. Results of PCA analysis aromatic precursors in green coffee samples of different origin



Graph 13 - PCA Projection of all variables on the factor-plane relative to aromatic precursors in green coffee samples of different origin

3.4 Conclusions

The analysis of the aromatic precursor of the six different green coffees studied by analytical techniques showed that their content precursors are determined mostly by the characteristic botanical variety, harvesting, post harvesting treatment and different climatic conditions. In particular way it was evident that these factors affected the quality in terms of quantity proportional contents there. In details the Brazil resulted to be more characterized of protein content, Kenya and Ethiopia more sucrose and trigonelline, Guatemala more glucose and chlorogenic acids and Honduras, Nicaragua more caffeine and fructose. If we compare the results seen in chapter 2 the fraction volatile plays a role to discriminate different green coffee, but a score variability of lesser than content aromatic precursors. At this point the content precursors seem to have a more significant weight about characterization of the single coffee origin. This hypothesis also explain the higher value of variance obtained in analysis principal component during elaboration and processing of statistical data. It is evident that the factors seen are the main critical points about content precursors and is necessary to apply proper manufacturing practices during harvest and overall post harvesting stages. It is been known that the content of the precursors play a important key during roasting process in the formation of specific aromatic compounds formed as referenced to Maillard reaction and Strecker degradation.
The high variability of individual precursors present in the origins of coffee is useful to perform a qualitative characterization of single origin and development of rapid methods (example near-infrared spectroscopy) capable of evaluating the quality of green coffee on objective data and build predictive mathematical models able to evaluate the quality of roasted coffee.

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FOURTH CHAPTER

"Sensory properties of espresso coffee from roasted coffee of different origins according to the degree of roasting applied"

4.1 Introduction

Coffee is consumed by a large proportion of the human population (about 70–80%) (Schilter *et al*., 2001). The extraction methods for coffee beverage preparation vary significantly on a geographical basis when soluble instant coffee is not used. In general in addition to the espresso coffee method there exist other modes of preparation. Some other coffee beverage preparation methods include drip filter coffee, emulsion-like (Nordic boiled coffee) and also thick suspensions (Turkish style brew) (Petracco, 2001). Conditions normally used in the espresso method, with phenomena such as foam and emulsion formation and stabilization strongly affect the organoleptic beverage properties. Italian espresso coffee is properly the beverage (not a coffee roasting degree or a coffee blend) prepared on request from roasted and ground coffee beans (6,5 ± 1,5 g) by means of the addition of hot water (90 ± 5,8 C°) at pressure (9 ± 2 bar) applied for a short time (30 ± 5 sec) to a compact roast and ground coffee cake by a percolation machine. The espresso extraction method results in a polyphasic beverage constituted by a foam layer of small bubbles with a particular tiger-tail pattern, and in this complex system, interfacial phenomena such as foam and emulsion formation and stabilization are of crucial importance in contributing to greater sensory satisfaction. To obtain a good espresso it is necessary to consider the variables during the preparation stages (coffee blend, roasting, grinding, water temperature and pressure, percolation time and/or beverage volume, etc.). Any or all of these possible variations can dramatically alter the properties of taste, flavour, mouth-feel (chemical composition) and the different phases (foam, emulsion, suspension and solution) present in the beverage (Andueza *et al*., 2002; Maetzu *et al*., 2001). Variations induced by preparation conditions (beverage volume/percolation time) on chemical composition can affect the foam characteristics in particular a change in colour, the texture and the life of the espresso foam. Espresso coffee's long-lasting after-taste, a sensation perceived for a while (up to 15 min) after having swallowed and emptied the

mouth, has been connected to the beverage surface properties (Petracco, 2001). Espresso coffee is produced by using coffee beans of various species and origins (blends), followed by roasting, grinding and brewing. However, preparing the "perfect" cup of espresso can still be considered a ritual, a combination of art and science. A cup of well-prepared fine espresso should have a bitter-sweet taste with an initial slightly acidic note, it should have a strong body and an intense aroma and should be pleasantly persistent (illy and Viani, 1995). The preparation method of beverage espresso coffee exalt the very strong aromatic properties and the taste of the coffee itself. All of these desirable sensory properties derived from a profound chemical complexity are affected by a huge number of factors. Aromatic components are particularly important in coffee beverages as they are the main constituents of the sensory experience for coffee drinkers. Illy and Viani (2005) summarized that there are six factors that affect the sensory properties of coffee: plant varieties, growing region/conditions, composition of green beans, processing methods (from coffee cherries to green coffee beans), roasting levels, grinding size, storage conditions and brewing methods. Extensive studies have been conducted since the beginning of the twentieth century to discover the volatile compounds responsible for coffee aroma and flavour in roasted bean, ground coffee, and brewed coffee (Czerny *et al*., 1999; Sarrazin *et al*., 2000; Semmelroch & Grosch *et al*., 1996). Many studies have investigated the impact of the origin of the coffee, the degree of roasting and roasting time and temperature combinations on the formation of volatile compounds responsible for coffee flavours and aromas (Baggenstoss, Poisson, Kaegi, Perren, & Escher, 2008; Mayer, Czerny, & Grosch, 1999; Schenker *et al*., 2002). However, finding the relation between the content of the hundreds of volatile compounds present in coffee and the complex aroma of coffee is not an easy task (Nebesny & Budryn, 2006). Sensory analysis has always been extensively used as a method to evaluate the quality of both espresso coffee and raw coffee. Beverage quality is strictly related to the chemical constituents of the roasted beans, whose

composition depend on the composition of green beans. Raw coffee beans contain the precursors of aroma volatiles that are developed during the roasting process. It is evident that the different content and ratio of precursors (chlorogenic acids, carbohydrates, proteins, trigonelline, caffeine, lipid) contribute at least in part to the distinctive aromatic compounds unique to each type/origin of green coffee. Unroasted coffee beans contain a wide range of different chemical compounds, which react and interact with each other throughout at all the stages of coffee roasting, resulting in an even greater number of final outcomes (Ribeiro *et al*., 2010).

The acidity or sourness of a coffee brew in general has always been recognized as an important attribute of its sensory quality. At tastings of espresso coffee, the level of acidity is not highly appreciated because its high concentration amplifies this character giving it an unbalanced feeling. The acids present in roasted coffee are responsible for 6% of the weight (Maier *et al*., 1987). Especially in high quality beans (arabica), roasted to light or medium roast degrees, a major taste is sourness (Clifford *et al*., 1989). Dark roasted arabicas, on the other hand, characteristically show less acidity, so that bitterness becomes the dominating taste. Nevertheless, washed coffees which become too dark in roasting will develop a pungent, burnt character usually judged as unpleasant. An acidic taste may also be the consequence of very fast roasting: it resembles a metallic tone, probably due to the presence of a residue of chlorogenic acid (CGA) that has not been involved, for lack of time, in the reactions leading to the formation of flavour components. Wet processed coffees are higher in acidity than unwashed ones (dry processed). In arabica coffee varieties, the pH of the brew is between 4.80 and 5.80 (Brollo *et al*., 2008). Although there is no doubt that hydrogen ion concentration is associated with perceived acidity, many studies have shown that there is only moderate correlation between a sour taste and the pH value. Total acidity of a coffee brew, expressed in terms of acidity titration, has been demonstrated to show better correlation to sourness than pH (Bähre & Maier *et al*., 1996). However, there

has been some debate about the best end point for the pH titration but according to the titration curve of individual acids, a titration of phenolic protons starts at pH values greater than 8. A poor correlation was found with the pH value, with a correlation coefficient of a linear regression of 0,53. (Maier *et al*., 1983). Studies on the acidity of different acids indicate that free protons (as represented by the pH) contribute to acidity as well as bound protons (Shallenberger *et al*., 1996). The roasting conditions and the bean type are important, but green bean processing and age also influence the pH (Werner & Kohley *et al*., 1965). The roasting intensity can also be used to change the bitterness-acidity ratio. The perceived acidity depends on the presence of aliphatic carboxylic acids and chlorogenic acids, although their contribution to perceived acidity is secondary (Esteban *et al*., 2004). The acids content is affected also by factors such as age, processing and fermentation of green coffee. An important part of acidity generated in coffee roasting can be attributed to the formation of the four aliphatic acids: formic, acetic, glycolic and lactic. These acids present a roast kinetics in function roast temperature where in particular acetic acid and formic acid are formed upon roasting up to a maximum at 240 °C roast temperature and decrease on further roasting. This can be explained by the high volatility of both compounds (Ginz *et al*., 2000). The formation of acids derived from various carbohydrates present in green coffee has been researched (Beck *et al*., 1990; Barlianto *et al*., 1990; Maier *et al*., 1994). In a paper by Ginz (2000) the presence of sucrose was confirmed as the principal green bean precursor of acids. The parameter that governs the contribution of a particular acid to pH and titration acidity is the pK_a value. The lower the pK_a value, the stronger the acid, the lower pH at which the molecule dissociates to give protons and anions in solution. Citric acid, phosphoric acid, quinic acid, chlorogenic acid and malic acid are the most acidic ones (Clifford *et al*., 1989). When looking at citric, malic and phosphoric acids, more than one proton has to be considered. Although most of the acids in coffee extracts are present as anions, which are not perceived as sour, they already contribute to acidity by providing

protons. Thus, an increase in the content of any of the coffee acids leads to a lowering of pH and an increase in titration acidity. Sweetness, on the other hand, is a characteristic which everybody appreciates, and is positively correlated with value. A fine *espresso* should, however, taste bitter-sweet with an initial slightly acidic note. It should display strong body and intense aroma and should also be pleasantly persistent. The sweetness sensation is provided by tiny amounts of monosaccharides, in particular reducing sugars, which can be found in the brew, and not sucrose because it has been transformed by the Maillard reaction (Trugo & Macrae, 1985). Instead according to Viani (1986) the sucrose content is 0,4%-2,8% of the dry weight. Light roast generally yielded a sweet sensation (Bhumiratana *et al*., 2011).
The body (mouth-feel) generally has a closer association with a high caffeine content, and the lipids where the viscosity is increased and the density is decreased (Esteban *et al*., 2004). The beverage body could also be related to the protein and chlorogenic acid content (Ribeiro *et al*., 2011). According to Illy and Viani (2005) the body of espresso coffee is closely related to emulsified lipids and proteins. The protein content is related to the viscosity of the coffee brew and could be related to the body of the coffee (Esteban-Diez *et al*., 2004). It is well known that the body is a peculiarity of robusta espresso brews, and, as a matter of fact, those beverages "fill the mouth". It is difficult to believe that oil droplets of colloidal size are present in higher amounts in robusta than in arabica espresso, given that the lipid content of roast and ground robusta is much lower than that in arabica. Several experiments indicate that freshly percolated Arabica espresso has a sensation of body perceived only in a second moment, during the sip and after two minutes when it displays a stronger body. It has been suggested that the reason for this strange behaviour could lie in rapidly disappearing gas bubbles of colloidal size-possibly more evident in robusta – and as such behaving as a viscosity enhancer (Petracco *et al*., 1989). The polysaccharides present in coffee are known to contribute to the organoleptic characteristic of the drink, such as the creamy

sensation perceived in the mouth known as "body" (Nunes and Coimbra *et al*., 2001).
In general, the more intense the roasting, the greater the aroma of the coffee but if it is too intense, the coffee turn bitter (Debry, 1994). Bitterness is closely connected to the total dissolved solids of a coffee. The substances are carbohydrates and emulsified lipids. Initial investigation revealed that the alkaloids caffeine and trigonelline, already present in the raw coffee bean, account for a maximum of 10-30% of the bitterness of a coffee beverage (Chen *et al*., 1979). The simple fact that decaffeinated instant coffee also tastes bitter suggested that substances other than caffeine might be contributing to the bitter taste. Quinic acid (degradation product of CGA) and caffeine content are partly responsible for the perceived bitterness (Esteban *et al*., 2004). More precisely, several heterocyclis are suggested as potential bitter-tasting agents in roasted coffees such as the furfurylalcohol (Shibamoto *et al*., 1981), 5-hydroxymethyl-2-furanaldehyde, pyrazines and various trigonellin thermolysis products (Belitz *et al*., 1975). In addition, a series of cis and trans configured 2,5-diketopiperazines have been reported as bitter constituents of roasted coffee (Ginz *et al*., 2001) and cocoa (Pickenhagen *et al*., 1975). Other research groups found some evidence that *O*-caffeoylquinic acids (chlorogenic acids) and their roast products may be a cause of the perceived bitter taste of coffee (Rizzi *et al*., 2004). Although a bitter-tasting fraction isolated from roasted coffee was reported to contain lactones of such chlorogenic acids (Ginz *et al*., 2001). Ginz and Engelhardt (2000) suggested that bitter tasting compounds might be formed by roasting protein. The presence of cyclic peptides called diketopiperazines identified also in beer (Gautschi *et al*., 1997) and cocoa (Pickenhagen *et al*., 1975) are responsible for the bitter taste. In addition, quinic acid, a well-known thermal degradation product of chlorogenic acids, was reported to exhibit an aspirin-like bitter taste at a threshold level of 10 ppm. In a recent work on the study of bioresponse-guided decomposition of roast coffee beverage and identification of key bitter state compounds found compounds formed from *0*-hydroxycinnamoyl quinic acid derivates upon

coffee roasting as the key compounds contributing to the bitter state of roasted coffee beverage. In detail the 3-*0*-caffeoyl-γ-quinide, 4-*0*-caffeoyl-γ-quinide, 5-*0*-caffeoyl-*epi*-δ-quinide, 4-*0*-caffeoyl-*muco*-γ-quinide, 5-*0*-caffeoyl-*muco*-γ-quinide, 3-*0*-feruloyl-γ-quinide, 4-*0*-feruloyl-γ-quinide present intense bitter coffee tastes. Besides the bitter taste it also gives complex quinic acid lactone isomers multiply esterified with *p*-coumaric acid, caffeic acid, ferulic acid, 3,4-dimethoxycinnamic acid, and quinic acid, respectively where as representatives of this fraction, 3,4-*0*-dicaffeoyl-muco-γ-quinide, 3,5-*0*-dicaffeoyl-*epi*-δ-quinide, and 4,5-*0*-dicaffeoyl-*muco*-δ-quinide identified as strongly bitter-tasting (Frank & Hofmann *et al*., 2005). Hofman (1999) identified different compounds aminohexose reductions from the formation of specific amino acids that take place in the Maillard reaction, and the bitter taste was drastically reduced. It follows that the bitter taste may be the result of a combination of caffeine, trigonelline, and phenolic compounds originally present in green coffee beans with compounds melanoidin or polymeric formed by roasting, such as cyclic peptides and aminohexose reductones.
Another tactile sensation is the astringency of the beverage. This has always been considered a negative quality. This defect has been related to the presence of immature beans containing dicaffeoyl quinic acids, which are astringent to mucous membranes by the precipitation of soluble proteins from saliva (Ohiokpehai *et al*., 1982). The astringency may be followed from bitter taste and developed by the combination of caffeine, trigonelline and phenolic compounds originally present in green coffee beans with compounds formed by roasting, such as cyclic compounds reported.
Astringency, on the other hand is caused by compounds tannin (GCA) that can precipitate salivary proteins on the tongue. Several attempts have been made to correlate the levels of chlorogenic acids with beverage quality and to correlate specific sensory attributes, such as astringency, to the presence of specific chlorogenic isomers. There is little evidence in the literature to support these ideas, but it is generally accepted that

Arabica coffee, with a lower chlorogenic acid content, is superior to that in Robusta coffee (Clifford *et al*., 1976).
Few studies have concentrated on the value of aroma sensorial attributes especially from different varieties of coffee bean. As generally accepted among various authors, the major descriptors perceived in espresso coffee are the following: malt, roasty, burn/acrid, buttery, caramel, brown, beany, nutty, cocoa, ashy/sooty, earthy/musty, sweet aromatic, sour aromatic and pungent (Sanz *et al*., 2002; Bhumiratana *et al*., 2011). The principal aim in this study is to identify, by means of sensory analysis, the specific sensorial descriptors resulting from the degree of roasting for every typology of green coffee. Not all the present volatile compounds in a substance are easily perceived. Further, to understand how the roasting process varies the colour and contributes to intensifying specific descriptors from a single origin. The roasting process was controlled by the control lightness value. The second aim of this work is to characterize different roasted coffees using sensory analysis and to study the possible correlation between specific aroma compounds evolved (see chapter 5) with specific descriptors for every single origin.

4.2 Material and methods

4.2.1 Coffee samples

Roasted coffee types

RAW COFFEE	LIGHT ROAST	MEDIUM ROAST	DARK ROAST
KENYA U.R 8,9 %	KENYA (1,5 Kg)	KENYA (1,5 Kg)	KENYA (1,5 Kg)
	KENYA (1,5 Kg)	KENYA (1,5 Kg)	KENYA (1,5 Kg)
	KENYA (1,5 Kg)	KENYA (1,5 Kg)	KENYA (1,5 Kg)
ETHIOPIA U.R 8.3 %	ETHIOPIA (1,5 Kg)	ETHIOPIA (1,5 Kg)	ETHIOPIA (1,5 Kg)
	ETHIOPIA (1,5 Kg)	ETHIOPIA (1,5 Kg)	ETHIOPIA (1,5 Kg)
	ETHIOPIA (1,5 Kg)	ETHIOPIA (1,5 Kg)	ETHIOPIA (1,5 Kg)
HONDURAS U.R 9,3 %	HONDURAS (1,5 Kg)	HONDURAS (1,5 Kg)	HONDURAS (1,5 Kg)

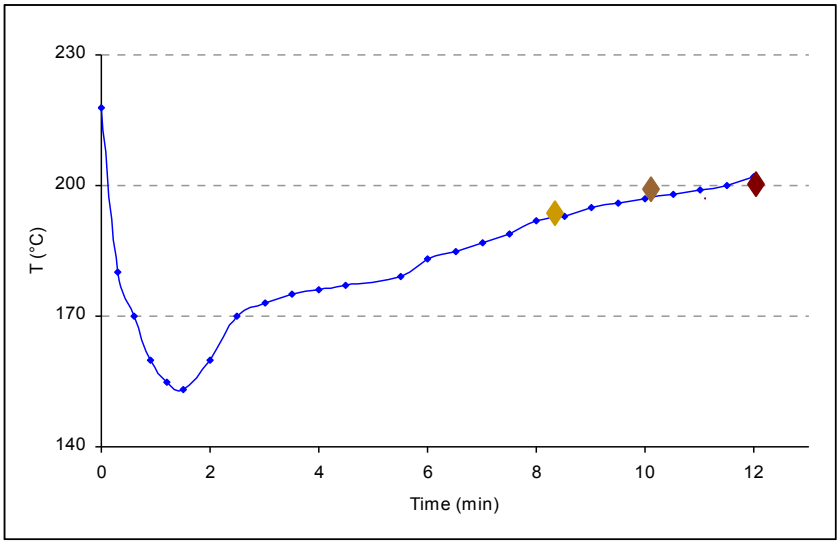
	HONDURAS (1,5 Kg)	HONDURAS (1,5 Kg)	HONDURAS (1,5 Kg)
	HONDURAS (1,5 Kg)	HONDURAS (1,5 Kg)	HONDURAS (1,5 Kg)
GUATEMALA U.R 8,9%	GUATEMALA (1,5 Kg)	GUATEMALA (1,5 Kg)	GUATEMALA (1,5 Kg)
	GUATEMALA (1,5 Kg)	GUATEMALA (1,5 Kg)	GUATEMALA (1,5 Kg)
	GUATEMALA (1,5 Kg)	GUATEMALA (1,5 Kg)	GUATEMALA (1,5 Kg)
NICARAGUA U.R 9,1 %	NICARAGUA (1,5 Kg)	NICARAGUA (1,5 Kg)	NICARAGUA (1,5 Kg)
	NICARAGUA (1,5 Kg)	NICARAGUA (1,5 Kg)	NICARAGUA (1,5 Kg)
	NICARAGUA (1,5 Kg)	NICARAGUA (1,5 Kg)	NICARAGUA (1,5 Kg)
BRAZIL U.R 9,3 %	BRAZIL (1,5 Kg)	BRAZIL (1,5 Kg)	BRAZIL (1,5 Kg)
	BRAZIL (1,5 Kg)	BRAZIL (1,5 Kg)	BRAZIL (1,5 Kg)
	BRAZIL (1,5 Kg)	BRAZIL (1,5 Kg)	BRAZIL (1,5 Kg)

The six green Arabica coffee samples from different countries of origin were roasted separately with three replicates of treatment in a gas-fired convective drum roaster oven Petroncini model capacity 1500 g (fig 1) at 200 °C. The model selected, a Petroncini machine, reproduces the same conditions as in industrial production.

Fig 1 - Petroncini coffee roaster machine

The samples were manually weighted (1500 g) and roasted under uniform roasting condition (same roasting curve, graph 1). The roaster was programmed with different time at the same temperature. For the light roast, coffee beans were roasted at 200 C° for 8 min, medium roasted for 10 min and dark roasted for 12 min. Once the roasting process was terminated, cool air was immediately blown in to the chamber for 4 min to cool down the roasted beans.

The samples were removed from the roaster, weighed and placed in a metal container and left to cool down to room temperature (24 C°). For each subsequent roasting treatment, the colour was measured with a colorimeter (model CR-200 MINOLTA). Before carrying out the measurements, a calibration was performed using a small blank tile. The roasted coffee samples were stored vacuum-packed in special plastic films and aluminum foil with a CO_2 degassing valve, to avoid a loss of aroma and contamination from external substances. The samples were stored at -5 C° for a maximum of 48 hours before being ground and analyzed in GC-MS and GC-O and were subsequently subjected to sensory analysis.



Graph 1 - Model Roasting curve used for all the samples

♦ = light roast
♦ = medium roast
♦ = dark roast

4.2.2 Determination of pH

The determination of pH of roasted coffee measurements was carried out by instrument called CRISON (AOAC Official method 1995). The samples of roasted coffee were ground for 1 minute. 0,5 g ground sample was placed in a test tube and 50 mL of deionised water was added. The pH analysis was carried out using a simple pH meter. The electrode used was HAMILTON Flushtrode/P at 25 C°

4.2.3 Determination of total acidity

The total acidity of roasted coffee measurements was carried out by means of automatic titration using an instrument called CRISON (AOAC Official method 1995). The samples of green coffee were ground for 1 minute. 10 g of the prepared ground

sample was placed in a Erlenmeyer test tube and 75 mL of 80% alcohol was added, the test tube was closed with a stopper, and it was left to stand for 16 hours being shaken occasionally. Filtered, transferred aliquot of filtrate (10 mL for roasted coffee) to a beaker, diluted to ca 100 mL with deionized water and titrate with 0,1N of NaOH, using phenolphthalein. Express results as ml 0,1N of NaOH required to neutralize acidity of 100 g sample. The end value is taken at pH 8. For this analysis 50 mL of sample was taken.

4.2.4 Sensory analysis

All of the 54 roasted samples were submitted to sensory evaluation by 15 judges with expert knowledge in sensorial analysis of food products. Sensory analysis was conducted by a panel of expert wine tasters. Before testing these judges were trained by an expert taster of coffee. The choice of the expert was based on the fact that coffee is a food matrix different from wine and the purpose was to guide the panel in the correct choice of descriptors and to give them a score as close as possible to that of the expert teacher. In the first step, the judges were subjected to different tastes of espresso blends and single origin to identify and choose the most relevant descriptors together with the expert teacher. The second step was the intensity perceived by the panel when testing Arabica and Robusta coffees, which contained different combinations of chemical substances and odors. The final stage involved first the comparison to and then the correction of the judges' scores with those of the expert teacher. During these four training sessions the panel acquired the knowledge and ability to differentiate between different coffees. After every three sessions of sensory analysis, one sample was repeated randomly in order to evaluate the repeatability of the judges responses. Various statistical tests were performed to evaluate the standard of the panel's responses. Evaluation of repeatability (graph 3), their ability in the sensorial analysis by using an average correlation coefficient calculation (table 2-3) and a comparison of their evaluations during the training between samples tasted during first session

and fourth session (graph 2). Regarding repeatability, every box plot (graph 3) showed the average, the standard deviation and the minimum and the maximum calculated for every judge for all the sensory analysis performed. The red line shows the general average deviation calculated for all the judges. Tables 2 and 3 show the average correlation coefficients (agreement) obtained with the software Senstools 2.3. Concerning agreement on values between the judges, an analysis of correlation between the score of each judge against the average score of the rest of the panel was carried out, except that the judge for each descriptor (tab 2). Then the average of the correlation coefficients for the various descriptors for each judge was calculated (tab 3).
After training the sensorial table was created, focusing more on flavour and taste descriptors (see Sensory Analysis Report). Thus, for the 27 sensory attributes selected for evaluation, 9 point scales were adopted. For each attribute an assignment of one point was considered a very low perception and nine points were considered a high perception.

SENSORY ANALYSIS REPORT

Name Judge_____Sample codex_____Date_____

Individualize the descriptors and express the sensation taste and after taste perceived

TASTE SENSATION

ACIDITY	0	1	2	3	4	5	6	7	8	9
SWEET	0	1	2	3	4	5	6	7	8	9
BITTER	0	1	2	3	4	5	6	7	8	9
EQUILIBRIUM/ BALANCING (+ sweet, + bitter)	0	1	2	3	4	5	6	7	8	9

TACTILES SENSATION

BODY	0	1	2	3	4	5	6	7	8	9
SOFTHESS	0	1	2	3	4	5	6	7	8	9
ASTRINGENCY	0	1	2	3	4	5	6	7	8	9

AFTER TASTE SENSATION

MALT	0	1	2	3	4	5	6	7	8	9
FLOREAL	0	1	2	3	4	5	6	7	8	9
CITRUS	0	1	2	3	4	5	6	7	8	9

FRUITY	0	1	2	3	4	5	6	7	8	9
SPICY	0	1	2	3	4	5	6	7	8	9
NUT	0	1	2	3	4	5	6	7	8	9
ROASTED BREAD	0	1	2	3	4	5	6	7	8	9
MILK CHOCOLAT	0	1	2	3	4	5	6	7	8	9
DARK CHOCOLAT	0	1	2	3	4	5	6	7	8	9
MAPLE SYRUP	0	1	2	3	4	5	6	7	8	9
CARAMEL	0	1	2	3	4	5	6	7	8	9
RESINOUS	0	1	2	3	4	5	6	7	8	9
TOBACCO	0	1	2	3	4	5	6	7	8	9
HERBACEOUS	0	1	2	3	4	5	6	7	8	9
COAL TAR	0	1	2	3	4	5	6	7	8	9
SMOKY	0	1	2	3	4	5	6	7	8	9
CARBONIZE	0	1	2	3	4	5	6	7	8	9
PHENOLIC	0	1	2	3	4	5	6	7	8	9
RANCID	0	1	2	3	4	5	6	7	8	9

PLEASANT INDEX	0	1	2	3	4	5	6	7	8	9

Scheme used for tasting sensory analysis with different descriptors

4.2.5 Sample preparation, grinding and serving Espresso coffee

It is the beverage in the cup, conforming to strict production regulations drafted by National Italian Espresso and approved by a third party operating under the ISO standard 45011 (CSQA Certificate nr. 214 of 24 September 1999). Each espresso coffee was prepared immediately before tasting in a white porcelain coffee cup. It is a white ceramic cup and without interior decoration, elliptical in form, with a capacity of 50 to 100 milliliters. Only with such a cup can the appearance of an excellent cream be fully appreciated. The precious perfume and warm softly expressed taste served with a special coffee spoon called *"goùte café"* (fig 2) without sugar and served one sample at a time for every judge. The "*goùte café"* are spoons used by special expert tasters allowing the coffee to fully penetrate the oral cavity and ensuring proper mixing of the coffee.

Each judge was also provided with a glass of water and some puffed rice to clean the palate after tasting each sample. The judges assessed six coffee samples per day, (except for the calibration sample) resulting in 12 sessions in all to taste all the samples, tasting being performed two times a week. Before each tasting session there was a panel test or calibration (adjustment) with one blend of roasted coffee. The amount of all coffee roasted samples were prepared following the rules of the Espresso Italian Certificate. The samples weighed 6,5 g of finely ground roasted coffee with a coffee bean grinder, ANFIM model. The grinding varied considerably between different origins and agreement roast for a volume 25 mL ± 5 using an espresso coffee machine (FAEMA Model). Prior to the extraction each sample coffee was ground at a pressure of 25 kg. Fixed espresso coffee preparation conditions were followed using a pressure in the espresso machine pump equal to 9 atm. And a water temperature of 90 ± 1 C°, extraction times ranged from 20 to 30 seconds and were

measured by a chronometer. Hot deionised water was used to rinse the filter support between each individual espresso coffee sample.

Fig 2 - Coffee spoon and cup for espresso coffee used for sensory analysis.

4.2.6 Data treatment and analysis

Upon completion of the sensory analysis the performance (repeatability) of the judges was evaluated using the program STATISTICA 7 and the statistical software called "Senstools 2.3" to evaluate the level of judge agreement and sample discrimination. In the second step the data were collected using only software STATISTIC 7. The descriptive data were analyzed using analysis variance (at two-way factorial ANOVA) with post-hoc mean separation, using (*Least Significant Difference)* test-LSD. ANOVA test was analyzed at 5% level of significance for all the evaluated attributes considering the (independent variables or factors) varieties coffee, light, medium, dark roast treatments, and the replicate of treatment roast. The value of the variance was analysed and of the homogeneity of variance which consists in a

comparison of the Fischer value as in the table and calculated one. To determine the relationship between the variables considered among degrees of roasts, types of coffee beans, and stages of preparation, Principal Component Analysis (PCA) was performed on all coffee samples. The analyses were performed in order to closely examine the effects of degrees of roasting and coffee varieties at each stage.

4.3 Results and Discussion

Table 1 (below) reports the data concerning the colour measurement from the colorimeter (MINOLTA).

Coffee	degree roast	Level brown (measure ±SD)	Level red (measure ±SD)	Level yellow (measure ±SD)	TINT red/yellow
NICARAGUA	light	89,5 ± 0,12	0,366 ± 0,001	0,334 ± 0,001	1,094
NICARAGUA	medium	90,6 ± 0,10	0,356 ±0,0017	0,328 ± 0,001	1,088
NICARAGUA	dark	91,1 ± 0,09	0,353 ± 0,001	0,325 ± 0,001	1,084
GUATEMALA	light	90,3 ± 0,15	0,363 ± 0,001	0,332 ± 0,001	1,094
GUATEMALA	medium	91,0 ± 0,17	0,356 ± 0,002	0,327 ± 0,001	1,089
GUATEMALA	dark	91,5 ± 0,11	0,347 ± 0,001	0,322 ± 0,001	1,079
BRAZIL	light	90,6 ± 0,13	0,359 ± 0,002	0,329 ± 0,001	1,093
BRAZIL	medium	91,0 ± 0,09	0,355 ± 0,001	0,326 ± 0,001	1,088
BRAZIL	dark	91,7 ± 0,05	0,346 ± 0,001	0,329 ± 0,002	1,052
ETHIOPIA	light	90,5 ± 0,08	0,364 ± 0,001	0,331 ± 0,001	1,099
ETHIOPIA	medium	90,9 ± 0,12	0,359 ± 0,004	0,328 ± 0,001	1,095
ETHIOPIA	dark	91,9 ± 0,07	0,347 ± 0,001	0,320 ± 0,001	1,082
HONDURAS	light	90,7 ± 0,07	0,363 ± 0,001	0,330± 0,003	1,099
HONDURAS	medium	91,1 ± 0,09	0,359 ± 0,005	0,328 ± 0,001	1,095
HONDURAS	dark	91,5 ± 0,11	0,355 ± 0,008	0,325± 0,004	1,091
KENYA	light	90,8 ± 0,13	0,363 ± 0,001	0,330 ± 0,001	1,100
KENYA	medium	91,6 ± 0,11	0,353 ± 0,01	0,324± 0,008	1,089
KENYA	dark	92,3 ± 0,05	0,344 ± 0,001	0,318 ± 0,001	1,080

Table 1-Value average (three measure replicates ± SD) obtained by colorimeter MINOLTA (level brown, level red, level yellow, tint) of different roasted samples

During roasting process every beans coffee in function of the origin present a different structure, in particular the density cellular, where are present different characteristic physical, for example the colour. A requisite important for this work was to obtained samples roasted more possible homogeneous coffee. We can observed in table 1 that the value of the tint obtained are resulted very similar.

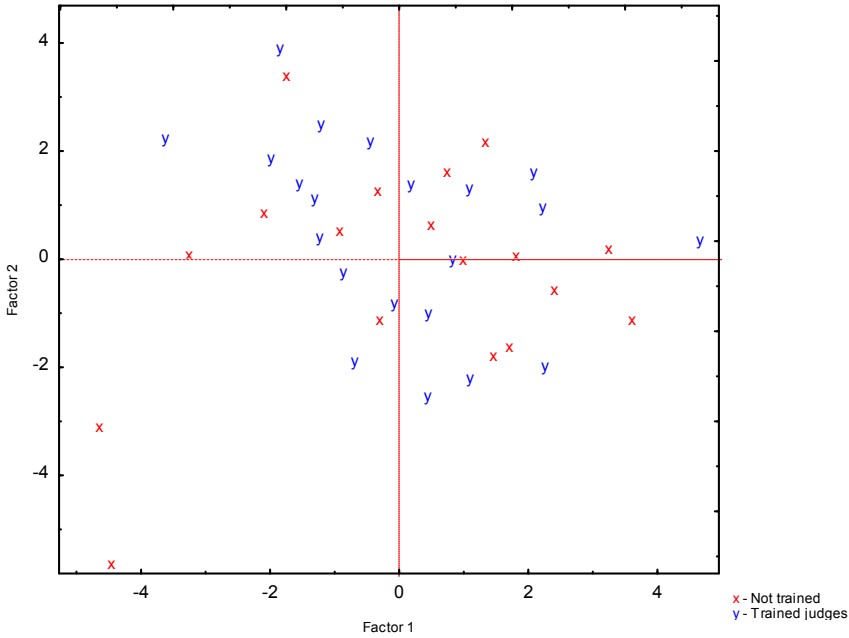
4.3.1 Panel evaluation

Before proceeding to an analysis of the data, the performance of the panel was evaluated applying various statistical tests. As regards the experts evaluation, this was performed on the basis of two criteria:

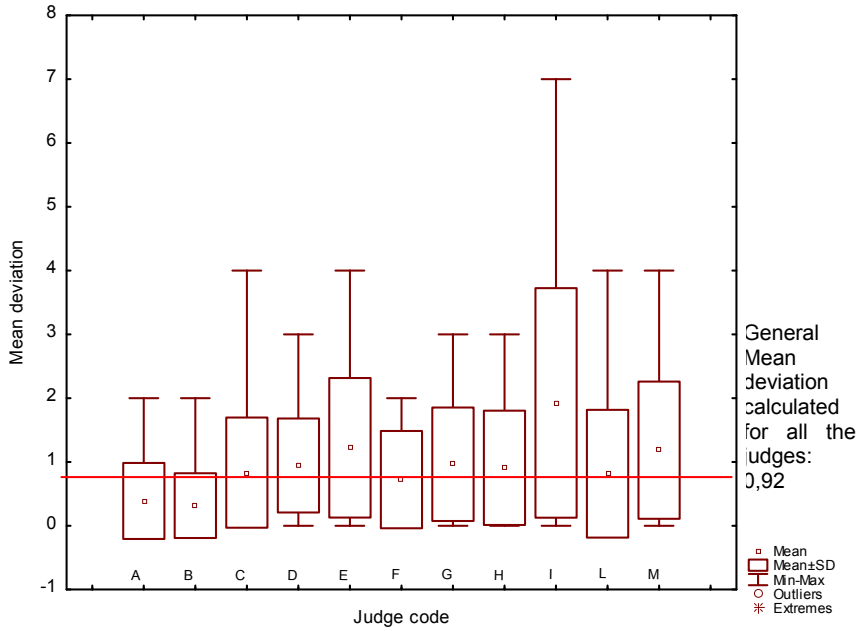
- judge's agreement with the average of the group, which means the judges ability to give the same or approximately the same evaluation to the average of the group for a specific series of coffees

- judges "repeatability" which means the judges ability to evaluate the same sample at two different times in the same sensorial session and perceive quite similar results (graph 3)

This graph illustrates the results on panel evolution. The PCA in graph 2 showed that judges after training are more compact in their evaluation between the first and the fourth sessions of sensorial descriptors.



Graph 2 - The Principal Component Analysis of all judges evalutation between first session and final training



Graph 3 – Box plot presentation of all judges: tests of repeatability during sensory analysis

This analysis showed a high level of repeatability, as we can see in fig 3 where the interval on the axis Y displays how far each judge is from the general average deviation for all the judges. The judge whose standard deviation is completely outside the mean deviation is rejected from further analysis due to its low repeatability level. The closer to 0 (zero) value the taster are, bigger are their repeatability. In our case only 1 was removed. The agreement of single experts computed with the software "Senstools 2.3" gave both negative and positive values. Judges who received negative values would eventually be excluded from further statistical analysis due to their different mean evaluation from the mean of the group. As an example see the table 2-3 reported below:

Judge	1	2	3	4	5	6	7	8	9	10	11	12	Average
A	-0,03	-0,03	-	0,17	-	-0,15	0,33	-	0,05	0,14	0,29	0,32	0,121
B	0,05	-	-	-	-	-	-	-	-	-	-	-	-
C	-0,03	-0,1	0,04	0,23	0,13	-0,17	0,3	-0,07	0,22	0,04	0,19	0,22	0,083
D	-	-	0,02	-0,08	0,13	-0,21	-0,04	0,08	-0,21	-0,09	0,01	0,01	-0,038
E	0,08	0,04	0,2	0,11	0,11	-0,07	0,08	-0,05	-0,14	-0,06	0,12	0,18	0,050
F	-0,25	0,08	-0,03	0,09	0,11	0,11	0,08	-0,05	0,23	0,16	0,29	0,41	0,103
G	-0,14	-	0,09	-	-0,19	-	-0,04	-	0,12	-	-	-	-0,032
H	-0,04	-	-0,05	-	0,01	-	0,13	-0,06	-	-	-	0,25	0,040
I	0,08	0,16	-0,07	-0,12	-0,03	-0,04	0,31	-0,07	0,14	0,07	-	-0,21	0,020
L	0,28	0,14	0,11	0,1	-0,06	-0,09	-0,08	-0,09	0,14	0,08	-0,14	0,14	0,044
M	-	-	-0,17	-0,13	0,15	-	0,1	-	-	-0,05	0,2	0,18	0,040
N	-0,06	-	-0,02	-0,16	0	0	0,13	0,08	0,08	0,03	0,31	-0,13	0,024

Table 2 – Average correlation coefficients (agreement) between the score of each judge against the average score of the rest of the panel, except that the judge for each descriptor correlation. The number 1 to 12 indicate the sessions. Letters stay for code of the experts. In red the values which involve the exclusion of judges.

Judge	1	2	3	4	5	6	7	8	9	10	11	12	Average
A	-0,11	-0,05	-	0,05	-	-0,04	0,27	-	0,03	0,11	0,23	0,25	0,082
B	-	-	-	-	-	-	-	-	-	-	-	-	-
C	-0,06	-0,04	-0,01	0,1	-0,06	-0,13	0,26	-0,11	0,22	0,04	0,19	0,2	0,047
D	-	-	-0,02	-0,12	0,04	-0,11	-0,04	0,03	-0,16	-0,09	0,02	0,05	-0,038
E	-0,03	0	0,2	0,14	0,02	-0,09	0,05	-0,06	-0,14	-0,06	0,13	0,13	0,027
F	-0,29	0,09	-0,05	0,04	-0,04	0,14	0,08	-0,03	0,27	0,16	0,27	0,33	0,075
G	-0,17	-	0,02	-	-0,26	-	-0,07	-	0,13	-	-	-	-0,070
H	-0,09	-	-0,03	-	-0,18	-	0,17	-0,08	-	-	-	0,28	0,012
I	0,1	0,17	-0,09	-0,07	-0,05	-0,03	0,28	-0,09	0,14	0,07	-	-0,19	0,023
L	0,16	0,18	0,1	0,15	-0,09	-0,1	-0,1	-0,08	0,14	0,08	-0,12	0,08	0,036
M	-	-	-0,07	-0,1	0,03	-	0,06	-	-	-0,05	0,14	0,14	0,024
N	-0,05	-0,02	-0,04	-0,1	-0,14	-0,12	0,08	0,03	0,13	0,03	0,22	-0,11	-0,006

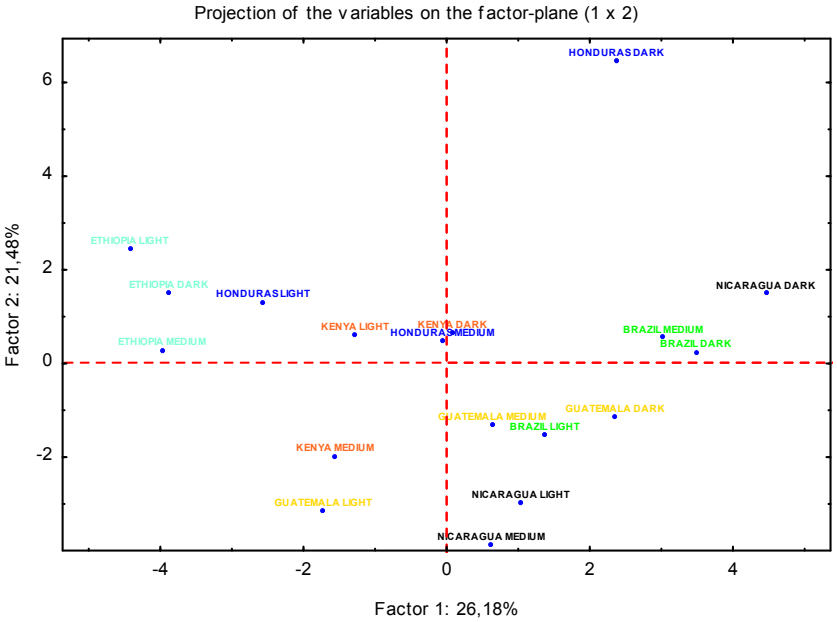
Table 3 - Average correlation coefficients (agreement) for the various descriptors for each judge The number 1 to 12 indicate the sessions. Letters stay for code of the experts. In red the values which involve the exclusion of judges.

At the end of the application of the statistical test three judges were deleted (I, D, G) not judge N, despite the fact that the judge presented a negative value of average correlation average and that it was very low. Besides during the elaboration of the data sensory analysis it was seen that the presence or absence of judge N showed no difference in terms of results. Therefore, judge N has not been cancelled for the purposes of the elaboration of sensorial data.

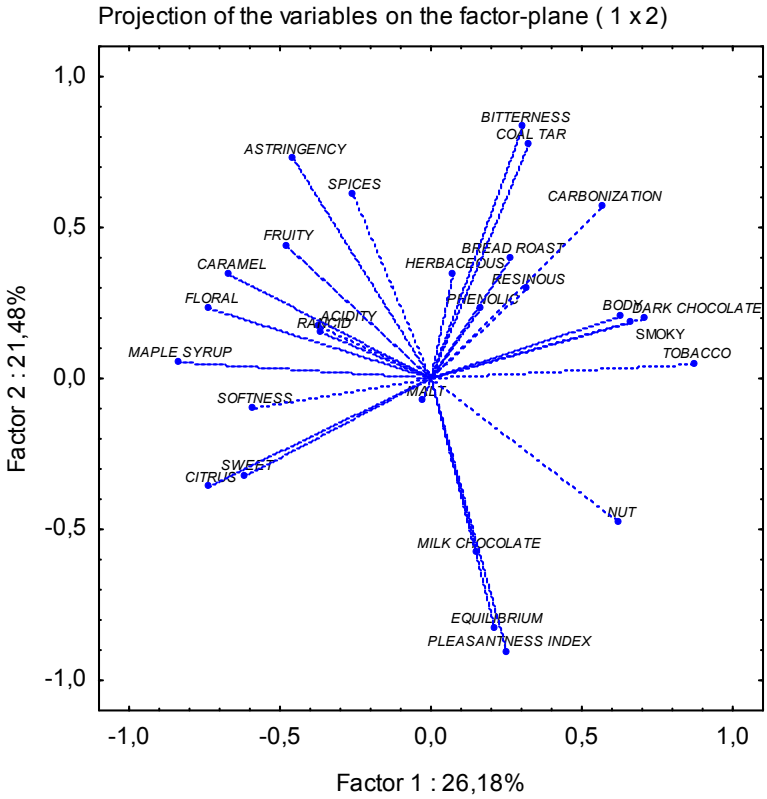
4.3.2 Results of sensorial analysis

As mentioned in the introduction, there are many variables that effect the quality of espresso coffee during the preparation stage. The sensory analysis has been conducted with precision, seeking standardization of the conditions for all the samples. Also the different grindings for each typology of coffee have been carried out with maximum precision in order to obtain a suitable extraction whilst respecting the conditions as reported in paragraph 3.2.3. After specific training of the members of the sensorial panel, whose performance is described in the previous paragraph, an analysis of their samples evaluation was elaborated with STATISTIC 6 program and the results are reported in the following graphs. Having calculated the average of the three replicates of treatment, the data was analysed by PCA (graph 1-2), explaining the similarities and differences between the various samples. Graph 2 shows the relation between different descriptors: the higher the angle is between the relative vectors in two attributes then the more they have been used independently by the judges. An angle of 180° means that two descriptors have an inverse relation. Instead vectors of smaller dimensions indicate inadequate discrimination between samples. Considering both graphs 1 and 2, the inertia percent is not high (< 50%). Despite the fact that the samples were of different geographical origin (represented by different colours), with different roast and well-trained judges the samples did not follow a satisfactory grouping order either in terms of their geographical origin or in terms of the treatment undergone (graph 1). Is seems likely that roasting process effect (light, medium, dark) can

influence favour or reduce in some way the light or strong determined descriptors for only a few coffees of different origins. Graph 1 demonstrates that Honduras coffee if dark roasted is more distant as respect to Honduras light and medium roasts and also much more than other coffees. The same observation applies to Nicaraguan coffee. Brazilian coffee behaves in a completely different mode – where a dark or medium roast display no great difference in terms of taste, instead the Brazilian light roast presented the opposite situation. Observing graph 1 it can be seen that Ethiopian coffees either light, medium or dark roasted, are more compact. This behaviour can be explained by different chemical compositions in each single origin sample. Nevertheless the variability is low, observing PCA graph 2 we can select a few important points. The following descriptors: acidity, rancid, bitterness, coal tar, herbaceous; body, dark chocolate and smoky; citrus and sweet; equilibrium, milk chocolate and pleasantness index all present angles for vectors which are very small and, therefore, they are strongly correlated. This means that the compounds responsible of these attributes carry the same weight or that one of the two descriptors has not been fully understood and, therefore, confused the testing panel. This kind of analysis is useful to identify which descriptors are relevant to differentiate between different samples and above all to shed greater light on the sensorial report card. In our coffee samples, looking the graph 2, the attributes which resulted most significantly are bitterness, coal tar, body, dark chocolate, smoky, citrus, sweet, equilibrium and milk chocolate.



