



UNIVERSITÀ  
DEGLI STUDI  
FIRENZE

DOTTORATO DI RICERCA IN  
Gestione Sostenibile delle Risorse Agrarie, Forestali e  
Alimentari

CICLO XXVIII

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**Factors affecting bitterness perception  
and preference for coffee**

Settore Scientifico Disciplinare AGR/15

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Anni 2012/2015

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## **Abstract**

The instinctive rejection of bitter taste has been crucial to the survival of our species and continues to influence food choices. In fact, the bitter taste receptors have evolved to protect the body from the ingestion of foods potentially harmful to health. However, some foods are widely consumed despite their high intensity of bitterness, for example coffee, one of the world's most popular beverages. Coffee is drunk for its pleasant flavour and aroma as well as for the stimulatory properties arising from its caffeine content. Such properties, as well as the addition of sugar and/or milk, are most likely responsible for our learned preferences for coffee.

The aim of this work was to study the relationship between the preference patterns for coffee and the perception of its bitterness. For this purpose, factors relating to both the product – degree of roasting – and the individual – bitterness perception and caffeine metabolism rate – were studied.

First, the effect of the roasting temperature on coffee sensory profile and preference was investigated, initially by describing the sensory properties of coffee brews prepared with both under-roasted (140-165°C) and standard roasted (220°C) beans. In addition, seventy-five coffee consumers rated their liking for unsweetened and freely sweetened under-roasted coffee samples. Increasing the bean processing temperature positively affected both the perceived intensity of some sensory attributes specific to coffee and also consumers' liking. In particular, coffee processed at temperature higher than 150°C were described by the terms "coffee", "roasted", "burnt". Furthermore, no significant differences in the intensity of these attributes were found between samples processed at temperatures higher than 155°C and samples roasted at the standard temperature. In general, samples processed at temperatures higher than 150°C were preferred. However, a subgroup of subjects who liked the samples roasted at lower temperatures was found. These subjects were on average less neophobic than the others, that is, more inclined to consume unfamiliar/unconventional foods.

Later, the effects of bitterness sensitivity and caffeine metabolism rate on perception of, and liking for coffee were examined. One hundred and thirty-five regular coffee consumers participated in the study. Subjects were characterised in terms of bitterness sensitivity (fungiform papillae – FP – number and sensitivity to the bitter compound 6-n-propylthiouracil –PROP-); caffeine metabolism rate (slow and fast metabolizers); attitudes

and food behaviour (frequency – number of coffees per day- and modality - black, with sugar, with milk- of coffee consumption; preference for and familiarity with bitter foods; self perceived effect of caffeine intake). Caffeine metabolism was investigated by competitive immuno-enzymatic assay. Subjects provided saliva samples after 12 hours of caffeine abstinence (T0) and at 30 and 90 minutes after ingestion of caffeine (100 mg). The individual caffeine metabolism rate in 90 minutes was expressed as a caffeine metabolism index (CmI), computed as the ratio between the amount of residual caffeine in saliva 60 minutes after the adsorption peak and the amount of caffeine at the adsorption peak corrected with the baseline.

Subjects rated their liking for six unsweetened and freely sweetened coffee samples selected on the base of their roasting degree, caffeine content and bitterness. The immersive test technique was applied to evaluate the effect of the context on coffee liking. In particular, just before starting the test, subjects were asked to describe the most preferred situation for consuming coffee (time of the day, place, company, etc.) and to imagine that they were doing coffee tasting in that particular situation. In a separate session, subjects evaluated the sourness, bitterness and astringency in the coffee samples. Psychophysical curves were constructed for caffeine and quinine-HCl over 6 concentrations. No significant relationships were found between the FP number, the PROP taster status and the caffeine metabolism rate. Subgroups of subjects were identified based on these indices and differences in preference and sensory responses were explored independently in the subgroups.