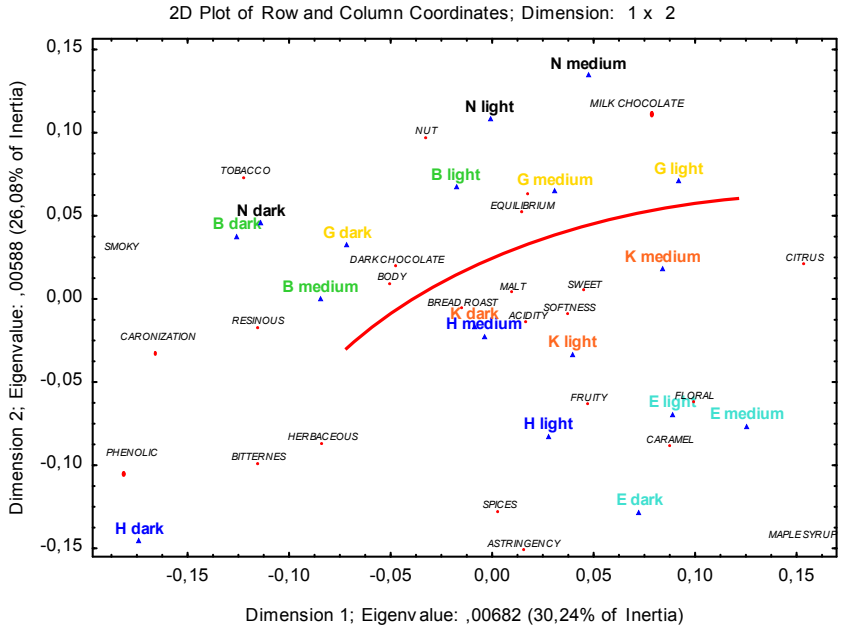
Graph 1 – PCA date sensory analysis with replicates obtained in espresso coffee. The graph explain the roasted coffees of different provenience and degree roasting.



Graph 2 – PCA date sensory analysis obtained in espresso coffee. The graph explain the variables (perceived descriptors) in roasted coffees of different provenience and different roasting (light, medium, dark). The descriptors with long vectors are the most important .

The superimposition of descriptors and samples (correspondence analysis in graph 3) show different sensory characteristics for each origin. Tle red line shows a separation of descriptors between coffee origin Africa and America. Ethiopian coffee is characterized as having in prevalence floral, fruity, caramel, maple syrup, spices odours and differing astringency levels between samples. Kenyan coffee, on the other hand has fruity,

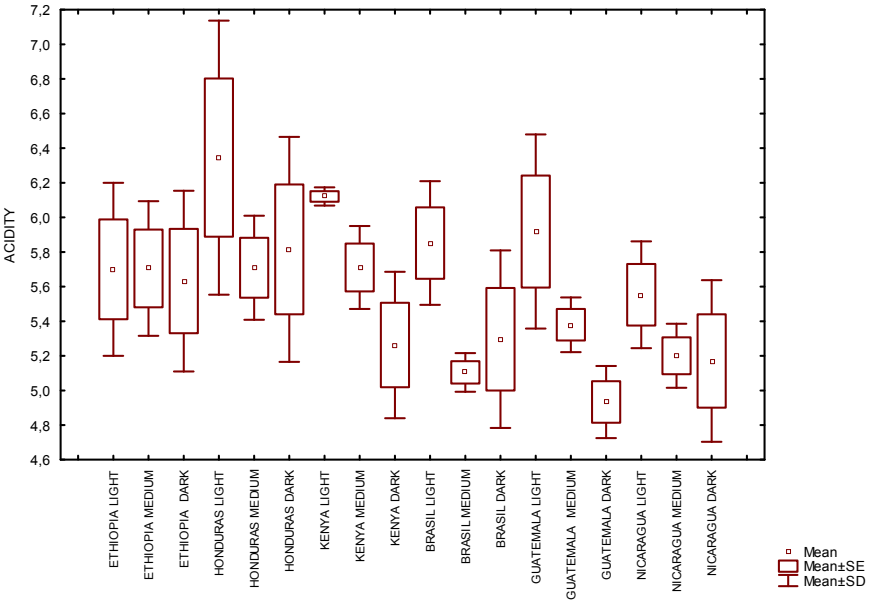
malt, citrus odours and acidity, sweet, and a degree of softness when looking at the sensation testing. Coffees coming from central America are characterized as having in prevalence descriptors such as dark chocolate, nut, milk chocolate, bread roast, herbaceous, resinous but with evident differences between single coffees. Brazilian, Nicaragua and Guatemala coffees present similar attributes near to dark chocolate, resinous, tobacco, nut, equilibrium and body. It appears, therefore, that coffees originating from the Americas present similar characteristics. Honduras coffee is an exception as it displays characteristics similar to coffees of African origin. In particular it presented phenolic, herbaceous, spices, fruity, baked bread, malt and acidity, bitterness and astringency in terms of taste.



Graph 3 – PCA (correspondence analysis) date sensory analysis obtained in espresso coffee. The graph explain the roasted coffees of different provenience and degree roasting (B=Brazil, H=Honduras, N=Nicaragua, E=Ethiopia,

K=Kenya). The red line show a separation of descriptors between coffee origin Africa and America.

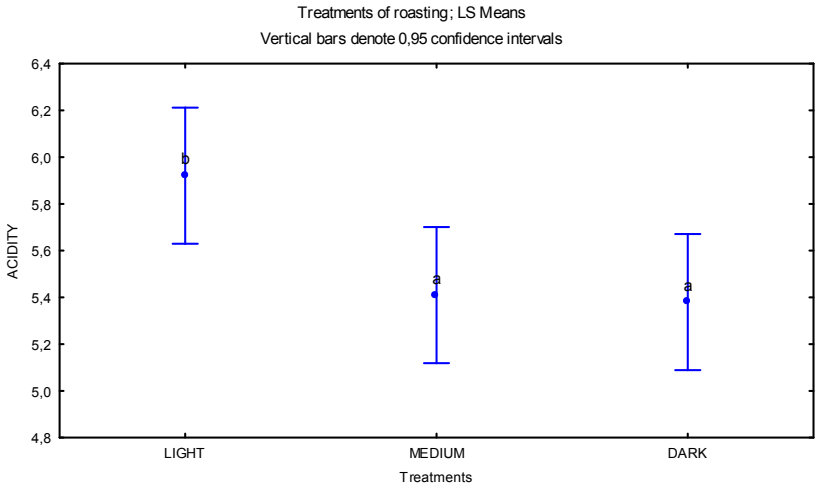
Specific aromas compounds, are responsibles of notes sensory of coffee, that are derived are a lot of reactions and the amount of these aromas in the final product depends on the initial quantity of precursors but also, possibility, of the extent of loss of aromas, once formed, which occurs by volatilization during the grinding process and for the heat of the thermal process, including in relation to the structure more or less dense granules of coffee. The same situation its seen in wood chips in wine (Tat L. *et al*., 2000).
Considering the averages for all descriptors for coffee it becomes apparent that a significant difference is hard to identify in sensory analysis particularly from a statistical point of view. A factorial analysis by the ANOVA test and the LSD test have been carried out to individualize the important differences between the variables or factors considered (origin, roasting treatment and replicates of treatment). The results of this analysis are explained in graphs 4 to 45. These report the descriptors that presented a significant difference with a confident interval, which is a better representation of the variance analysis. Lesser overlapping of the bars indicates a greater significant difference between variables. These significant differences are marked with letters located next to the bars. Before each graph explaining variance analysis box-plot graphs have been inserted to give a clearer picture of the different general characteristics for each typology of coffee.



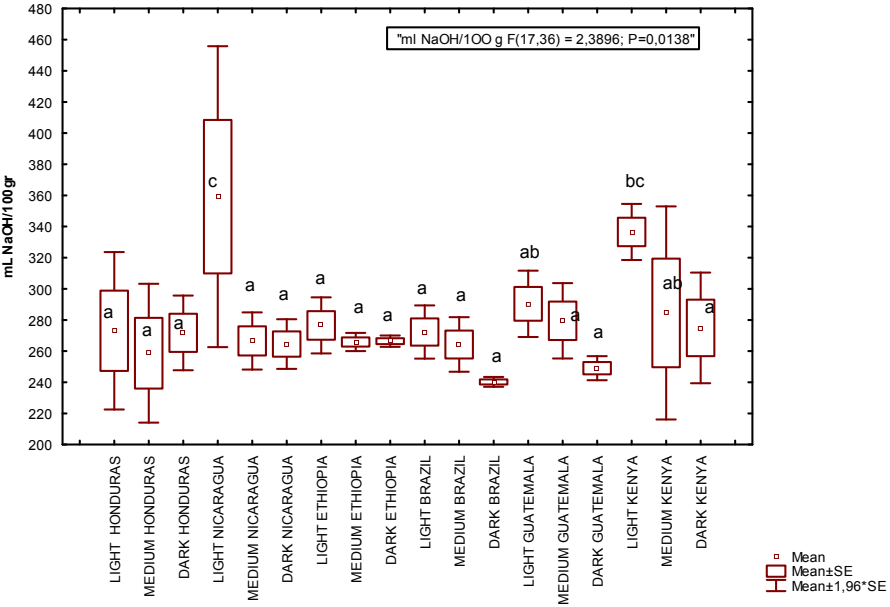
Graph 4- Acidity content (score detected by sensory analysis) explain by box-plot in the coffee espresso: effect of increasing in base treatment roasting process and origin.

Observing the graph 4 we can see the variation of acidity perceived during sensory analysis for different coffees based on the origin and the roasting process. When analysing the variance it is evident that the significant difference occurs because of the effect of treatment – the difference between light and dark roasts (graph 5) – this is not so in any single case regarding the origin of the coffee. Graph 4 highlights the fact that the coffees with a light roasting process are characterized as having high acidity levels, in particular, in coffees from Honduras, Kenya and Guatemala. In agreement with the results found in the chapter about the analysis of acidity in green coffee, these coffees presented high levels of acidity from the beginning. The level of acidity depends also on the quantity and typology of sugars present in green coffee, where these precursors take place in Maillard reaction.

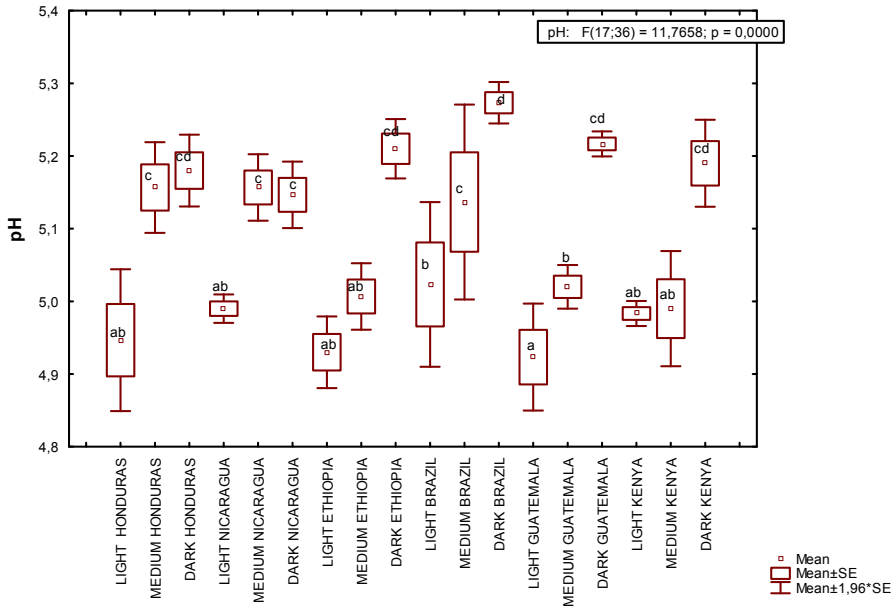
Observing the content of sugars analysed using the enzymatic kit (chapter 3) the same coffees i.e. Guatemala, Honduras and Kenya came out as having a generally good level of sugars.
The determination of acidity by titration and pH generate a different answer if compared with the result of sensory analysis. Observing graph 6, the titration analysis did not present a significant difference between roasted coffee samples, instead the sensorial analysis was better able to discriminate between different samples. The coffees from Nicaragua, Kenya and Guatemala presented a high content of acidity as seen in the sensory analysis, with the exception of the coffee from Honduras. This coffee even if a low acidity content was detected, and the pH value was in agreement with the sensory analysis, the acidity/sourness perceived probably in this case is correlated only to hydrogen ion concentration – as explained in the introduction about the mechanisms regulating perceived acidity. Concerning the trend towards acidity and the pH detected in sensory analysis, and the chemical analysis seen in graphs 4-6-7, the value decreased in accordance with the roasting process for all of the coffees, even though the behaviour was different for every coffee from each origin.



Graph 5 – Confidence of interval, results of ANOVA and LSD test detected during sensorial analysis; different letter mark significant difference for $p < 0,05$.

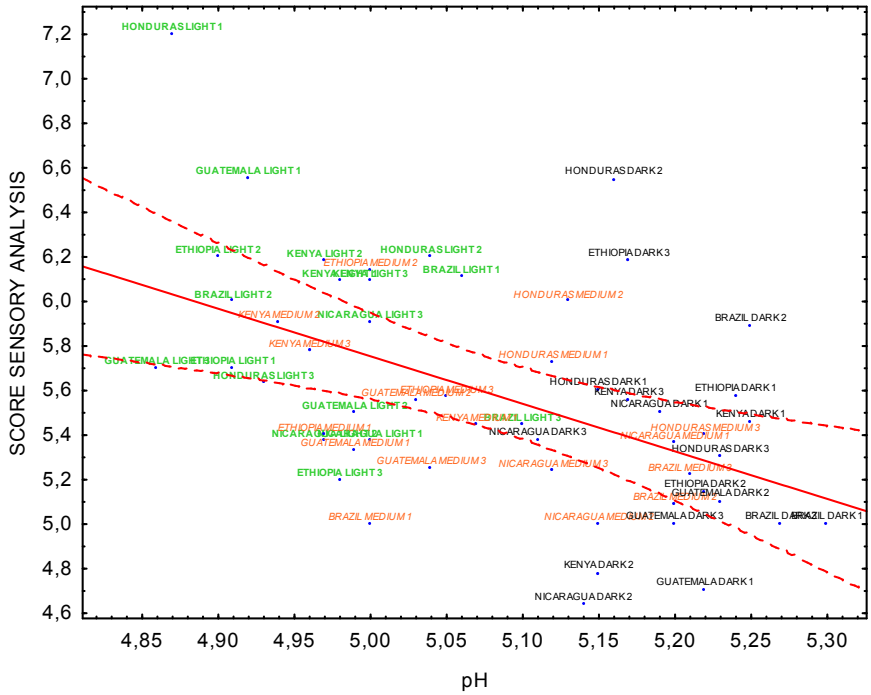


Graph 6 - Acidity content by titration explain by box-plot in the coffee espresso: effect of increasing in base of treatment roasting process and origin.

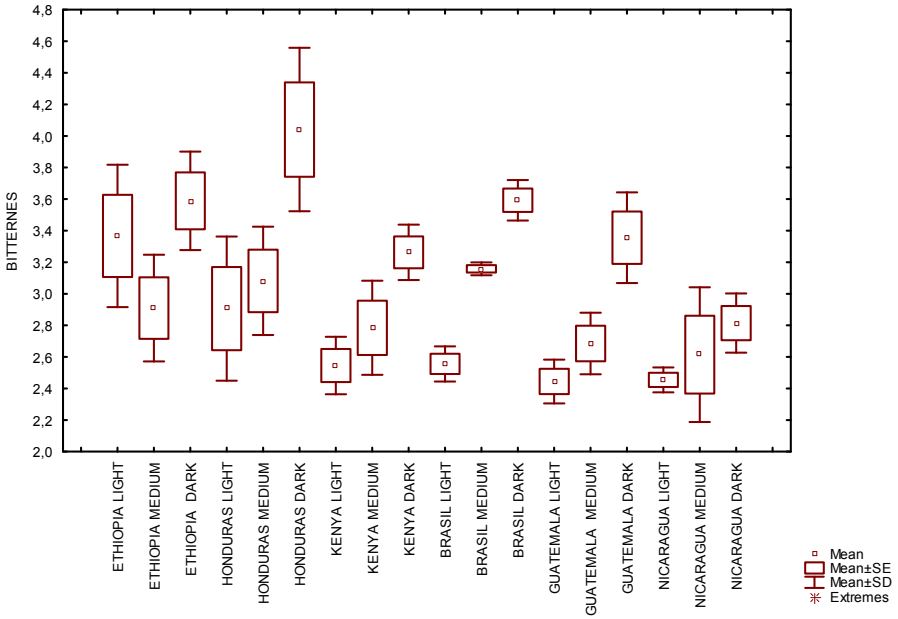


Graph 7 – pH analysis explain by box-plot in the coffee espresso: effect of increasing in base of treatment roasting process and origin.

In agreement with several authors (Clarke *et al*., 2001), the arabica coffee varies, the pH measured of the brew was between 4.85 and 5.30 (graph 7) and a poor correlation was found with the pH value and the sensory analysis, the correlation coefficient (r^2) of linear regression is 0,51 as reported in the introduction (Maier, 1987). Graph 8 shows three separate zones in different colours according to the relevant roasting process: light, medium and dark for all samples, including the treatment replicates. The roasted coffee samples from different origins are separable and it is easy to observe the range and the minimum variation of the pH in the different coffees. However the acidity and pH showed that the coffee light roast together sensory analisys are resulted more acids (graph 5 and 8).



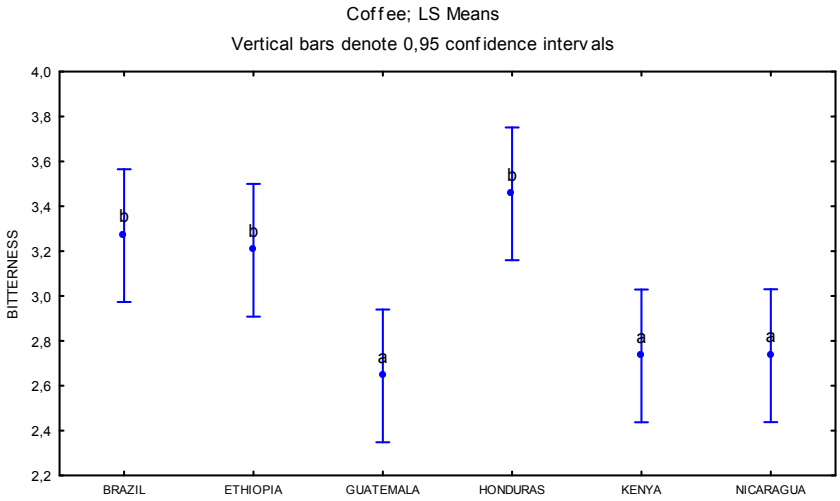
Graph 8- Correlation between score sensory analysis and pH measure. The samples with replicates of treatment are subdivide in different colour in function agreement roast light, medium and dark.



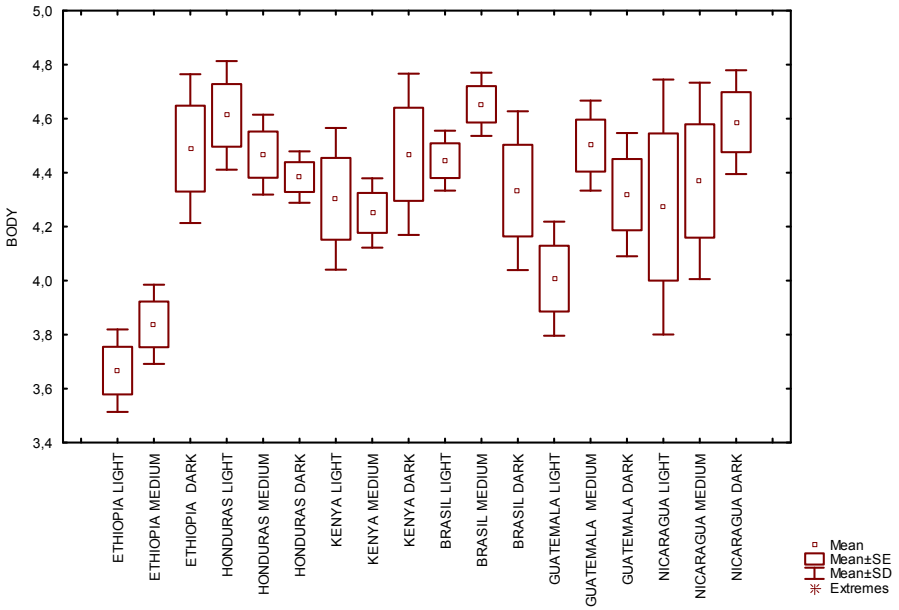
Graph 9 - Bitterness content (score detected by sensory analysis) explain by box-plot in the coffee espresso: effect of increasing in base treatment roasting process and origin.

Graph 9 concerns the perception of bitter coffee and, as accepted in previous studies (Debry, 1994), all coffees demonstrate both positive and diverse trends according to the roasting process adopted. Dark roasted Honduras coffee presented a high level of perceived bitterness, but considering graph 10, with the ANOVA test, there is a significant difference only in terms of typology of origin and not in terms of treatment of roasting. Two large groups of coffees emerge. Looking at the results there appear to be few factors linking the different types of coffee, as stated in the introduction. For example, the coffees from Brazil, Ethiopia and Honduras showed higher levels of bitterness. These coffees contained high levels of caffeine, trigonelline, and the chlorogenic acid was distributed in different way. However, looking at coffees from Guatemala, Kenya and Nicaragua the results were

contradictory. Although these coffees showed low values of bitter taste, they contained high levels of alkaloids and chlorogenic acids. As stated in the bibliography, the formation of the specific compounds "aminohexose reductones" together with specific amino acids present in different coffees, may give rise to a decrease in bitterness.



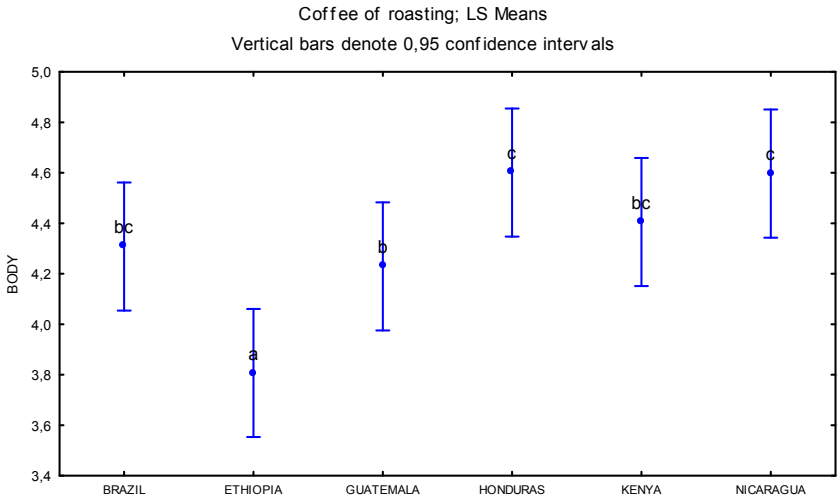
Graph 10 - Confidence of interval, results of ANOVA and LSD test detected during sensorial analysis; different letter mark significant difference for $p < 0,05$.



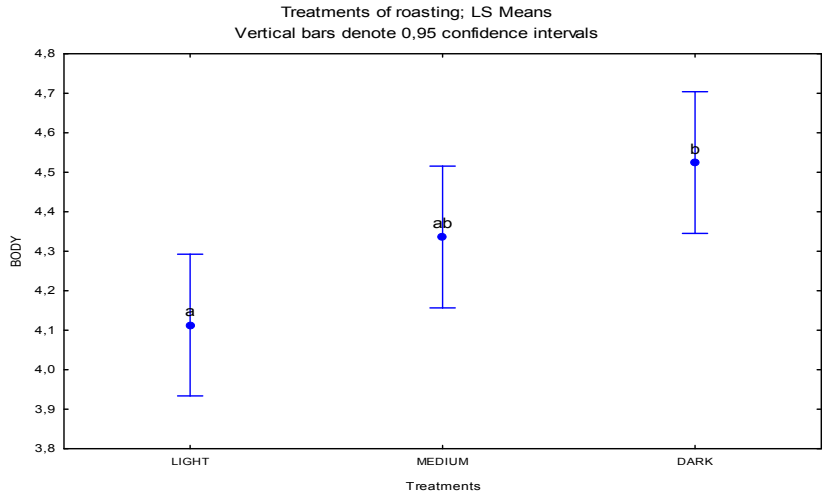
Graph 11 - Bitterness content (score detected by sensory analysis) explain by box-plot in the coffee espresso: effect of increasing in base treatment roasting process and origin.

Graph 11 shows how coffee is perceived in terms of "body". Just a few typologies of coffee presented a positive or negative trend in relation to the effect of roasting treatment, but is evident that Ethiopian light and medium roast coffees achieved low scores in respect to coffees from all other origins. The "body", as reported in literature, depends on the content of lipids, caffeine, protein and CGA. With the analysis of variance we notice how the body depends on the effect of various origins, and the roasting treatment, (see graphs 12-13). Graph 11 shows that coffee from Honduras presented a high content of body as to respect other coffees, being followed by coffees from Kenya and Nicaragua. These raw coffees showed discreet level of CGA, caffeine and protein in according with bibliography. As far as the effect of

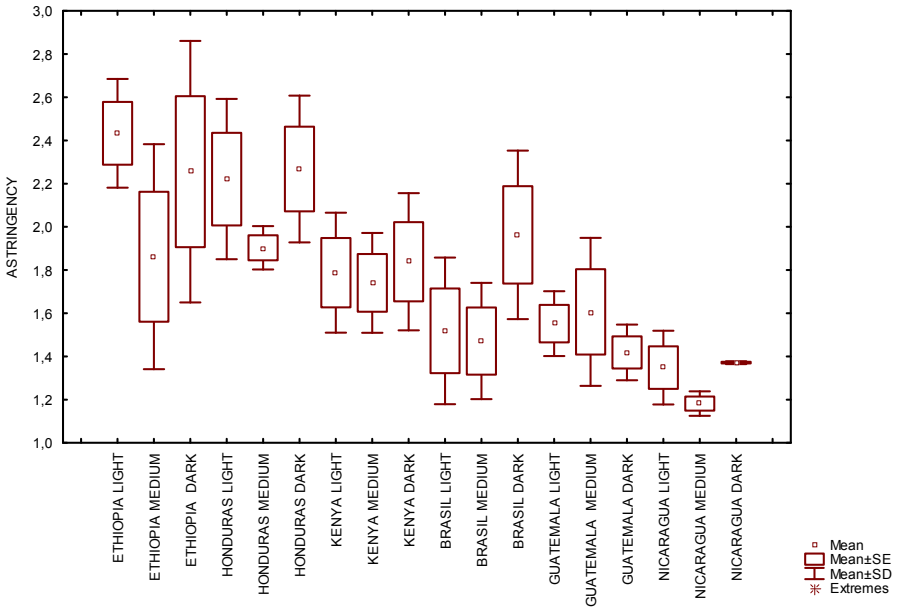
thermal treatment is concerned, the perceived body increased in line with the degree of the roasting process adopted.



Graph 12 - Confidence of interval, results of ANOVA and LSD test detected during sensorial analysis; different letter mark significant difference for $p < 0,05$.

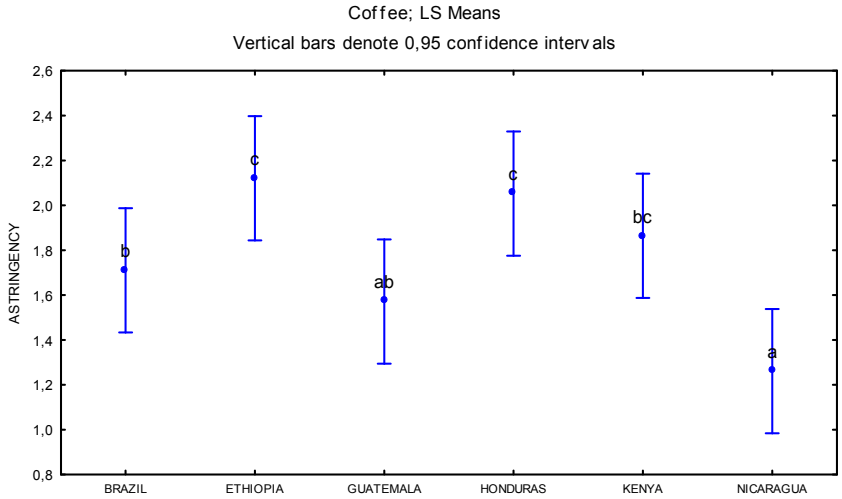


Graph 13 - Confidence of interval, results of ANOVA and LSD test detected during sensorial analysis; different letter mark significant difference for $p < 0,05$.



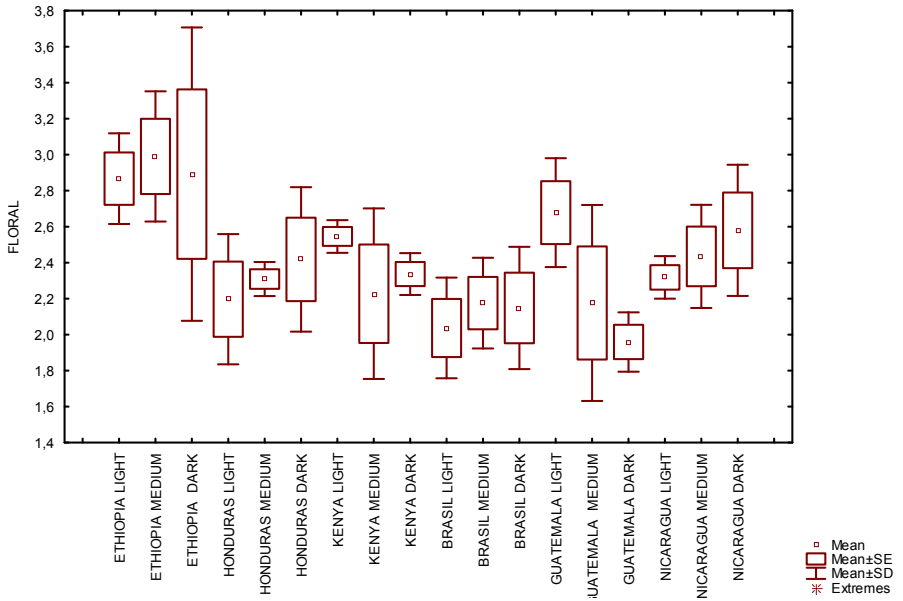
Graph 14 - Astringency content (score detected by sensory analysis) explain by box-plot in the coffee espresso: effect of increasing in base treatment roasting process and origin.

The astringency is another tactile sensation which is always considered as being negative, since an astringent coffee beverage reminds one of medicine and is a very unpleasant sensation. As stated in the introduction, this depends on the quality of the green coffee, in particular presence of immature green beans, and the content of caffeine, trigonelline and overall CGA. The astringency is generally recognised as being due to the presence of polymerised phenols, having a high molecular weight (500-3000), at least two 1,2-dihydroxyphenyl residues and an ability to precipitate salivary proteins. The degree of astringency is determined by the concentration of these phenols and by their degree of polymerisation. Such substances are collectively known as tannins. Unlike tea, in coffee there are no tannins or polymerised phenols. Clifford (1985) concludes that some of the CGA (the dicaffeoyl quinic acids) are the main astringent compounds of a coffee brew. Graph 14 shows the differing trend from each origin and one observes how it increases with dark roasting, but considering the analysis of variance there are no significant differences resulting from the roasting treatment adopted. Therefore, the various origins of the coffee have an important effect on the astringency. Graph 13 illustrates that African origin coffee is characterized by high levels of astringency. Part of this phenoma can be explained by the high content of phenolic compounds in green coffee.

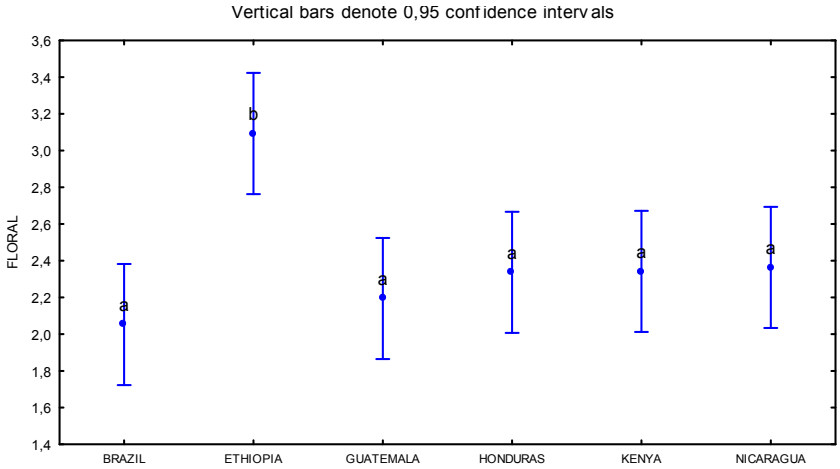


Graph 15 - Confidence of interval, results of ANOVA and LSD test detected during sensorial analysis; different letter mark significant difference for $p < 0,05$.

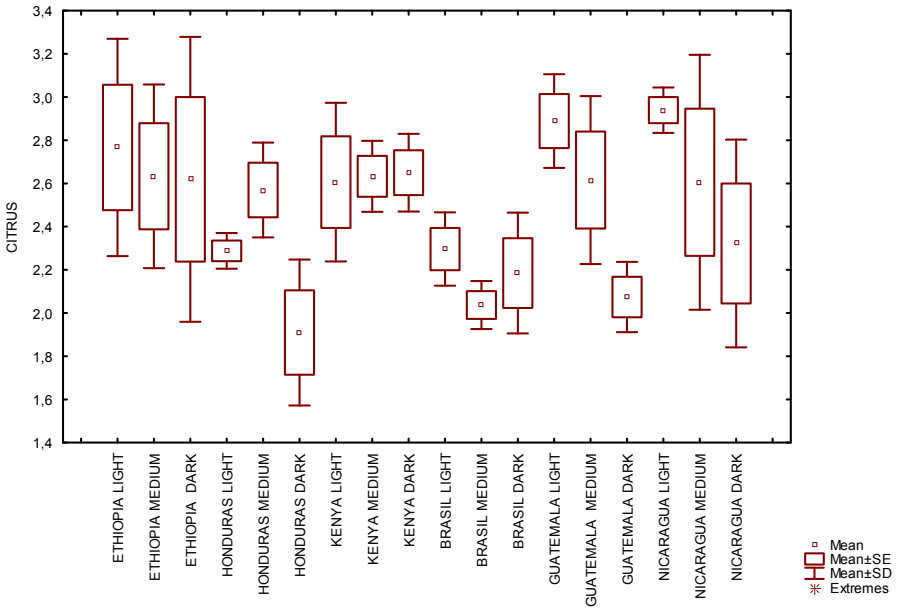
The "floral" descriptor perceived during sensory analysis is influenced by the origin of the coffee and treatment. Graph 16 shows that it is not always the roasting process that increases or reduces the floral tone, in fact every single coffee presented a different trend. A confirmation of the this is illustred in graph 51-52-53 relative chapter 5. Graph 17 shows that only coffee from Ethiopia detected a high level of the floral tone. In accordance with the data obtained in chapter 2, the PCA of graph 20 explained how green coffee from Ethiopia is characterized by relevant floral and, more fruity tones. Besides the analysis of the aromatic fraction of coffee roasted the Ethiopia coffee shows to have the highter contents terpenes compounds and a positive trend of degree roasting treatment.



Graph 16 - Floral content (score detected by sensory analysis) explain by box-plot in the coffee espresso: effect of increasing in base treatment roasting process and origin.

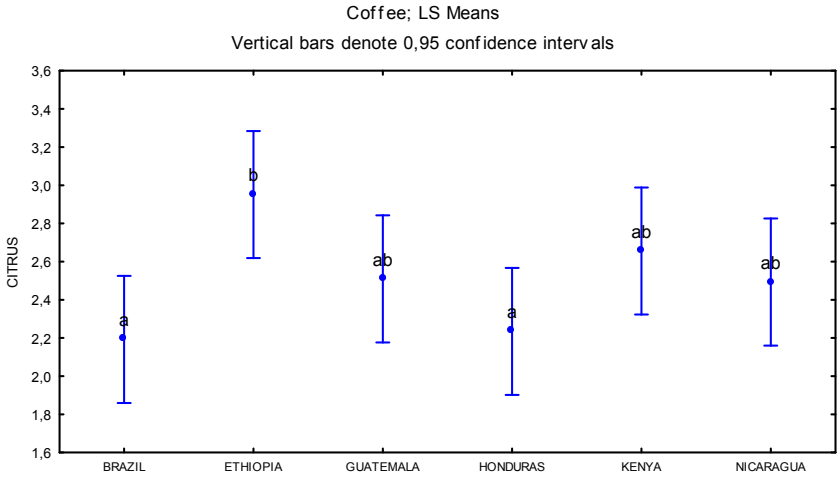


Graph 17 - Confidence of interval, results of ANOVA and LSD test detected during sensorial analysis; different letter mark significant difference for $p < 0,05$.

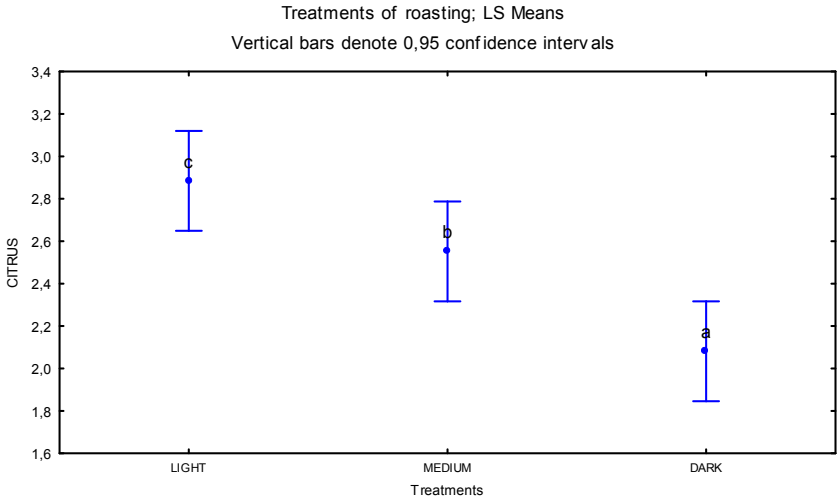


Graph 18 - Citrus content (score detected by sensory analysis) explain by box-plot in the coffee espresso: effect of increasing in base treatment roasting process and origin.

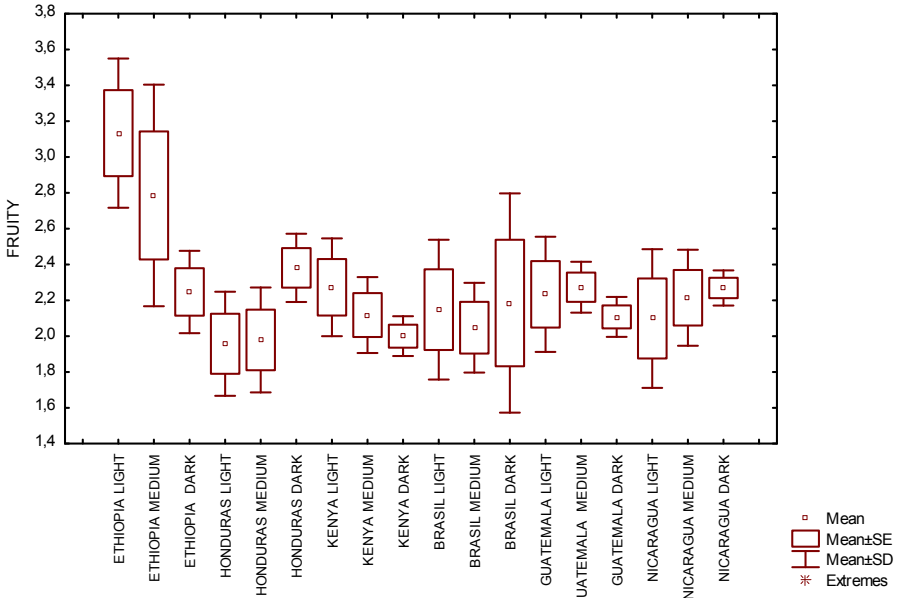
The "citrus" tone: as in graph 18. It is apparent that African and central America coffees presented notable levels of this descriptor but with different behaviour as a result of the degree of the roasting process. The opposite was true in Brazilian coffee. The coffees from Ethiopia and Kenya presented a constant perception of citrus tones in light, medium and dark roasts. Whereas coffee from Central America gave a negative trend in this area. Graphs 19 -20 show that the perceived citrus tone is highly influenced both by origin and by the type of roasting treatment used. Also the coffee from Ethiopia is characterized as having intense citrus tones. In general, however, as in graph 20, the effect of the roasting process changes and influences the perceived intensity of the citrus tone.



Graph 19 - Confidence of interval, results of ANOVA and LSD test detected during sensorial analysis; different letter mark significant difference for $p < 0,05$.

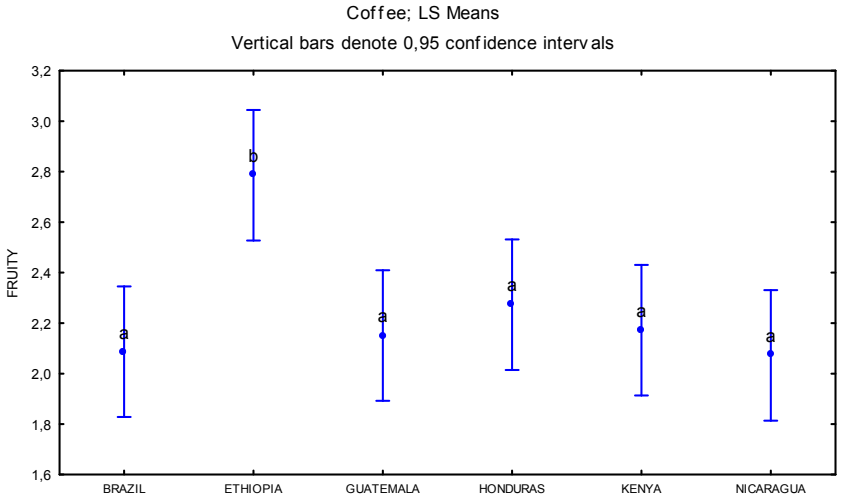


Graph 20 - Confidence of interval, results of ANOVA and LSD test detected during sensorial analysis; different letter mark significant difference for $p < 0,05$.

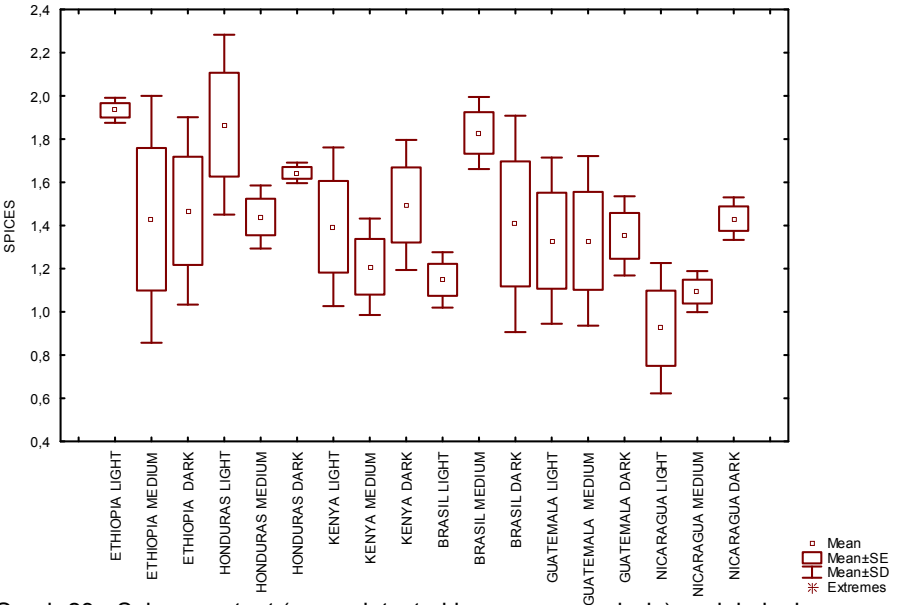


Graph 21 - Fruity content (score detected by sensory analysis) explain by box-plot in the coffee espresso: effect of increasing in base treatment roasting process and origin.

The "fruity" tone presented the same trend as the floral tone. Once Ethiopia coffee presented both the characteristic fruity tone together with a floral tone. The perception and intensity of a fruity tone depend on the presence of esters and aldehydes in particular acetaldehydes, propanal, and Strecker aldehydes 2-methylpropanal, 2-methylbutanal, 3-methylbutanal that originate from the Maillard reaction (Semmelroch & Grosch *et al*., 1995). It is clear, looking the graphs 22, that the fruity tone might depend only on origin, considering variance analysis and not depending on the degree of the roasting process. Considering that coffee Ethiopia has showed to have a hight content of floral notes showed a low content of esters and aldehydes.

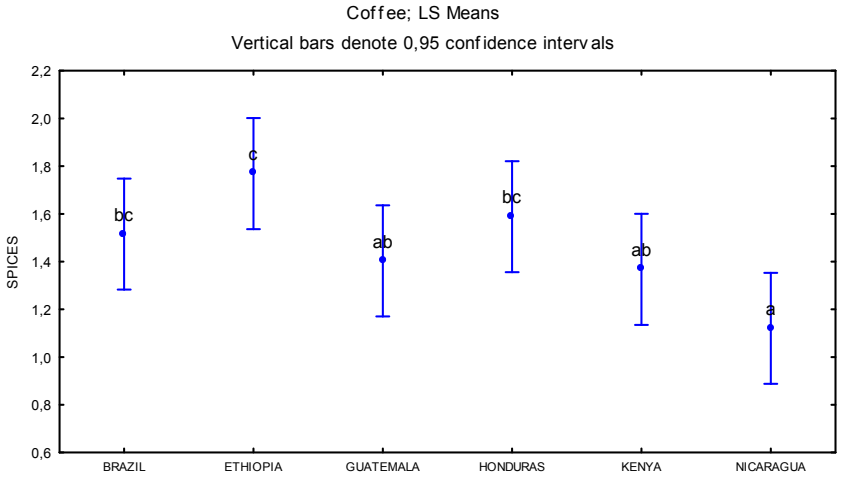


Graph 22 - Confidence of interval, results of ANOVA and LSD test detected during sensorial analysis; different letter mark significant difference for $p < 0,05$.

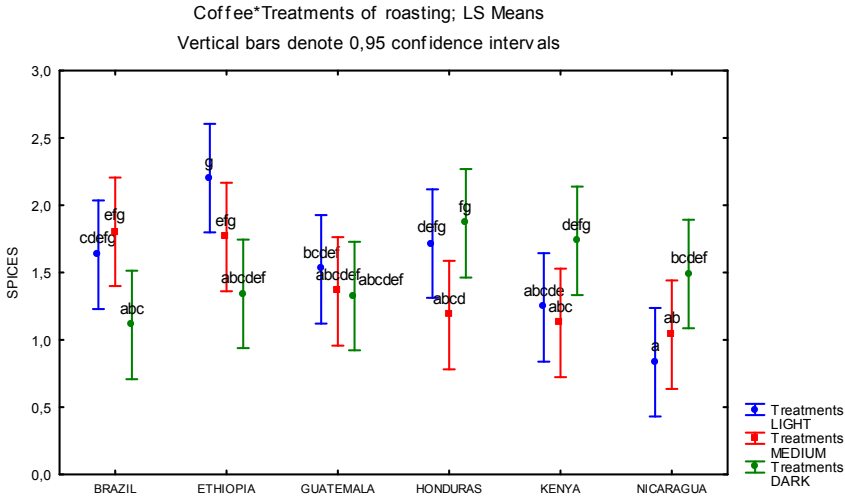


Graph 23 - Spices content (score detected by sensory analysis) explain by box-plot in the coffee espresso: effect of increasing in base treatment roasting process and origin.

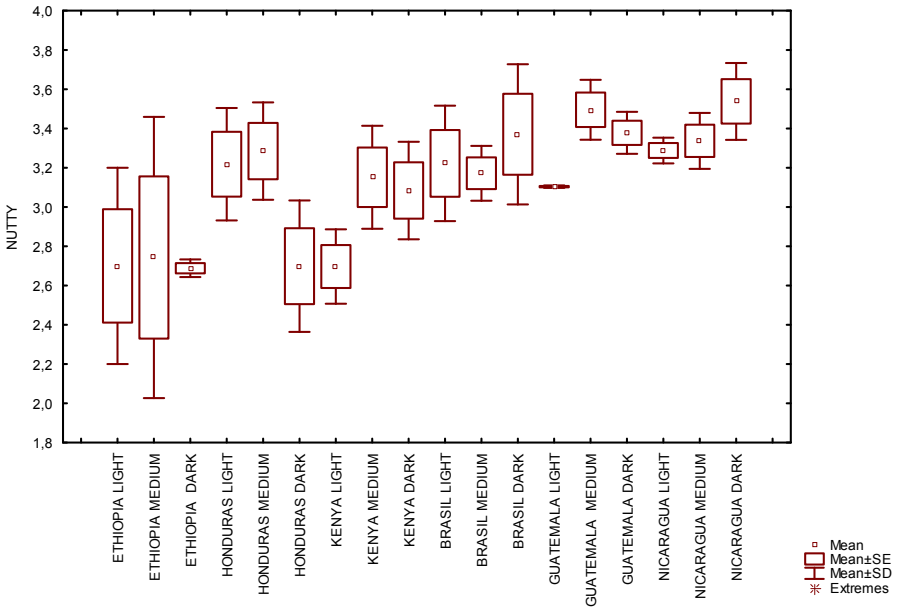
Graphs 23 and 24 describes the influence of roasting treatment on the descriptor "spices", a descriptor originating from the Maillard reaction. The spices tones have been perceived in all coffees with different intensities. The compounds responsible spicy notes are aromatic aldehydes (vanillic aldehydes, siringaldehydes) and votatile phenols (eugenol, 4-ethyl-guaiacol, guaiacol). Some level of pungency, induced by specific components in spices such as capsicum, pepper, ginger and mustard, is a positive attribute in the flavour profile. Graph 24 demonstrates that Ethiopian coffee has superior perceived intensity, being followed, in order, by coffees from Honduras, Brazil, Guatemala, Kenya and Nicaragua. All these coffees showed a good content of phenols compounds and aromatic aldehdes. The origin of the coffee plays an important role in the perception of the spices tone. Graph 25 indicates that the spices attribute depends largely on the interaction of two factors – the origin of the coffee and the type of roasting treatment used. There are significant differences and different behaviours stemming from coffees of different origins. For example, with Ethiopian and Guatemalan coffees it is the light roasting treatment which triggers an increase in the spices descriptor. Whereas with Honduran, Nicaraguan and Kenyan coffees it is the dark roasting treatment which amplifies the intensity of this tone. With Brazilian coffee the opposite situation is true.



Graph 24 - Confidence of interval, results of ANOVA and LSD test detected during sensorial analysis; different letter mark significant difference for $p < 0,05$.



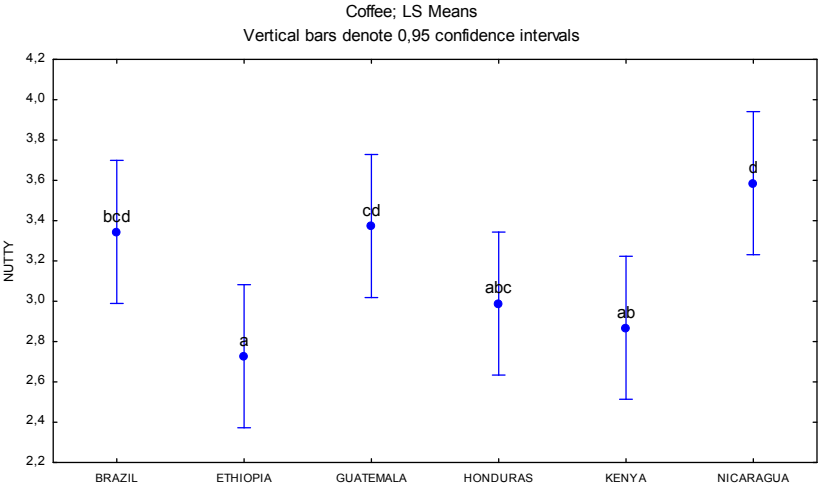
Graph 25 - Confidence of interval, results of ANOVA and LSD test detected during sensorial analysis; different letter mark significant difference for $p < 0,05$.



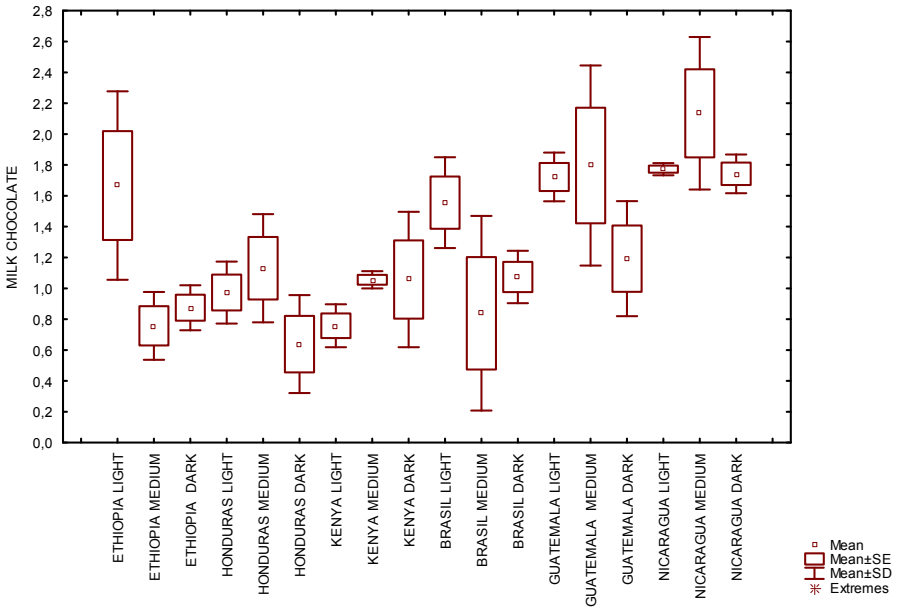
Graph 26 - Nutty content (score detected by sensory analysis) explain by box-plot in the coffee espresso: effect of increasing in base treatment roasting process and origin.

As regards the "nutty" tone: coffees from Nicaragua, Guatemala and Brazil score particularly highly in this area in comparison to coffees with other origins. The perception of a nutty tone is a result with different intensity which might be expected as it stems from the formation of compounds during the roasting process. The aromatic sensation which is perceived as a nutty tone originates from the caramelised sugar, and could be related to the content of pyrazines (2-methylpyrazine, 2-ethylpyrazine, 2,3-dimethylpyrazine), and some furans (Clarke and Macrae *et al*., 1985). In particular the Honduras, Nicaragua coffee showed a highter content of pyrazines where the amino acids, particularly threonine, appear to be the source of pyrazine during coffee roasting. Instead the coffee Honduras, Nicaragua and Brazil showed a highter content of furans. The furans are formed by various systems, namely the reaction of asparagine with sugars, such as glucose, fructose, sucrose and arabinose (Odell *et al*.,

1974). It can also be formed by Strecker degradation of pyruvaldehyde with glyoxal (Wang *et al*., 1969). The Nicaragua beans coffee presented a hight content of sucrose, glucose and fructose. The nutty descriptor is influenced only by different origins of the coffee.



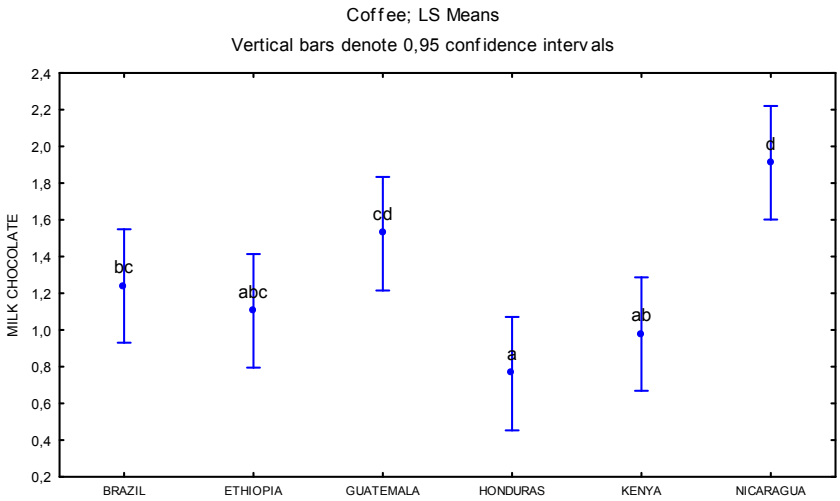
Graph 27 - Confidence of interval, results of ANOVA and LSD test detected during sensorial analysis; different letter mark significant difference for $p < 0,05$.



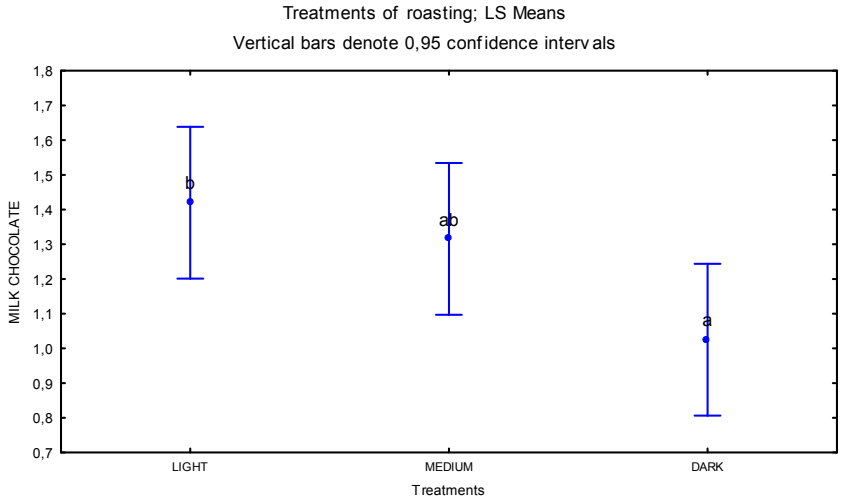
Graph 28 – Milk chocolate content (score detected by sensory analysis) explain by box-plot in the coffee espresso: effect of increasing in base treatment roasting process and origin.

Graph 28 looks at the "milk chocolate" descriptor. This descriptor has a wider range of variables across the range of coffees tested although a greater intensity of this tone was found in coffees from Nicaragua and Guatemala. Coffees originating from the Americas, particularly Central America are also characterized as having chocolates tones. Honduran coffee, however, presented the converse tendency. This perceived descriptor is a result of the Maillard reaction. The low content of sucrose in Honduran coffee might explain the lowering of this perception in that coffee. The compounds causing the chocolate tones have been well catalogued by various authors: 2-methyl propanal, 2 methyl-pentanal (Chemisis *et al*., 1994), the 2,3-dimethyl-pyrazine, trimethylpyrazine (Calabretta *et al*., 1978), 2,5-dimethyl-pyrazine (Fors *et al*., 1983), and 2,3-diethyl-5,6-dimethyl pyrazine (Chemisis *et al*., 1978). It becomes evident that this attribute is

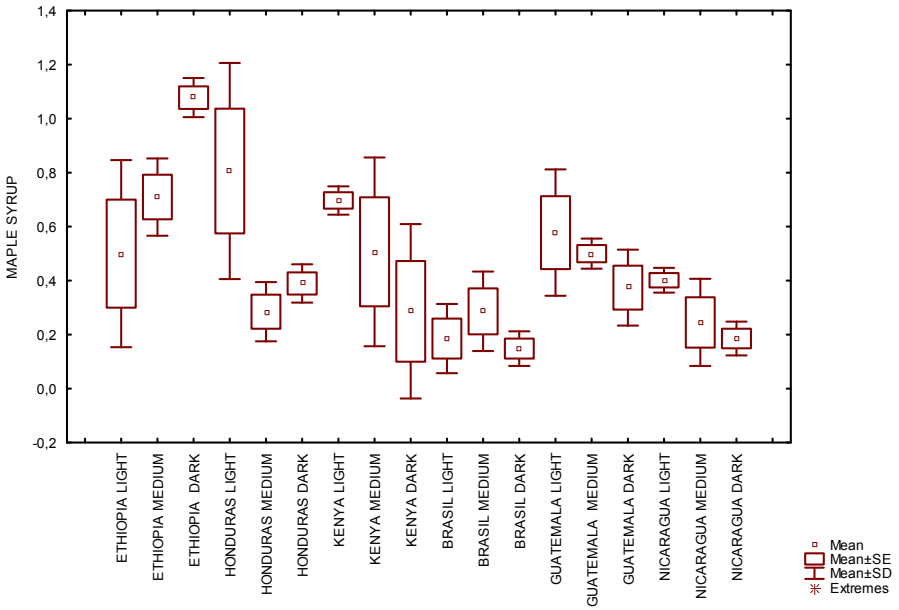
more highly influenced by variables origins and degrees of the roasting process. Graph 29 shows that the average perception of this descriptor is slightly lower when looking at light and medium roasts, wheras there is a dramatic reduction in its perception when looking at dark roasts.



Graph 29 - Confidence of interval, results of ANOVA and LSD test detected during sensorial analysis; different letter mark significant difference for $p < 0,05$.

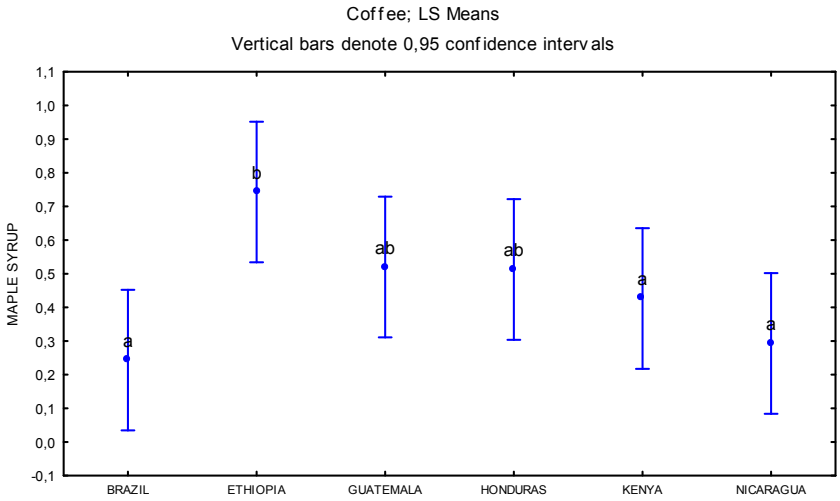


Graph 30 - Confidence of interval, results of ANOVA and LSD test detected during sensorial analysis; different letter mark significant difference for $p < 0,05$.

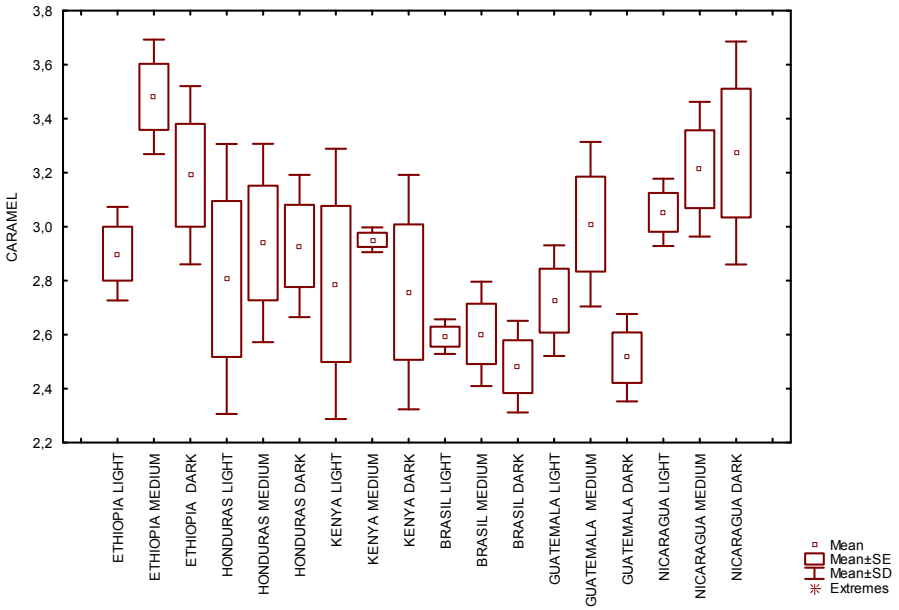


Graph 31 – Maple syrup content (score detected by sensory analysis) explain by box-plot in the coffee espresso: effect of increasing in base treatment roasting process and origin.

The "maple syrup" descriptor is similar to that of a sugar syrup or caramel tone and it is always formed during the Maillard reaction. The compounds responsible for this perception are: the α-cyclopentanediones (2-cyclopenten-1-one, 2-hydroxy-, 2-cyclopenten-1-one, 2-hydroxy-3-methyl, 2-cyclopenten-1-one-3-ethyl-2-hydroxy-, 2-cyclopenten-1-one, 2-hydroxy-3,4-dimethyl) and a few anhydrides (2,5-furandione, 3-ethyl-4-methyl-), furan with ketones (ethanone, 1-3-hydroxy-2-furanyl), 3-furanones (3 (2H)-furanone, 2-ethyl-4-hydroxy-5-methyl and 1-(2-furanyl)-1-propanone (Stoll et al., 1967). Ethiopian coffee demonstrated a high perception of this sensorial descriptor also if Honduras, Nicaragua coffee risulted to have hight content of ketones but about Ethiopia coffee this elevated level probably stems from the high levels of sucrose present in green coffee (see chapter 3).

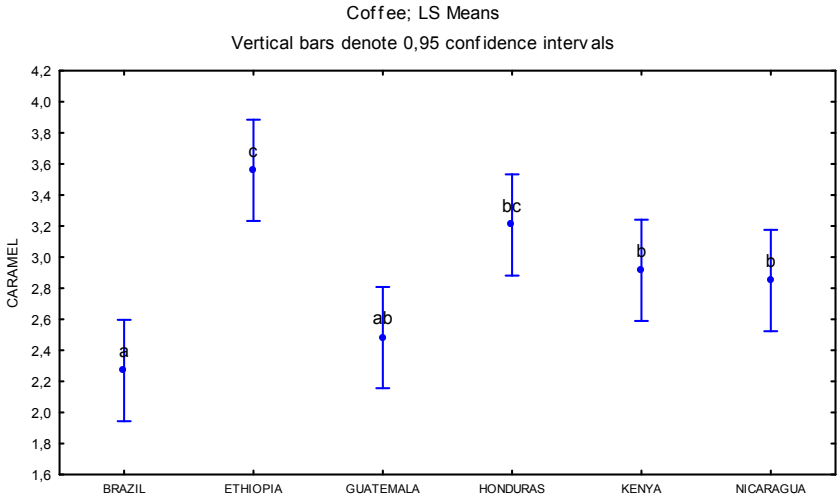


Graph 32- Confidence of interval, results of ANOVA and LSD test detected during sensorial analysis; different letter mark significant difference for $p < 0,05$.

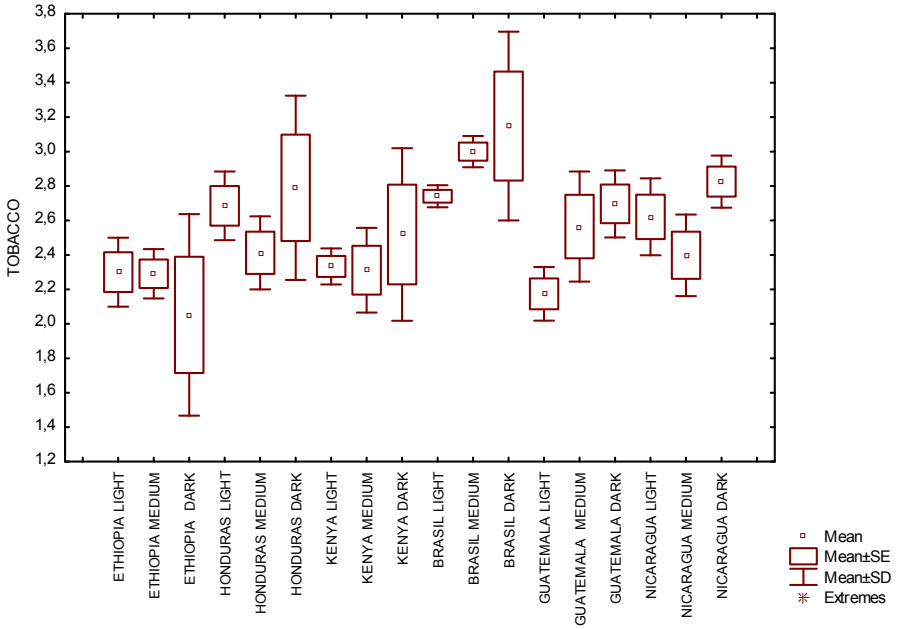


Graph 33 - Caramel content (score detected by sensory analysis) explain by box-plot in the coffee espresso: effect of increasing in base treatment roasting process and origin.

The "caramel" descriptor (graph 33-34) is perceived to a high intensity in Ethiopian coffee to a lesser extent in Guatemalan and Honduran coffees and at exactly the same level of intensity in coffees from Kenya and Nicaragua. Therefore, the origin of the coffee might well influence the perception of this descriptor. It is known that the furan compounds in coffee, which are responsible for the burnt sugar and caramel tones are formed during the caramelization reaction (Baltes *et al*., 1987; Clarke *et al*., 1985). According to Lingle (1996) the effects of caramelised sugars and the Maillard reaction to the coffee aroma are difficult to assess due to the fact that there are so many of them, their level of complexity and the renowned in stability of aromatic compounds. The compounds responsible for this descriptor: 2-methyl butanal, 3-ethyl-2-hydroxy-2-cyclopenten-1-one, 2-hydroxy-3,5-dimethyl-2-cyclopenten-1-one (Flament, 2002), and 3-methyl-2-cyclohexen-1-one (Ganturco *et al*.,1963).



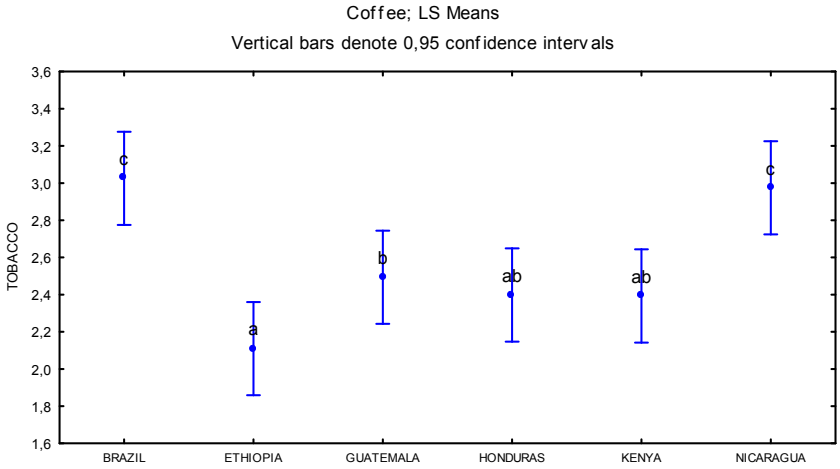
Graph 34 - Confidence of interval, results of ANOVA and LSD test detected during sensorial analysis; different letter mark significant difference for $p < 0,05$.



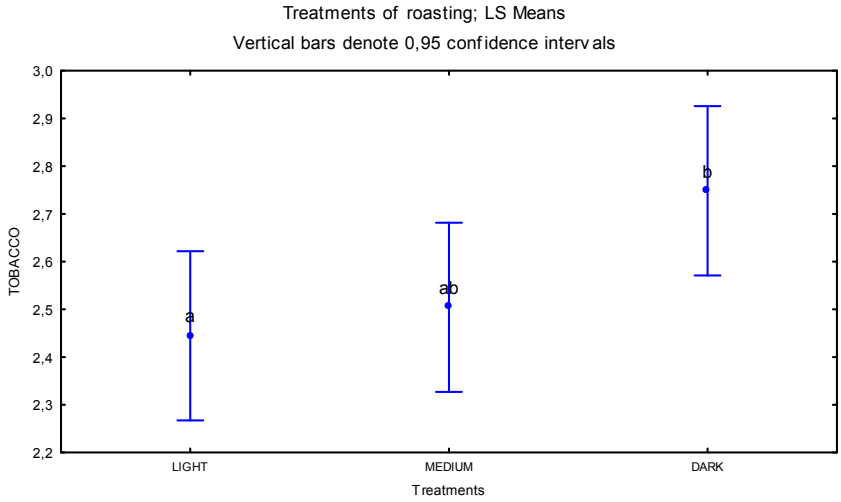
Graph 35 - Tobacco content (score detected by sensory analysis) explain by box-plot in the coffee espresso: effect of increasing in base treatment roasting process and origin.

The "tobacco" sensorial perception descriptor has close connection to the degree of roasting involved. The compounds responsible for this olfactive are caused by the presence of the 2-acetylpyridine (pyridines, quinolines with oxygenated function) identified by Vizthum and Werhhoff (1974). It has been identified in the degradation rearrangement products of glucose/glycine and fructose/glycine during the Maillard reaction. Other possible compounds include: methyl pyridine-3-carboxylate with mildly tobacco-like tones. The 3 ethyl pyridine is characterized as the tobacco flavour and it is one of the pyrolysis products of trigonelline (Viani and Homan *et al.*, 1974). This perception of this descriptor increases in line with the roasting process adopted (graph 37) and is, statistically, more marked with dark roasting

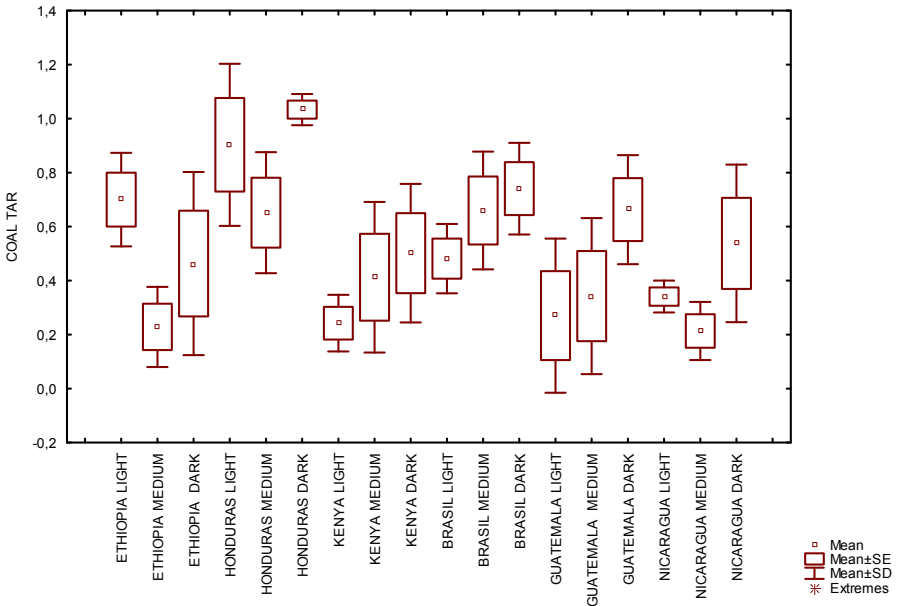
treatments. Although the effect of the roasting treatment adopted is significant, also the origin of the coffee affects the level of perception of this descriptor. Graph 36 shows that coffees from Brazil and Nicaragua demonstrated high levels of the tobacco tone in according with results found in chapter 5 where the pyridines are major classes compunds found in Brazil, Honduras and Nicaragua coffee.



Graph 36 - Confidence of interval, results of ANOVA and LSD test detected during sensorial analysis; different letter mark significant difference for $p < 0,05$.

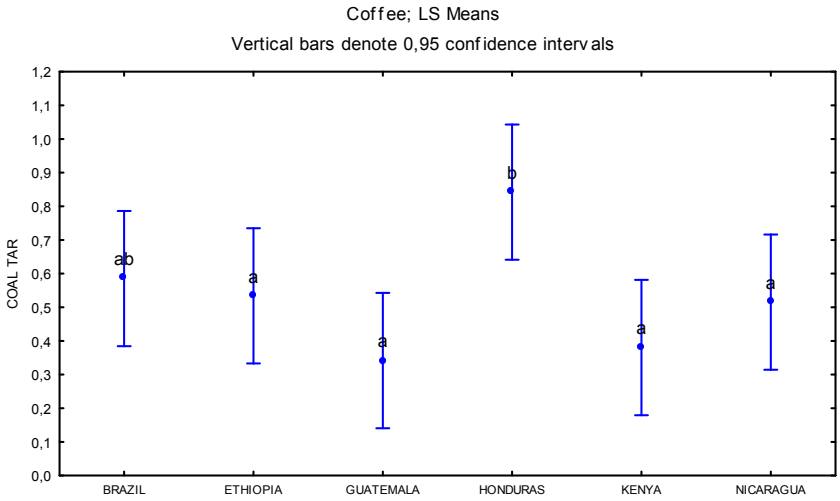


Graph 37 - Confidence of interval, results of ANOVA and LSD test detected during sensorial analysis; different letter mark significant difference for $p < 0,05$.

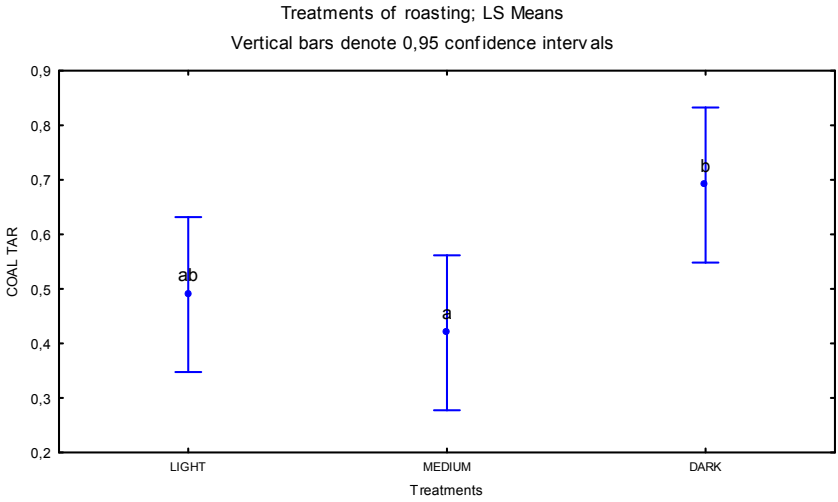


Graph 38 – Coal tar content (score detected by sensory analysis) explain by box-plot in the coffee espresso: effect of increasing in base treatment roasting process and origin.

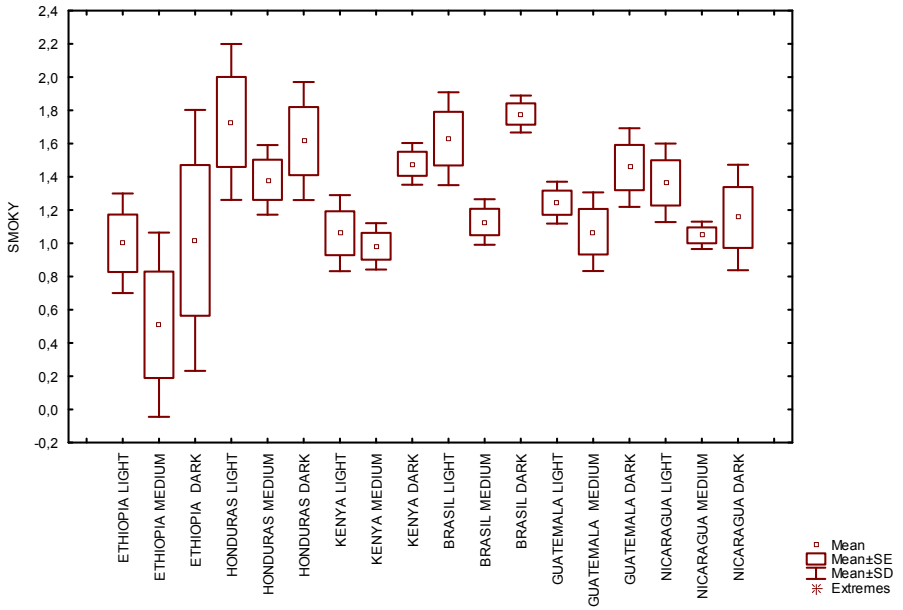
The "coal tar" tone is usually regarded as a defect, particularly when the perception of it is at a high or concentrated level. All typologies of coffee affected a positive trend according to the degree of roasting treatment. This descriptor behaved in a similar way to the "tobacco" descriptor – both to roasting process adopted and the origin of the coffee affected the results in some way. It is probable that this descriptor has been classified as a simple generic defect rather than being taken as a descriptor in its own right. The panel of judges perhaps did not fully understand this descriptor.



Graph 39 - Confidence of interval, results of ANOVA and LSD test detected during sensorial analysis; different letter mark significant difference for $p < 0,05$.

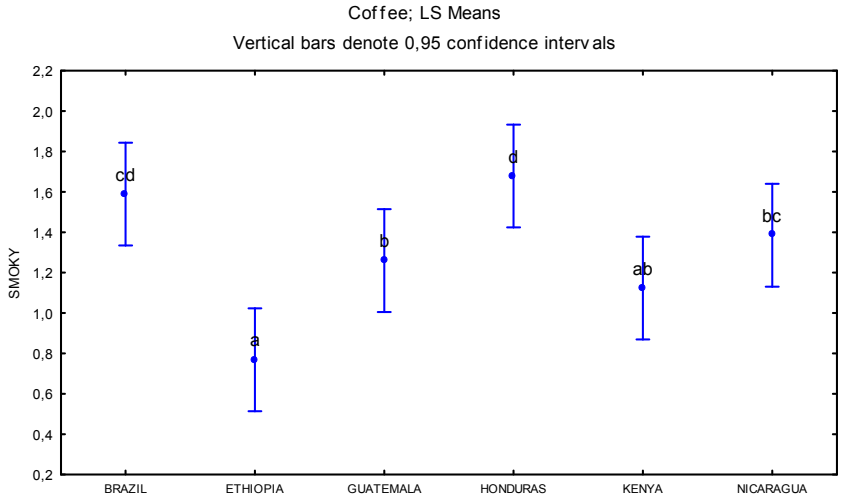


Graph 40 - Confidence of interval, results of ANOVA and LSD test detected during sensorial analysis; different letter mark significant difference for $p < 0,05$.

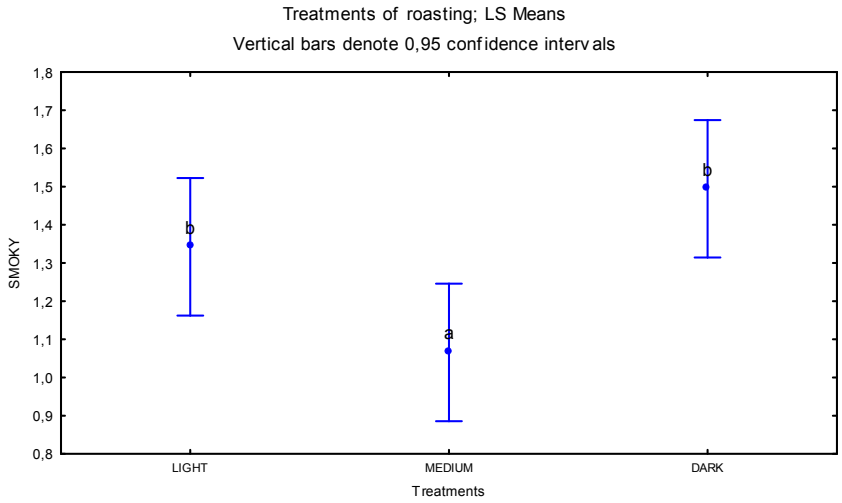


Graph 41 - Smoky content (score detected by sensory analysis) explain by box-plot in coffee espresso: effect of increasing in base treatment roasting process and origin.

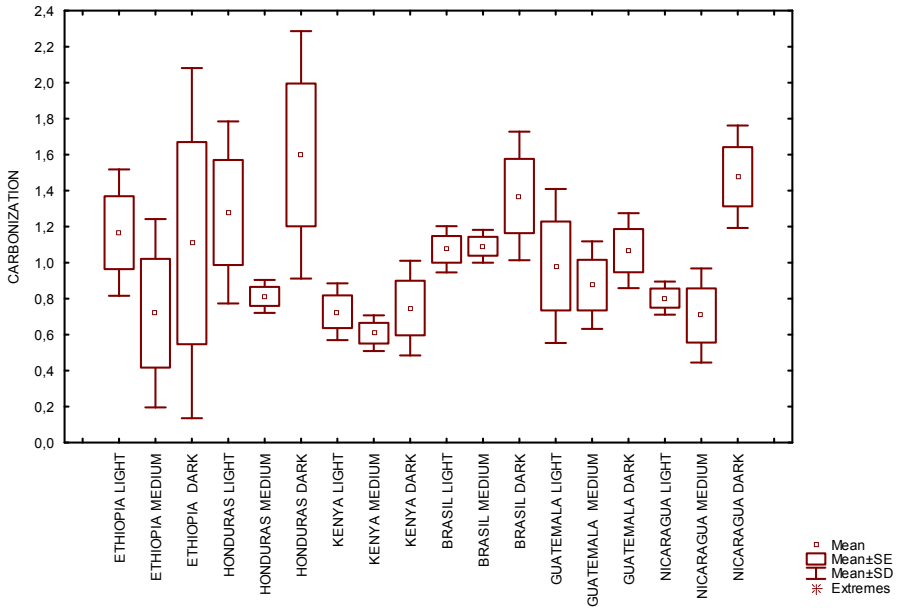
The "smoky" descriptor is the result of various compounds similar seen about spicy note: 2-ethylphenol (Aratandei *et al*.,1967), the 4-vinylphenol described as phenolic, "smoky" and "medicinal" (Chemisis *et al*., 1999), and Guaiacol has a powerful smoke-like, somewhat medicinal odour (Arctander *et al*., 1967). All of these compounds increase with roasting (graph 43). The origin of the coffee also has a significant affect on this descriptor. This descriptor behaved in a similar manner to the "coal tar" descriptor and, therefore, the same points raised for "coal tar" apply here.



Graph 42 - Confidence of interval, results of ANOVA and LSD test detected during sensorial analysis; different letter mark significant difference for $p < 0,05$.

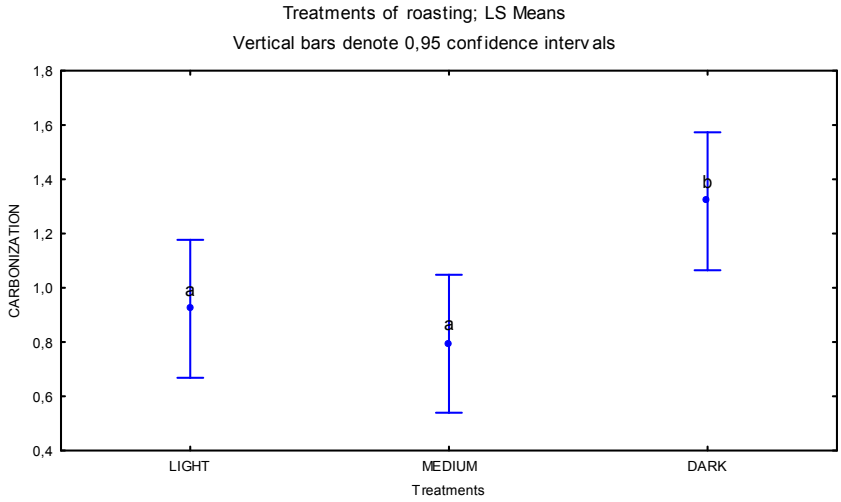


Graph 43 - Confidence of interval, results of ANOVA and LSD test detected during sensorial analysis; different letter mark significant difference for $p < 0,05$.

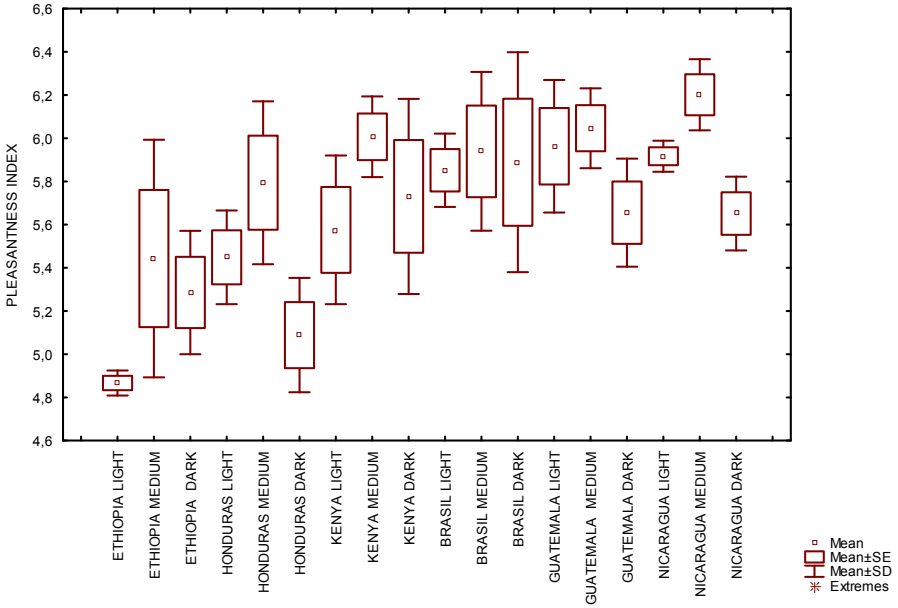


Graph 44 - Carbonization content (score detected by sensory analysis) explain by box-plot in the coffee espresso: effect of increasing in base treatment roasting process and origin.

The "carbonisation" descriptor, as in graph 44 showed a positive trend in relation to all typologies of coffee concerning the roasting process adopted. Graph 45 highlights the effect of light, medium and dark roasts on the intensity of perception of this descriptor. Significant differences are shown in coffees from Honduras and Nicaragua.

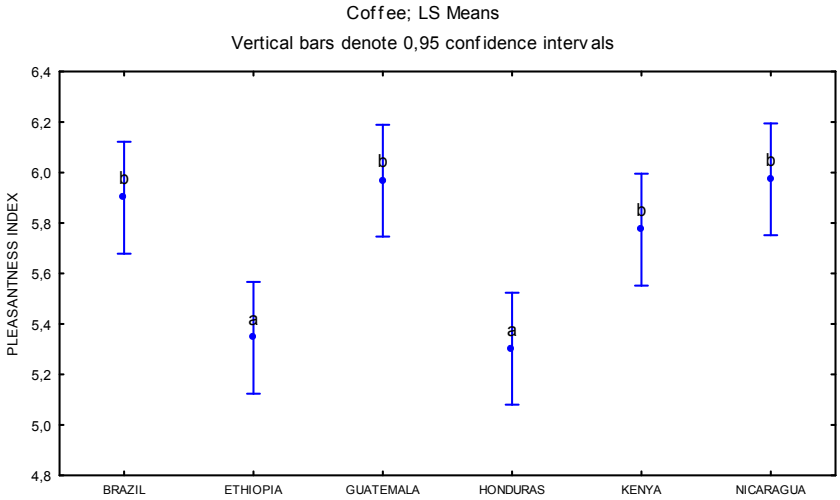


Graph 45 - Confidence of interval, results of ANOVA and LSD test detected during sensorial analysis; different letter mark significant difference for $p < 0,05$.

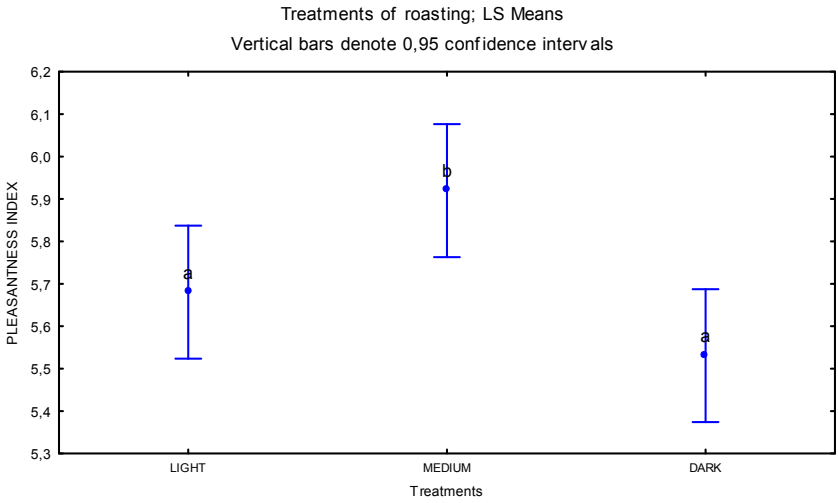


Graph - 46 Pleasantness index content (score detected by sensory analysis) explain by box-plot in the coffee espresso: effect of increasing in base treatment roasting process and origin.

The "pleasant index" (graph 46) shows a low reading for Ethiopian light roasted coffee, whereas coffees originating from Brazil, Guatemala and Nicaragua had high readings. Apart from seeing this variation from the point of view of the origin of the coffee, the degree of roasting treatment is also a significant factor. Graphs 46 and 48 show that the "pleasant index" increases to its maximum value with medium roasted coffee.



Graph 47 - Confidence of interval, results of ANOVA and LSD test detected during sensorial analysis; different letter mark significant difference for $p < 0,05$.



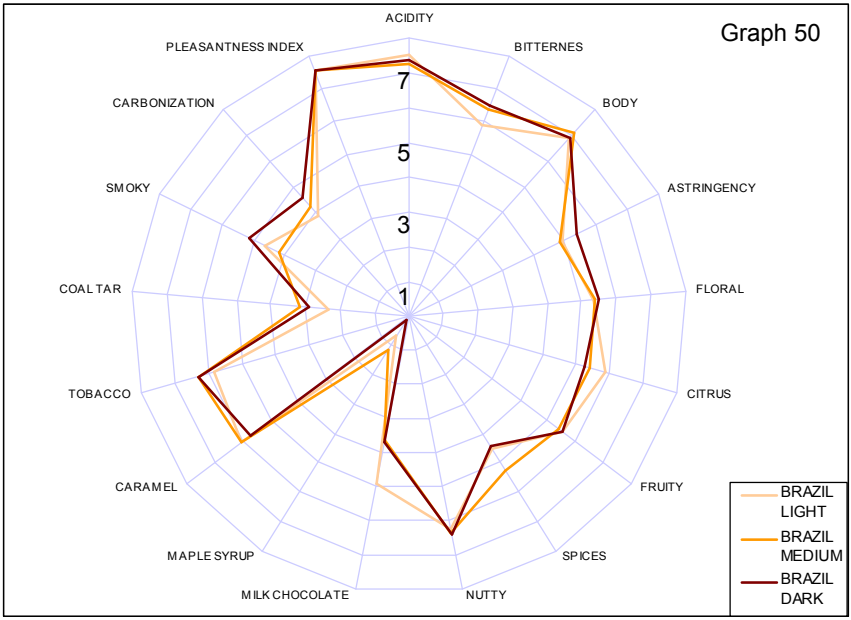
Graph 48 - Confidence of interval, results of ANOVA and LSD test detected during sensorial analysis; different letter mark significant difference for $p < 0,05$.