Both FP density and PROP taster status affected taste sensitivity for bitter compounds in general (caffeine; quinine-HCl) and the use of sugar in coffee. In particular, subjects with a higher FP number rated the bitterness of caffeine and quinine-HCl solutions and sourness of coffees stronger than subjects with a lower FP number. They also added more sugar to, and gave higher liking ratings for, the coffee samples. While coffee liking was unrelated to PROP taster status, PROP non-tasters added more sugar to the coffee samples than did PROP super-tasters. However, super-tasters rated sourness, bitterness and astringency as stronger than non-tasters, both in coffee samples and in standard solutions. Caffeine metabolism was related to bitterness perception both in coffee samples and in standard solutions of caffeine, but not of quinine-HCl. In particular, subjects with a lower caffeine metabolism index rated the bitterness of coffees and of caffeine solutions as stronger than

subjects with a higher caffeine metabolism index. Moreover, they added more sugar in coffees and consumed less coffees daily.

In general, the results demonstrate that even in high coffee consuming cultures such as Italy, there are clear sensory variations in the coffees that are consumed. Coffee consumption and preference, in terms of frequency and modality, are influenced both by the sensory properties of coffee and the psychological, physiological and metabolic characteristics of consumers. In the coffee production process, the roasting degree is a crucial step towards the development of the sensory properties affecting consumer preference. Furthermore, both the physiological indices – FP number and PROP taster status – and the caffeine metabolism rate play a significant role in taste sensitivity for bitter compounds in general and also in the preference for coffee with or without sugar.

## **Acknowledgments**

This study was carried out during 2012-2015 at the Department of Agricultural, Food and Forestry System Management, University of Florence, Italy. The research was supported by illycaffè S.p.A. (Trieste, Italy).

I wish to express my sincere gratitude to my supervisor Prof. Erminio Monteleone and to my co-supervisor Dr. Caterina Dinnella. Without both of you, I would not be where I am now. Thanks Erminio, in particular for your guidance and your encouragement. Thanks Caterina, in particular for your patience and your support.

I am also very grateful to Prof. John Prescott (TasteMatters Research & Consulting, Sydney, Australia) visiting professor at the Department of Agricultural, Food and Forestry System Management, University of Florence, Italy, for nine months from 2013-2014. Thanks John for providing me with additional constructive criticism that helped to put my work into a wider perspective. Thanks also for all the time you have spent to interpret my research data and to revise my manuscripts.

Warm thanks go to Dr. Margrethe Hersleth (Nofima, Ås, Norway). Thanks Margrethe for welcoming me as part of your team and supervising me during my stay abroad. Thanks also to Dr. Valérie Lengard Almli, Dr. Jens Petter Wold, Dr. Paula Varela Tomasco for the great collaboration.

Sincere thanks to Prof. Bruno Zanoni (Department of Agricultural, Food and Forestry System Management, University of Florence, Italy) for his valid support and encouragement throughout these years.

I wish to thank illycaffè S.p.A. for the financial support for my research and Dr. Luciano Navarini and Dr. Massimo Barnabà for their cooperation.

I would like to thank the people who have shared their knowledge with me.

Sincere thanks to Dr. Sara Spinelli for precious contribution to my work by analysing and interpreting data regarding context.

Thanks to Dr. Nicola Pirastu for performing the genetic analysis at the Institute for Maternal and Child Health - IRCCS "Burlo Garofolo" (Trieste, Italy).

Thanks also to the team of the Department of Health Sciences (University of Florence), in particular to Dr. Roberta Felici, for the hospitality and the free access to the equipment in performing analysis of saliva samples.

I wish to thank past and present colleagues at the Sensory Science group at the University of Florence. Special thanks to Annamaria, Monica, Maria, Danny, Veronica, Isabella.

Finally, I want to thank the consumers who participated to my studies, for their vital contribution.

Florence, 19 December 2015

Camilla Masi

### **List of original publications**

This study is based on the following original publications, referred to in the text by Roman numerals I, II, and III.

**I** C. Masi, C. Dinnella, M. Barnabà, L. Navarini, and E. Monteleone (2013). Sensory properties of under-roasted coffee beverages. *Journal of Food Science*, vol. 78 (8), pp. S1290-S1300.

**II** C. Masi, C. Dinnella, E. Monteleone and J. Prescott (2015). The impact of individual variations in taste sensitivity on coffee perceptions and preferences. *Physiology and Behavior*, vol. 138, pp. 219-226.