For all the samples roasted coffee the pleasant index showed a significantly positive correlation with sweet (r^2=0,47), equilibrium (r^2=0,64), nut (r^2=0,53), milk chocolate (r^2=0,48), instead a negative correlation with bitterness (r^2=0,68), astringency, (r^2=0,78) spices (r^2=0,52), coal tar (r^2=0,54) and carbonization (r^2=0,54). Surely all these descriptors are related both to the effect of treatment of roasting a single origin and effect at the different chemical composition of precursor content. In particular the attributes bitter, coal tar, and carbonization are felt more in dark roast, that might depreciate and condition of the pleasantness of the beverages. Responsibles are the phenols compounds where take place respect at the key odours most represented (aldehydes, ketones, sulphur compounds, esters)

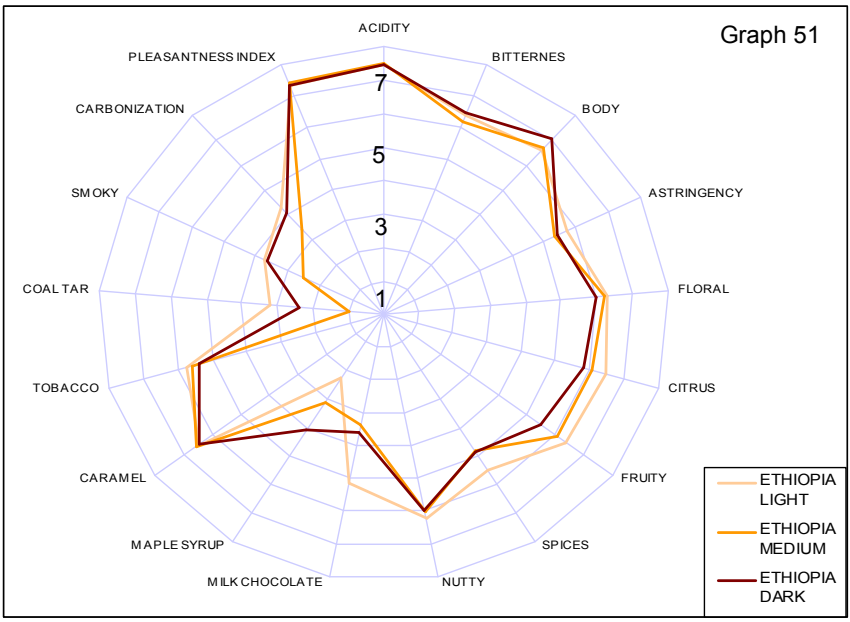
4.3.3 Simplifying approach

One of the main aims of this work is to provide key information on the choice of coffee typology with a view to formulating new blends to present on the market. The results can be interpreted in various ways; the "easy" interpretation of the ANOVA results of

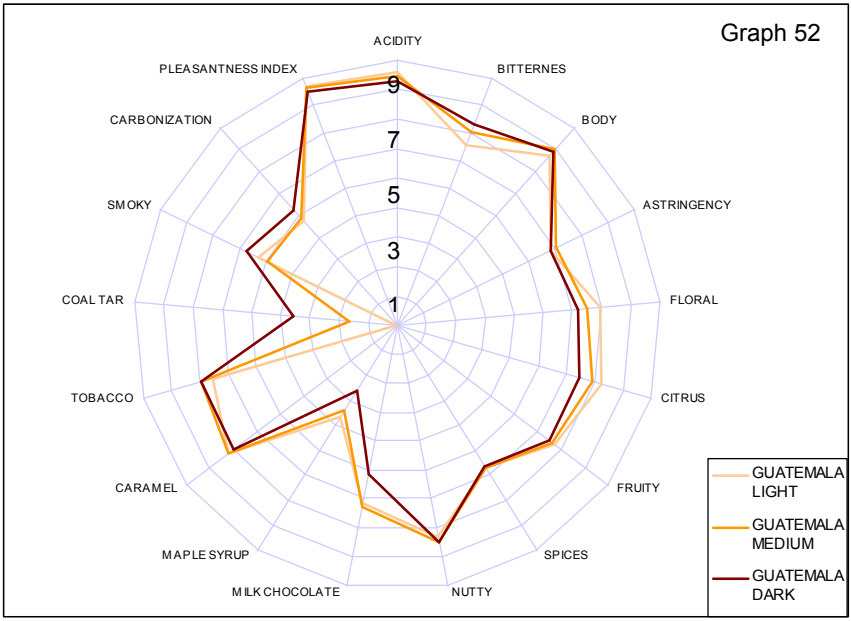
sensory analysis, or a "graphic" representation which observes the behaviour of the single origin of the coffee with the values being reported in terms of averages according to the roasting treatment adopted in each case. This second approach is clearly more instructive – to follow there are various "cobweb" graphs which highlight the most significant descriptors. Graph 49, which looks at Brazilian coffee, shows that the most evident descriptors are the following: "body", "floral", "fruity", "nutty", "caramel", "tobacco" and "smoky". These olfactive descriptors changed once thermal treatment was introduced, but these alterations were minimal in nature. The most significant variation concerning light roasts shows how the acidity and the "milk chocolate" tone increased whilst the bitterness and "citrus" tone fell. The medium roasting process, conversely, has a tendency to increase the body, the "spices", "maple syrup", "caramel" and "coal tar" tones. The dark roasting process causes an increase in bitterness, astringency and "floral", "smoky" and "carbonisation" tones. It is evident that the effect of roasting might bring about minimal variations to the characteristics of coffee - exalting different descriptors in different situations. However, the global "pleasure index", for example, failed to distinguish and significant differences between Brazilian coffee subjected to either light, medium or dark roasting treatments.



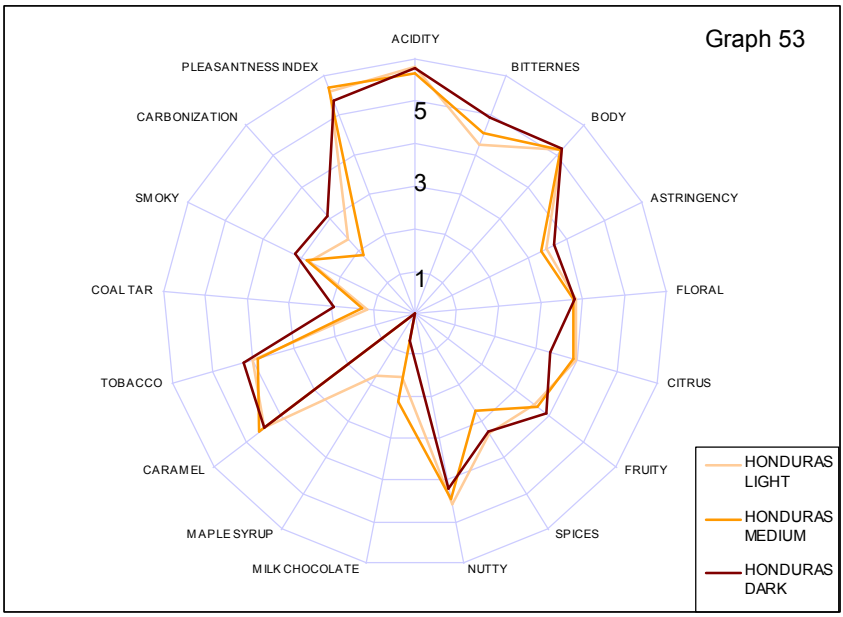
Graph 50 looks at coffee from Ethiopia. It is clear to see that this typology is characterized by the following descriptors: high levels of acidity and body with "floral", "citrus", "fruity", "nutty", "caramel" and "tobacco" tones. The differences become apparent when the results are approached from the effects of the roasting process. With light roasted coffee the following prevail: the astringency taste, "floral", "citrus", "fruity", "spices", "nutty", "milk-chocolate", "tobacco", "coal tar" and "smoky" tones. These attributes were greatly reduced when approached through the dark roasting process. The global "pleasure index" is orientated towards an average situation with several attributes.



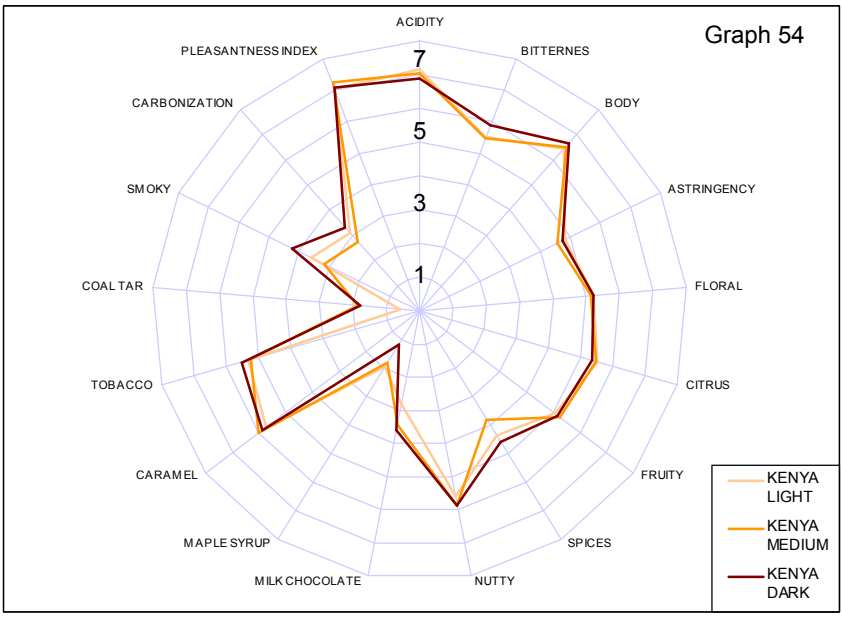
Graph 51 shows coffee from Guatemala and here the following prevail: increased acidity, body, "nutty", "milk chocolate", "caramel", "tobacco" and "smoky" tones. Approaching the results from the roasting process angle, few descriptors change. For example, for light roasts acidity, "floral" and "citrus" tones prevail. In medium roasts both body and taste along with "caramel" and "milk chocolate" tones prevail. Dark roasts introduce the bitterness taste and "coal tar", "smoky", "carbonisation" tones prevail whilst the tones of "maple syrup", "milk chocolate", "floral" and "citrus" are dramatically reduced. The global "pleasure index" is placed in medium roasts presumably due to the lower "carbonisation", "coal tar" and "smoky" tones.



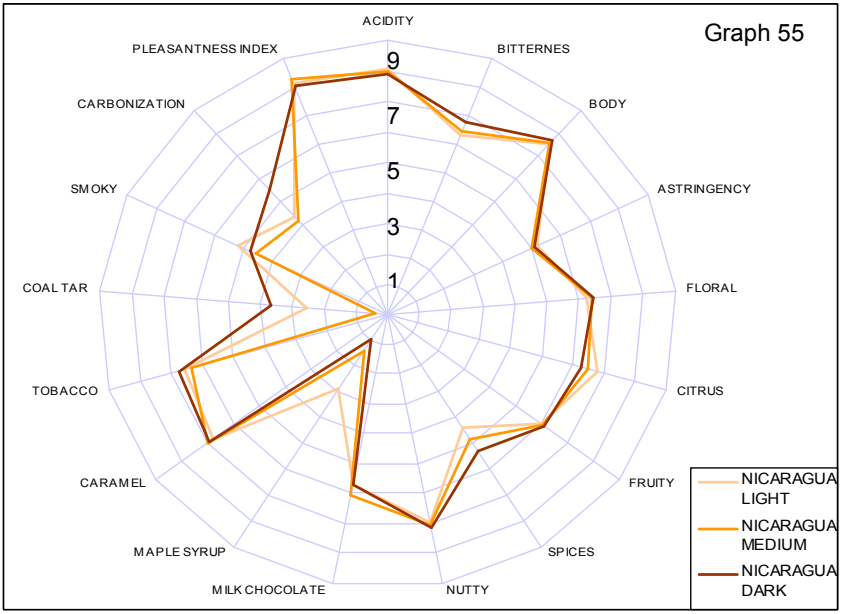
Graph 52 deals with coffee from Honduras, which is characterized by its acidity, body tastes and its "nutty", "caramel", "tobacco" and "smoky" tones. The light roast treatment brings about a reduction in only the bitterness taste, whilst causing an increase in "nutty", "maple syrup" tones. Medium roast treatment gave the coffee a small amount of the astringency taste and the tones of "spices", "tobacco" and "carbonisation". The dark roast treatment, however, modified the perceived intensity of numerous descriptors (as with coffees of different origin) and greatly increased the tastes of bitterness and astringency. The tones of "fruity", "tobacco", "coal tar" and "carbonisation" increased whilst those of "citrus", "nutty", "milk chocolate" and "caramel" were somewhat reduced in terms of their perceived intensity . The medium roast version of this coffee was the one most highly appreciated and this was probably due to enhancement of the "citrus" and "milk chocolate" tones and the lowering of the tastes of bitterness and astringency and a reduction in "spices", "tobacco", "coal tar" and "carbonisation" tones.



Graph 53 shows coffee from Kenya which is characterized by its acidity and body, along with the tones of "nutty", "caramel", "tobacco", "smoky", light "fruity" and "floral". With this coffee the changes caused by light, medium or dark roasting were negligible. However, graph 53 highlights the differences brought about in the taste of bitterness and the tones of "spices", "nutty", "maple syrup", "coal tar" and "smoky". The global "pleasure index" for Kenyan coffee falls dramatically, however, with the introduction of a medium roast.



Graph 54 represents coffee from Nicaragua. This is characterized by the tastes of acidity and body along with the tones of "floral", "citrus", "nutty", "milk chocolate", "caramel" and "tobacco". The body and astringency tastes and the tones of "floral", "citrus" and "fruity" remained largely unaltered as a result of the roasting process. Differences occurred, however, as regards the tones of "spices", "tobacco", "coal tar", "smoky" and "carbonisation", which increased in intensity in line with the degree of roasting. where increasing in degree roasting. In Nicaraguan coffee, as with others, the global "pleasure index" was further satisfied with the medium roast.



4.4 Conclusions

The results of this study indicate that the aroma and taste characteristics of coffee, as detected by the descriptive panel, were affected principally by either the origin of the coffee and/or by the degree of roasting applied to the coffee. The preparation stage for an espresso coffee surely also has an influence on the aroma perceived, although the conditions used were always meticulous. The study shows that there is an evolution and change in the aroma characteristics when passing from green beans to brewed coffee. It looks closely into the effects each typology of coffee or origin of the coffee have. The characteristics are noted for their differing content of aromatic precursors and classes of aromatic compounds in particular, aldehydes, esters, phenols, furans, and pyrazines. The effect of environmental features and external influences greatly affect the formation of specific compounds that characterize the range of different

coffees available. However, many consumers in this and other countries prefer dark roasted coffee while others prefer medium or lighter roasts. The differences brought about by degrees of roasting in terms of bitterness and acidity are highly significant. Further, the differences in the amount and proportion of volatile compounds, especially those really contributing to the aromatic character, are equally significant. The effects of roasting, be the treatment either light, medium or dark, cannot be ignored although the roasting factor is secondary to the coffee origin factor. The roasting process will either enhance or suppress certain descriptors that behave differently according to the type of coffee used. The global "pleasure index" is clearly more satisfied when dealing with medium roast coffee, this is due to the fact that the judges detected greater equilibrium for all descriptors when applying them to medium roast coffee. Taking an overall view of the results it can be said that the coffees originating in Africa are more floral with citrus tones, whereas coffee from Central American are more concentrated in terms of nutty, milk chocolate, maple syrup and tobacco tones. Brazilian coffee has no significant or overriding descriptor, it is considered to be a coffee with a great deal of balance and due to this characteristic, it is often used in blends and introduced to balance the taste and olfactory tones.

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FIFTH CHAPTER

"Characterization of volatile compounds in coffee roasted Arabica of different geographic origins"

5.1 Introduction "Roasting process"

In general the quality of roasted coffee depends on a large number of factors and parameters explained followed in fig 1.

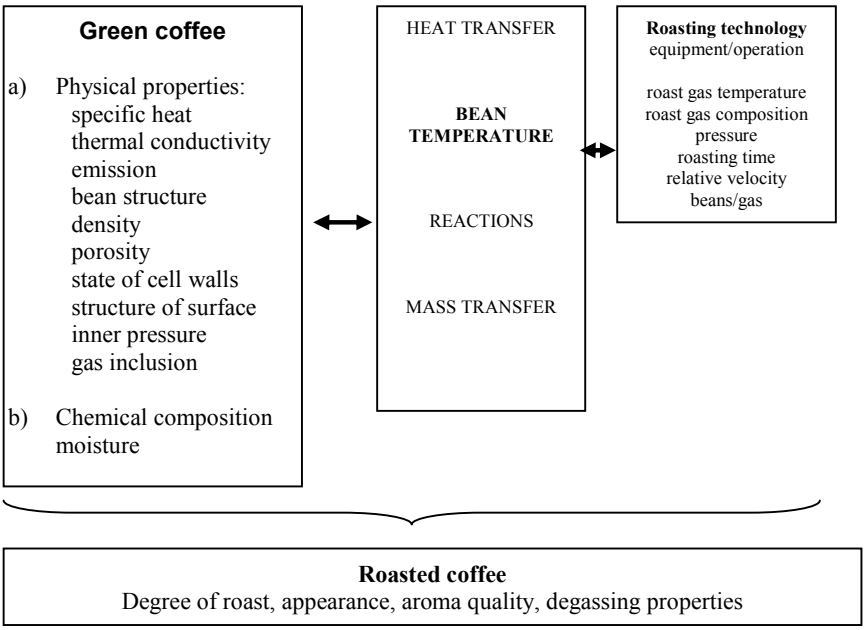


Fig 1: The roasting process some parameters and influences

It is well known that the coffee is appreciated and consumed for its pleasing aroma, which is the result of roasting. process. From the technical point of view, the roasting process is complex and several parameters and processes influence each other (fig 4). The most important step in coffee processing, causing marked chemical, physical, structural (tab 1), and sensorial changes (Sievetz & Desrosier, 1979). During this process take place many reactions because coffee beans are subjected to high temperatures (in the case of an Italian-style roasting it ranges

between 200 and 240 °C bean temperature) for different times depending on the characteristic of the final product (Lerici & Nicoli, 1990). A possible causes of the relevant change physical of the texture during roasting, could be attributed to the noticeable reduction of density due to the volume increase and to the corresponding increase of porosity of the bean structure determined by the increase of the pressure of the internal gases, products of the heat-induced reactions (Massini *et al.*, 1990; Gutierrez *et al.*, 1993). It is known, in fact, that the overall mechanical properties of a food could be related to the cell structure which is typical of various vegetables and fruits or they may result from the physical state, or porosity (Roos, 1995).

Some average physical properties of arabica coffee beans (initial mass 0.15 g).

	Mass (g)	Moisture (wt%)	Roast loss (wt%)	Dry matter loss (wt%)	Density (g/ml)	Volume (ml)	Radius[1] (mm)	Porosity (—)
Green	0.15	10–12	0	0	1.2–1.4	0.11–0.13	3	<0.1
Medium roast	0.13	2–3	15–18	5–8	0.7–0.8	0.16–0.19	3.5	0.5

Table 1 (Coffee - Recent Developments edited by Clarke and Vitzthum 2001)

The coffee roasting process consists essentially of receiving, cleaning, sorting, weighing, conveying, storage, roasting, cooling, grinding, and packaging operations. Bags of green beans are hand or machine-opened (fig 2), dumped into a hopper, and screenend to remove debris. The green beans are then weighed and transferred by belt or pneumatic conveyory to storage hoppers. From the storage hoppers, the green beans are conveyed to the roaster.

Fig 2 - Raw coffee cleaning machines before storage. Plants to automatize the receiving of raw materials that can reach the production facility in different modes or containers, such as:

big bags of ~ 600 kg;
standard bags of 60÷70 kg on pallets;
standard bags of 60÷70 kg loose in container;
loose (into tank truck or container)

www.petroncini.com

Roasters typically operate at temperatures between 370 °C and 540 °C and beans are roasted for a period of time ranging from a few minutes to about 30 minutes (Clifford *et al*., 1985; Ostendorf, 1992; Plenninger, 1993; Clarke *et al*., 1987; Wasserman *et al*., 1992). More in detail the roasting process can be divided into a drying phase, during which moisture is eliminated, a roasting phase, transforming the precursors into the components of roasted coffee. During the drying step, which is endothermic phenomena the smell of the beans slowly changes from green to peasy, to bread-like, and the colour turns yellowish (fig 3). During the actual roasting step pyrolytic reactions take place within each

cell, which, given the thickness of their walls, with internal pressures about 25 bar. The chemical composition of the beans is drastically modified with release of large amounts of carbon dioxide (5-12 litres of CO_2 Kg^{-1}) and formation of the many hundreds of substances which give roasted coffee characteristic aroma and taste. The beans: initially, at about 160 °C, the process is exothermic and pyrolysis reactions reach a maximum between 190 °C and 210 °C (Raemy & Lambelet, 1982). It then becomes endothermic with release of volatile compounds and in its final stage, around 210 °C, it once again becomes exothermic. The final temperature, which can reach 220 °C for the very dark roasts. At the change from endothermic to exothermic reaction it is possible to hear the coffee beans pop; the second popping, when cells explode from the increase in internal pressure, indicates the end of roasting and water sprays are used to "quench" the beans. When the desired degree of roast (color, flavor, roast mass loss) is reached, the beans are discharged from the roaster and cooled rapidly using air or water as cooling agent. The cooling is necessary for stopped chemical process inside the beans and the freshly roasted coffee is brought back to room temperature.

Brazil *Mundo Novo*

Fig 3 - Changing of colour, volume to green to darker brown in Brazil coffee

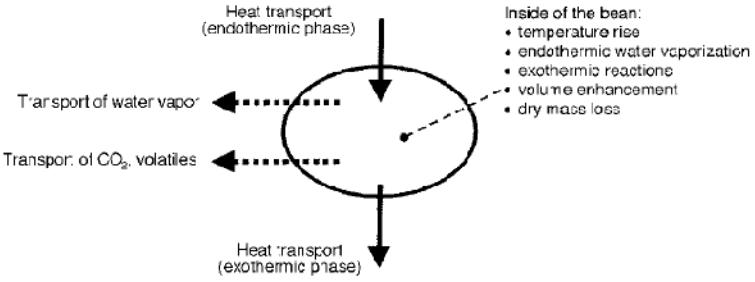


Fig 4: Roasting of coffee beans- main aspects (Source: Coffee - Recent Developments edited by Clarke and Vitzthum 2001)

The two system of cooling presents a few disadvantages and critical for quality.
About air cooling:

- a loss of aroma due to the stripping of volatile substances
- the emission of fumes laden with organic substances
- the carbon dioxide formed during roasting is trapped within the beans and influences packaging

About water cooling:

- the oil on the surface of the beans is easily oxidized in the presence of water, decreasing the keeping period of roasted coffee dark
- water vapour the pores on the surface of the bean open, allowing most of the gas in the bean to escape, so stripping off most of the volatile substances, the aroma
- excessive quantity of water decreasing cup yeld

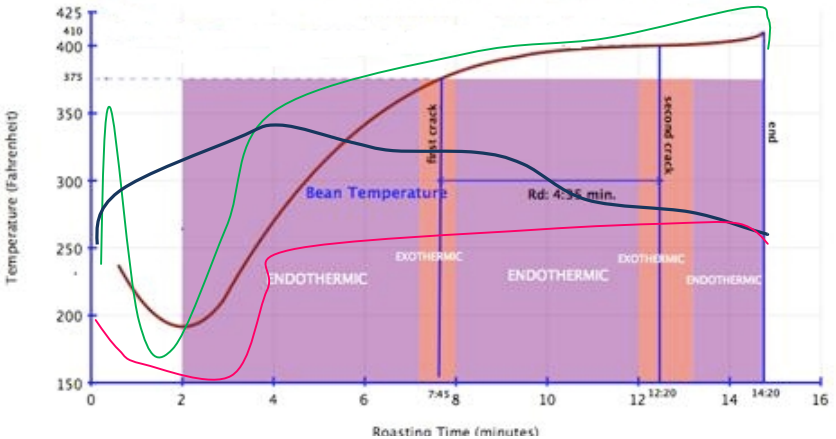
Following end process the coffee through a destoners pneumatically convey the beans to a hopper, where the beans are stabilize and dry. Subsequently the coffee, before packaging

will be on trial the equilibrium and stabilization called "degassing" (to rest). This step last for different hours in function type of coffee and roast condition. The carbon dioxide formed during Maillard reaction, Strecker degradation and pyrolysis of sugars is trapped in the coffee and slowly diffuses out after roasting and grinding. The diffusion mechanism of CO_2 is a combination of pressure-driven viscous flow, surface diffusion, and interactions between the CO_2 molecules and the coffee matrix (Anderson *et al*., 2003). Besides the cooling speed and final bean temperature influence the carbon dioxide loss. In fact, in winter, coffee cooled using air drawn from the outside contains more carbon dioxide than in summer, because the lower air temperature can changes in the porosity of the cell-walls.

5.1.1 Process parameters (emission and controls)

The most important parameter of the roasting process is the quantity of heat transferred to the beans, which can be determined from the temperature of the coffee beans and the roasting time. Beans are introduced in a roaster machine, typically horizontal rotating drums that tumble the green coffee beans in a current of hot combustion gases; the roasters operate in either batch or continuous modes and can be indirect or direct-fired. There is a burner produce hot air at temperature about 550 °C. Obviously roasting temperature and roasting time depend also strongly on the heat transfer system by burner and therefore the technology applied. At the start the process there is a preheating phase in drum followed from roast curve profile built in function also of the desired color. Traditionally the roasting times is 8 to 13 minutes and the might also to be manage by a system in PLC (graph 1). Every kind of coffee must be roasted with a specific curve that follows a correct profile roasting time and temperature. A parameter often used for to know the end process is the colour. Infact the intensity of the colour of the beans is correlated to the final roasting temperature (illy 1998). In addition to the colour control executed at the end of roasting, there is moisture control that must not exceed 5%. Further additional analysis can be performed to verify the volume of coffee and

grain size (correct grind of coffee). Roasting time may take as long time or short time where the longer roasting periods produce bitter taste and not very aromatic kinds coffee, but permit the use of low quality beans. Instead shorter periods produce a coffee with an underdeveloped aroma because not all pyrolytic reactions can be completed and leads to coffee with underdeveloped organoleptic characteristics (Buffo & Cardelli-Freire, 2004). An example are the CGA where if the degradation is incomplete the cup tastes bitter and metallic teste (illy, 1998). Other roasting curve designed in function parameters time and temperature, there are others indicators that manage the process and coming keep under control. Observing the graph 1 is clear that the process is also drive from control the temperature smoke and principal emissions (particulate matter, volatile organic compounds, organic acids and combustion products CO and CO_2) in exit from drum.



Graph 1 – Example of roasting curve with different endothermic and exothermic phases (Source: www.bootcoffee.com/roastprofiling.html)

---- TEMPERATURE SMOKE

---- ROAST CURVE

---- SMOKE BURNER

---- BURNER

All these variables have to be continuing observed and monitored by roasting operator responsible of the process and overall to prevent fires in the roasting plant. Fig 5 show different components of a coffee roaster machine.

1 DRUM

2 SMOKE BURNER

3 HOPPER GREEN COFFEE

4 COOLING ROASTED COFFEE

5 DESTONING

6 HEAT ASPIRATION

7 WEIGHTING SYSTEM

Fig 5 – Roaster machine: Tecnology of coffee, tea and other beverages (Source: Antoniazzi, 2008)

Particulate matter emissions from the receiving, storage, cleaning, roasting, cooling, and stoning operations are typically ducted to cyclones before being emitted to the atmosphere. Gaseous emissions from roasting operations are typically ducted to a thermal oxidizer or thermal catalytic oxidizer following particulate matter removal by a cyclone. Some facilities use the burners that heat the roaster as thermal oxidizers. However, separate thermal oxidizers are more efficient because the desired operating temperature is typically between 650 °C and 816 °C, which is 93 °C to 260 °C more than the maximum temperature of most roasters. Some facilities use thermal catalytic oxidizers,

which require lower operating temperatures to achieve control efficiencies that are equivalent to standard thermal oxidizers. Catalysts are also used to improve the control efficiency of systems in which the roaster exhaust is ducted to the burners that heat the roaster. Emissions from spray dryers are typically controlled by a cyclone followed by a wet scrubber.

5.1.2 A few concept key of physical about Heat transfer

Fluidization of the beans is achieved by high velocity hot gas directed towards the beans, with good control of the process parameters and high uniformity of the products. High speed roasting with high hot air temperature led to different formation and elimination kinetics and in many cases to different concentrations of aroma compounds at roast color (Baggenstoss *et al*., 2008). The control the gas the heat and the flow are a point crucial and approximately 1000-1500 KJ Kg^{-1} of green coffee are necessary for heating from burner. This energy can be transmetted to the coffee beans by conduction (from contact with the hot metal surfaces), by radiation (from the heated surfaces), and by convection (laminar or turbolent flow). Exist a equation that explain the relathionship between the heat necessary to roast a given quantity of coffee, and the flow and temperature:

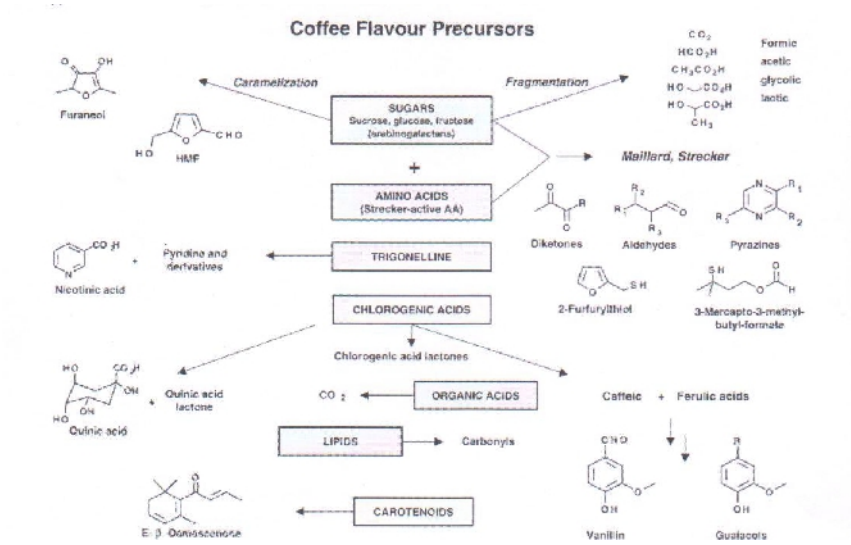
$$Q=CG \int [T_E-T_u(t)]\, dt-P$$

Where :

Q = energy (KJ)
G = gas mass flow (Kg s^{-1})
C = specific heat of the gas (KJ Kg^{-1} K^{-1})
Te = temperature of incoming gas (K)
Tu = temperature of outgoing gas (K)
T = roasting time (s)
P = heat loss from the roaster (KJ)

5.1.3 Maillard reaction

In coffee during roasting process take place two important reaction, Maillar and Strecker degradation and in following graph are reported the principal reaction.



A simplified scheme showing the main classes of volatile compounds formed from non-volatile precursors in the green beans during roasting (Source: Ribeiro, 2009)

Make a complete list of all the substances that make up the aroma of coffee is impossible, are reported only the main categories of these compounds and their mode of formation:

- Aldehydes and volatile acids are formed by pyrolysis of carbohydrates and caramelisation (Yeretzian *et al*., 2002).
- Proteins and free amino acids react with carbohydrates to give the products of the Maillard reaction. Amino acids are involved in the formation of flavor and color of coffee brew; both quantity and types of amino acids affect the intensity and quality of aroma and specific odor (tab 2).

AMINO ACID	100 °C	180 °C
Aspartic acid	CARAMELIZED SUGAR	CARAMEL
Threonine	CHOCOLATE	BURNT
Serine	MAPLE SYRUPE	
Glutamic acid	CARAMEL	BURNT SUGAR
Proline	BURNT PROTEIN	OVEN-BAKED
Glycine	CARAMEL	BURNT SUGAR
Alanine	CARAMEL	BURNT SUGAR
Valine	RYE BREAD	PENETRATING CHOCOLATY
Isoleucine	MOUDLY, TASTY AROMATIC	BURNT CHEESE
Leucine	CHOCOLATE, TOAST	BURNT CHEESE
Tyrosine	ROSE, PERFUME, CARAMEL	VIOLET, LILAC
Phenylalanine	VIOLET, ROSE, CARAMEL	VIOLET, LILAC
Lysine		BREAD
Histidine		CORNBREAD
Arginine	BUTTERY	BURNT SUGAR

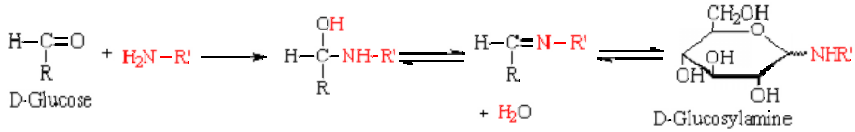
Tab 2: Aromas produced by heating amino acids together with glucose at various temperatures (Source: Carle & Montanari 1987).

- Chlorogenic acids degrade to phenols, quinic acid and quinic acid lactone
- The trigonelline is degraded via the Maillard reaction in pyridines, alchilpirazine and pyrroles
- Some terpene compounds, in particular several monoterpenes (linalool, limonene, geraniol, alpha-terpinolo) are the compounds responsible for some of the main positive notes of the aroma of coffee, particularly floral, citrus and fruity. These compounds are present in the bean in free form, is stored in the form glycosylated. These are released from the glycosidic component during the roasting process, while in the physiology of the seed will be the presence of specific glucosidase hydrolyzing this bond releasing the aromas (Mizutani *et al*., 2002).

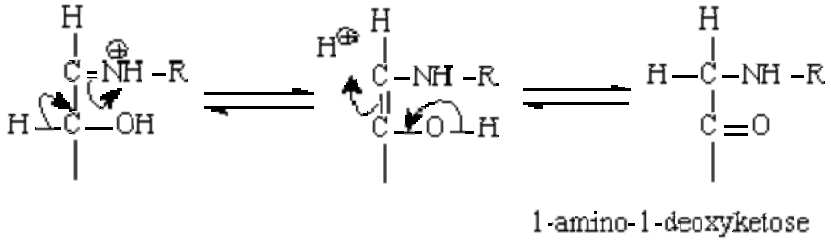
The aroma of roasted coffee is dependent on a quality and quantity aromatic precursors. Reichstein and Staudinger carried out the first exhaustive research in the years 1920-1930. In the first place this progress is due to the work of several authors

(Gianturco *et al*., 1963, 1964, 1966; Bondarovich *et al*., 1967; Goldman *et al*., 1967; Stoll *et al*., 1967; Friedel *et al*., 1971; Vitzthum & Werkhoff *et al*., 1974a,b, 1975, 1976, Tressl *et al*., 1978a,b, 1981; Tressl & Silwar 1981; and Silwar *et al*., 1987). Details of these studies have been reviewed by Dart and Nursten (1985), Flament (2002) as well as by Nijssen (1996). During Maillard reaction free amino acids, peptides and proteins with free amino groups, react with reducing sugars to form glycosylamines and/or aminoaldoses and/or aminoketones by condensation. Glycosylamines rapidly rearrange into aminoaldoses and/or aminoketones through Amadori. After reaction with other coffee constituents containing hydroxyl-groups, many volatile aroma constituents and coloured pigments are formed.

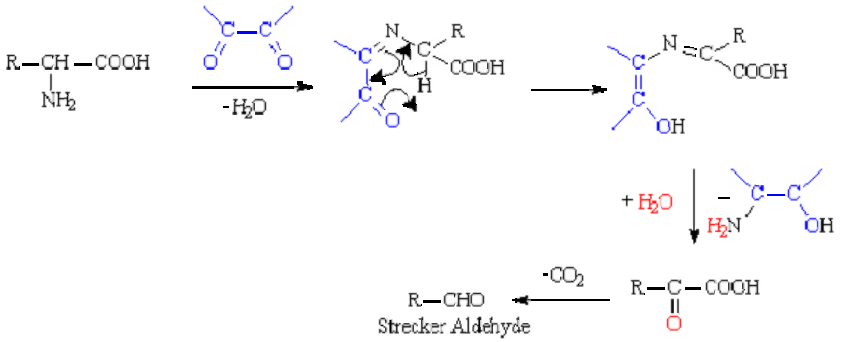
In detail
1) initial step: formation N glycoside



2) After formation of N glycoside the immonium ion is formed and then isomerize, this reaction is called Amadori rearrangement and forms a compound called ketosamine:

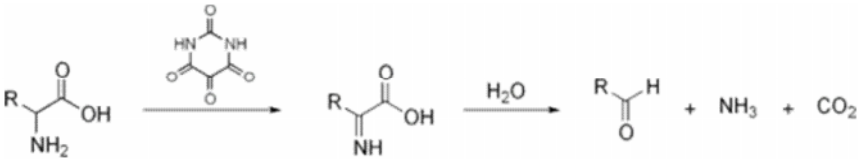


3) The ketosamine products then either dehydrates into reductones and dehydro reductones, which are caramel, or products short chain hydrolytic fission products such as diacetyl, acetol or pyruvaldehyde which then undergo the Strecker degradation.



5.1.4 Strecker degradation

The amino acids participate in the generation of flavour and colour via dehydration, fragmentation, cyclisation and polymerisation reactions (Hodge, 1953; Namiki & Hayashi, 1982). In detail it's a reaction of an amino acid with an α-dicarbonyl, with formation of an aminoketone. Subsequently, aminoketone molecules may condense to form pyrazines and other nitrogen heerocyclic compounds, or react with formaldehyde to form oxazoles. These two last compounds are the main classes present in coffee aroma.



Amino acid α-dicarbonyl aminoketone

5.2 Volatile compounds

As already seen in chapter 4 the quality coffee espresso in mainly related to the organoleptic characteristics, where aroma plays the most important role. The mechanism of formation saw in precedent paragraphs, about the volatile compounds in coffee, are extremely complex and the parameters that influencing the browning reaction and aroma compounds are: temperature roasting process, pH, moisture content in green coffee (different kinetics reactions) and the concentration relative precursor. There have been years of studies about aroma compounds. During the roasting process of the green coffee beans, the aroma compounds are formed by a number of complex pyrolitic reactions, while different degrees of roasting (light, medium, dark) produce various aroma profiles (Mondello *et al.*, 2005). Mayer and others (1999) investigated the influence of geographical coffee bean origin and the degree of roast on concentrations of aroma compounds in coffee Arabica. Schenker and others (2002) investigated the impact of roasting conditions on the formation of aroma compounds in coffee beans and the water content and temperature are the major factors which determine whether the potential of green coffee beans will lead to the formation of the distinct and desirable profile of aroma compounds. Is necessary to remember that the basic taste sensation of coffee is given by non-volatile compounds (caffeine, polysaccharides and chlorogenic acids), which determine bitterness, sourness and astringency.
The major mechanisms that including the formation of aroma compounds are:

- Maillard reaction reactions between proteins, amino acids, trigonelline, carbohydrates, hydroxyl-acids and phenols
- Strecker degradation
- Degradation of individual amino acids sulphur amino acids, hydroxyl amino acids
- Degradation of trigonelline
- Sugar degradation
- Degradation of phenolic acids

The importance of each of these reaction depends on the pool of reactive precursors available. More than 800 volatile compounds have been identified in coffee aroma (Clarke, 1986; Van Straaten *et al*., 1986) and the table 2 shows a compilation of the classes involved (illy, 1998).

CLASS	NUMBER OF COMPOUNDS
Acids	22
Alcohols	20
Aldehydes	29
Aliphatic nitrogen compounds	22
Aliphatic sulphur compounds	17
Anhydrides	3
Benzofurans	3
Esters	29
Ethers	2
Furans	112
Hydrocarbons	72
Ketones	68
Lactones	9
Oxazoles	28
Phenols	40
Pyrans	2
Pyrazines	81
Pyridines	15
Pyrroles	67
Thiazoles	26
Thiophenes	30

Table 2: Classes of volatile compounds identified in roasted coffee (Illy 1998).

Furthermore, coffee contains different chemical families, including sulphur compounds, pyrazines, pyridines, pyrroles, oxazoles, furans, carbonyl compounds, phenols (Buffo & Cardelli-Freire, 2004) and the degree of roast had also an impact on the concentrations of a series of important compounds of thermal origin such as ketones, furans, pyrazines and pyridines that increasing in line with the degree of roasting (Gonzales-Rios *et al*., 2007). Different compounds are also responsible of odour

notes. As shown in previous studies (Lopez-Galilea *et al*., 2006; Maeztu *et al*., 2001; Grosch *et al*., 2000; Semmelroch & Grosch 1995), only a relatively small group of components is responsible for the coffee aroma called key odorants. The principle classes of volatile compounds identified in roasted coffee are:

1) Sulphur compounds are among the most important aroma compounds in coffee. Methanethiol, dimethyltrisulphide, 3-mercapto-3-methylbutyl formate, and especially 2-furfurylthiol were cited as being impact compounds of coffee aroma (Blank *et al*., 1992; Czerny *et al*., 1999). May be formed by the natural metabolic pathways of the plant or in mostly the case, be produced during the roasting process. In detail the methanethiol is believed to result from the pyrolysis of methionine (Merritt *et al*., 1966) and it is likely that dimethyl sulfide and dimethyl trisulfide are further oxidation and disproportionation products of the same reaction sequence (Parliament *et al*., 1982). The degradation of cysteine, methionine during roasting in the presence of reducing sugars produce different compounds that are: furfurylmethylsulphide, difurfurylsulphide, and difurfuryldisulphide. The presence also of the 2-furfurylthiol, compounds developed in an unhindered way in Colombian and Kenyan coffees, and greatly increased with increasing degree of roast way, produce freshly roasted odor. This compound is formed by reactions of cysteine with arabinose (Baggenstoss *et al*., 2008). The thiophenes produce onion-like odor, and thiazoles produce grassy and roasted odor. In table 3 are reported different odorant Thiols compounds detected in Colombian Arabica (Grosch, 1999).

Odorant	Concentration (µg/kg)
2-Furfurylthiol	914
3-Methyl-2-buten-1-thiol	12
3-Mercapto-3-methylbutanal	800
3-Mercapto-3-methylbutyl formate	13
Methanethiol	1310

Table 3: Bounds thiols in medium roasted Colombian arabica coffee *Source:* Grosch (1999)

As discussed in the section on the evaluation of key odorants, 2-furylthiol (FFT) is the outstanding odorant of the sulphur-containing fraction of roasted coffee. Model experiments performed under controlled roasting conditions revealed that pentoses were effective and significantly more effective than hexoses as precursors of FFT (Parliament & Stahl, 1995; Grosch, 1999). Further model experiments confirmed that free and peptide-bound cysteine as well as arabinose, occurring as building blocks of polysaccharides (Bradbury & Halliday, 1990), are the most active precursors of FFT (Grosch, 1999). 3-methyl-2-buten-1-thiol and 3-mercapto-3-methylbutyl formate (MMBF) are potent odorants due to their very low odour thresholds (Holscher *et al*., 1992). Phenyl alcohol, of which about 0,5 mg/kg occurs in raw coffee, has been proposed as a precursor of the two thiols (Holscher *et al*., 1992). Hydrogen sulphide liberated from free and bound cysteine may substitute the hydroxy group of the phenyl alcohol to form 3-methyl-2-buten-1-thiol. On the other hand hydrogen sulphide may react with the double bond of the phenyl alcohol yielding 3-mercapto-3-methylbutanol.

2) Pyrazines are present in coffee at level exceeding their threshold value and have a high odour value (Clifford, 1975). Many pyrazines are recognized as the volatiles contributing to roasted aromas of cooked foods (Shimoda *et al*., 1990). There are 81 pyrazines already identified in coffee aroma, whose concentrations are variable depending on the time and temperature of the thermal treatment (Dart *et al*., 1985). The pyrazines are derived particularly from Maillard reaction and responsible of bitter-sweet note, the alkylpyrazines responsible nutty odour and methoxypyrazines present in nature are responsible of peasy note (Flament, 2002). Among the most aroma-active alkylpyrazines, 2-ethyl-3,5 dimethylpyrazine (EDMP) and 2,3-diethyl-5- methylpyrazine (DEMP) occur in high-concentrations in coffee and other roasted foods (Grosch, 1998b). The formation of the two last pyrazines has been studied at pH 5.6 by heating mixtures of sugars and amino acids at 180 °C (Cerny & Grosch, 1994). Hashim (1996) published that the alkyplyrazines are generally associated with heated food flavours

and the ratio between 2-methyl-2,5-methylpyrazine and 2-methyl-2,6-methylpyrazine are of high interest in controlling roasted coffee beans because increasing with temperature of roasting. Clarke and Macrae (1985) reported that the nutty roasted aroma could be responsible for the caramel ones. The correlation of the methylpyrazines quantity with the sensory analysis of the beans has shown the possibility of monitoring the roasting process of coffee beans (Hashim & Chaveron, 1996).
3) Pyridines derived pyrolysis products of model reactions involving amino acids and sugars and are responsible of green, bitter, roasty, astringent (2-methylpyridine), ethylpyrazines (buttery, caramel) notes (Flament, 2002).
4) Pyrroles present N whose concentration increases with roasting degree, (furfurylpyrrole) smells, mushrooms, (acylpyrroles) cereal smells. There a few important compounds: pyrroles, 2-acetylpyrrole, 2-acetyl-1-methylpyrrole, 2-acetyl-1-ethylpyrrole, 1-furfurylpyrrole and 2-propionylpyrrole. Pyrroles are primarily formed thermally, with other formation pathways existing and they are not present in fresh, raw coffee (Flament, 2002).
5) Oxazoles present green sweet, earthy and vegetable-like odor, nutty, sweet, green, herbal and vegetable-like notes (Flament, 2002).
6) Thiazoles are eteroatomi N and S are responsible of meat note, potatoes, peanuts and nutty. Different compounds are 2-isobutylthiazole, 4-methyl-5-thiazole ethanol benzothiazole, 2,4-dimethyl-5-acetylthiazole, 4-methyl-vinylthiazole, 2-acethyltiazole, 2-ethoxytiazole, 2-sec-buthylthiazole and 4-methyl-5-vinylthiazole (Tateo and Bononi, 1999).
7) Furans formed pyrolityc degradation of sugars present in green coffee and have caramel-like or burnt sugar aromas (Leino *et al*., 1991). They also result from thermal oxidation of lipids, from degradation of thiamine and terpenic precursors already present in the green beans (Flament, 2002). Heating of hexoses or hexose-phosphates directly produces 4-hydroxy-2,5-dimethyl-3(2H)-furanone (HDMF) (Schieberle, 1992). HDMF and 2(5)-ethyl-4-hydroxy-5(2)-methyl-3(2H)-furanone (EHMF) are also generated from pentose sugars in the presence of glycine and

alanine, respectively (Blank & Fay *et al*., 1996; Blank *et al*., 1997). The furans in base concentration might to gives different odours. For example the furfuryl-mercaptano (detection 0,005 ppb) gives aroma toasted fresch instead present at concentration 0,01 ppb is responsible sulphureous notes. Others important compounds are 2,5-dimethyl-4-hydroxy-3(2H)-furanone (HDMF, known as furaneol), intense maltol note (Tateo and Bononi 1999) and caramel-like aroma (Buttery, Takeoka, Krammer, & Ling, 1994; Rapp, Knipser, Engel, Ullemeyer, & Heimann, 1980; Rodin Himel Silverstein, Leeper, & Gortner, 1965; Steinhaus & Schieberle, 2007; Tei & Yamanaishi, 1974; Tressl, Bahri, KÖppler, & Jensen, 1978). At low pH, HDMF has been shown to react with cysteine or hydrogen sulphide in generating meat-like aroma compounds (Shu & Ho, 1998; Zheng, Brown, Ledig, Mussinana, & Ho, 1997). Other the HDMF there is the 2(5)-ethyl-4-hydroxy-5(2)-methyl-2-(2H)-furanone (EHMF). The furans are found to be the most predominant group of compounds amongst the coffee aromatics in particular the furfural and 2-acetylfuran (sweet balsamic-cinnamic, fruits and flowers note (Adams, 1995, Buttery, Stern & Ling, 1994; Nishimura, Yamaguchi, Mihara, & Shibamoto, 1989, Yamaguchi & Shibamoto, 1979). Furfural is a aldehydes precursor of 2-furfurylthiol, a key roast aroma in coffee (Cerny & Davidek, 2004).

8) Carbonyl compounds are aldehydes and ketones. Many aldehydes are important key odorants of coffee in particular acetaldehydes and propanal have been reported to have fruity notes (Semmelroch *et al*., 1995). The Strecker derivate 3-methylbutanal has been reported by Blank (1996) be the key odorant of roasted Arabica ground coffee and to have malty notes. Semmelroch and Grosch (1995) also reported the Strecker aldehydes 2-methylpropanal, 2-methylbutanal and 3-methylbutanal to have malty and fruity notes. The aldehydes are formed partially by Strecker degradation of amino acids. Aldehydes are formed also by the oxidative degradation of amino acids during their interaction with sugars at high temperatures and during the interaction of amino acids and polyphenols in the presence of polyphenol oxidase at normal temperature (Motoda,

1979). Many cyclic ketones are associated with food aromas and may be formed in coffee from carbohydrates in particular glucose and fructose (Flament, 2002). The sucrose come destroyed by roasting and it's a precursor formation of diketones (Feldman *et al*., 1969). The 5-methylfurfural is responsible caramel note, furfuryl formate and 3-methyl-2-cyclopenten-1-one are responsible floral note (Gonzales-Rios *et al*., 2007). The ketones for example 2,3-butanedione and 2,3-pentanedione are responsible of buttery odour in agreement with the results reported by Holscher and Steinhart (1992). They developed to a maximum concentration for a medium degree of roast and exhibited lower concentrations in dark-roasted coffee beans (Mayer *et al*., 1999). The hydroxy-2-propanone is been identified how a possible marker substances to monitor the course of roasting (Baggenstoss *et al*., 2008).
9) Phenols they are often qualified as having a somewhat negative character as tarry, smoky, woody, spicy, leathery or medicinal. Nevertheless most of them, at low concentrations, are described as having a sweeter, warm, floral, balsamic with pleasant vanilla, clove-like aromas with bitter astringent and medicinal notes (Flament, 2002). Model experiments indicated that 4-feruloyl quinic acid is the precursor of guaiacol, 4-ethyl and 4-vinylguaiacol (Tressl, 1989). The guaiacol compounds increased with increasing degree of roast way.
10) Esters are essential contribution in fruit flavors (Flament, 2002). The quantities of esters, which the compounds present in green coffee or which are produced during post-harvest processing, decreased as the degree of roasting increased (Gonzales *et al*., 2007)

Quantitative and qualitative analyses of aroma compounds in coffee aroma are difficult, because they are mainly found at trace levels and are volatile (Costa Freitas & Mosca, 1999). However gas chromatography coupled with mass spectrometry offers a sensitive analytical method that is commonly used for the analysis of coffee aroma. GC and MS complement each other the former is well known as an excellent tool for the separation of

different components in a wide range of sample types and the latter is an established technique for the identification of unknown analytes (Grob & Barry, 2004). For the GC-MS analysis of coffee aroma, the static headspace technique is predominately used for sample preparation (Bicchi, Bello & Cid, 2001). It is the most suitable method for studying volatiles because the prepared sample is near to a realistic representation of the coffee aroma perceived by the consumer (Mondello *et al*., 2005). Most of the studies analyzing the aroma of conventional ground coffee and coffee brew were done in order to compare the effects of different sample preparation methods (Bicchi *et al*., 2002; Sanz *et al*., 2001). Other investigations have been focused on the aroma compounds of espresso coffee from different botanical varieties (Maeztu *et al*., 2001) and on the aroma differences in the brew caused by the preparation with a filter coffee maker and an espresso machine, respectively (Lopez-Galilea *et al*., 2006). The impact of time-temperature (HTST, LTLT) combinations of roasting processes on the kinetic of aroma formation in coffee was been investigated. Compared to low temperature-long time roasting, high temperature-short time roasting resulted in considerable differences in the physical properties and kinetics of aroma formation. Excessive roasting generally led to decreasing or stable amounts of volatile substances (Baggenstoss *et al*., 2008).

5.3 Main goals of the research

The aim of this work was to identify the aroma compounds formed during roasting process which could distinguish different six coffee origins (Ethiopia, Kenya, Honduras, Nicaragua, Guatemala and Brazil). In the present study, the same developed SPME method seen in chapter 2 relative characterization green coffees was applied to coffees samples roasted. Another objective of the present study was to investigate the evolution of aroma compounds during roasting light, medium and dark with different time conditions (no temperature) for every coffee. An

other objective is to find possible marker substances to monitor the course of roasting and to identify which are compounds responsible of odours for single coffee through GC-O technique. Furthermore in order to find possible markers of quality will be search possible correlation with sensorial data and analytical data. Study of the characteristic coffee aroma depending on much variables could help food chemist and flavorist to create new and more desirable blend coffee flavours for processed foods and beverages.

5.4 Material and methods

The analysis have been performed in the same samples used for the sensory analysis in espresso coffee seen in chapter 4. For the analysis of aromatic fraction and identification of compounds responsible of odors were used the same conditions seen in chapter 2 relative the analysis fraction aromatic of green coffee grinded. The only modify was the split ratio. In green coffee grinder the analysis in GC-MS and GC-O was made in splitless, instead in roasted coffee was used split ratio 1 to 5. The choice of this ratio was subject to the fact that the coffee roasted was very rich of compounds aroma and with injection in splitless mode give problems of saturation of the detector.

5.5 Results and discussion

The SPME analysis connected with GC-MS and GC-O technique was used for the identification and quantification of the volatile compounds of six roasted coffee samples reported in table 4. Besides the compounds were divided for classes. In qualitative analysis, of important volatile compounds were identified by the comparison with mass spectra in the NIST mass spectra library and selected by GC-O tecnique.

Table 4. The main compounds and odour/flavour descriptors identified in samples roasted coffee as a result of the SPME/GC-MS and GC-O.

Compound Name	I	I Ref. [a]	Odour/Flavour detection by SPME-GC-O analysis	Odour description (literature) [a]	Group of compounds
Acetaldehyde	809	690			ALDEHYDES
Carbon disulfide 76(100); 38(20); 44(10)	817	n.d			SULPHUR COMPOUNDS
Dimethyl sulfide	823	n.d			SULPHUR COMPOUNDS
Methyl formate 60(100); 54(45); 43(32)	829	n.d			ESTERS
Propanal	842	n.d			ALDEHYDES
3-Methyl-2,5-furandione	844	n.d			FURANS
2-Methyl-propanal	852	n.d			ALDEHYDES
2-Propanone	854	n.d			KETONES
Methyl acetate	862	827		Pleasant [b]	ESTERS
2-Methyl-furan	887	876		Unpleasant [b]	FURANS
Glutaraldehyde 44(100); 40(70); 53(30); 82(30)	906	n.d			ALDEHYDES
2-Butanone	910	909			KETONES
2-Methyl-butanale **	919	913	Sweet	Sweet [b]	ALDEHYDES
3-Methyl-butanal	923	916		Sweet [b]	ALDEHYDES
2,5-Dimethylfuran	955	953		Coffee [b]	FURANS
2-Methyl-2-cyclopenten-1-one	970	n.d			KETONES
2-Pentanone 43(100); 57(20); 86(15); 71(10); 41(10)	979	n.d			KETONES
2,3-Butanedione **	984	979	Vanilla	Buttery, creamy [h]	KETONES
2-Butanolo	1015	1027			ALCOHOLS
Toluene	1041	1043		Unpleasant [b]	BENZENIC COMPOUNDS
3-Hexanone	1055	1053			KETONES
2,3,5-Trimethyl-furan	1060	1056			FURANS
2,3-Pentanedione **	1068	1062	Buttery	Buttery [b]	KETONES
3-Methylpyridazine	1079	n.d			PYRAZINES
Hexanal	1084	n.d			ALDEHYDES
2-Methyl-thiophene	1090	n.d			THIPHENES
2-Methyl-2-butenal	1094	1102			ALDEHYDES
2-Methyl-1,6-heptadiene 41(100); 69(60); 39(20); 25(10);	1111	n.d			ALKENES
3-Penten-2-one	1127	1138			KETONES
2,4-Dimethyl-3-pentanone	1139	1137		Pleasant [b]	KETONES
1-Methyl-1*H*-pyrrole	1141	1139		Coffee [b]	PYRROLES
2,3-Hexandione	1148	n.d			KETONES
1-Methylpyrrole	1157	n.d			PYRROLES
Myrcene	1167	n.d			TERPENES
Pyridine	1180	1198		Burnt [b]	PYRIDINES
Limonene	1190	1198			TERPENES
Pyrazine	1209	1212		Coffee [b]	PYRAZINES
2-Pentyl-furan	1233	1229			FURANS
Furfuryl methyl ether **	1243	1243	Soya bean/potato		FURANS
3-Methyl-3-buten-1-ol	1253	1258		Unpleasant [b]	ALCOHOLS
Tetrahydro-3-methylfuran	1259	n.d			FURANS
2-Methylpyrazine	1265	1264			PYRAZINES
1,3-Thiazole	1276	1270		Toasted [b]	THIAZOLES
2-Methyltetrahydrofuran-3-one	1280	1283		Nutty [b]	FURANS

3-Hydroxy-2-butanone	1283	1289		Buttery [b]	KETONES
3-Methylpyridine	1289	1323			PYRIDINES
1-Hydroxy-2-propanone	1297	1300		Nutty [b]	KETONES
2,5-Dimethylpyrazine **	1320	1322	Little nut/sweet	Toasted [b]	PYRAZINES
2,6-Dimethylpyrazine **	1327	1334	Little nut/toasted	Nutty [b]	PYRAZINES
2-Ethylpyrazine	1332	1338		Toasted [b]	PYRAZINES
3-Hidroxy-2-pentanone	1340	1366			KETONES
2,3-Dimethyl-pyrazine	1346	1345		Toasted [b]	PYRAZINES
2-Cyclopenten-1-one	1354	1385			KETONES
4-Methyl-2-pentanol 45(100); 41(30); 57(20); 69(15)	1358	n.d			ALCOHOLS
2-Methyl-2-cyclopentenone	1365	1366		Toasted [b]	KETONES
1-Hydroxy-2-butanone	1373	1375		Toasted [b]	KETONES
3-Ethyl-pyridine	1378	1387		Toasted [b]	PYRIDINES
2-Ethyl-6-methyl-pyrazine **	1383	1391	Caramel/sweet	Toasted [b]	PYRAZINES
2-Ethyl-3-methyl-pyrazine **	1388	1406	Butter/sweet/vanilla	Nutty [b]	PYRAZINES
2-Ethyl-5-methyl-pyrazine	1401	1411		Toasted [b]	PYRAZINES
Maleic anhydride	n.d	n.d	Unpleasant		ALDEHYDES
2-Vinyl-5-methylpyrazine	1463	1433		Toasted [b]	PYRAZINES
2,6-Diethylpyrazine	1445	1433		Toasted [b]	PYRAZINES
Acetic acid	1450	1440		Sour	ACIDS
Furfurale	1466	1461		Almond/bitter [b]	ALDEHYDES
1-Acetoxy-2-propanone **	1473	1470	Roast/woody		KETONES
2-Furfuryl methyl sulfide **	1484	1481	Roasted	Coffee [b]	FURANS
Furfuryl formate	1499	1504		Floral [b]	FURANS
2-Acetylfuran	1503	1498		Spicy [b]	FURANS
Formic acid	1509	1501		Unpleasant [b]	ACIDS
Pyrrole **	1514	1518	Vanilla/sweet	Toasted [b]	PYRROLES
1-(2-Furyl)-2-propanone	1521	1548		Pleasant [b]	FURANS
1-(Acetyloxy)-2-butanone	1535	1539			KETONES
Propanoic acid	1539	1536			ACIDS
Furfuryl acetate	1541	1539		Nutty [b]	FURANS
Linalool	1553	n.d			TERPENES
1*H*-Methylpyrrole	1561	1542			PIRROLES
5-Methylfurfural **	1574	1566	Vanilla/sweet/roasted	Caramel [b]	FURANS
4-Cyclopentene-1,3-dione	1584	1573			KETONES
2,2'-Bifuran 134(100); 78(95); 105(35); 135(10)	1593	n.d			FURANS
Furfuryl propionate	1599	1599			FURANS
2-Formyl-1-methylpyrrole	1616	1607		Buttery [b]	PIRROLES
γ-Butyrolactone **	1623	1609	Caramel/butter	Sweet [b]	LACTONES
Butanoic acid	1629	1634			ACIDS
2-Acetylpyridine	1634	1644		Toasted [b]	PYRIDINES
3-Methyl-3-Hexen-2-one 41(100); 43(80); 69(40); 112(25) **	1645	n.d	Roasted		KETONES
2-Acetyl-1-methylpyrrole	1652	1645		Coffee [b]	PYRROLES
Furfuryl alcohol **	1664	1668	Little nut toasted	Burnt [b]	FURANS
Isovaleric acid	1670	1680			ACIDS
3-Ethyl-2-hydroxy-2-cyclopenten-1-one	1676	n.d			KETONES
2,3-Dimethyl-3-pyrazolin-5-one	n.d	n.d	Unpleasant		KETONES
2-Formylthiophene	1690	1679			THIOPHENES
Pyrazinamide	1719	1733			PIRAZINES
2-Hexene	1749	1763			ALKENES
2-Methyl-cyclopentanone	1759	1795			KETONES

3-Hydroxypyridine monoacetate	1775	n.d			PYRIDINES
3-Methyl-2-butenoic acid **	1794	1802	Little nut/butter/spicy		ACIDS
Hexyl ester-acetic acid 56(30); 61(20); 69(20); 84(15); 73(15)	1802	n.d			ESTERS
2-Hydroxy-3-methyl-2-cyclopenten-1-one	1825	1829			KETONES
2,3-Dihydro-5-hydroxy-6-methyl-4*(H)*-pyran-4-one	1853	1848			KETONES
2-Methoxy-phenol	1856	1859		Burnt [b]	PHENOLS
3-Octyne-2-one	1864	1851			KETONES
3-Ethyl-2-hydroxy-2-cyclopenten-1-one **	1889	1894	Caramel/sweet		KETONES
2,4-Hexanedione 57(45); 85(30); 114(25); 99(5)	1939	n.d			KETONES
2-Ethyl-hexanoic acid	1949	1959			ACIDS
3-Hydroxy-2-methyl-4*H*-Pyran-4-one	1958	1959			FURANS
2-Acetylpyrrole	1966	1969		Unpleasant [b]	PYRROLES
Difurfuryl ether	1981	1977		Unpleasant [b]	FURANS
3,4-dimethyl-2,5 furandione	1997	2007			FURANS
2-Formylpyrrole	2016	2013		Unpleasant [b]	
4-Ethylguaiacol	2025	2023		Spicy [b]	PHENOLIC
Furaneol	2029	2131	Honey/caramel/sweet		FURANS
2-Nitro-hexane	2058	n.d			
Valeric aldehyde	2077	2187			ALDEHYDES
4-Vinylguaiacol	2189	2187		Spicy [b]	PHENOLIC
2-Metossi-4-acetato vinylphenyl	2217	2199			PHENOLIC
2,3-Dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one	2258	2238			FURANS
2-Furancarboxaldehyde-5-(hydroxymethyl)	2385	2343			FURANS

I: Kovats' retention index. [a] Reported by different authors: [b] Gonzales-Rios (2007); [c] A.T.Toci (2008), [d] C.Sanz (2002); [e] G. Budriyn (2011); [h] B. Zellner (2008); [f] reported in http://www.nysaes.cornell.edu/flavornet/chem.html. [g] Isomers according to Holscher (1990), Cantergiani (2001), Sanz (2001); * Odor compound detected in the headspace by GC-O of a few roasted coffee samples; ** Odor compound detected in the headspace by GC-O of the roasted coffee in all the roasted samples. N.d not detected.

The formation of many aroma compounds is justified thanks complete transformation of the raw coffee beans. The following fig 6-7 represent gas-chromatographic analysis on the volatile components (thus potentially responsible for a contribution to the aroma of the product) before a green coffee and then the same coffee after roasting. In green coffee can be seen a few peaks and in low concentration instead in second chromatogram on the roasted coffee, there are many peaks. The roasting process creates a product complex than the product initial.

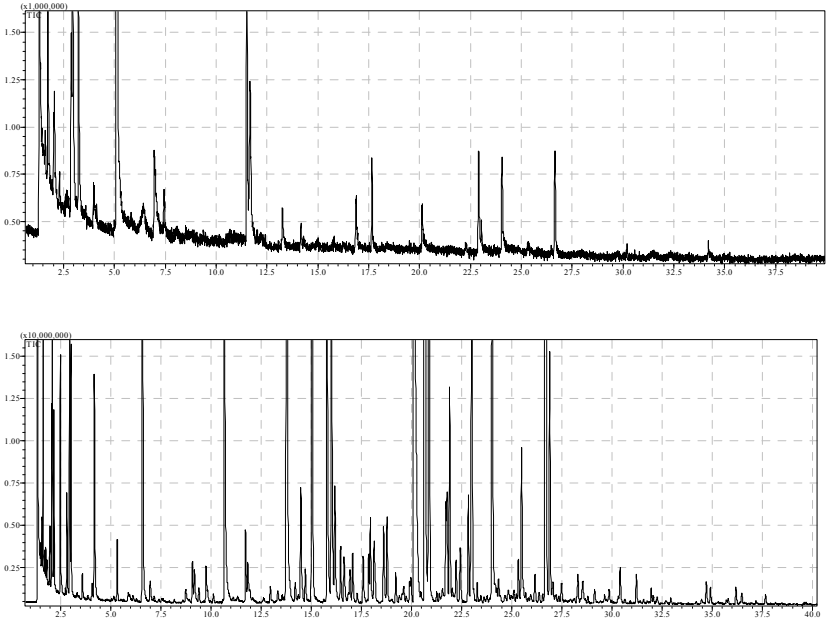
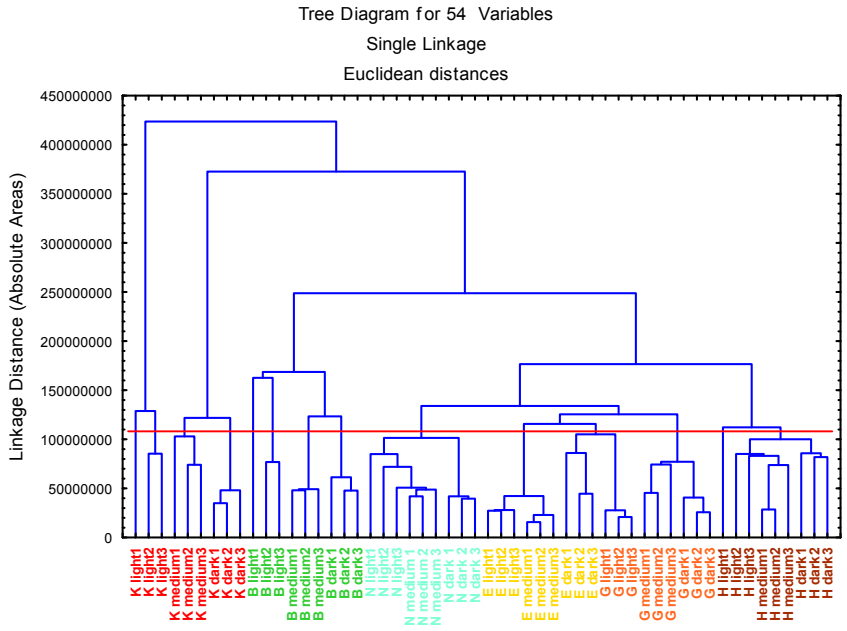


Fig 6-7 – Comparison between aroma compounds in green coffee Guatemala (above) and roasted coffee light Guatemala (below) evaluation obtained by SPME/GC-MS



Graph 2 results of Cluster analysis carried out on the absolute areas in detected by SPME - GC-MS analysis of roasted coffee samples with replicates of treatment (E-Ethiopia, K-Kenya, G-Guatemala, H-Honduras, B-Brazil, N-Nicaragua).

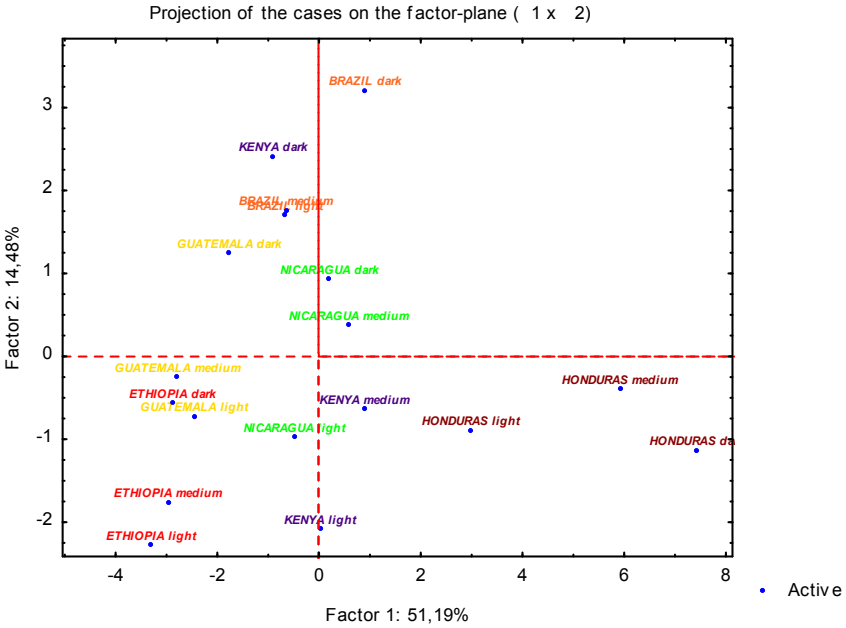
On the basis of what has been said, considering a comparison of green coffee (chapter 2) and roasted coffee volatile fraction, can declare that the roasted coffee is affected a fraction total volatile changed respect in green coffee volatile fraction. In this work were not identified specific compounds already present at the origin. In according with Kwang-Geun Lee (2002), the heterocyclic compounds, which provide characteristic flavour to coffee, their presence in green coffee beans was quiete different from those of roasted coffee beans. However, these heterocyclic compounds were not found in the samples from green coffee beans, except for 2-isobutyl-3-methoxy-pyrazine. Graph 2 showed the dendrograms and the cluster analysis was able to

distinguish all the groups of roasted coffee. Looking at the graph 2 it is easy to see that all coffees showed differences in relation treatment roasting process light, medium and dark. It is interesting to observe that the aromatic fraction in Kenya coffee, presented a marked difference with roasting light respect to the other groups of roasted coffees; probably this phenomena is related at the ratio of specific aromatic precursors that develop different aroma compounds. Red line designed separate the graph 2 in two subgroups important, and its explained the origin effect and treatment light, medium and dark effect. Its possible deduce that origin effect is able to characterize better the single coffee origin than treatment roasting effect. Franca (2008), and others investigated the preliminary evaluation of the effect of processing temperature on coffee roasting degree assessment confirming the significant effect of roasting on the coffee volatile profiles. The PCA statistical analysis was performed to indicate similarities or differences within the complex composition, to establish the relation between varieties and particular volatile compounds. Standard multivariate statistical methods, like principal component analysis (PCA), are widely used to analyze results of chemical measurements. The main objective of PCA is to reduce high-dimensional data sets by preserving as much as possible of the variation, contained in the data and the results of PCA, both loadings and scores, can be advantageously visualized using biplot with the corresponding interpretation. As it is known, coffee aroma is composed of an extensive group of volatile compounds. Chemical composition of individual coffee products, accompanying coffee aroma, depends on several factors including species of green coffee beans, geographical origin, processing conditions, especially roasting, packaging and stocking of products. Applying standard PCA to the data matrix consisting of the relative amounts of the all aroma compounds the first two principal components were extracted (graphs 3-4). The total variance of initial data explain for 65,67%, where the first principal component factor 1 accounts 51,19 % and the second principal component accounts 14,48%, see graph 3. The scores illustrate showed a moderate resolution of groups of

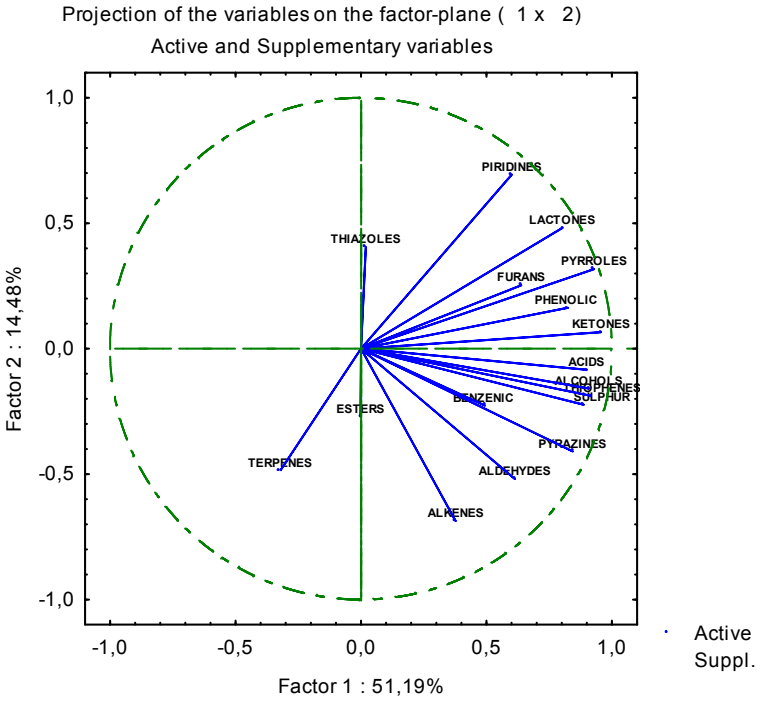
coffee distinct for origin and treatment. It is well known that the reliability of the result is supported by high proportion of explained variability expressed by the Factor 1 and Factor 2 in this case exceeds of 50%. In details the factor 1 has a greater weight (51,19%) and by focusing attention on this factor, its possible to observe the coffee Honduras more separated for origin and treatment than other coffees. The fraction volatiles of Honduras coffee is higher than other origins. In consideration Factor 2 the Brazil, Guatemala, and Ethiopia coffee showed the same behavior in relation degree roasting, where the coffees with treatment light and medium are resulted more close than treatment dark roasted. Kenya coffee showed a clear separation for treatment light, medium and dark and Nicaragua medium and dark are resulted more close.

The distribution of the variables represented in graph Biplot (graph 4), displays different projections of aroma compounds classes for a single type of roasted coffee. Looking at graphs 3 and 4 it can be said that most of the content of aroma compounds is present in Honduras coffee areas. In the other coffees, instead, the content in terms of quality and quantity of the aromatic fraction is present in low concentrations, except for classes terpenes compounds that characterize Ethiopia coffee, prevalently. For all the samples was executed Levene test for to verify the homogeneity of variance and then variance analysis for to understand which classes of compounds characterize the single coffees and over all to simplify a comparison given the large number of aroma compounds. Inside every group of compounds was investigate by the ANOVA test. As can be seen in the following graphs 6 to 50 the trend of classes compounds of major interest are represented by acids, alcohols, aldehydes, alkenes, benezenic compounds, esters, furans, ketones, lactones, phenolic compounds, pyrazines, pyridines, pyrroles, sulphur compounds, terpenes, thiophenes and thiazoles. The main compounds, in terms of the number of compounds and their quantity, were the furans, the ketones and the pyrazines, which partly came from the caramelization of sugars in the case of furans, and from the Maillard reaction between sugars and amino

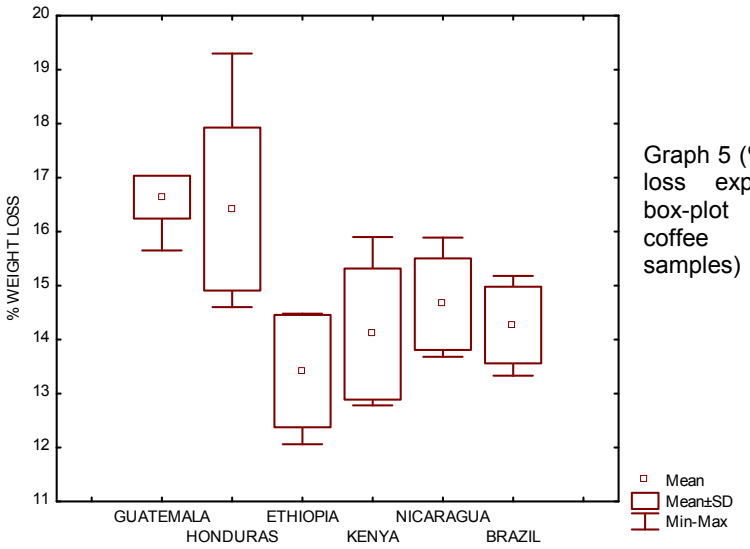
acids for ketones and pyrazines (Knoch and Baltes, 1992; Baltes and Knoch, 1993). Light roasting gave the lowest concentrations of volatile compounds. Medium and dark roasting gave very similar total extracted quantities. All the classes aromatic compounds detected by analysis showed different trend for origin and treatment This phenomena is always correlated at content different aromatic precursor and weight loss. Franca (2009) showed during roasting process a significant increase in the weight loss rate and that can be attributed to an intensive release of organic compounds, carbon dioxide, and water resultant from pyrolysis and other roasting reactions. This behaviour has been reported by previous studies on coffee roasting (Sivez *et al*., 1979; Dutra *et al*., 2001; Oliveira *et al*., 2005). Graph 5 showed the high % weight loss for Honduras coffee and lightly inferior level for Guatemala coffee. The higher concentration aroma compounds detected in Honduras coffee might be probably explained by higher % weight loss. The variation in weight loss behaviour is a indication that the roasting temperature is affecting the volatiles profile by flavoring different reactions and product removal pathways at different processing conditions (Franca *et al*., 2009).



Graph 3: Results of PCA analysis carried out on the absolute areas in detected by SPME-GC-MS analysis of roasted coffee samples.

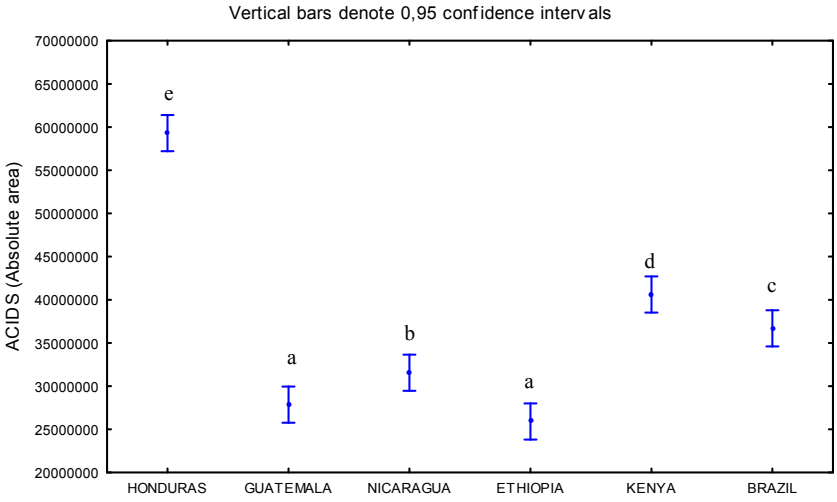


Graph 4: PCA Projection of all variables on the factor-plane relative to classes aroma compounds in roasted coffee samples of different origin.



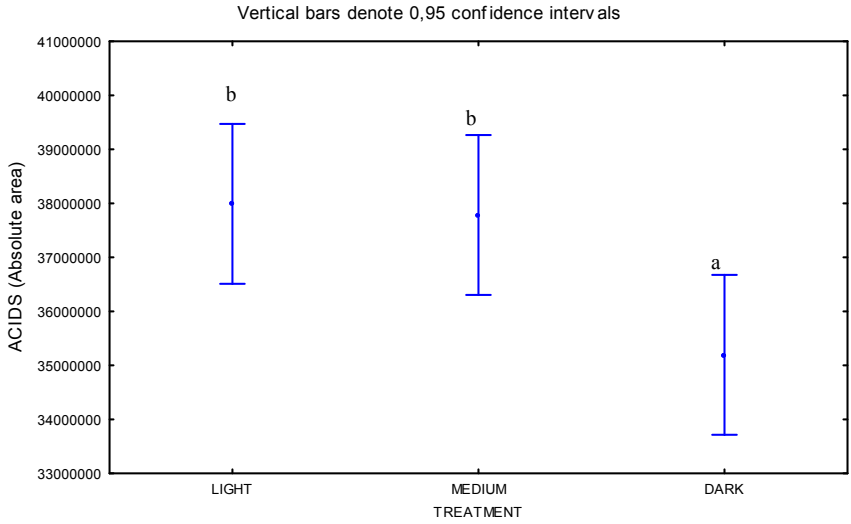
Graph 5 (% weight loss explain by box-plot in the coffee roasted samples)

The objective of the following graphs was to investigate the formation of aroma impact compounds during roasting as influenced by different effects of origin, treatment and possible interactions between effects take in consideration. Most of classes of compounds were affected by a treatment effect, origin effect and interaction between treatment and origin how reported in literature.



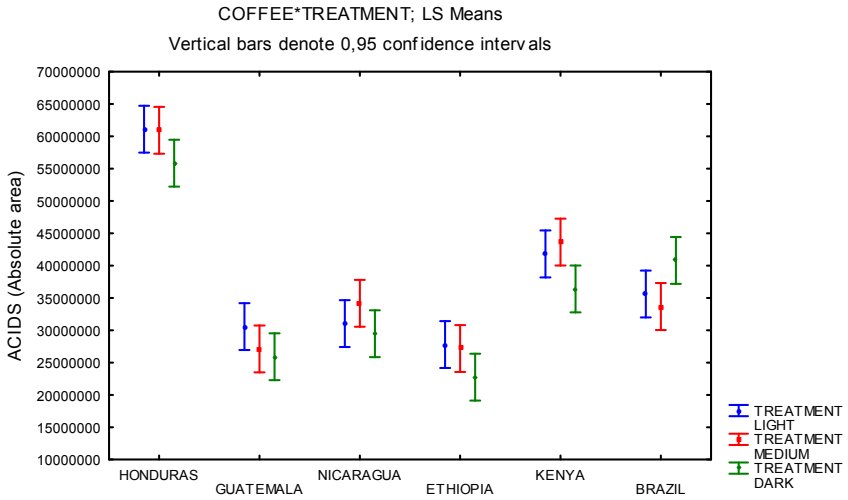
Graph 6 - Confidence of interval, results of ANOVA and LSD test detected by SPME-GC-MS technique; The different letter mark significant difference for $p < 0,05$.

As shown in this graph those coffee samples with Honduras origins shows the highest level of acids compounds while the rest of the samples demonstrated a significant lower degree, among them and as a second place we can find the Kenya samples.



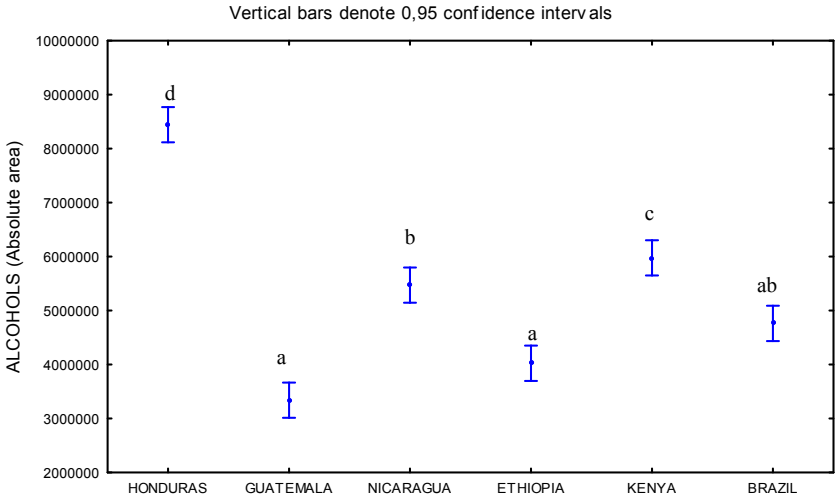
Graph 7 - Confidence of interval, results of ANOVA and LSD test detected by SPME-GC-MS technique. The different letter mark significant difference for $p < 0,05$.

The effects of the temperature showed a slight decrease negative in medium roast, but a marked decline for the dark roast.



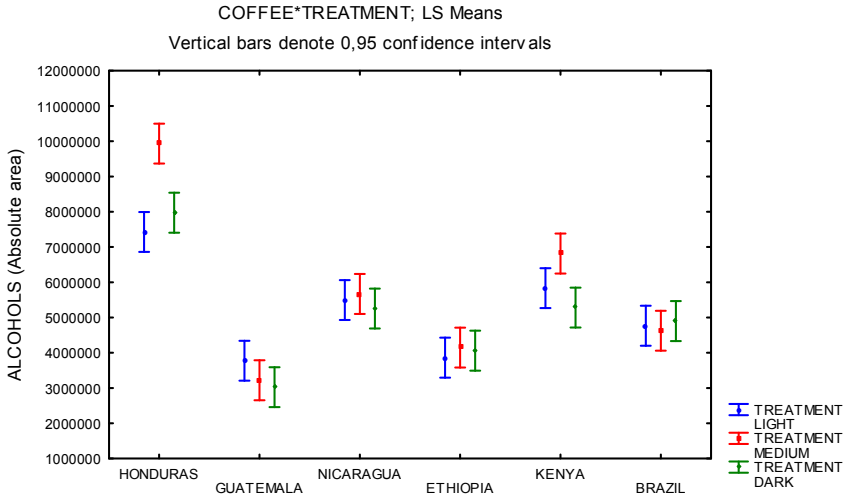
Graph 8 - Confidence of interval, results of ANOVA and LSD test detected by SPME-GC-MS technique.

The acids (graph 8) content were very variables between single origins with a maximum for Honduras coffee and a minimum in Ethiopia and Guatemala coffee. The termic treatment reduces the amount of acids with a trend negative for all coffees, instead opposite situation for Brazil coffee. Almost all acid compounds identified in this kind of samples have shown a relevant difference (ANOVA test) among them taking in consideration the coffee bean origin and the kind of treatment. The 3-methyl-2-butenoic acid, acetic acid, butanoic acid, butanoic acid-2-propenyl ester, formic acid, 2-ethyl-hexanoic acid, isovaleric acid and propanoic acid are the compounds that showed relevant differences about origin effect and the treatment. In details the greater concentrations for 3-methyl-2-butenoic acid is resulted in coffee of origin central America, the acetic acid in Honduras and Guatemala coffee, butanoic acid in Honduras and Nicaragua coffee, 2-propenyl ester-2-butanoic acid in Kenya and Guatemala, formic acid in Brazil and Nicaragua, isovaleric acid in Honduras and Nicaragua and propanoic acid in Honduras coffee.



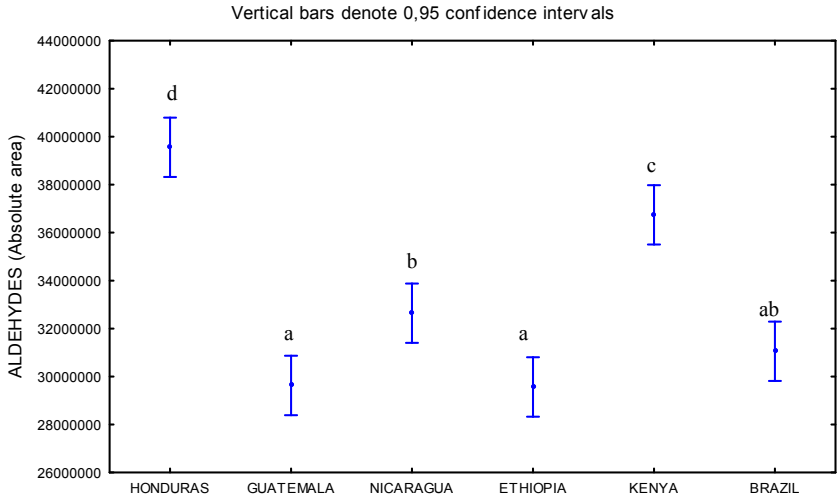
Graph 9 - Confidence of interval, results of ANOVA and LSD test detected by SPME-GC-MS technique. The different letter mark significant difference for $p < 0,05$.

The alcohol compounds are presents in roasted coffee and take place during roasting process. In graph 9 the alcohols compounds are in high concentration in Honduras coffee and in low concentrations in Guatemala coffee. These trends are different in origin and treatment of roasting.



Graph 10 - Confidence of interval, results of ANOVA and LSD test by SPME-GC-MS technique.

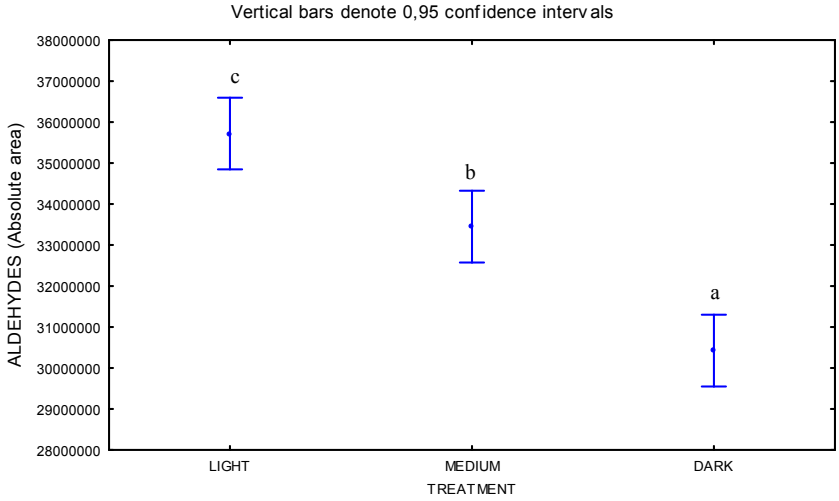
The treatment effect for every single origin (graph 10) reduces the content alcohols compounds, but only the Brazilian coffee have shown an increasing in their alcohol compounds. The 2-butanol, 3-methyl-3-buten-1-ol, 4-methyl-2-pentanol are the main compounds that showed relevant differences either of the coffee origin effect, the treatment and their interaction. In details the 2-butanol is mostly present in concentrations level in Honduras coffee, the 3-methyl-3-buten-1-ol in Honduras, Kenya followed by Nicaragua, Ethiopia and Brazil coffee. The 4-methyl-2-pentanol is present in greater content in Honduras followed by Nicaragua and Kenya coffee.



Graph 11- Confidence of interval, results of ANOVA and LSD test detected by SPME-GC-MS technique. The different letter mark significant difference for $p < 0,05$.

The aldehydes content as reported in the graph 11 varies depending on the origin and especially by the effect of heat treatment for every coffee (graph 13). The aldehydes have higher concentration in Honduras and Kenya coffee instead low concentration for Ethiopia, Guatemala and Brazil coffee. The aldehydes to their chemical nature are very volatile at high temperatures (graph 12). The aldehydes detected by SPME are 2-methyl-2-butenal, methyl-propanal, 3-methyl-butanal, acetaldehydes, furfural, glutaraldehydes, hexanal, propanal and valeric aldehydes. The aldehydes 2-methyl-2-butenal is more contained in the Nicaragua and Honduras coffee, the 3-methyl-butanal in Guatemala, methyl propanal in Brazil coffee, the acetaldehydes in Honduras coffee, and the furfural was detected in all the coffees in particular in Kenya coffee. The hexanal showed highter content in coffee Ethiopia. Hexanal is not formed by the Maillard reaction but results from the oxidation of lipids (Flament, 2002). Considerable amounts of hexanal were found in

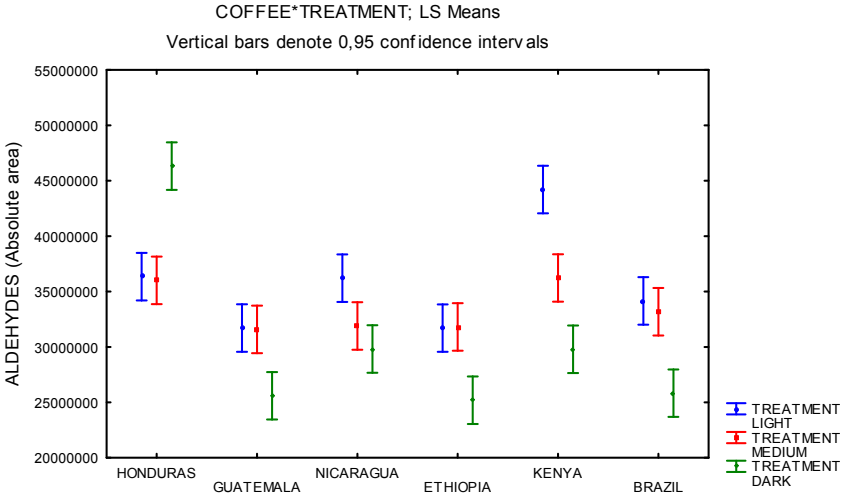
green coffee Ethiopia (see chapter 2) and roasted coffee. The extent of formation and maximum concentration depend on roasting temperature (HTST and LTLT) where was significantly higher in HTST roasted coffee than in LTLT roasted coffee (Baggenstoss *et al*., 2008). Coffee Ethiopia is characterized by abundant floral notes thanks the hight presence in green beans and to scarce degradation at light and medium temperature. The propanal was abundant in Honduras, Nicaragua and Ethiopia coffee and valeric aldehydes in Guatemala, Honduras and absent in Nicaragua coffee.



Graph 12 - Confidence of interval, results of ANOVA and LSD test detected by SPME-GC-MS technique. The different letter mark significant difference for $p < 0,05$.

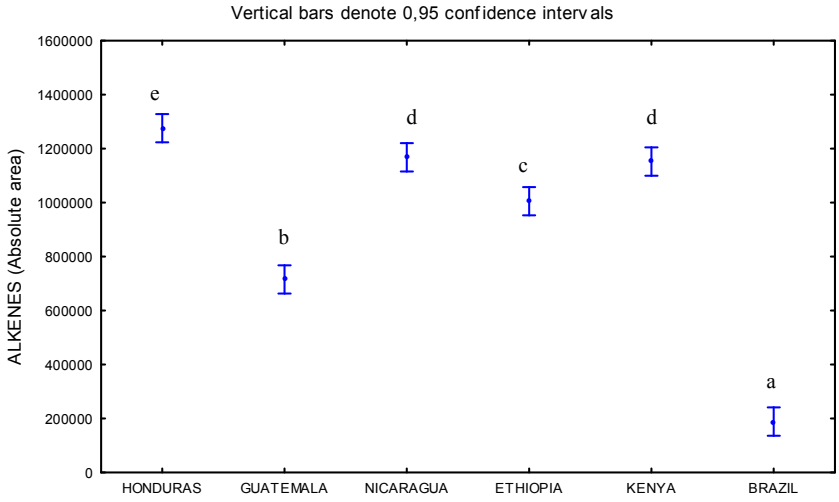
The aldehydes decreases in agreement roasting processes and can be stinging and pungent or sweet, fruit-like, and floral note. Some aldehydes combine with acids under high heat conditions to form esters, which can be identified as having distinct aromatic qualities, like pineapple, pear, or peach. The best Arabica coffees typically have a higher concentration of these aldehydes and the coffee Kenya and Honduras are resulted be abundant of these

compounds. The more agressive the treatment the less aldehydes degree due to its high volatility situation.