**III** C. Masi, C. Dinnella, N. Pirastu, J. Prescott , E. Monteleone. Caffeine Metabolism Influences Coffee Perception, Preferences and Intake. *Submitted to PloS one*.

## **1. Introduction**

### **1.1. Food preference**

A variety of social, cultural and economic factors contribute to the development, maintenance and change of dietary patterns. Intra-individual determinants such as physiological and psychological factors, acquired food preferences and knowledge, can be distinguished from interpersonal or social factors such as culture and family influences (Glanz & Mullis 1988). Liking, or the affective response to the sensory properties of a certain food (Rozin 1990), is believed to play a major role, and to a greater extent, to be the best predictor of human food choice or intake in the absence of economic and availability constraints (Cowart 1981; Rozin & Zellner 1985; Rozin & Schulkin 1990).

#### **1.1.1. Innate and acquired food preferences**

Researchers investigating innate flavour preferences have mainly depended on facial reflexes of the neonate offered tastes or smells, as reflections of hedonic responses towards these stimuli (Steiner et al. 2001). Neonates' expressions suggest an innate preference for sweet and rejection of bitter and sour tastes. Salty taste seems to be innately appealing to humans, neonates start to recognize salty taste at four months old and from four to twenty-four months of age show heightened acceptance of saline solution relative to water (Beauchamp et al. 1986). Innate preferences and rejections were related to survival and still determines the choice of food (Hofmann 2009). In fact, the bitter taste receptors have evolved to protect the body from the ingestion of substances which are potentially harmful to health (Scott & Mark 1987). Similarly, sour taste receptors are thought to have evolved to guard against the ingestion of unripe and/or spoiled foods (Lindemann 2001). In contrast, sweet taste is associated with carbohydrates and its preference depends on energy needs. Similarly, positive responses occur for salty and umami tastes. Sensitivity to salty taste has probably evolved to ensure the cell homeostasis of ions and water (Lindemann 1996). Umami taste, contained in meat extracts and fermented products, is an indicator of protein resources (Bellisle 1999).

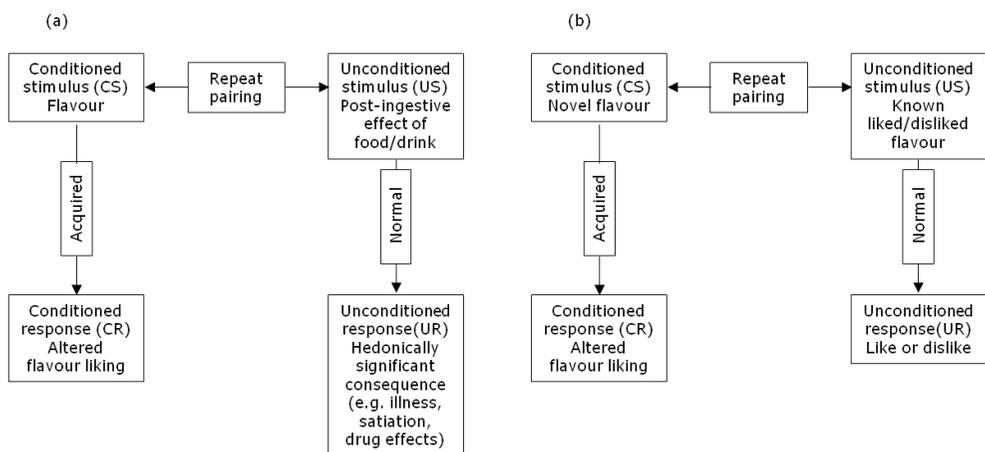
Some instinctive reactions, such as aversion to bitterness, can be reversed: the majority of human food preferences are learned via experience with food and eating (Yeomans 2009).

In fact, bitter taste is generally rejected in food (Steiner et al. 2001), yet many individuals enjoy a certain amount of bitterness in products such as beer, dark chocolate or coffee (Guinard et al. 1996; Harwood et al. 2012; Varela et al. 2014). Various mechanisms contribute to acquired preferences, including mere exposure (Methven et al. 2012) and Pavlovian conditioning (Birch 1993; Zellner et al. 1983; Letarte et al. 1997; Rozin & Schulkin 1990).