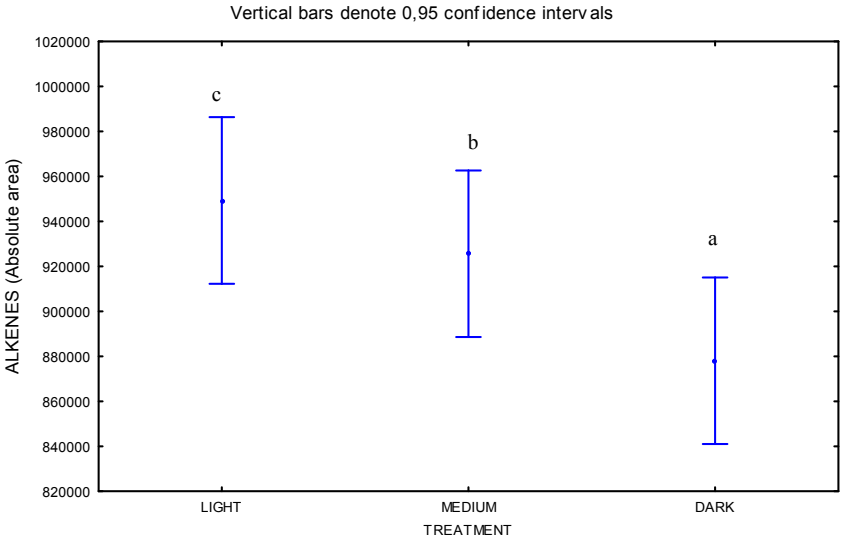
Graph 13 - Confidence of interval, results of ANOVA and LSD test detected by SPME-GC-MS technique.

Only Honduras coffee presented a positive trend by roasting in comparison with other coffees. Then the major part of aldehydes decreased when a dark treatment is applied.
All the aldehydes identified in all samples have shown a high sensitiveness of heat treatments.

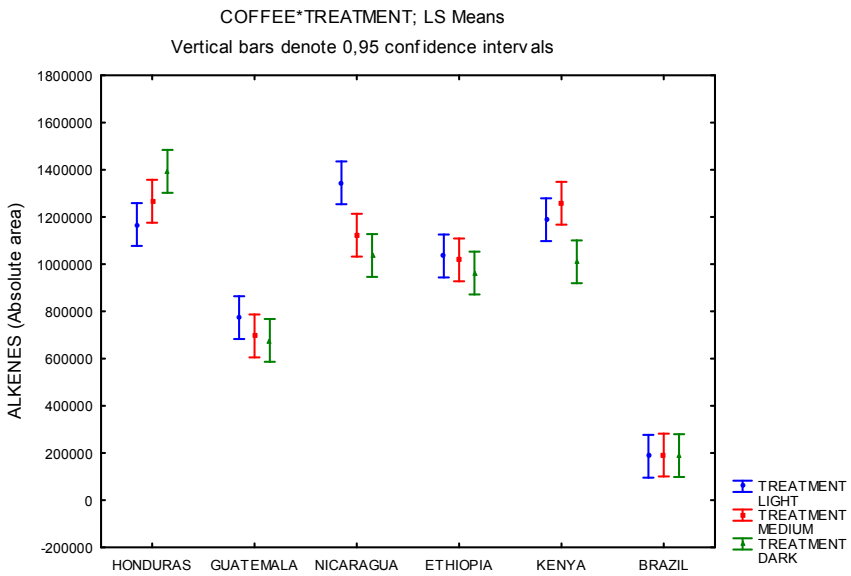


Graph 14 - Confidence of interval, results of ANOVA and LSD test detected by SPME-GC-MS technique. The different letter mark significant difference for $p < 0,05$.

The class alkenes compounds are in high concentrations in Honduras, Nicaragua, Ethiopia and Kenya coffee, less concentration in Guatemala coffee, instead very low concentration in Brazil coffee.

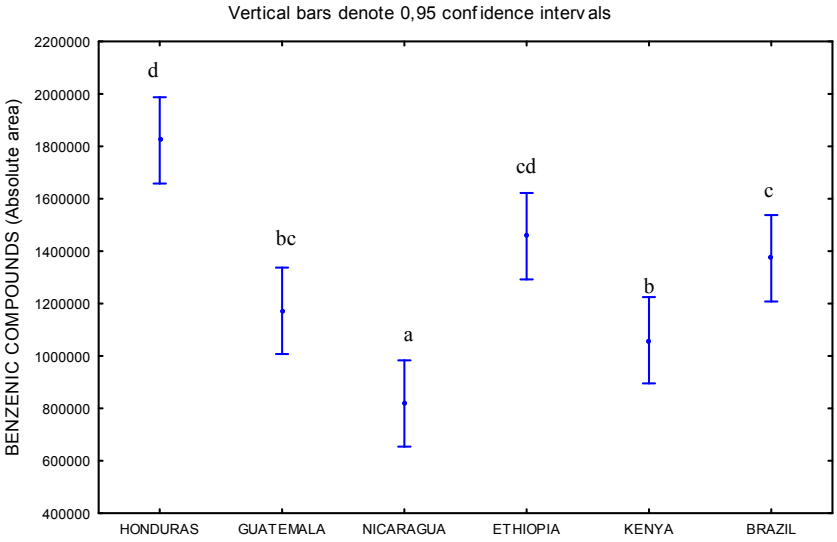


Graph 15 - Confidence of interval, results of ANOVA and LSD test detected by SPME-GC-MS technique. The different letter mark significant difference for $p < 0,05$.



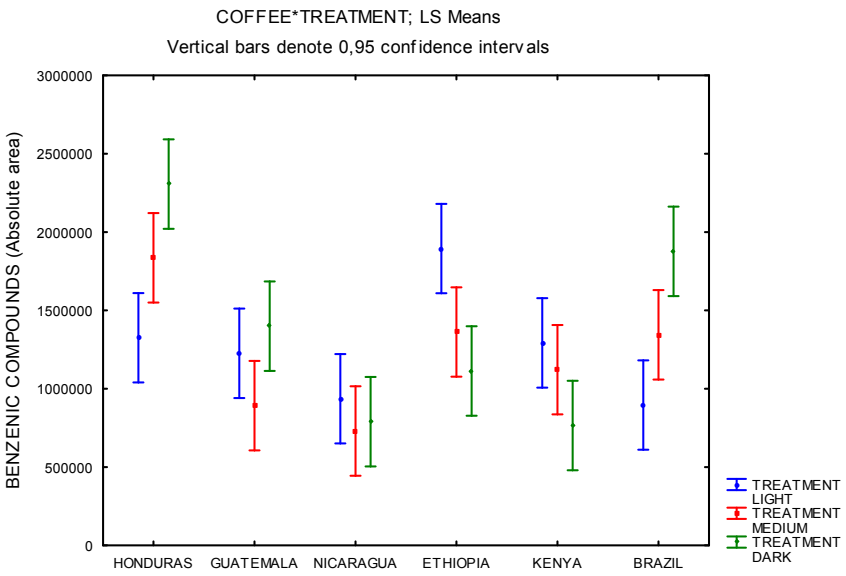
Graph 16 - Confidence of interval, results of ANOVA and LSD test detected by SPME-GC-MS technique.

The alkenes showed a negative trend of concentration in agreement roasting process. All results showed that the content of alkenes decreased when a tougher temperature treatment is applied but only the Honduras coffee showed an increasing trend. The alkenes compounds that presented a significant difference were 2-methyl-1,6-heptadiene and 2-hexene.



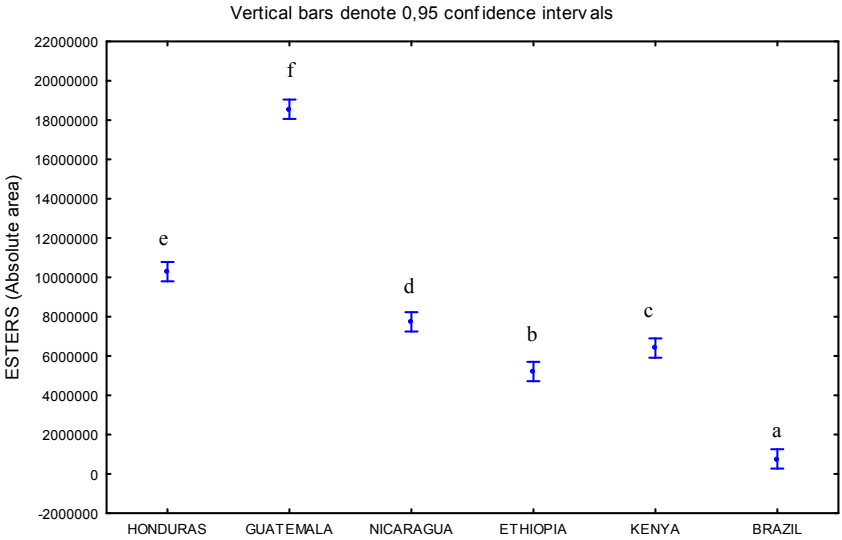
Graph 17 - Confidence of interval, results of ANOVA and LSD test detected by SPME-GC-MS technique. The different letter mark significant difference for $p < 0,05$.

The benzene compounds are derived from the roasting process and are present in higher amounts in all coffees in particular in the Honduras coffee followed by Ethiopia and Brazil (graph 17). The principal compounds identified was the toluene, present a greater concentration in Brazil coffee, followed by Honduras, Guatemala, Ehiopia, Nicaragua and Kenya coffee.



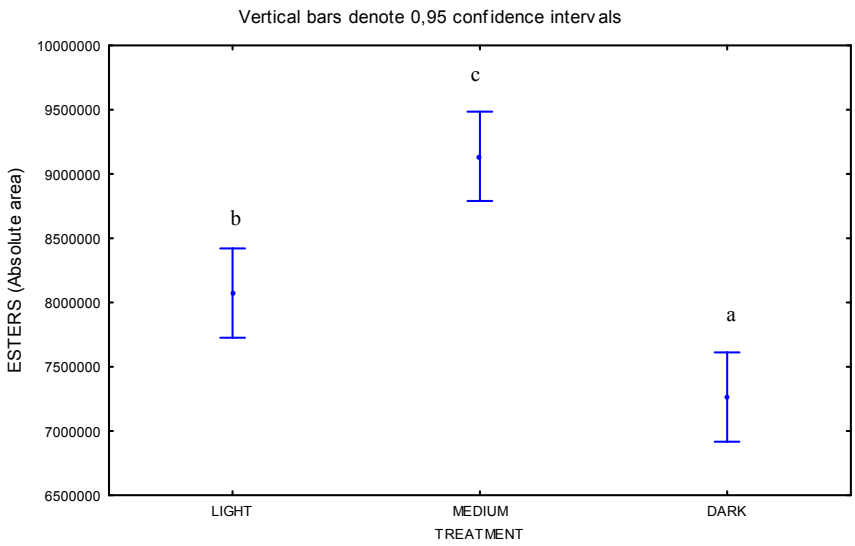
Graph 18 - Confidence of interval, results of ANOVA and LSD test detected by SPME-GC-MS technique.

Among the different benzenic compounds found, with different treatments the Honduras, Guatemala and Brazil samples showed a positive trend while the other coffees showed a negative trend.



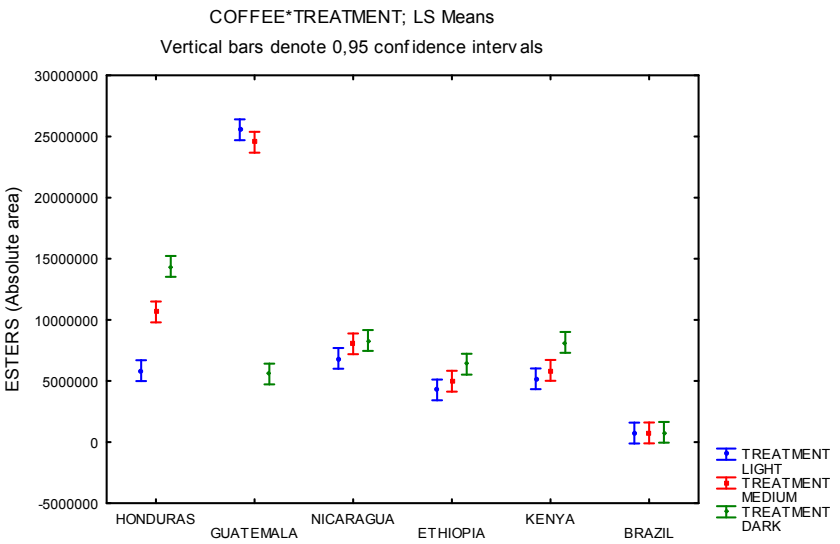
Graph 19 - Confidence of interval, results of ANOVA and LSD test detected by SPME-GC-MS technique. The different letter mark significant difference for $p < 0,05$.

The esters compounds are presented in high level in Guatemala and Honduras coffee, less level for Nicaragua, Ethiopia and Kenya coffee. The lowest degree has been found in Brazil samples. Nevertheless there is a high level of esters found in the green coffee mostly in Kenya and Brazil samples and after roasting it decreased considerably.



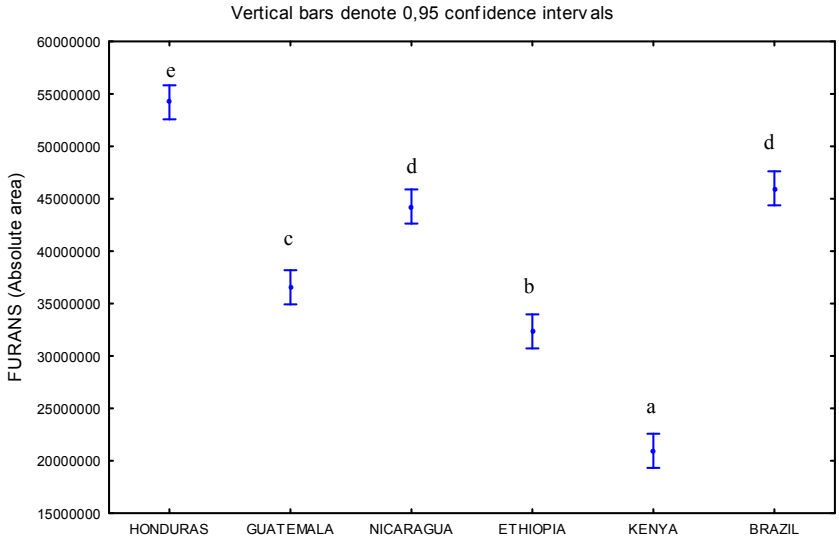
Graph 20 - Confidence of interval, results of ANOVA and LSD test detected by SPME-GC-MS technique. The different letter mark significant difference for $p < 0,05$.

The formation of esters increased with the roasting treatment. In particular, the highest concentration of esters production was observed during the medium treatment roasting process. The acetic acid-hexyl-ester is the only one that has presented a relevant difference either from the point of view that the origin or the treatment. The esters have been identified that have a significant statistical diffeence are: 2-methyl-ethenylester-2-propenoic acid, hexyl ester-acetic acid, methyl acetate, methyl formate and 2-hydroxy-ethyl ester propanoic acid.



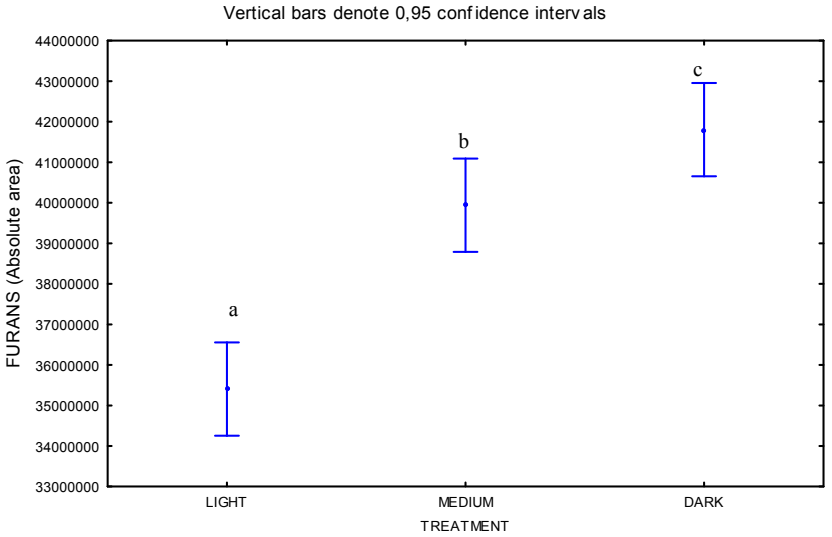
Graph 21 - Confidence of interval, results of ANOVA and LSD test detected by SPME-GC-MS technique.

All results showed that the content of esters increased when the temperature treatment is applied but only the Guatemala coffee showed a decreasing trend. Only the Brazil sample has demonstrated no changes with effect of treatment.



Graph 22 - Confidence of interval, results of ANOVA and LSD test detected test detected by SPME-GC-MS technique. The different letter mark significant difference for $p < 0,05$.

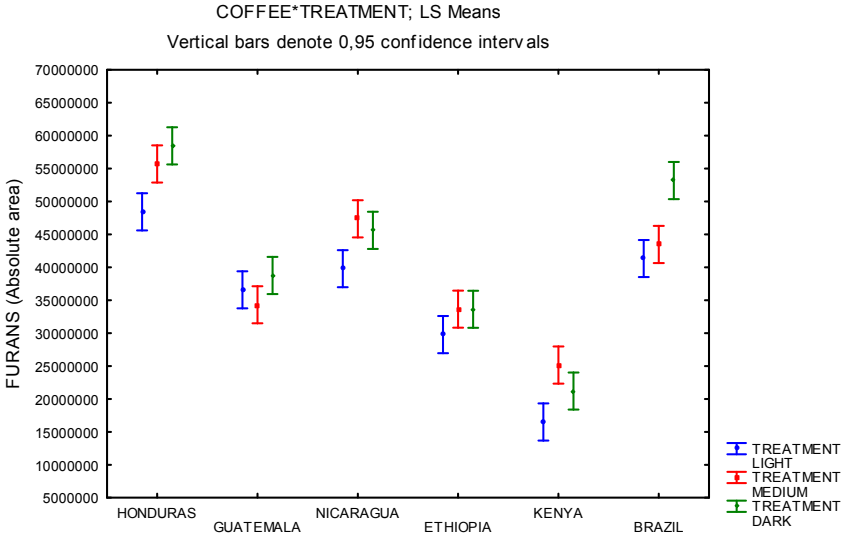
Graph 22 shows the different trend for every typology of coffee and is easy to observe that all coffees provenience American are more rich than coffee of origin African.
In the group of furans are incluse compounds called 2-furyl-methanethiol and 5-methyl derivates. They are very important compounds because conditioning the quality of coffee (Tateo and Bonomi 1999) in base concentration their. Graph 22 shows the different trend for every typology of coffee and is easy to observe that all coffees provenience American are more rich than coffee of origin African.



Graph 23 - Confidence of interval, results of ANOVA and LSD test detected test detected by SPME-GC-MS technique. The different letter mark significant difference for $p < 0,05$.

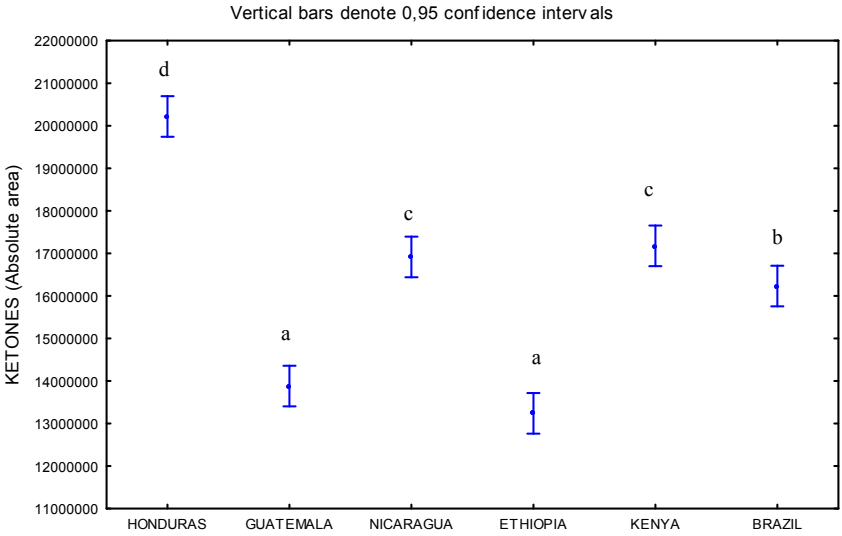
As an ANOVA test result, were revealed the differences of the furans content through the different treatment roasting process. The furans detected were: furfuryl formate, furfuryl alcohol, furfuryl acetate, 3,4 dimethyl-2,5 furandione, 5-methyl furfurale, 2-methyl furan, 2,5 dimethyl furan, furfuryl propianate, furfuryl methyl ether, 2,5-dimethylfuran, 3-methyl-2,5-furandione, 2-acetylfuran, 2-furancarboxaldehyde, 2-furfurylmethylsulfide, 2-pentyl furan, and furaneol. The volatile compounds that have shows significant effect impact by GC-O were the furfurylmethylether (soya bean/potato) present in all the coffee but at higher concentration in Honduras and Nicaragua coffee, the 2-furfurylmethylsulfide (roasty) abundant in Brazil coffee, the 5-methylfurfural (vanilla, sweet, roasted) present in all the coffee but at higher concentration in Honduras and Kenya coffee, furfuryl alcohol (little nut toasted) absent in Kenya coffee instead in others coffee the level were medium and furaneol (honey,

caramel, sweet) particularly abundant in Honduras, kenya and Brazil coffee.



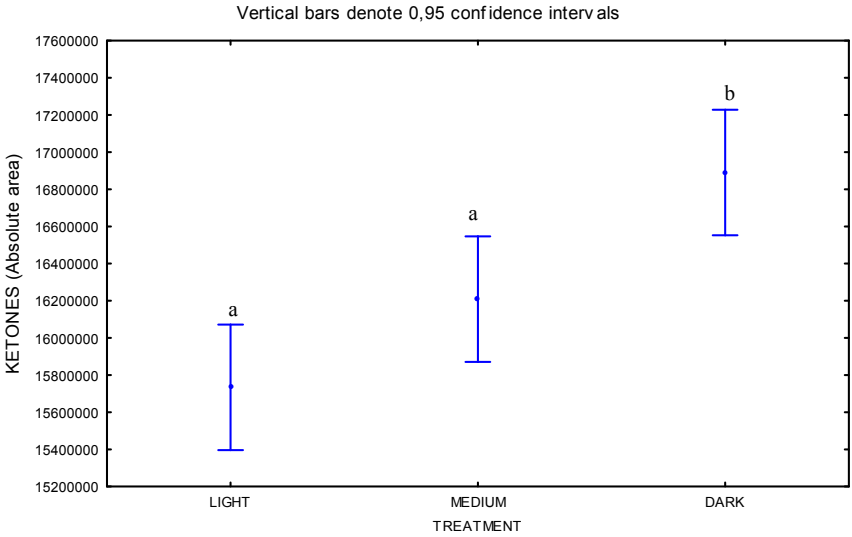
Graph 24 - Confidence of interval, results of ANOVA and LSD test detected by SPME-GC-MS technique.

The furans in Honduras and Brazil coffee were showed an increasing trend. The Guatemala coffee sample showed a decreasing in medium treatment and an increasing in the dark treatment. The Nicaragua, Ethiopia and Kenya coffee showed a higher level in medium treatment.



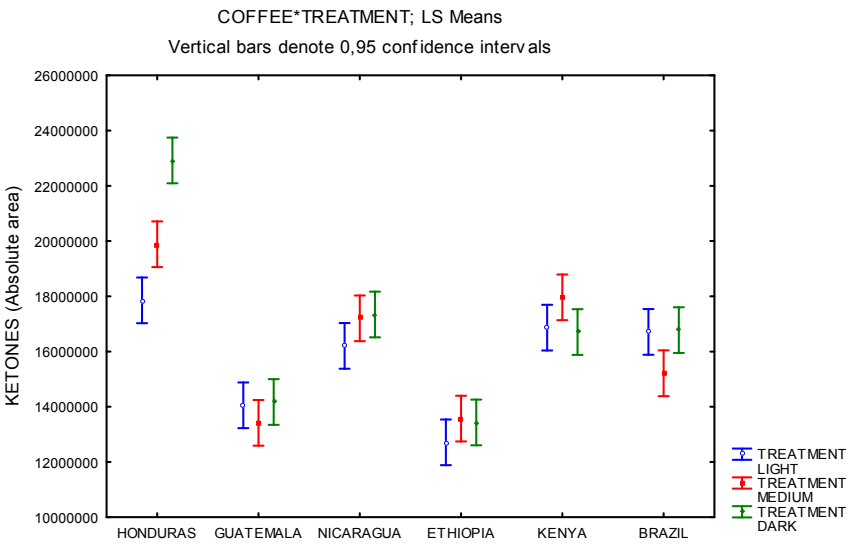
Graph 25 - Confidence of interval, results of ANOVA and LSD test detected by SPME-GC-MS technique. The different letter mark significant difference for $p < 0,05$.

Also the ketones compounds are a classes responsible of important olfactive notes in roasted coffee. Graph 25 shows that are presents in high level in Honduras coffee, and then follows presence in Nicaragua, Kenya and Brazil coffee. The main volatile compounds perceived were the 2,3-butanedione (vanilla) detected in all the coffee samples, the 2,3-pentanedione (buttery) particular abundant in Nicaragua, Honduras and Kenya coffee, 1 acetoxy-2-propanone (roasty, woody), butyrolactone (caramel, butter) and 3-methyl-3-hexen-2-one (roasted) in maximum concentrations in Honduras coffee, 3 ethyl-2-hydroxy-2-cyclopenten-1-one (caramel, sweet) major present in Brazil and Honduras coffee.



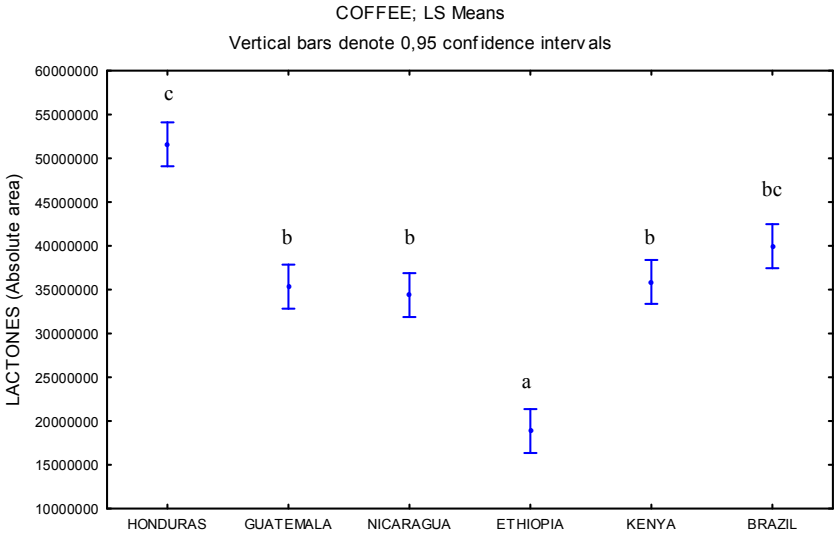
Graph 26 - Confidence of interval, results of ANOVA and LSD test detected by SPME-GC-MS technique. The different letter mark significant difference for $p < 0,05$.

The ketones subjected to heat have the same behaviour of aldehydes. Their trend is in line with the notice reported by Mayer (1999).



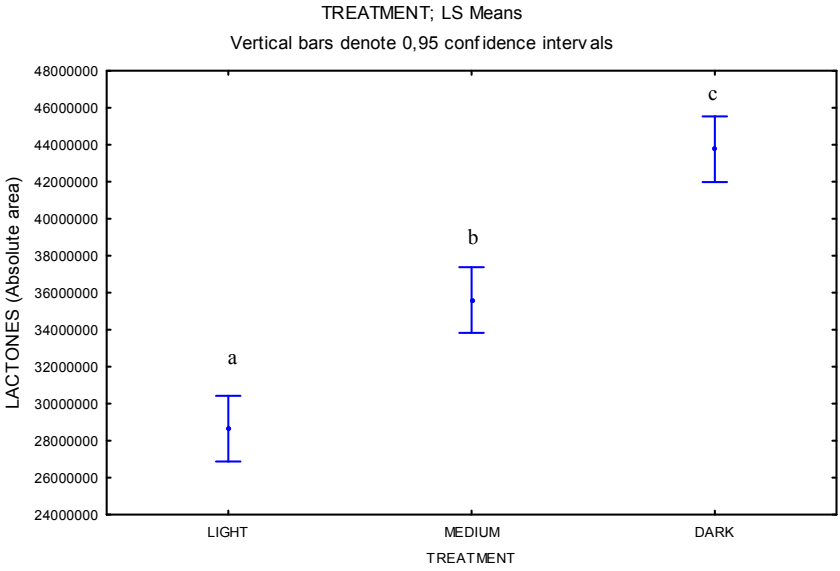
Graph 27 - Confidence of interval, results of ANOVA and LSD test detected by SPME-GC-MS technique.

The Honduras, Ethiopia coffee showed an increasing ketones content in agreement roasting process, Guatemala and Brazil showed a negative trend only medium roast, instead Kenya coffee showed a positive trend only medium roast.

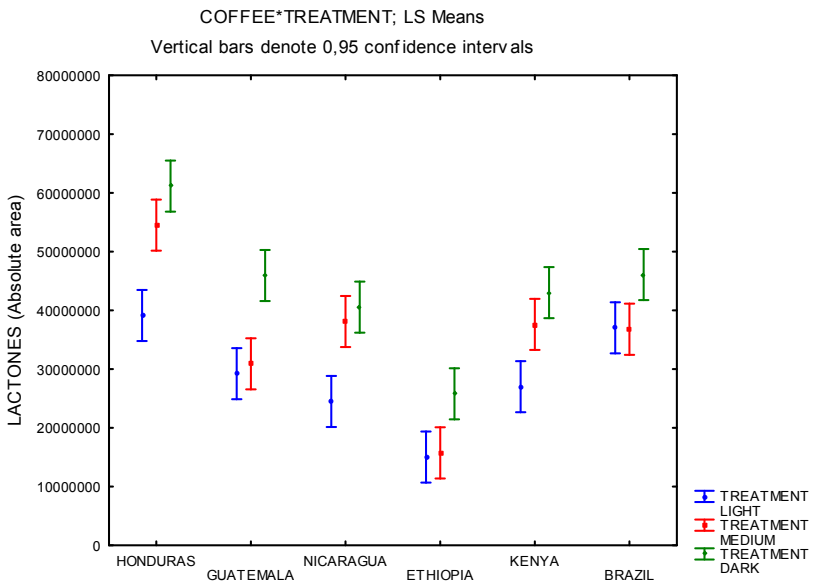


Graph 28 - Confidence of interval, results of ANOVA and LSD test detected by SPME-GC-MS technique. The different letter mark significant difference for $p < 0,05$.

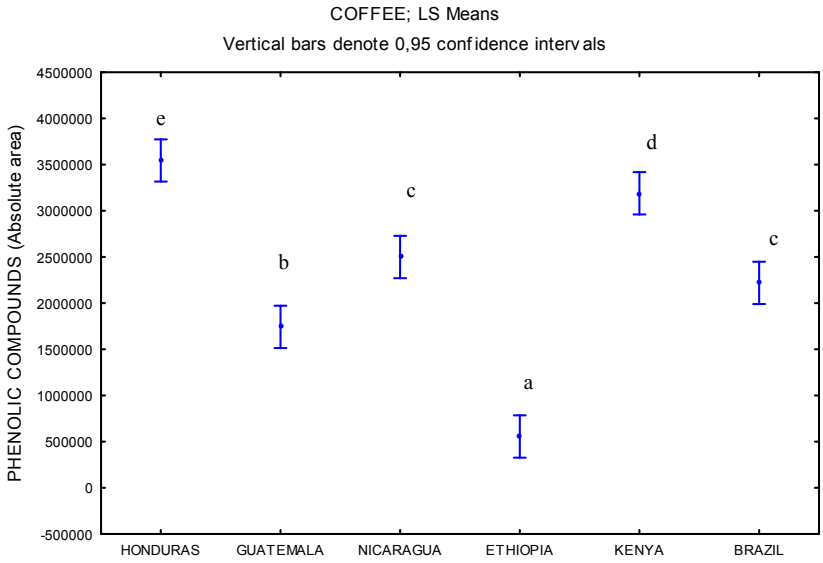
The lactone compound detected by analysis in GC-MS is γ-butyrolactone, as shown in the graph 28, its present in all the origin coffee in particular in Honduras coffee. γ-butyrolactone gave notes caramel, butter. Graph 29 shows the lactones increase with the roasting treatment with a significant growth. All results showed that the content of lactones increased when a temperature treatment is applied (graph 30).



Graph 29 - Confidence of interval, results of ANOVA and LSD test detected by SPME-GC-MS technique. The different letter mark significant difference for $p < 0,05$.

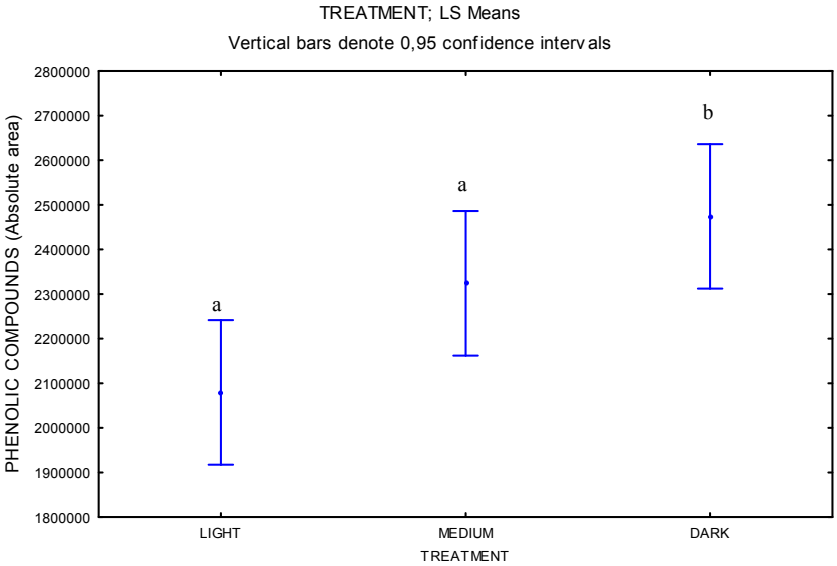


Graph 30 - Confidence of interval, results of ANOVA and LSD test detected by SPME-GC-MS technique.

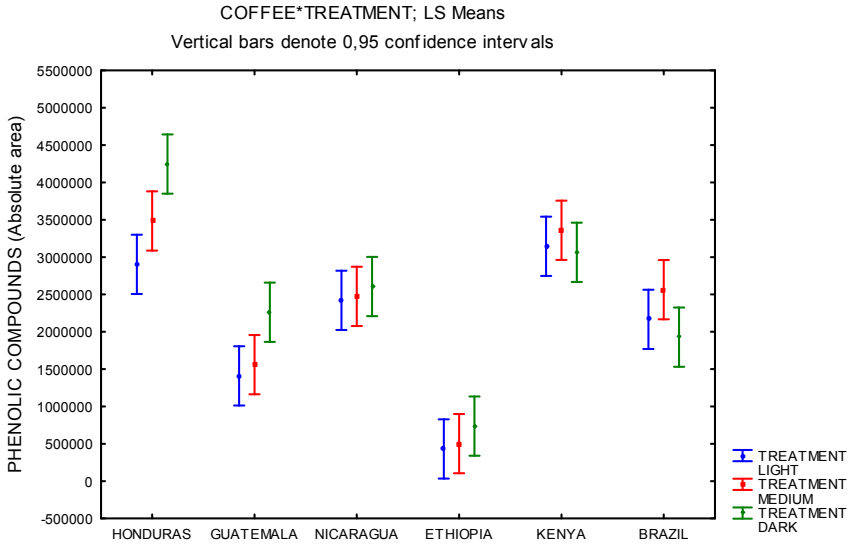


Graph 31 - Confidence of interval, results of ANOVA and LSD test detected by SPME-GC-MS technique. The different letter mark significant difference for $p < 0.05$.

The phenolic compounds are responsible spicy/clove-like, astringent notes and they tend to evaporate quickly. They are typical roasting products, resulting from the Maillard reaction in particular thermal decomposition of ferulic acid (guaiacols). The 4 vinyl guaiacol is a key important of phenolic, burnt note in roaste coffee (Baggenstoss *et al*., 2008) and guaiacol spicy, harsh, earthy (Zellner *et al*., 2008). In this scientific work are not considered how possible *markers* of quality because probably their high concentrations only gave negative notes. Surely these two compounds may greatly contribute to the aroma of dark-roasted coffees seen the positive trend their in degree of roast (graph 32). The phenolic compounds increase with the roasting treatment and often give some typical negative aromatic odours like smoky, burn or medicinal and specially when a dark roasting treatment has been applied. Graph 31 shows the phenolic compounds are presents in all coffee at different quantitative level.

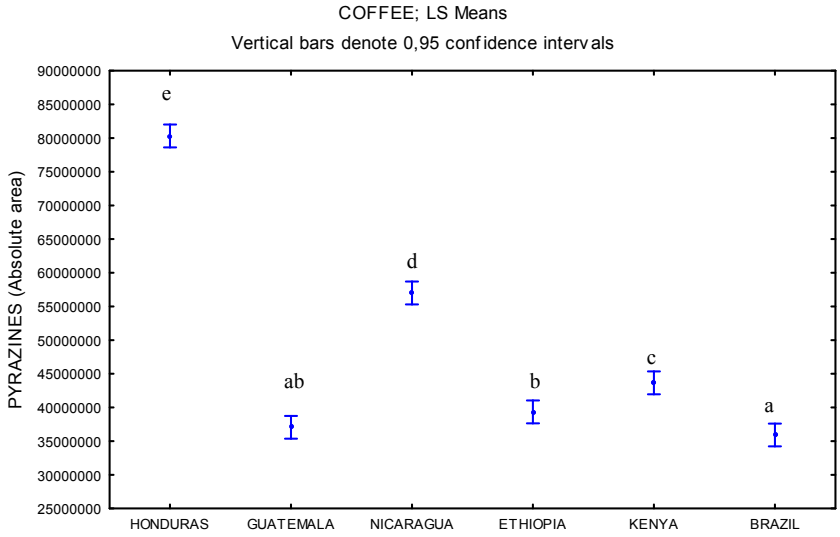


Graph 32 - Confidence of interval, results of ANOVA and LSD test detected by SPME-GC-MS technique. The different letter mark significant difference for $p < 0,05$.



Graph 33 - Confidence of interval, results of ANOVA and LSD test detected by SPME-GC-MS technique.

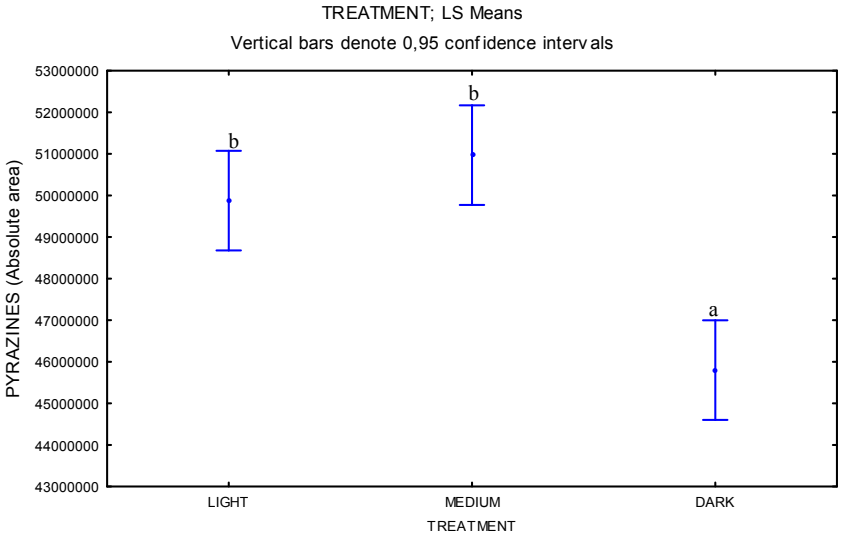
Almost all results showed that the content of phenolic compounds increased degree of roasted coffee. Only the Kenya and Brazil samples showed a lightly decreasing trend.



a

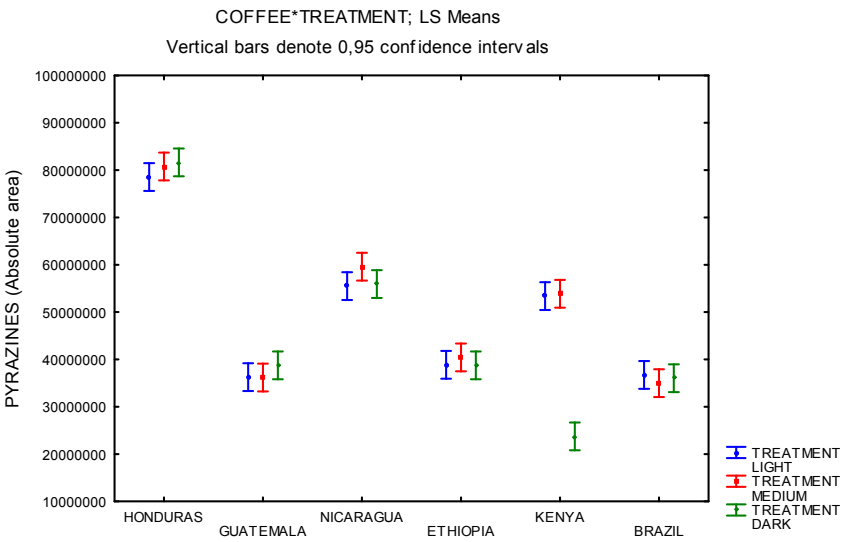
Graph 34 - Confidence of interval, results of ANOVA and LSD test detected by SPME-GC-MS technique. The different letter mark significant difference for p < 0.05.

The pyrazines compounds (earthy/musty/nutty/sweet) are presented in a considerable high level in Honduras coffee while the rest of the coffee origins maintain their degree in the medium to low content. Baggenstoss et al., (2008) reported only 2-ethyl-3,5-dimethylpyrazines is a key importance for coffee aroma, instead in this scientific work are reported other pyrazines of key importance by GC-O technique (tab 4).



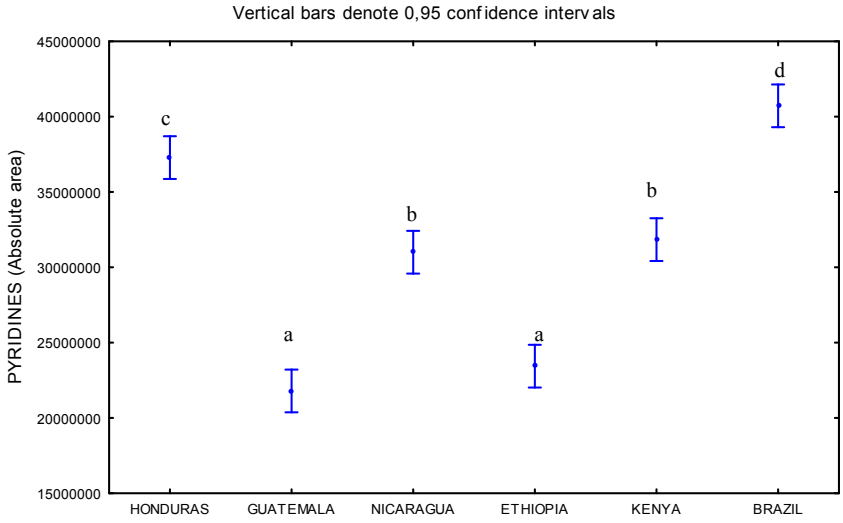
Graph 35 - Confidence of interval, results of ANOVA and LSD test detected by SPME-GC-MS technique. The different letter mark significant difference for $p < 0,05$.

The pyrazines compounds show a bell behaviour where are considered as a markers of the effects of roasting treatment, in particular the alkylpyrazines (Are usually linked with heated food flavours and often are used for monitoring the roasting process (Hashim, 1996). Pyrazines, some of which are key odorants of Arabica coffee, are highly volatile and subject to dissipation upon exposure to air and non-enzymatic browning. The pyrazines found by GC-MS were 2,5 dimethylpyrazine (little nut, sweet), 2,6 dimethylpyrazine (little nut, toasted), 2 ethyl-6-methyl-pyrazine (caramel, sweet) detected in high concentration in Honduras and Brazil coffee, and 2-ethyl-3-methyl-pyrazine (butter, sweet, vanilla) detected in hight concentration in Honduras, Nicaragua and Brazil coffee.



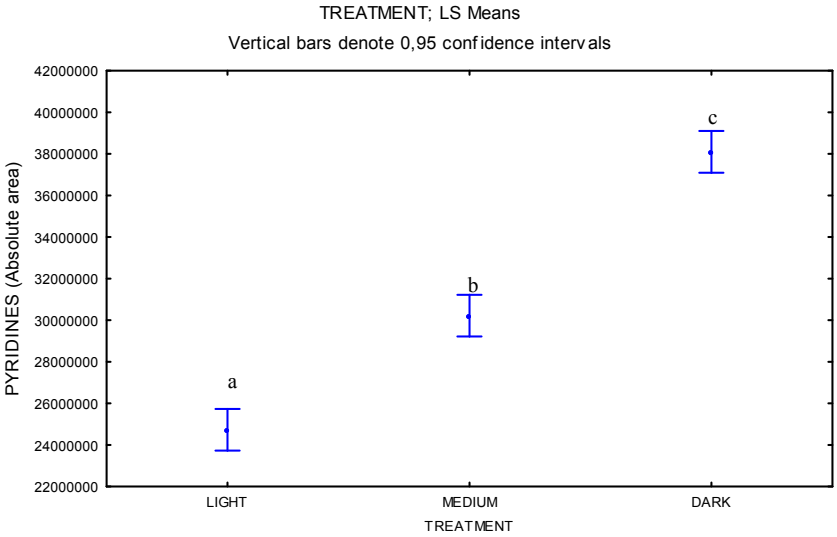
Graph 36 - Confidence of interval, results of ANOVA and LSD test detected by SPME-GC-MS technique.

The content of pyrazines was found to increase with a high degree of roasting for treatment, in particular for Kenya dark roast the level of pyrazine is very low.



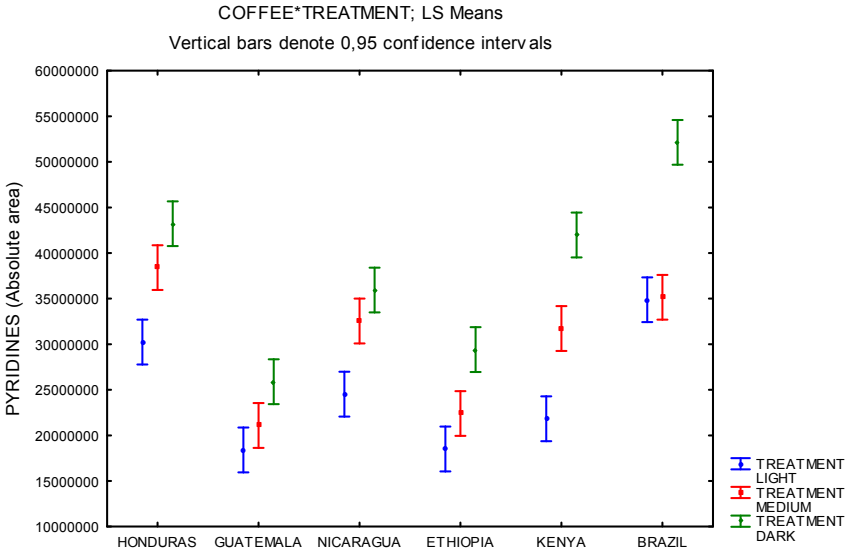
Graph 37 - Confidence of interval, results of ANOVA and LSD test detected by SPME-GC-MS technique. The different letter mark significant difference for $p < 0,05$.