*Mere repeated exposure* to a stimulus enhances the affective response towards it (Zajonc 1968; Zajonc et al. 1974; Moreland & Zajonc 1977). Food preference is an increasing function of exposure frequency: the more frequently a food has been tasted, the better it is liked (Pliner 1982). Evidence suggests that exposure of the unborn child to flavours in the amniotic fluid and may contribute to later preferences for such flavours (Mennella et al. 2001). Thus, this mechanism starts to determine food preference in early human life.

*Classical or Pavlovian conditioning* (Pavlov 1927) is a learning process in which an innate response to a potent stimulus comes to be elicited in response to a previously neutral stimulus; this is achieved by repeated pairings of the neutral stimulus with the potent stimulus. Classical conditioning occurs when a conditioned stimulus (CS) is paired with an unconditioned stimulus (UCS). Usually, the conditioned stimulus (CS) is a neutral stimulus (e.g., the sound of a tuning fork), the unconditioned stimulus (UCS) is biologically potent (e.g., the taste of food) and the unconditioned response (UCR) to the unconditioned stimulus is an unlearned reflex response (e.g., salivation). After pairing is repeated, the organism exhibits a conditioned response (CR) to the conditioned stimulus when the conditioned stimulus is presented alone (Pearce 1987; Rescorla 1988).

Changes in the liking of a stimulus are usually interpreted within an evaluative conditioning framework, occurring when the stimulus has been paired with other, positive or negative stimuli. Evaluative conditioning can happen under conditions that do not support other forms of Pavlovian conditioning (De Houwer et al. 2001). The two most important theories of flavour learning are *Flavour-Consequence Learning* and *Flavour-Flavour Learning* (Figure 1).



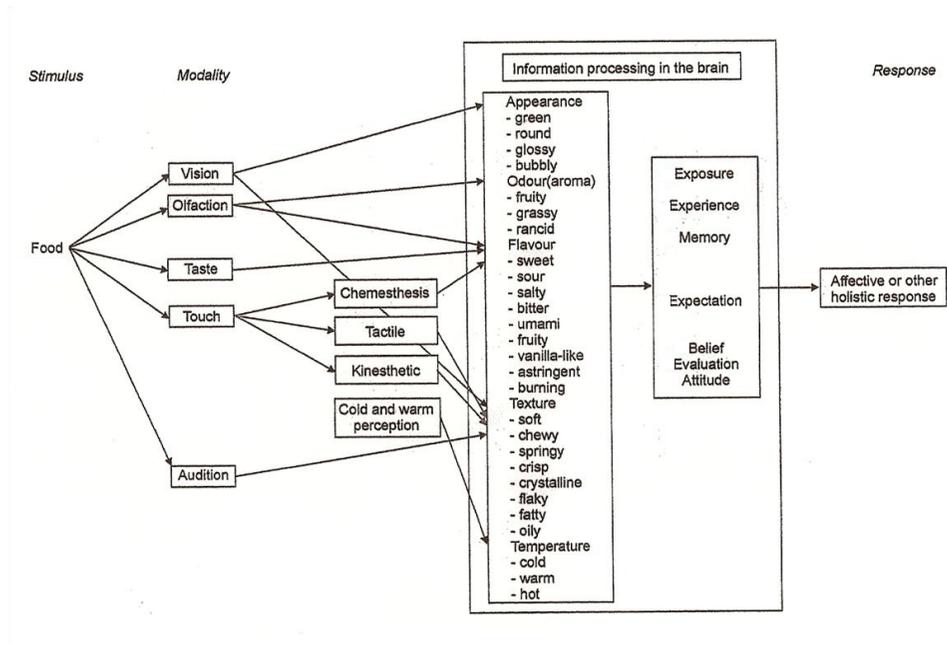
**Figure 1.** A Pavlovian model of the nature of the associations in (a) flavour-consequence learning and (b) flavour-flavour learning (Yeomans 2007, p.84).