Impact of roasting produce an other classes of compound that are the pyridine. Graph 38 shows high level content in Brazil coffee and Honduras coffee.



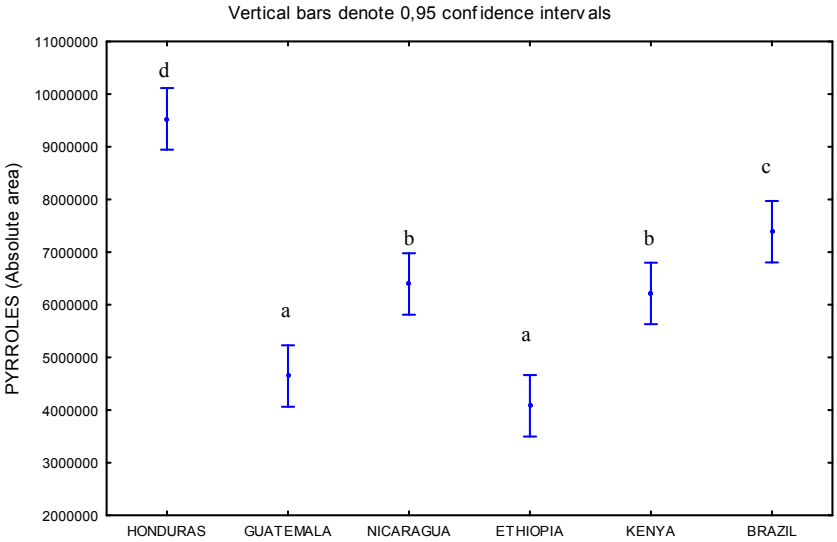
Graph 38 - Confidence of interval, results of ANOVA and LSD test detected by SPME-GC-MS technique. The different letter mark significant difference for $p < 0,05$.

The pyridines as seen before on aldehydes and furans, are products of thermal origin therefore the dark roasting helps their content increasing in according with Baggenstoss et al., (2008).



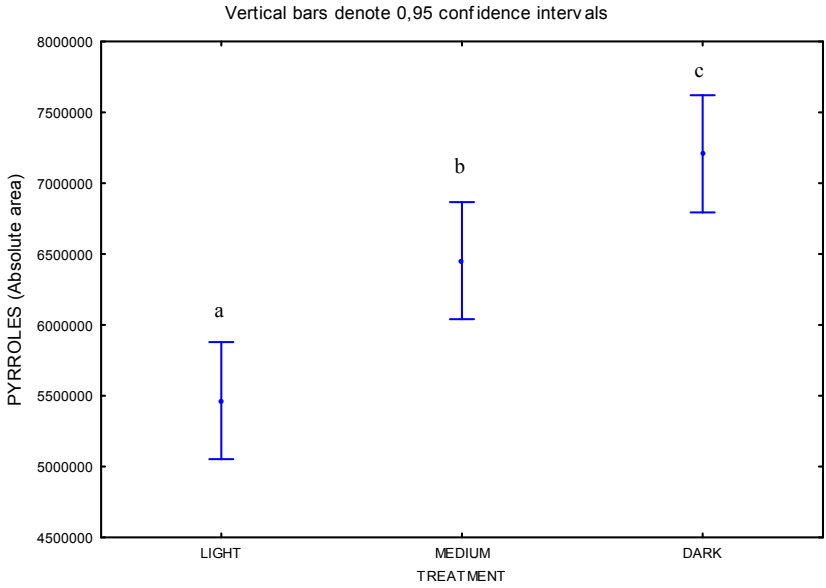
Graph 39 - Confidence of interval, results of ANOVA and LSD test detected by SPME-GC-MS technique.

All results showed that the content of pyridines increased when a temperature treatment is applied. Pyridines for all kind of coffees show always a positive and homogenous trend. Pyridines are more stable, but are negatively perceived in too great a concentration.



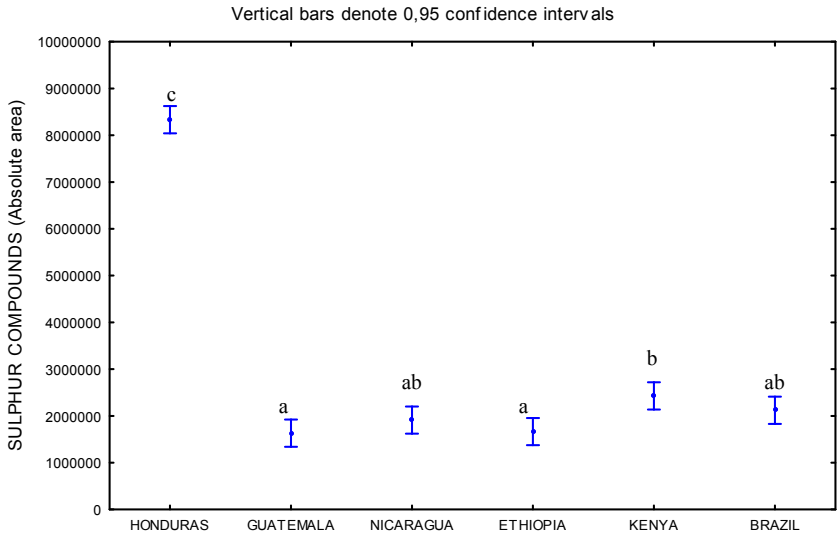
Graph 40 - Confidence of interval, results of ANOVA and LSD test detected by SPME-GC-MS technique. The different letter mark significant difference for $p < 0,05$.

The pyrroles show a significant high level in Honduras coffee while the rest stand for the medium to low content level. The pyrroles present specific sensorial property that are sweet, licorice stick, bread and coffee.



Graph 41 - Confidence of interval, results of ANOVA and LSD test detected by SPME-GC-MS technique. The different letter mark significant difference for $p < 0,05$.

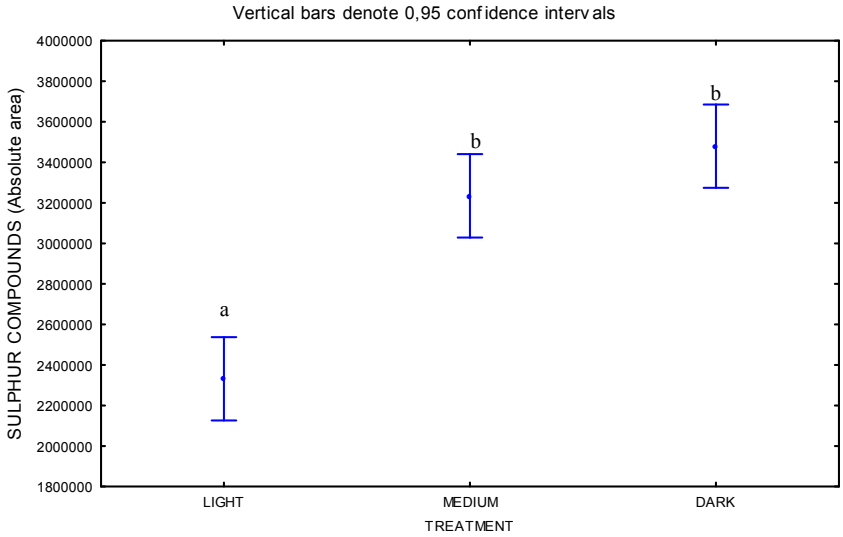
The pyrroles increase degree the roasting process in agreement with Flament (2002). Pyrroles are dissolved in the naturally occurring coffee oils and subject to oxidation. These compounds are not present in large amounts compared to other aromatics, but they are strong in aroma and slight deterioration can have a dramatic effect on flavour.



bc

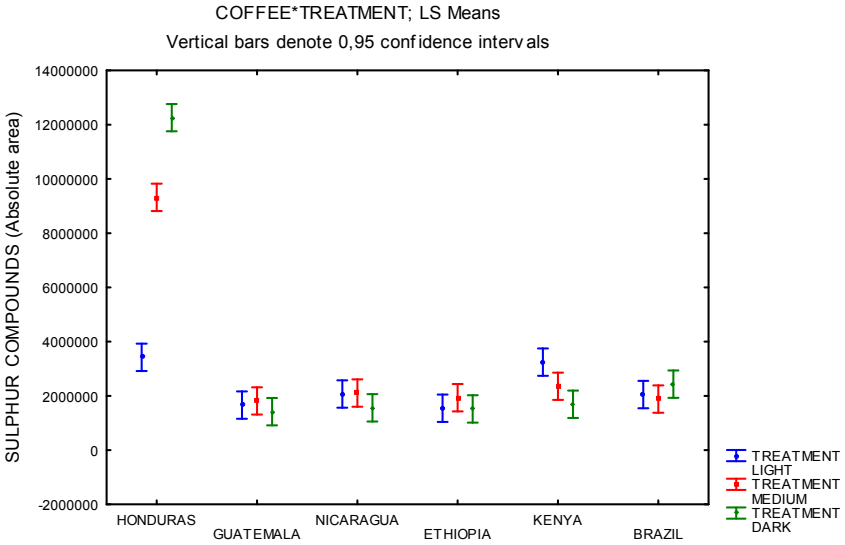
Graph 42 - Confidence of interval, results of ANOVA and LSD test detected by SPME-GC-MS technique. The different letter mark significant difference for $p < 0,05$.

The sulphur compounds are among the most important aroma compounds in coffee, responsible as impact compounds of coffee aroma (Blank *et al*., 1992; Czerny *et al*., 1999). They shows a considerable high level in Honduras coffee but in the other origin is detected low level content. They might present positive or negative notes (rotten egg-like, sulfitic, cooked vegetable-like, onion-like, garlic-like, raw potato-like and mushroom-like) depending on concentration theresold. In this cases might derived by way methabolic, roasting process in particular effect of temperature, further oxidation and disproportionation products of the same reaction sequence (Parliament *et al*., 1982) or pyrolysys of methionine (Merritt *et al*., 1969). The characterisation of the desirable coffee aroma is a challenging task as many of the important odorants are just present in trace amounts and/or are quite reactive and unstable.



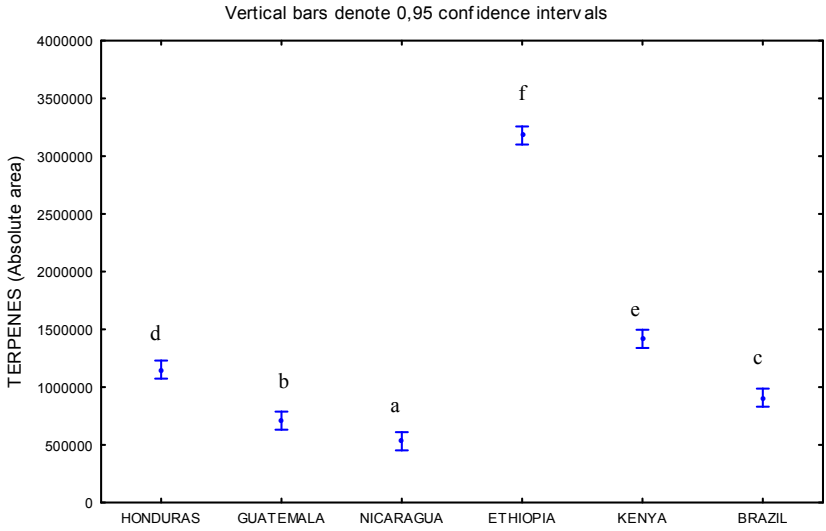
Graph 43 - Confidence of interval, results of ANOVA and LSD test detected by SPME-GC-MS technique. The different letter mark significant difference for $p < 0,05$.

The sulphur compounds in agreement with Blank *et al* (1992) and Czerny (1999), increase with the roasting process.



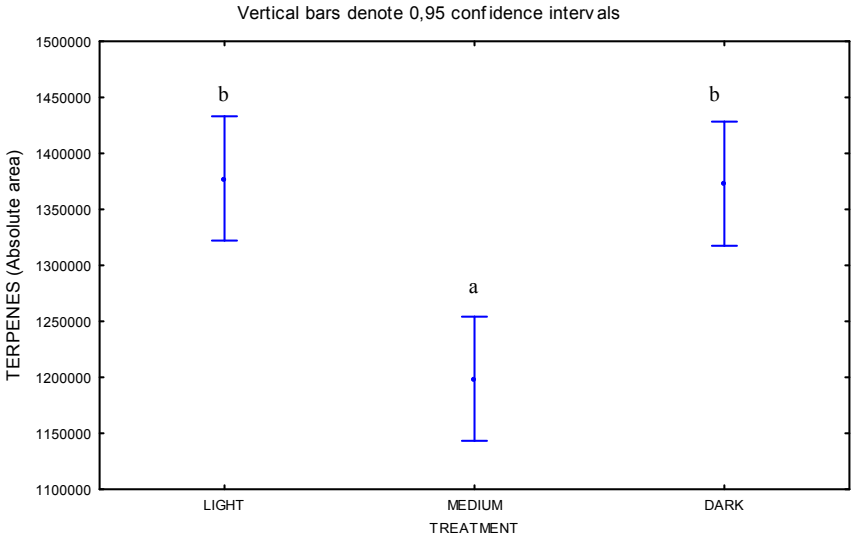
Graph 44 - Confidence of interval, results of ANOVA and LSD test detected by SPME-GC-MS technique.

As a result almost all samples showed a content of sulphur compounds in the average strip with slightly variances. Only the Kenya sample showed a decreasing trend, but the Honduras one showed a significant increasing degree. It is also important to notice that however the Honduras green coffee has a very lower level of proteins, after roasting process it shows a very high content of sulphurs, there is probably that the high content of sulphur compound is given by sulphured aminoacid compounds.



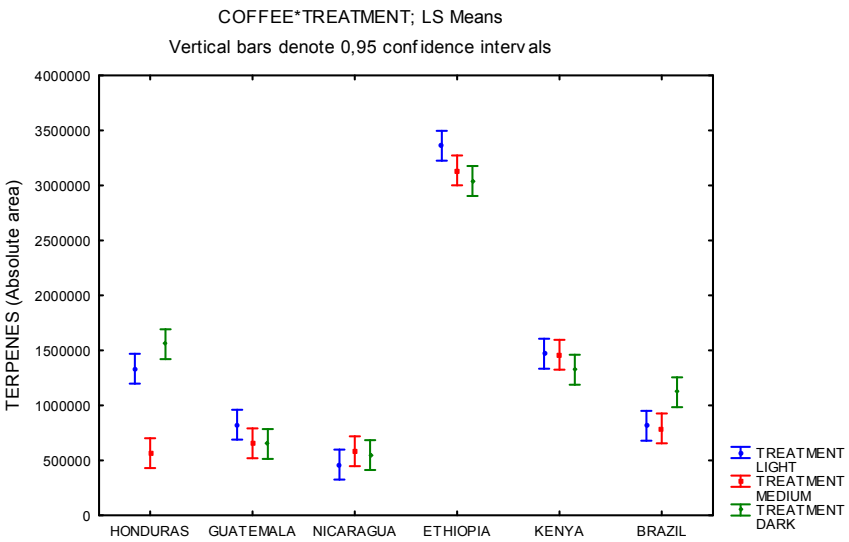
Graph 45 - Confidence of interval, results of ANOVA and LSD test detected by SPME-GC-MS technique. The different letter mark significant difference for $p < 0,05$.

The content of terpenes compounds is higher in Ethiopia coffee. The rest of the coffees presented an average level content. Most probably is given the effect of the content of compounds responsible of pleasant flavours, see graph 20 second chapter.



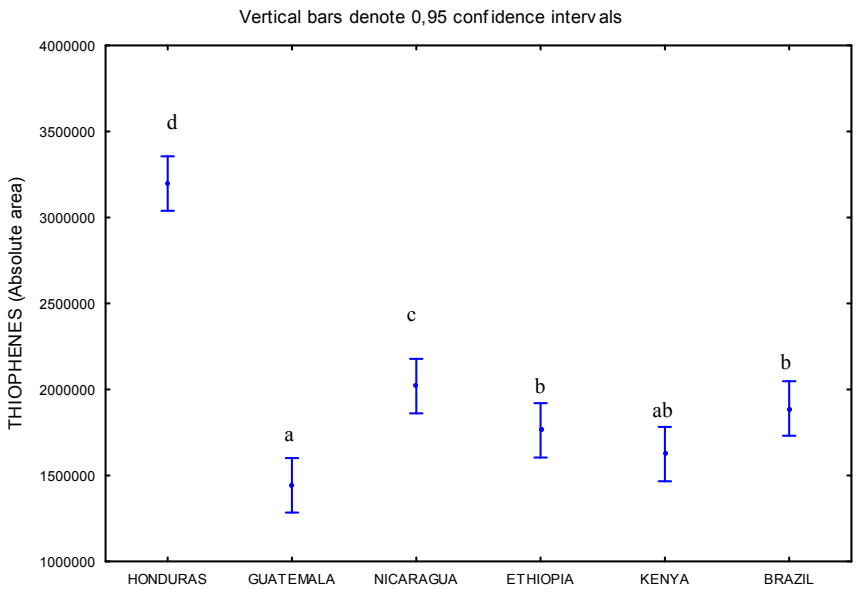
Graph 46 - Confidence of interval, results of ANOVA and LSD test detected by SPME-GC-MS technique. The different letter mark significant difference for $p < 0,05$.

The terpenes compounds was presented a decreasing trend with medium roast and then a increasing with the dark roast (graph 46).



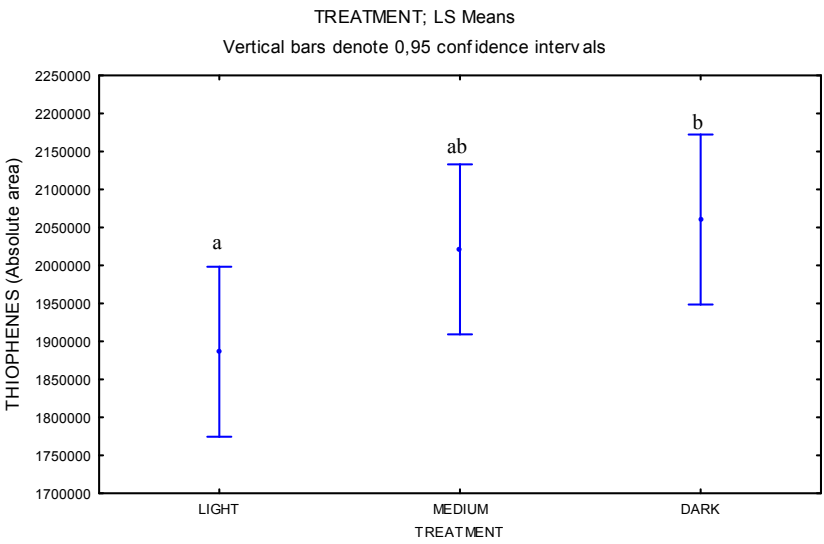
Graph 47 - Confidence of interval, results of ANOVA and LSD test detected by SPME-GC-MS technique.

In general the content of terpenes compounds decrease. Only in the Honduras and Brazil samples coffee showed an increasing trends.



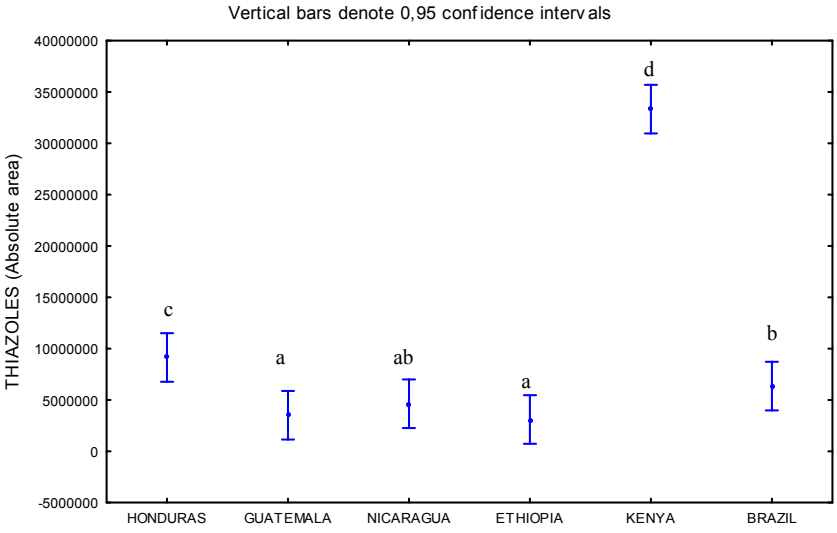
Graph 48 - Confidence of interval, results of ANOVA and LSD test detected by SPME-GC-MS technique. The different letter mark significant difference for $p < 0,05$.

The highest content of thiophenes was detected in Honduras coffee, while the others coffee showed a lower content level.



Graph 49 - Confidence of interval, results of ANOVA and LSD test detected by SPME-GC-MS technique. The different letter mark significant difference for $p < 0,05$.

The content of thiophenes change with treatment of roasting process with slightly differences. Thiopenes are compounds heterociclic with S and often shows a profile not desirable for example 2-acetiltiophene with onions note and yours derived 5-metil with sweet and floreal note.

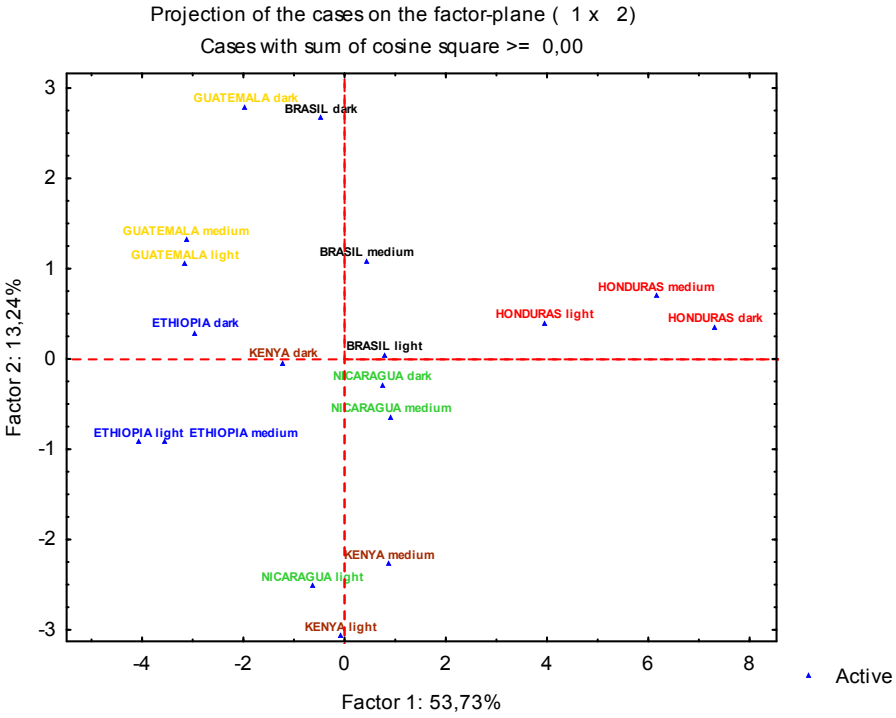


Graph 50 - Confidence of interval, results of ANOVA and LSD test detected by SPME-GC-MS technique. The different letter mark significant difference for $p < 0,05$.

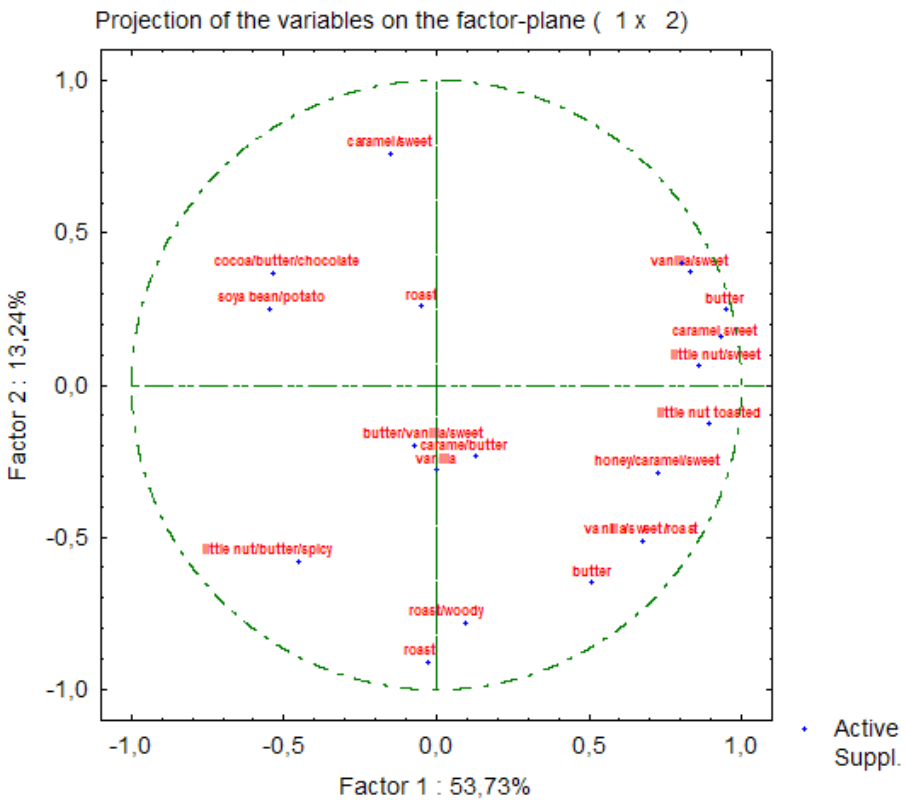
The highest content of thiazoles is in Kenya coffee while others coffees maintain their stability. The thiazoles does not vary during thermal treatments. Thiazoles are more associated a flavour meat, chips, peanuts and nut-like.

5.5.1 Odour compounds

Then characterized for classes of compounds the single coffee samples of different origin using SPME-technique the GC-O based to the sniffing mode was applied. Olfactory characterization of coffees is one the most important criteria for distinguishing between the coffees of different provenience. Using combined SPME-GCO was detected 19 odorous (see table 4 **) compound for all samples examined. The 19 most frequent aromatic notes in terms of the numbers of compounds were coffee, roasted, caramel, honey, sweet, vanilla, butter, spicy, soya bean, potato and little nut. The most frequent aromatic notes in terms of the numbers of the quantities perceived were little nut, roasted, caramel and vanilla. The PCA reported in graph 51 contain (53,73% + 13,24%) of the information and was carried out to differentiate between the origin coffee with three degrees of roasting. For each degree of roasting its been possible found specific aroma differences between the coffee of provenience Central America, Africa and Brazil. The graph 52 relative projection of all variables on the factor-plane seemed to discriminate between the six samples roasted (Central America, Africa and Brazil) by geographic origin group: for Guatemala coffee the samples were characterized by cocoa, buttery, chocolate, and caramel/sweet, while the Ethiopia coffee was characterized by little nut/buttery/spicy, Nicaragua coffee buttery, vanilla, sweet caramel, the Kenya coffee roasted woody, the Brazil only roast note and Honduras coffee presented a lot of aroma compounds (butter, caramel, honey, sweet).



Graph 51 - Results of PCA analysis of the 19 olfactory notes identified by GC-O for the six coffee roasted samples.



Graph 52 – PCA Projection of all variables on the factor-plane relative to odor detected in roasted coffee samples of different origin by SPME-GC-O analysis.

5.5.2 Correlation sensory analysis with odor detection by SPME-GC-O analysis

A Correlation analysis was performed using scores for the various descriptors of sensory analysis and GC peaks areas of GCO active compounds. Correlations were identified for each coffee origin and some examples are reported below (see attachments at the end chapter 5).

There are some compounds who change their concentration as a function of heat treatment, some increase and then tend to predominate in the dark roasted samples, others have the opposite trend, while some increase by the heat treatment, but are lost due to volatilization or thermal degradation and therefore reach their greater concentration in the medium-roasted coffees.
Some of the compounds that increase with heat treatment have a typical olfactory note, and so they contribute to characterize the aroma of the roasted coffee; these compounds can be considered "*markers of treatment*" as well as markers of quality, if their note is positively judged by the panel. Fig 53 is an example of this situation: 2-furfurylmethyl sulfide increases with roasting and contributes to bread roast flavour, because it has a "roasted" note detected by GCO analysis. Fig 54-55 scatter plots representing the correlation between a volatile compounds positive, detected by GCO analysis, and descriptors used during the sensory analysis; the regression coefficient and the equation interpolating points are below reported. The points labels indicate coffee origin and degree of roasting.

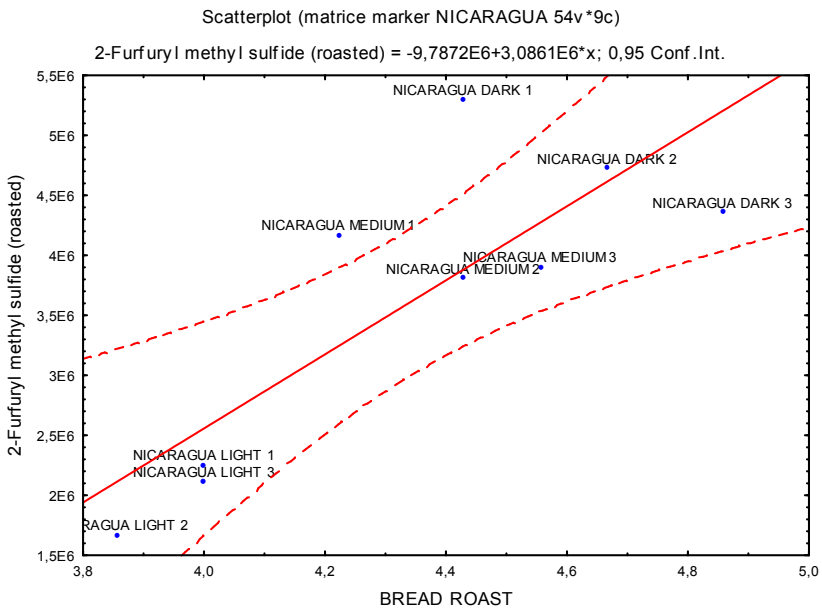

Scatterplot (matrice marker NICARAGUA 54v*9c)
2-Furfuryl methyl sulfide (roasted) = -9,7872E6+3,0861E6*x; 0,95 Conf.Int.
NICARAGUA DARK 1
NICARAGUA DARK 2
NICARAGUA DARK 3
NICARAGUA MEDIUM 1
NICARAGUA MEDIUM 3
NICARAGUA MEDIUM 2
NICARAGUA LIGHT 1
NICARAGUA LIGHT 3
RAGUA LIGHT 2
2-Furfuryl methyl sulfide (roasted)
5,5E6
5E6
4,5E6
4E6
3,5E6
3E6
2,5E6
2E6
1,5E6
3,8
4,0
4,2
4,4
4,6
4,8
5,0
BREAD ROAST

Fig 53

Other compounds characterize the light roast products, because decreasing degree in roasting process. For example 2,3-pentandione, buttery note (fig 54) is more abundant in light roast product than medium and dark roast. This "buttery" note, may remind a sweet and full flavour as like "maple syrup" note.

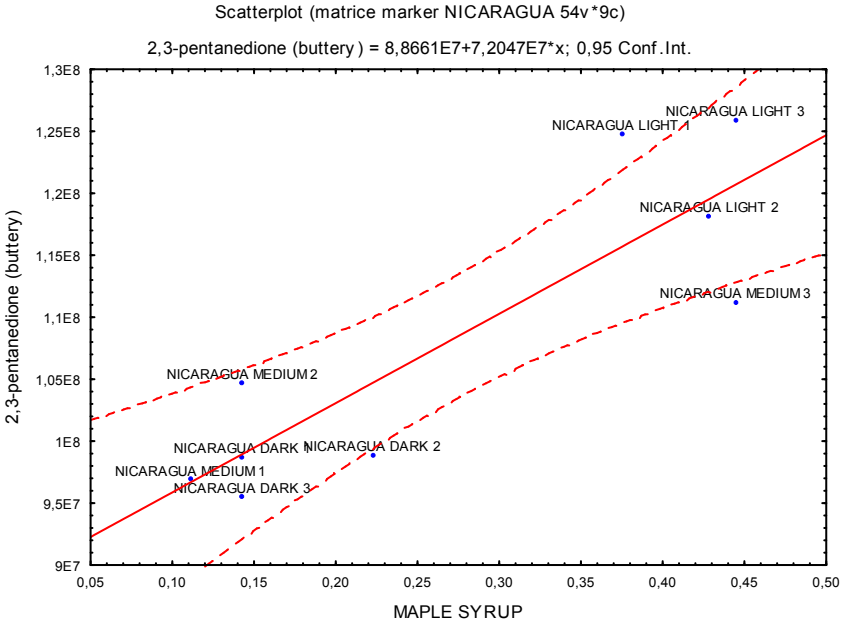


Fig 54

Among the compounds that reach their maximum concentration in medium roast coffees, the 2-ethyl-3-methylpyrazine (fig 55) has a "buttery, vanilla, sweet" aroma, that surely contribute and condition to the preference of judges (global index) for all the coffees medium samples.
Another compound that surely represents a *quality marker*, is the 5-methylfurfural; its reaches its maximum concentration in the medium treated products, and it is positively correlated with the global pleasantness of coffee (fig 56).

Otherwise, some flavour notes contribute in a contrasting way to the global aromatic result, with a masking or suppression effect. Furaneol may be an example: its "sweet, honey, caramel" note, but may be in contrast with the bread roast one; so in dark roast products, the bread roast note can emerge (fig 57). However this is only a possible hypothesis, because the decreasing of furaneol compounds, by the thermic treatment, simply may be accompanied with a increasing of the compounds with a roast note.

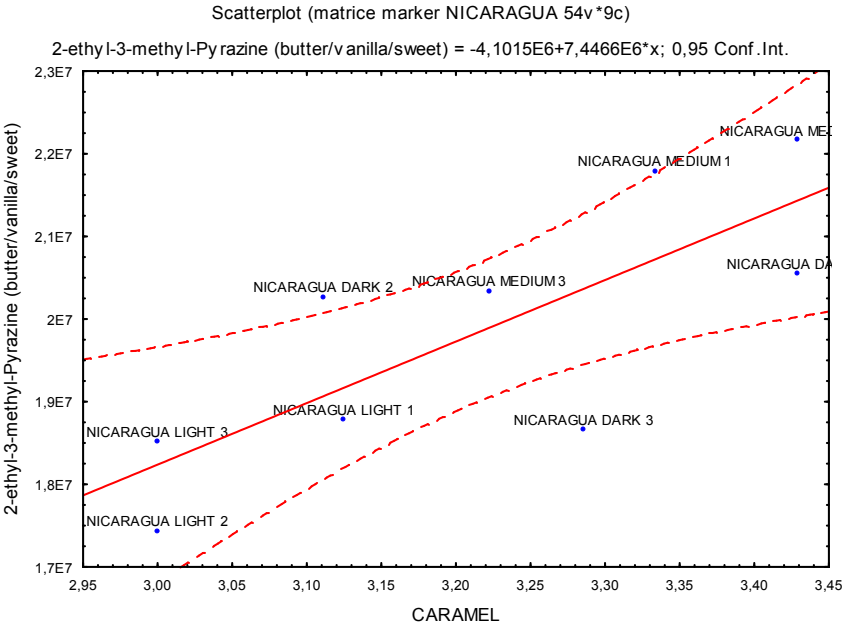
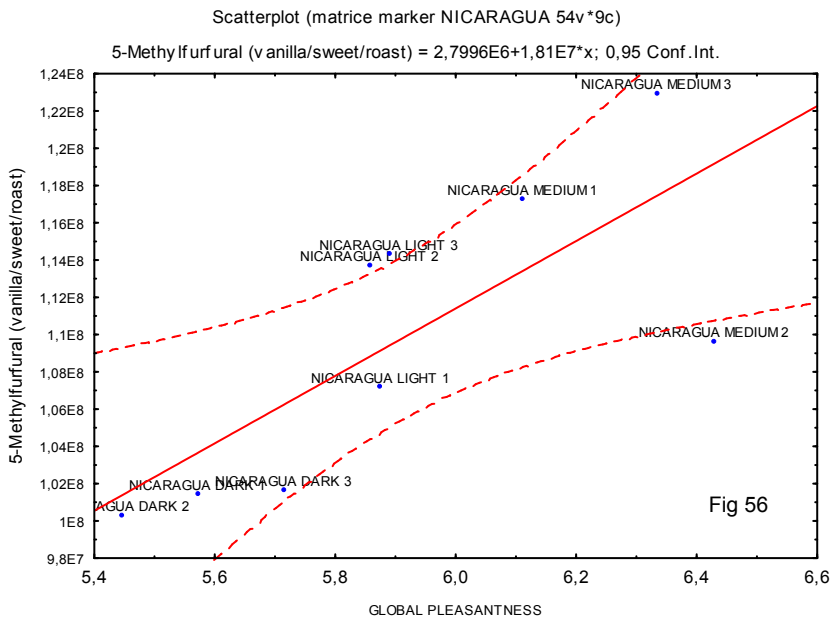
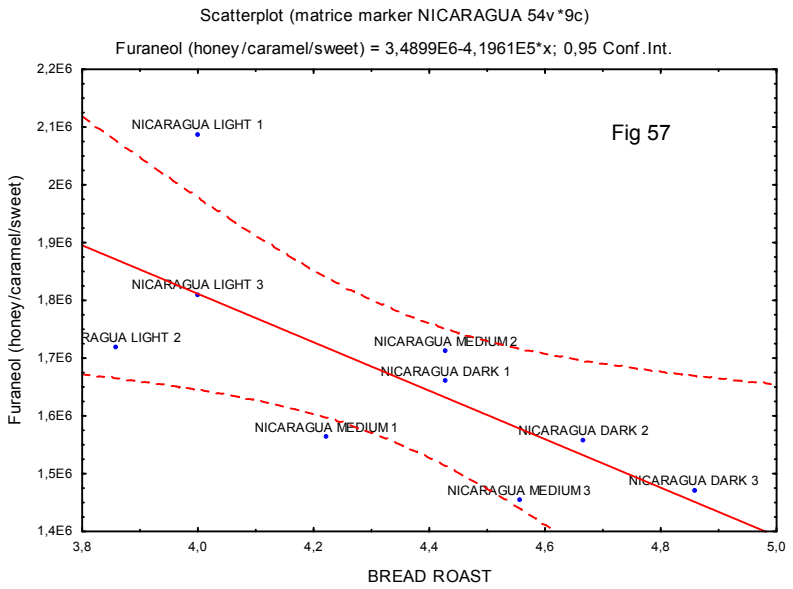
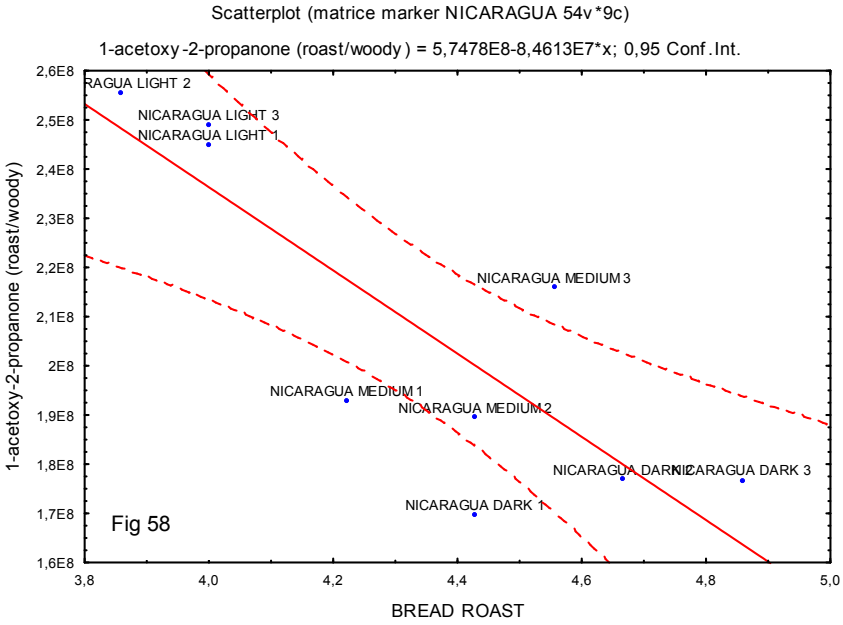


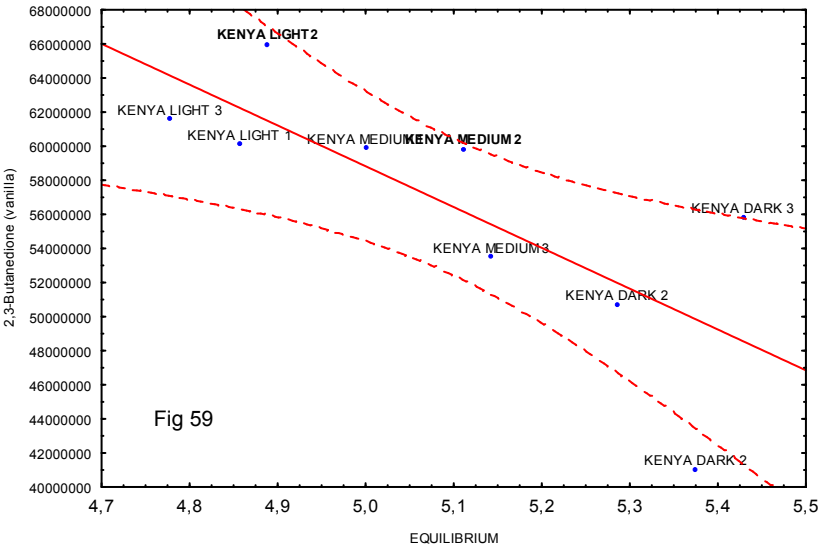
Fig 55

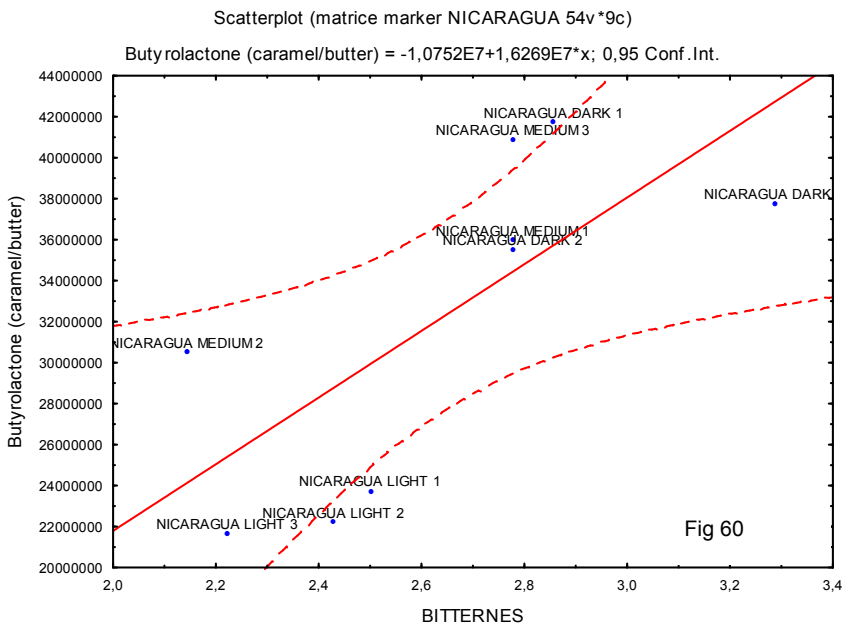

Scatterplot (matrice marker NICARAGUA 54v*9c)
5-Methylfurfural (vanilla/sweet/roast) = 2,7996E6+1,81E7*x; 0,95 Conf.Int.
NICARAGUA MEDIUM 3
NICARAGUA MEDIUM 1
NICARAGUA LIGHT 3
NICARAGUA LIGHT 2
NICARAGUA MEDIUM 2
NICARAGUA LIGHT 1
NICARAGUA DARK 1
NICARAGUA DARK 3
NICARAGUA DARK 2
Fig 56
5-Methylfurfural (vanilla/sweet/roast)
GLOBAL PLEASANTNESS


Scatterplot (matrice marker NICARAGUA 54v*9c)
Furaneol (honey/caramel/sweet) = 3,4899E6-4,1961E5*x; 0,95 Conf.Int.
NICARAGUA LIGHT 1
Fig 57
NICARAGUA LIGHT 3
NICARAGUA LIGHT 2
NICARAGUA MEDIUM 2
NICARAGUA DARK 1
NICARAGUA MEDIUM 1
NICARAGUA DARK 2
NICARAGUA DARK 3
NICARAGUA MEDIUM 3
Furaneol (honey/caramel/sweet)
BREAD ROAST

In fact, except for some simple examples, it is extremely difficult to rationalize and identify the responsible components for composition changes that lead to a change in the sensorial global result in any food. The balances between synergy, suppression and masking effects among the sensorial notes, belonging to volatile compounds, are extremely complex and as yet unknown and not understand. So much so that, even in the case of this thesis, some behaviours do not seem understandable (example, negative correlations between some compounds that carry a certain note and the same sensory analysis descriptor, fig 57) and sometimes inconsistent among the same kind of products.
Also compounds, do not always represent the real cause-effect but are only indicators of a process leading to a number of other components as a whole, that lead in turn to changes in sensory profile. For example, 2,3-butanedione (fig 59) can not lead alone to a loss of equilibrium of a product, but may only contribute or only accompany the formation of compounds, non necessarily volatile ones, that are not welcome; in the same way, butyrolactone (fig 60), can not be responsible of the increasing of bitterness, but it increases with the increasing of the heat treatment, like the real not volatile bitter compounds.
Given the complexity, this approach does not seem the right one to find, if not in some lucky cases, doubtless the real markers of quality.


Scatterplot (matrice marker NICARAGUA 54v*9c)
1-acetoxy-2-propanone (roast/woody) = 5,7478E8-8,4613E7*x; 0,95 Conf.Int.
1-acetoxy-2-propanone (roast/woody)
BREAD ROAST
RAGUA LIGHT 2
NICARAGUA LIGHT 3
NICARAGUA LIGHT 1
NICARAGUA MEDIUM 3
NICARAGUA MEDIUM 1
NICARAGUA MEDIUM 2
NICARAGUA DARK 2
NICARAGUA DARK 3
NICARAGUA DARK 1
Fig 58


Scatterplot (Spreadsheet1 53v*10c)
2,3-Butanedione (vanilla) = 1,7856E8-2,3947E7*x; 0,95 Conf.Int.
2,3-Butanedione (vanilla)
EQUILIBRIUM
KENYA LIGHT 2
KENYA LIGHT 3
KENYA LIGHT 1
KENYA MEDIUM 1
KENYA MEDIUM 2
KENYA DARK 3
KENYA MEDIUM 3
KENYA DARK 2
KENYA DARK 1
Fig 59



5.6 Conclusions

Among the coffee roasting process brings about numerous and diverse affects upon each single type of coffee. As previously noted, these affects, which are normally put down to the origin of the coffee, are in fact most probably due to the very composition of the green coffee. Furthermore, the green coffee is subject to variable growing conditions even within the country of origin causing even further variations to appear. The most notable variations occur when considering Honduras coffee. Honduras SHG coffee has shown itself to be particularly sensitive to the roasting process, whereas from the point of view of volatile fraction, coffees originating from elsewhere display far lower degrees of variation following the same roasting process. A system is already in place to assess the intensity of the roasting process placing it alongside a sensorial analysis. Compounds like

these are true examples of "cause and effect". They show that the variable degree of intensity of a process brings about a variation in the sensorial profile. With this in mind, a further series treatment markers has been established, although there is still some way to go before a definitive system can be set up due to the complexity of the markers. Certainly having seen the different behaviour for single class of compound the same coffee bean using different time-temperature conditions during roasting does not necessarily mean that coffees are equivalent in terms of aroma properties. The degree of roast is ultimately a question of definition, and the definition should depend on the specific requirements. In industial practice, where constant quality of green is roasted on the same roasting equipment, color measurement is surely an adequate, fast, and simple method for determining the degree of roast. In addition, an ideal definition of a degree of roast should also be independent from variations in raw material. Concentrations and ratios of different reaction products and remaining amounts of green coffee precursors are potentially well suited indicators; however, their analysis is usually complex but possible by analysis near infrared for industrial practice.

5.7 References

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5.8 GENERAL CONCLUSIONS TO THE THESIS

In this study six coffee samples were analysed. Each coffee sample had a different geographical origin and each sample possessed characteristics which were already recognised by the market. It is well known that the coffee market bases its comparison of these products almost exclusively on the origin of the product and the "in-the-cup" taste of the product. This somewhat simplified approach is clearly effective in terms of commercial interests and for empirical reasons. However, this standard approach leaves a lot to be desired in terms of standardisation of the various strands of production and in particular that regarding the roasting process.
The 6 samples displayed enormous differences in terms of their composition, particularly as regarded the contents of sugars, proteins and alcaloids – these being used as the aromatic degree markers in the roasting process. One must also consider that these 6 samples were only examples of coffee from those different regions.
Contrary to what is generally stated, no significant differences were noted between the aromatic quality of green coffee and roasted green coffee – the latter being the quality benchmark of the final product. It is important to keep in mind the fact that the 'quality' given to the product through the roasting process is relevant only in relation to the composition of the green coffee used.
Proof of this is found in the observed differences of behaviour resulting from the three different degrees of roasting process adopted on coffees of different origin. The most notable reaction to the roasting process came with the effect on coffee from Honduras.
The development of rational systems of roasting need to be directed towards an assessment of the macro composition of the primary matter (the green coffee itself). This also needs to be performed rapidly and to focus on an assessment of the reactivty of the system adopted.

The complexity of the subject of aroma differentiation in toasted coffee, as shown in the bibliography, and also the complexity of the tasting systems (not present in the bibliography) make it essential to adopt a system which unites the simplicity of the information available with the global nature of that information. The way forward is not based on the traditional chromatographic techniques, but instead using near infrared spectrometry in order to assess the composition of the primary material in real-time during the roasting process. However, for this technique to operate successfully an enormous wealth of samples (hundreds) would need to be worked on, but for this thesis that was not the case.
As regards the matter of composition, the calibration of results could still be carried out using traditional methods of analysis: this has been the topic of previous works. Approaching the subject from the point of view of the roasting process, however, the way forward would be to unite the results obtained from near infrared spectrometry with the sensorial analysis of the product.
The assessment of individual specific compounds would be possible only as regards some of the defects contained in the primary material, this is the case also for many other products, not just coffee. The parameters to detect a "no quality" assessment are easily identifiable. The parameters to assess "quality" are a great deal more complicated to establish as they emerge from sets of characteristics in the product and from various other threads.

Attachment I - Ethiopia coffee. Correlations of quality markers and sensorial descriptors. Marked red correlations are significant at p < ,05000.

	2-methyl-butanale (cocoa-butter/chocolate)	2,3-Butanedione (vanilla)	2,3-pentanedione (buttery)	Furfuryl methyl ether (soya bean/potato)
GLOBAL PLEASANTNESS	0,11	0,33	0,19	0,13
RANCID	0,26	**0,75**	0,42	-0,34
PHENOLIC	0,00	0,00	0,00	0,00
CARBONIZATION	0,36	-0,19	0,02	-0,19
SMOKY	0,38	-0,01	-0,07	-0,03
COAL TAR	0,03	-0,35	-0,02	-0,29
HERBACEOUS	-0,45	0,24	0,29	-0,53
TOBACCO	0,62	0,29	**0,67**	**-0,72**
RESINOUS	-0,35	-0,58	**-0,77**	**0,85**
CARAMEL	0,13	0,36	0,31	-0,45
MAPLE SYRUP	-0,26	-0,39	**-0,71**	0,62
DARK CHOCOLATE	-0,10	0,04	0,19	-0,49
MILK CHOCOLATE	-0,21	-0,35	0,18	-0,43
BREAD ROAST	-0,12	0,36	0,57	-0,49
NUT	0,29	0,51	0,31	-0,21
SPICES	0,10	0,07	0,17	-0,36
FRUITY	-0,05	0,12	0,51	**-0,72**
CITRUS	-0,05	-0,20	0,26	-0,54
FLORAL	0,43	0,16	0,49	-0,67
MALT	0,08	0,22	-0,35	**0,70**
ASTRINGENCY	-0,15	0,17	0,07	-0,34
SOFTNESS	-0,05	0,18	0,25	-0,13
BODY	-0,14	-0,21	**-0,82**	**0,97**
EQUILIBRIUM	0,16	0,18	0,29	0,01
BITTERNESS	-0,15	-0,14	-0,39	0,31
SWEET	-0,18	0,20	0,09	0,13
ACIDITY	0,24	-0,18	0,02	-0,18

2,5-dimethylpyrazine (little nut/sweet)	2,6-dimethylpyrazine (little nut toasted)	2-ethyl-6-methyl-Pyrazine (caramel sweet)	2-ethyl-3-methyl-Pyrazine (nutter/vanilla/sweet)	1-acetoxy-2-propanone (roast/woody)
0,45	0,42	-0,19	0,34	0,20
-0,11	-0,34	-0,03	-0,06	-0,37
0,00	0,00	0,00	0,00	0,00
-0,56	-0,51	0,29	-0,23	-0,24
-0,73	-0,60	0,22	-0,15	-0,19
-0,55	-0,53	0,24	-0,46	-0,44
0,16	-0,08	-0,01	-0,31	-0,49
-0,30	**-0,67**	-0,30	**-0,66**	**-0,74**
-0,12	0,32	0,10	0,40	**0,67**
0,14	-0,06	-0,15	-0,13	-0,15
0,07	0,41	-0,09	0,30	**0,74**
0,10	-0,09	0,18	-0,23	-0,25
0,04	-0,11	0,41	-0,43	-0,58
0,45	0,20	0,46	0,18	-0,39
-0,04	-0,12	0,14	0,05	-0,28
-0,30	-0,54	0,12	-0,55	-0,57
0,36	-0,02	0,54	-0,32	**-0,68**
0,15	-0,18	0,37	-0,55	-0,61
-0,12	-0,48	0,16	-0,54	-0,64
-0,21	0,08	-0,51	0,44	0,60
-0,27	-0,40	0,03	-0,36	-0,39
0,12	-0,05	**-0,76**	-0,30	-0,15
-0,25	0,27	-0,10	**0,57**	**0,86**
0,41	0,26	-0,37	0,06	0,04
-0,47	-0,19	0,37	0,20	0,14
0,49	0,47	-0,36	0,26	0,23
-0,07	-0,23	-0,44	-0,53	-0,11

2-Furfuryl methyl sulfide (roasted)	Pyrrole (vanilla/sweet)	5-Methylfurfural (vanilla/sweet/roast)	Butyrolactone (caramel/butter)	3-methyl-3-Hexen-2-one (roast)
0,07	0,07	0,29	-0,01	0,03
-0,42	-0,42	0,30	-0,58	-0,31
0,00	0,00	0,00	0,00	0,00
-0,19	-0,31	-0,22	0,00	-0,17
-0,10	-0,23	-0,31	0,02	-0,06
-0,26	-0,33	-0,41	-0,05	-0,29
-0,39	-0,35	0,22	-0,50	-0,36
-0,76	**-0,82**	-0,12	**-0,68**	**-0,79**
0,77	**0,83**	-0,39	**0,81**	**0,71**
-0,26	-0,34	0,41	-0,40	-0,18
0,74	**0,72**	-0,19	0,66	**0,75**
-0,30	-0,32	0,30	-0,30	-0,20
-0,44	-0,44	-0,29	-0,12	-0,49
-0,48	-0,48	0,66	-0,40	-0,44
-0,29	-0,42	0,09	-0,25	-0,26
-0,48	-0,38	-0,34	-0,40	-0,41
-0,72	**-0,70**	0,21	-0,47	-0,63
-0,58	-0,47	-0,20	-0,32	-0,52
-0,70	**-0,70**	-0,04	-0,54	-0,61
0,60	0,62	-0,06	0,30	0,55
-0,28	-0,26	-0,06	-0,35	-0,20
-0,12	-0,03	-0,07	-0,34	-0,24
0,92	**0,88**	-0,29	**0,78**	**0,90**
-0,08	0,01	0,16	-0,15	-0,15
0,27	0,22	-0,17	0,31	0,31
0,17	0,20	0,28	0,00	0,09
-0,13	-0,08	-0,35	-0,14	-0,14

Furfuryl alcohol (little nut toasted)	3,4-dimethyl-2,5 furandione (butter)	3-methyl-2-Butenoic acid (little nut/butter/spicy)	2-Cyclopenten-1-one, 3-ethyl-2-hydroxy- (caramel/sweet)	Furaneol (honey/caramel/sweet)
0,23	0,02	-0,42	-0,25	0,23
-0,38	-0,33	0,32	-0,25	-0,11
0,00	0,00	0,00	0,00	0,00
-0,40	-0,02	0,02	0,09	-0,03
-0,33	-0,03	0,12	0,18	-0,31
-0,46	-0,29	0,31	0,01	0,01
-0,29	-0,54	0,86	-0,24	0,20
-0,85	-0,57	-0,17	**-0,77**	0,39
0,70	0,63	-0,25	**0,72**	-0,50
-0,20	-0,15	0,37	-0,25	0,19
0,72	0,51	0,00	0,54	-0,48
-0,27	-0,17	0,51	-0,07	0,20
-0,47	**-0,70**	0,14	-0,33	0,40
-0,30	-0,33	0,22	-0,25	0,64
-0,27	-0,51	0,06	-0,33	0,08
-0,58	-0,52	0,18	-0,25	-0,19
-0,62	**-0,82**	0,22	-0,52	0,53
-0,58	**-0,68**	0,02	-0,42	0,26
-0,76	-0,56	-0,05	-0,53	0,24
0,58	0,66	-0,24	0,39	-0,55
-0,33	-0,34	0,70	-0,02	-0,20
-0,05	-0,15	-0,07	-0,46	0,16
0,84	**0,73**	-0,13	**0,78**	**-0,78**
0,05	0,01	-0,60	-0,41	0,32
0,14	0,24	0,37	0,60	-0,46
0,35	0,07	-0,13	-0,17	0,23
-0,20	-0,14	-0,26	-0,35	-0,05

Attachment II - Kenya coffee. Correlations of quality markers and sensorial descriptors. Marked red correlations are significant at p < ,05000.

	2-methyl-butanale (cocoa-butter/chocolate)	2,3-Butanedione (vanilla)	2,3-pentanedione (buttery)	Furfuryl methyl ether (soya bean/potato)
GLOBAL PLEASANTNESS	**-0,84**	-0,62	-0,48	0,23
RANCID	0,32	0,26	0,41	-0,52
PHENOLIC	0,32	0,17	0,06	0,49
CARBONIZATION	**0,79**	0,66	0,56	-0,07
SMOKY	**0,69**	0,43	0,05	0,04
COAL TAR	0,03	0,05	0,06	0,13
HERBACEOUS	0,31	-0,09	-0,13	0,54
TOBACCO	0,37	0,16	0,03	0,63
RESINOUS	0,18	0,35	0,38	0,30
CARAMEL	**-0,67**	-0,63	**-0,71**	0,28
MAPLE SYRUP	-0,23	-0,27	-0,04	0,49
DARK CHOCOLATE	-0,21	0,02	0,24	0,04
MILK CHOCOLATE	-0,58	**-0,68**	**-0,82**	0,26
BREAD ROAST	0,12	0,28	0,44	0,20
NUT	-0,36	-0,56	-0,46	**0,90**
SPICES	**0,73**	0,52	0,23	0,18
FRUITY	-0,30	-0,40	-0,41	0,50
CITRUS	-0,06	0,00	0,21	-0,28
FLORAL	0,17	-0,08	-0,43	-0,03
MALT	0,28	0,19	0,04	0,19
ASTRINGENCY	0,51	0,24	0,28	0,07
SOFTNESS	-0,41	**-0,69**	**-0,76**	0,50
BODY	0,31	-0,03	-0,08	0,53
EQUILIBRIUM	-0,45	**-0,76**	**-0,77**	0,57
BITTERNESS	0,62	0,17	-0,18	0,38
SWEET	-0,30	-0,43	-0,19	0,07
ACIDITY	0,30	0,48	**0,74**	-0,23

2,5-dimethylpyrazine (little nut/sweet)	2,6-dimethylpyrazine (little nut toasted)	2-ethyl-6-methyl-Pyrazine (caramel sweet)	2-ethyl-3-methyl-Pyrazine (butter/vanilla/sweet)	1-acetoxy-2-propanone (roast/woody)
-0,26	-0,24	-0,07	-0,07	-0,23
0,36	0,29	0,05	0,07	0,45
-0,15	-0,05	-0,13	0,15	-0,22
0,09	0,10	-0,11	0,01	0,27
-0,60	-0,59	**-0,75**	-0,56	0,10
-0,03	0,02	-0,19	0,22	0,26
-0,24	-0,18	-0,14	-0,03	-0,45
-0,01	0,10	0,08	0,25	-0,19
0,43	0,51	0,46	0,62	0,07
-0,45	-0,44	-0,16	-0,25	-0,42
0,45	0,52	0,53	0,61	0,00
0,15	0,17	0,08	0,16	0,43
-0,65	-0,64	-0,43	-0,39	-0,50
-0,02	0,04	-0,16	0,11	0,13
-0,04	0,09	0,22	0,45	-0,47
-0,17	-0,12	-0,30	-0,13	0,20
-0,31	-0,26	-0,09	-0,18	-0,29
0,61	0,56	0,61	0,48	-0,02
-0,08	-0,09	0,12	-0,05	-0,52
-0,44	-0,42	-0,35	-0,29	-0,57
0,36	0,38	0,25	0,33	-0,02
-0,60	-0,54	-0,51	-0,25	-0,09
-0,18	-0,08	-0,31	0,13	0,26
-0,47	-0,42	-0,19	-0,19	-0,58
-0,43	-0,37	-0,43	-0,20	-0,22
-0,20	-0,22	-0,20	-0,11	-0,25
0,83	**0,81**	**0,71**	0,56	0,40

2-Furfuryl methyl sulfide (roasted)	Pyrrole (vanilla/sweet)	5-Methylfurfural (vanilla/sweet/roast)	Butyrolactone (caramel/butter)	3-methyl-3-Hexen-2-one (roast)
0,07	0,28	-0,02	0,33	-0,04
-0,19	-0,40	0,15	-0,41	-0,18
0,70	**0,67**	-0,28	0,52	0,40
-0,10	-0,03	-0,11	-0,25	0,09
0,47	0,10	**-0,71**	0,25	-0,22
0,43	**0,74**	-0,04	0,47	0,47
0,14	0,20	-0,40	0,21	0,12
0,42	0,41	-0,11	0,35	0,46
0,14	0,57	0,40	0,13	**0,70**
0,07	0,16	-0,17	0,38	-0,08
0,03	0,14	0,41	0,13	0,49
-0,55	-0,01	0,27	-0,23	0,12
0,43	0,36	-0,45	0,63	-0,12
0,36	0,54	-0,15	0,17	0,16
0,27	**0,71**	0,01	0,62	**0,67**
0,42	0,07	-0,32	0,15	0,09
0,06	-0,13	-0,22	0,18	-0,14
-0,64	-0,14	0,57	-0,44	0,25
0,36	-0,02	-0,06	0,23	0,06
0,41	0,46	-0,55	0,25	-0,08
0,09	-0,01	0,09	-0,09	0,25
0,23	0,31	-0,43	**0,63**	0,09
0,29	0,45	-0,27	0,45	0,45
0,10	0,10	-0,36	0,42	-0,05
0,51	0,33	-0,56	0,44	0,18
-0,19	0,14	-0,25	0,05	-0,13
-0,68	-0,44	**0,69**	**-0,74**	0,18

Furfuryl alcohol (little nut toasted)	3,4-dimethyl-2,5 furandione (butter)	3-methyl-2-Butenoic acid (little nut/butter/spicy)	2-Cyclopenten-1-one, 3-ethyl-2-hydroxy- (caramel/sweet)	Furaneol (honey/caramel/sweet)
0,60	0,17	0,05	0,13	-0,29
-0,46	-0,56	0,30	-0,20	0,57
0,37	0,45	-0,04	-0,09	-0,36
-0,44	0,25	-0,29	-0,10	0,10
-0,08	-0,07	-0,55	-0,52	-0,50
0,56	0,15	0,28	-0,03	0,08
-0,03	0,46	**-0,77**	-0,02	-0,44
0,13	0,61	-0,15	0,20	-0,33
0,22	0,66	0,45	0,40	0,20
0,50	0,07	-0,27	0,08	-0,47
0,13	0,41	0,37	0,55	0,15
-0,04	0,48	-0,05	0,39	0,21
0,68	-0,17	-0,32	-0,22	-0,58
0,25	0,52	0,12	-0,11	-0,13
0,63	**0,78**	-0,24	0,47	-0,35
-0,19	0,13	-0,16	-0,17	-0,26
0,07	0,32	-0,41	0,17	-0,54
-0,25	-0,04	0,18	0,31	**0,68**
0,06	-0,48	0,05	-0,17	-0,12
0,19	0,32	-0,56	-0,42	-0,53
-0,29	-0,01	0,05	0,01	0,26
0,55	0,09	-0,53	0,05	-0,51
0,24	0,40	-0,34	0,13	-0,19
0,34	0,17	-0,67	0,09	-0,59
0,08	0,13	-0,61	-0,26	-0,47
0,15	0,06	-0,52	-0,13	-0,08
-0,67	0,26	0,39	0,45	0,71

Attachment III - Guatemala coffee. Correlations of quality markers and sensorial descriptors. Marked red correlations are significant at p < ,05000.

	2-methyl-butanale (cocoa-butter/chocolate)	2,3-Butanedione (vanilla)	2,3-pentanedione (buttery)	Furfuryl methyl ether (soya bean/potato)
GLOBAL PLEASANTNESS	-0,16	-0,28	0,03	-0,13
RANCID				
PHENOLIC				
CARBONIZATION	-0,16	-0,09	-0,28	0,36
SMOKY	-0,08	0,13	-0,13	0,63
COAL TAR	0,07	0,25	-0,23	0,41
HERBACEOUS	0,12	0,04	0,06	-0,07
TOBACCO	-0,09	0,02	-0,25	0,40
RESINOUS	0,04	-0,05	0,33	-0,08
CARAMEL	-0,08	-0,32	0,13	**-0,83**
MAPLE SYRUP	0,21	0,09	0,45	-0,46
DARK CHOCOLATE	0,64	**0,68**	0,38	0,09
MILK CHOCOLATE	0,05	-0,17	0,13	-0,56
BREAD ROAST	-0,20	-0,02	-0,57	0,45
NUT	0,14	0,26	0,05	0,49
SPICES	0,51	0,55	0,59	0,26
FRUITY	0,47	0,28	**0,67**	-0,58
CITRUS	-0,05	-0,25	0,36	**-0,68**
FLORAL	0,05	-0,17	0,38	**-0,75**
MALT	-0,09	-0,01	-0,44	0,10
ASTRINGENCY	0,20	0,16	0,17	0,05
SOFTNESS	-0,12	-0,16	0,28	-0,19
BODY	-0,37	-0,29	-0,50	0,13
EQUILIBRIUM	0,55	0,51	0,60	-0,15
BITTERNESS	-0,20	0,01	-0,45	0,36
SWEET	0,58	0,42	**0,73**	-0,30
ACIDITY	-0,35	-0,49	0,06	-0,42

2,5-dimethylpyrazine (little nut/sweet)	2,6-dimethylpyrazine (little nut toasted)	2-ethyl-6-methyl-Pyrazine (caramel sweet)	2-ethyl-3-methyl-Pyrazine (butter/vanilla/sweet)	1-acetoxy-2-propanone (roast/woody)
0,48	-0,31	0,01	0,12	-0,28
0,05	-0,13	-0,21	0,58	0,47
-0,07	-0,08	-0,02	0,42	0,55
-0,45	0,41	-0,19	0,16	0,62
-0,02	0,00	-0,25	0,00	0,26
-0,17	0,10	-0,31	0,40	**0,71**
0,46	-0,58	0,31	0,00	-0,49
0,02	-0,14	0,12	-0,45	-0,65
0,09	-0,15	0,29	-0,60	-0,62
-0,30	0,27	0,08	-0,37	0,01
0,19	-0,03	0,00	-0,31	-0,51
-0,29	**0,69**	-0,38	0,32	0,66
-0,05	-0,04	-0,18	0,26	0,61
0,03	-0,43	0,22	-0,11	0,07
0,07	-0,40	0,40	-0,61	**-0,78**
0,24	-0,48	0,37	-0,45	**-0,83**
0,15	-0,26	0,33	**-0,62**	**-0,87**
-0,36	0,85	-0,29	0,09	0,36
-0,03	-0,30	-0,12	0,16	0,29
0,22	-0,47	0,34	-0,21	-0,42
-0,24	0,34	-0,37	0,27	0,58
-0,08	-0,07	0,27	-0,57	-0,34
-0,44	0,62	-0,20	0,13	0,55
0,23	-0,43	0,30	-0,47	-0,57
0,35	-0,53	0,23	-0,04	-0,54

2-Furfuryl methyl sulfide (roasted)	Pyrrole (vanilla/sweet)	5-Methylfurfural (vanilla/sweet/roast)	Butyrolactone (caramel/butter)	3-methyl-3-Hexen-2-one (roast)
-0,29	-0,45	0,50	-0,48	-0,16
0,38	0,38	0,09	0,32	0,41
0,43	0,45	0,06	0,49	0,44
0,55	0,67	-0,46	**0,72**	0,44
0,03	-0,05	0,20	0,00	0,08
0,47	0,44	0,01	0,50	0,51
-0,42	-0,46	0,53	-0,55	-0,35
-0,56	-0,57	-0,04	-0,62	-0,48
-0,56	-0,59	0,16	-0,56	-0,55
0,08	0,26	-0,34	0,25	-0,20
-0,43	-0,49	0,10	-0,53	-0,41
0,66	0,65	-0,49	**0,74**	0,58
0,34	0,30	0,24	0,38	0,35
-0,09	-0,02	0,38	-0,02	-0,16
-0,66	-0,54	0,13	-0,64	**-0,74**
-0,74	**-0,76**	0,28	**-0,82**	-0,61
-0,74	**-0,76**	0,14	**-0,79**	**-0,67**
0,44	0,50	-0,62	0,54	0,32
0,07	0,09	0,25	0,06	0,09
-0,45	-0,51	0,39	-0,49	-0,28
0,41	0,32	-0,21	0,43	0,51
-0,32	-0,24	0,05	-0,22	-0,46
0,55	0,59	-0,58	**0,71**	0,49
-0,55	-0,51	0,40	-0,57	-0,64
-0,51	-0,61	0,41	-0,66	-0,28

Furfuryl alcohol (little nut toasted)	3,4-dimethyl-2,5 furandione (butter)	3-methyl-2-Butenoic acid (little nut/butter/spicy)	2-Cyclopenten-1-one, 3-ethyl-2-hydroxy- (caramel/sweet)	Furaneol (honey/caramel/sweet)
-0,31	-0,42	0,32	-0,56	0,33
-0,39	-0,50	-0,03	-0,54	0,33
0,29	0,43	-0,34	0,58	-0,47
-0,33	-0,38	-0,35	-0,30	0,03
0,38	0,33	0,01	0,41	-0,33
-0,25	-0,43	0,12	-0,49	0,25
0,37	-0,03	0,38	0,02	-0,29
0,12	0,39	-0,34	0,50	-0,37
-0,75	**-0,68**	-0,24	**-0,72**	0,29
-0,62	**-0,68**	0,02	**-0,76**	0,27
-0,57	**-0,68**	-0,13	**-0,67**	0,03
0,21	-0,19	0,19	-0,14	-0,09
0,54	0,26	0,21	0,32	-0,11
0,37	0,63	-0,25	**0,71**	-0,23
-0,49	-0,30	-0,15	-0,37	0,38
-0,08	-0,02	-0,43	0,12	-0,29
-0,58	-0,53	-0,33	-0,53	0,30
-0,57	-0,55	-0,14	-0,55	0,00
-0,22	-0,28	0,33	-0,46	0,54
0,61	0,37	0,24	0,44	-0,35
0,17	0,01	0,07	0,05	-0,02
0,45	0,37	-0,14	0,54	-0,66
0,56	0,34	0,29	0,37	-0,24
0,54	0,37	0,50	0,32	-0,18
-0,17	-0,08	0,26	-0,27	0,70

Attachment IV - Nicaragua coffee. Correlations of quality markers and sensorial descriptors. Marked red correlations are significant at p < ,05000.

	2-methyl-butanale (cocoa-butter/chocolate)	2,3-Butanedione (vanilla)	2,3-pentanedione (buttery)	Furfuryl methyl ether (soya bean/potato)
GLOBAL PLEASANTNESS	-0,01	-0,29	0,18	-0,05
RANCID				
PHENOLIC	0,21	0,57	-0,40	-0,10
CARBONIZATION	0,00	0,62	-0,66	0,35
SMOKY	0,55	0,35	0,36	**-0,74**
COAL TAR	0,19	0,67	-0,27	-0,13
HERBACEOUS	-0,50	0,11	-0,52	0,50
TOBACCO	0,40	**0,94**	-0,19	-0,14
RESINOUS	0,32	0,58	-0,18	-0,19
CARAMEL	**-0,72**	-0,11	**-0,71**	**0,76**
MAPLE SYRUP	0,56	0,05	**0,87**	**-0,67**
DARK CHOCOLATE	0,13	**0,71**	-0,44	0,28
MILK CHOCOLATE	-0,44	**-0,76**	-0,02	0,00
BREAD ROAST	-0,42	0,40	**-0,77**	0,65
NUT	-0,50	-0,15	-0,51	0,63
SPICES	-0,04	0,45	-0,47	0,52
FRUITY	-0,12	0,04	-0,38	0,48
CITRUS	0,19	-0,35	0,55	**-0,67**
FLORAL	0,02	0,17	-0,29	0,01
MALT	-0,27	0,34	**-0,75**	0,62
ASTRINGENCY	0,49	0,48	0,21	-0,40
SOFTNESS	-0,18	-0,58	0,35	-0,30
BODY	0,43	0,63	-0,22	0,12
EQUILIBRIUM	-0,15	**-0,80**	0,34	-0,23
BITTERNESS	-0,02	**0,86**	-0,66	0,38
SWEET	-0,31	**-0,72**	0,08	-0,16
ACIDITY	0,64	0,15	0,52	-0,51

2,5-dimethylpyrazine (little nut/sweet)	2,6-dimethylpyrazine (little nut toasted)	2-ethyl-6-methyl-Pyrazine (caramel sweet)	2-ethyl-3-methyl-Pyrazine (butter/vanilla/sweet)	1-acetoxy-2-propanone (roast/woody)
0,71	0,62	**0,72**	0,43	0,22
-0,44	-0,42	-0,33	-0,28	-0,34
-0,51	-0,37	-0,49	-0,09	-0,63
-0,65	**-0,73**	-0,43	**-0,73**	0,49
-0,80	-0,73	**-0,70**	-0,57	-0,23
-0,07	0,07	-0,04	0,19	-0,52
-0,84	**-0,73**	**-0,76**	-0,56	-0,07
-0,63	-0,61	-0,66	-0,51	-0,20
0,51	0,59	0,58	**0,78**	**-0,77**
-0,31	-0,40	-0,43	-0,64	**0,87**
-0,38	-0,28	-0,49	-0,06	-0,47
0,66	0,53	**0,72**	0,35	-0,10
0,03	0,16	-0,02	0,38	**-0,84**
0,21	0,26	0,03	0,45	**-0,72**
0,00	0,12	-0,23	0,29	-0,53
0,38	0,42	0,14	0,48	-0,49
0,06	-0,12	0,16	-0,41	0,54
0,16	0,10	0,18	0,13	-0,31
0,19	0,29	0,14	0,50	**-0,78**
-0,43	-0,46	-0,38	-0,48	0,27
0,27	0,17	0,48	0,00	0,40
-0,26	-0,18	-0,44	-0,03	-0,20
0,60	0,46	**0,68**	0,24	0,35
-0,53	-0,36	-0,45	-0,03	-0,59
0,32	0,21	0,49	0,08	0,07
-0,20	-0,26	-0,22	-0,38	0,60

2-Furfuryl methyl sulfide (roasted)	Pyrrole (vanilla/sweet)	5-Methylfurfural (vanilla/sweet/roast)	Butyrolactone (caramel/butter)	3-methyl-3-Hexen-2-one (roast)
-0,21	0,06	**0,75**	-0,06	-0,02
0,23	0,33	-0,39	0,26	0,29
0,62	0,53	-0,56	0,58	0,59
-0,59	-0,40	-0,12	-0,52	-0,43
0,17	0,25	-0,59	0,20	0,21
0,48	0,22	-0,57	0,35	0,27
0,11	0,41	-0,18	0,29	0,42
0,14	0,20	-0,43	0,16	0,20
0,75	0,64	-0,19	**0,70**	0,55
-0,76	-0,42	0,52	-0,56	-0,44
0,54	**0,67**	-0,15	0,66	**0,70**
-0,08	-0,31	-0,17	-0,28	-0,44
0,82	**0,74**	-0,41	**0,81**	**0,73**
0,73	0,36	-0,49	0,55	0,38
0,64	0,63	0,01	**0,70**	**0,74**
0,55	0,48	0,13	0,56	0,54
-0,68	-0,49	0,12	-0,64	**-0,67**
0,22	0,37	-0,01	0,28	0,26
0,79	**0,79**	-0,12	**0,82**	**0,79**
-0,23	0,21	0,26	0,01	0,11
-0,50	-0,54	0,05	-0,58	-0,65
0,31	0,41	0,19	0,43	0,57
-0,46	-0,64	0,19	-0,61	**-0,67**
0,62	**0,74**	-0,35	**0,72**	**0,77**
-0,26	-0,58	-0,36	-0,52	-0,66
-0,51	-0,15	0,62	-0,30	-0,14

0,05	0,24	-0,30	0,03		-0,03
0,16	0,32	0,05	-0,07		-0,38
0,42	0,35	0,39	0,20		-0,53
-0,41	-0,31	-0,13	-0,60		0,37
0,15	0,14	0,23	-0,21		-0,23
0,36	0,25	0,29	0,59		-0,29
0,39	0,21	0,51	-0,35		-0,37
0,10	0,01	0,32	-0,25		-0,35
0,49	**0,82**	-0,36	**0,75**		-0,34
-0,30	-0,60	0,21	**-0,82**		0,35
0,58	0,41	0,46	0,00		-0,65
-0,39	0,00	-0,66	0,37		0,25
0,69	**0,68**	0,26	0,57		**-0,72**
0,24	0,26	-0,03	0,52		-0,30
0,66	0,35	0,62	0,28		**-0,74**
0,42	0,29	0,27	0,34		-0,59
-0,58	-0,29	-0,53	-0,46		0,45
0,17	0,43	-0,22	0,07		-0,37
0,64	**0,73**	0,14	0,49		**-0,72**
0,04	0,10	0,00	-0,62		-0,07
-0,53	-0,19	-0,66	-0,03		0,66
0,45	0,08	**0,70**	-0,10		-0,62
-0,58	-0,34	-0,54	0,13		0,55
0,68	0,61	0,42	0,18		-0,61
-0,63	-0,25	**-0,67**	0,23		0,55
-0,22	-0,28	0,04	**-0,70**		0,19
Furfuryl alcohol (little nut toasted)	3,4-dimethyl-2,5 furandione (butter)	3-methyl-2-Butenoic acid (little nut/butter/spicy)	2-Cyclopenten-1-one, 3-ethyl-2-hydroxy- (caramel/sweet)		Furaneol (honey/caramel/sweet)

Attachment V - Honduras coffee. Correlations of quality markers and sensorial descriptors. Marked red correlations are significant at p < ,05000.

	2-methyl-butanale (cocoa-butter/chocolate)	2,3-Butanedione (vanilla)	2,3-pentanedione (buttery)
GLOBAL PLEASANTNESS	-0,65	-0,51	-0,58
RANCID	-0,20	-0,45	0,01
PHENOLIC	-0,48	-0,28	-0,40
CARBONIZATION	0,30	0,36	0,24
SMOKY	0,08	0,32	-0,03
COAL TAR	0,53	0,57	0,35
HERBACEOUS	0,26	0,44	0,08
TOBACCO	0,38	0,25	0,46
RESINOUS	0,09	0,34	-0,01
CARAMEL	-0,31	-0,04	-0,51
MAPLE SYRUP	-0,39	-0,49	-0,20
DARK CHOCOLATE	0,60	0,56	0,46
MILK CHOCOLATE	-0,43	-0,27	-0,37
BREAD ROAST	0,16	0,22	-0,03
NUT	**-0,82**	**-0,83**	**-0,68**
SPICES	-0,02	-0,14	-0,05
FRUITY	0,12	0,37	0,00
CITRUS	**-0,71**	**-0,70**	-0,57
FLORAL	0,32	0,03	0,42
MALT	-0,25	-0,50	-0,09
ASTRINGENCY	0,17	0,28	0,09
SOFTNESS	-0,51	-0,56	-0,38
BODY	0,32	0,33	0,27
EQUILIBRIUM	-0,26	-0,49	-0,18
BITTERNESS	0,62	**0,73**	0,46
SWEET	-0,26	-0,52	-0,15
ACIDITY	0,23	-0,13	0,44

0,09	0,32	0,28	0,03	0,03	0,37
-0,31	0,13	0,08	0,03	0,13	-0,42
-0,13	-0,60	-0,62	-0,45	-0,42	-0,17
-0,20	-0,32	-0,32	0,12	-0,17	0,06
-0,13	-0,39	-0,38	0,00	0,06	-0,16
0,27	-0,03	-0,02	-0,04	-0,16	0,29
0,30	0,09	0,08	-0,04	0,29	0,40
-0,12	0,22	0,20	0,63	0,40	0,19
0,26	-0,17	-0,19	-0,21	0,19	-0,06
0,25	0,20	0,20	-0,06	-0,06	0,05
-0,39	-0,04	-0,11	-0,03	0,05	-0,31
0,27	0,32	0,38	0,34	0,40	-0,61
0,34	0,00	-0,01	-0,22	-0,08	0,27
0,07	-0,03	0,01	-0,23	-0,07	-0,10
-0,45	-0,33	-0,35	-0,60	-0,31	0,22
-0,51	-0,55	-0,54	-0,51	-0,61	-0,41
0,01	-0,22	-0,22	0,02	0,27	0,00
-0,21	0,09	0,03	-0,08	-0,10	-0,17
0,39	0,43	0,48	0,11	0,22	-0,06
-0,34	-0,35	-0,32	-0,44	-0,61	0,16
-0,20	-0,46	-0,47	-0,11	-0,41	0,11
-0,70	-0,26	-0,29	-0,08	0,00	
-0,18	-0,20	-0,20	0,27	-0,17	
-0,09	0,32	0,34	-0,16	0,17	
0,29	-0,03	-0,02	0,32	-0,06	
-0,49	0,08	0,11	-0,10	0,16	
-0,17	0,24	0,22	0,38	0,11	
Furfuryl methyl ether (soya bean/potato)	2,5-dimethylpyrazine (little nut/sweet)	2,6-dimethylpyrazine (little nut toasted)	2-ethyl-6-methyl-Pyrazine (caramel sweet)	2-ethyl-3-methyl-Pyrazine (butter/vanilla/sweet)	

1-acetoxy-2-propanone (roast/woody)	2-Furfuryl methyl sulfide (roasted)	Pyrrole (vanilla/sweet)	5-Methylfurfural (vanilla/sweet/roast)	Butyrolactone (caramel/butter)
-0,67	-0,22	-0,45	0,21	-0,27
-0,35	-0,38	-0,39	0,15	-0,36
-0,50	-0,46	-0,06	**-0,72**	-0,37
0,35	0,09	0,24	-0,12	0,26
0,10	0,12	0,16	-0,18	0,24
0,72	0,51	0,48	0,14	0,51
0,40	0,54	0,21	0,32	0,44
0,24	0,11	0,05	0,33	0,21
0,16	0,27	0,39	0,01	0,40
-0,01	0,27	-0,23	0,20	0,05
-0,49	-0,49	-0,37	0,01	-0,33
0,56	0,52	0,51	0,40	0,60
-0,43	-0,17	-0,04	-0,28	-0,24
0,28	0,24	0,33	0,09	0,36
-0,89	**-0,74**	-0,55	-0,44	**-0,71**
0,02	-0,33	0,02	-0,43	-0,17
0,14	0,23	0,26	0,02	0,39
-0,67	-0,49	-0,62	-0,01	-0,53
0,08	0,01	0,51	0,11	0,16
-0,40	-0,64	-0,10	-0,64	-0,58
0,31	0,03	0,19	-0,27	0,17
-0,63	-0,62	-0,54	-0,15	-0,44
0,37	0,09	0,19	-0,07	0,23
-0,41	-0,28	-0,22	0,16	-0,27
0,78	0,59	0,41	0,11	0,51
-0,46	-0,43	-0,40	0,08	-0,38
0,04	-0,18	-0,14	0,17	-0,19

3-methyl-3-Hexen-2-one (roast)	Furfuryl alcohol (little nut toasted)	3,4-dimethyl-2,5 furandione (butter)	3-methyl-2-Butenoic acid (little nut/butter/spicy)	2-Cyclopenten-1-one, 3-ethyl-2-hydroxy- (caramel/sweet)
-0,42	-0,22	-0,05	0,49	-0,12
-0,32	-0,24	-0,22	0,28	-0,13
-0,45	-0,55	-0,60	-0,50	-0,46
0,33	0,08	0,06	-0,27	-0,01
0,23	0,01	0,28	-0,25	-0,08
0,55	0,49	0,35	0,09	0,59
0,41	0,47	**0,68**	0,38	0,61
0,34	0,24	0,19	0,04	0,03
0,29	0,21	0,40	0,26	0,47
-0,04	0,15	0,19	0,29	0,15
-0,36	-0,38	-0,29	0,25	-0,24
0,62	0,53	0,65	0,15	0,40
-0,35	-0,22	-0,41	-0,06	-0,10
0,27	0,17	0,39	0,18	0,26
-0,85	**-0,79**	-0,54	-0,06	-0,62
-0,16	-0,37	-0,24	-0,34	-0,29
0,33	0,14	0,49	0,04	0,15
-0,60	-0,44	-0,49	0,26	-0,34
0,12	0,15	-0,10	0,10	0,19
-0,57	-0,62	**-0,80**	-0,59	-0,63
0,21	-0,01	-0,07	-0,32	-0,01
-0,48	-0,60	-0,22	-0,04	-0,65
0,32	0,10	-0,01	-0,32	-0,08
-0,36	-0,22	-0,03	0,31	-0,16
0,64	0,56	0,30	-0,20	0,43
-0,40	-0,38	0,00	0,12	-0,44
-0,03	0,01	-0,26	-0,02	-0,09

Furaneol (honey/caramel/sweet)	0,25		
	0,00		
	-0,70		
	-0,12		
	-0,14		
	-0,03		
	0,10		
	0,20		
	-0,30		
	0,45		
	-0,06		
	0,39		
	-0,34		
	0,23		
	-0,23		
	-0,23		
	-0,06		
	0,10		
	-0,04		
	-0,41		
	-0,25		
	0,10		
	0,01		
	0,31		
	-0,04		
	0,33		
	0,01		

Attachment VI - Brazil coffee. Correlations of quality markers and sensorial descriptors. Marked red correlations are significant at p < ,05000.

	2-methyl-butanale (cocoa-butter/chocolate)	2,3-Butanedione (vanilla)	2,3-pentanedione (buttery)
GLOBAL PLEASANTNESS	0,05	-0,03	-0,03
RANCID	-0,01	0,34	0,18
PHENOLIC	-0,39	-0,40	-0,53
CARBONIZATION	-0,14	-0,46	0,06
SMOKY	0,23	-0,18	-0,56
COAL TAR	-0,18	-0,19	-0,06
HERBACEOUS	0,10	-0,46	-0,39
TOBACCO	-0,45	-0,52	-0,45
RESINOUS	**-0,67**	-0,50	0,19
CARAMEL	0,40	0,14	-0,11
MAPLE SYRUP	-0,64	-0,24	-0,12
DARK CHOCOLATE	-0,23	-0,59	0,43
MILK CHOCOLATE	0,58	0,33	-0,36
BREAD ROAST	-0,61	**-0,68**	-0,22
NUT	-0,22	-0,50	-0,26
SPICES	-0,26	-0,28	-0,51
FRUITY	-0,08	-0,26	-0,46
CITRUS	0,17	0,18	**0,82**
FLORAL	0,47	**0,77**	-0,16
MALT	0,20	-0,31	-0,27
ASTRINGENCY	-0,20	0,00	-0,09
SOFTNESS	0,42	-0,08	-0,43
BODY	-0,15	-0,53	-0,40
EQUILIBRIUM	0,42	-0,02	-0,41
BITTERNESS	0,26	-0,16	-0,54
SWEET	0,41	-0,13	0,09
ACIDITY	-0,60	-0,31	0,59

Furfurylmethyl ether (soya bean/potato)	2,5-dimethylpyrazine (little nut/sweet)	2,6-dimethylpyrazine (little nut toasted)	2-ethyl-6-methyl-Pyrazine (caramel sweet)	2-ethyl-3-methyl-Pyrazine (butter/vanilla/sweet)	1-acetoxy-2-propanone (roast/woody)
0,49	-0,62	0,26	-0,10	-0,49	-0,80
-0,37	0,34	0,21	0,41	-0,03	0,53
0,49	-0,22	0,13	0,22	0,04	0,30
-0,29	0,44	-0,50	-0,02	0,50	0,40
0,30	0,26	-0,44	-0,35	0,50	0,24
0,47	-0,44	0,35	0,42	-0,32	-0,23
-0,07	0,57	**-0,79**	-0,38	**0,79**	0,09
0,15	0,05	-0,18	-0,14	0,31	0,37
0,08	-0,40	0,30	0,43	-0,27	0,14
0,33	-0,29	-0,11	-0,45	-0,08	**-0,77**
-0,09	-0,11	0,27	0,08	-0,15	0,24
-0,16	-0,13	-0,30	-0,21	-0,08	-0,57
0,57	-0,27	0,00	-0,29	-0,06	**-0,69**
0,52	-0,49	0,27	0,42	-0,34	0,04
0,24	-0,11	-0,24	-0,18	0,19	-0,41
0,12	0,19	-0,17	-0,19	0,35	0,62
0,13	0,05	-0,32	-0,65	0,22	-0,14
-0,69	0,34	-0,14	0,35	0,09	-0,11
-0,08	0,23	0,14	0,04	0,21	0,38
0,38	0,01	-0,34	0,07	0,29	-0,07
-0,34	0,48	-0,07	0,30	0,35	**0,71**
0,36	0,05	-0,44	**-0,67**	0,30	**-0,73**
0,15	0,23	-0,44	-0,30	0,45	-0,05
0,13	0,26	-0,56	-0,66	0,46	-0,49
0,13	0,47	-0,57	-0,33	**0,73**	0,25
0,00	0,26	-0,47	-0,06	0,27	-0,57
-0,75	0,26	0,00	0,28	-0,01	0,51

2-Furfuryl methyl sulfide (roasted)	Pyrrole (vanilla/sweet)	5-Methylfurfural (vanilla/sweet/roast)	Butyrolactone (caramel/butter)	3-methyl-3-Hexen-2-one (roast)	Furfuryl alcohol (little nut toasted)
0,05	-0,12	0,28	0,48	0,35	0,45
-0,26	-0,21	0,18	-0,03	-0,07	0,14
0,54	0,16	0,03	0,19	0,36	-0,20
-0,01	0,30	-0,42	-0,62	-0,53	-0,64
0,61	0,71	-0,49	-0,32	-0,17	-0,50
0,31	-0,08	0,41	0,33	0,36	0,24
0,34	**0,69**	**-0,81**	**-0,68**	-0,60	**-0,93**
0,34	0,25	-0,27	-0,17	0,09	-0,48
-0,09	-0,41	0,37	0,17	0,29	0,05
0,21	0,19	-0,09	0,16	0,09	0,15
-0,16	-0,28	0,15	0,13	0,31	0,06
-0,43	-0,02	-0,12	-0,35	-0,39	-0,12
0,54	0,33	-0,04	0,32	0,23	0,19
0,16	-0,10	0,27	0,31	0,31	0,04
0,29	0,24	-0,24	-0,12	-0,01	-0,36
0,38	0,36	-0,29	-0,21	0,07	-0,40
0,14	0,37	-0,44	-0,14	-0,03	-0,27
-0,57	-0,36	0,06	-0,34	-0,52	-0,07
0,33	-0,01	0,05	0,16	0,23	0,07
0,48	0,39	-0,29	-0,10	-0,19	-0,44
0,01	0,01	-0,13	-0,26	-0,15	-0,36
0,48	**0,68**	-0,46	-0,23	-0,15	-0,24
0,40	0,58	-0,46	-0,47	-0,23	-0,57
0,34	0,60	-0,61	-0,30	-0,30	-0,42
0,66	**0,74**	-0,62	-0,49	-0,29	**-0,73**
0,04	0,36	-0,36	-0,34	-0,57	-0,33
-0,78	-0,50	0,07	-0,34	-0,31	-0,13

3,4-dimethyl-2,5 furandione (butter)	3-methyl-2-Butenoic acid (little nut/butter/spicy)	2-Cyclopenten-1-one, 3-ethyl-2-hydroxy- (caramel/sweet)	Furaneol (honey/caramel/sweet)
-0,36	-0,16	-0,16	-0,25
0,16	0,12	-0,32	0,32
0,57	-0,12	0,09	-0,39
0,48	0,15	0,35	-0,04
0,69	0,08	0,60	-0,43
0,79	-0,04	-0,15	-0,30
0,18	-0,63	**0,75**	-0,59
0,38	-0,04	0,23	-0,25
0,32	0,25	-0,32	0,16
-0,36	-0,29	0,25	-0,30
-0,09	-0,02	-0,37	0,09
-0,03	0,20	0,00	-0,03
-0,05	-0,51	0,33	-0,54
0,38	0,13	-0,22	-0,29
0,16	-0,62	0,29	-0,57
0,61	0,32	0,24	-0,11
-0,36	-0,12	0,28	-0,29
-0,27	-0,26	-0,10	0,37
-0,07	-0,22	0,14	0,19
0,16	-0,40	0,52	-0,49
0,28	-0,19	0,01	0,07
0,34	-0,50	0,57	**-0,75**
0,88	-0,24	0,45	-0,60
-0,24	**-0,72**	0,62	-0,61
0,71	-0,35	**0,75**	-0,54
-0,06	**-0,73**	0,41	-0,54
-0,29	0,42	-0,42	0,66