Associations between the flavour and the effects of the ingested food on body can produce aversions or preferences for it. When the post-ingestive effect is negative, an aversion can be developed through a conditioned taste aversion (CTA) (Garcia & Koelling 1966). When ingestion of a substance is followed by gastrointestinal distress and nausea, an association between the taste of the ingested substance and internal consequences of its ingestion is quickly established. This association is maintained in a long-term, so that people reject ingestion of the substance at subsequent exposures. On the contrary, when post-ingestive effects are positive – for example, providing the body with energy - preference is generated by flavour consequence learning (FCL) (Rozin & Zellner 1985). In FCL, there is an association between the perception of the sensory characteristics of a food and its effect on the organism. This association induces a change in preference during exposure to consumption (Yeomans 2007). FCL is dependent on the biological effect of nutrients, such as fatty substances (Yeomans & Mobini 2006) and the physiological / pharmacological effect, such as caffeine (Tinley et al. 2003). Positive post-ingestive effects include provision of valued nutrients such as glutamate (Prescott 2004), energy in the form of sugars or fats (Yeomans & Mobini 2006) as well as the physiological and behavioural effects associated with caffeine (Tinley et al. 2003). Flavour flavour learning (FFL) involves pairing of a neutral flavour (CS) with a liked or disliked flavour (UCS), that lead to changes in subsequent liking for the CS alone. Pairing novel odours in solution with bitterness has been reported to

reduce liking for those odours (Yeomans & Mobini 2006; Yeomans et al. 2006; Yeomans, Mobini, et al. 2007). On the contrary, sucrose has been reported to increase in overall liking for the sweet-paired flavour (Yeomans et al. 2006; Mobini et al. 2007). For this reason, people start drinking coffee by adding milk and sugar, or try alcohol with a sweet mixer.

### **1.1.2. The role of individual differences in food preference**

Food choice, liking and consumption depend on several variables, such as the sensory characteristics of the product (Tuorila & Cardello 2002); the characteristics of the consumers in terms of responsiveness to food cues (Rodin et al. 1976), restrained eating (Westenhoefer et al. 1999), and expectations (Cardello 1995); the environment in which the product is chosen and consumed, economic and social factors (Bell et al. 1995; Meiselman 1996; Wansink 2004; Stroebele & De Castro 2004; Rozin 2005). Some models of food choice and acceptance include just one or two of these variables, while other models consider all three classes of food, people, and environmental variables. The first model (Harper 1981) is basically a sensory model linking physical, sensory and affective variables. According to this model, the product acceptance depends on three factors: the physicochemical composition of the product, the sensory qualities of the product and the hedonic variables. Later in Land's (Land 1983) model of food acceptance personal and environmental variables such as experience, expectation, availability, advertising, price were added. Individual psychological and physiological variables were added in Cardello's acceptance model (Cardello 1996). In the latest model by Tuorila (Tuorila 2007) concerning the factors that influence consumer behaviour, the information induced by the product through the stimulation of the sensory system becomes integrated and connected with the information derived from previous experiences, together with the expectations and the attitudes towards the product, to generate the overall affective response (Figure 2). This model has been further improved considering the role of genetics in chemosensory preference traits and also the heritability of a range of personality traits, as underlined very recently by Tuorila (Tuorila 2015).



**Figure 2.** Tuorila's model of food preference.

Regardless of the model, sensory perception plays a crucial role in determining product acceptability. Sensory quality is considered a key factor in food acceptance because consumers look for food with palatable characteristics. Consumers tend to consume what they like and avoid what they dislike. In fact the first reason to eat is the biological need for nutrients. However, many foods are consumed almost entirely for pleasure, such as coffee, chocolate, ice cream and alcohol (Clark 1998).

Individual sensitivity to sensations induced by food shows considerable variability between subjects, and this variability significantly influences preference and consumption (Reed et al. 2006). However, the perception depends on physiological traits common to groups of subjects: individual sensitivity to sensations induced by food shows a normal distribution of variability among subjects on the basis of different physiological factors such as gender, age, hormonal status, exposure to pathogens, intake of medicines (Duffy 2007).

Genetic factors can explain individual differences in psychophysical responses to oral sensations. Genetic variations associated with individual differences in gustatory perception

are well known for bitterness, sweetness and umami, while the genetic variability associated with the perception of salty and sour tastes is less known (Kim & Drayna 2004; Shigemura et al. 2009). Genetic variations in the TAS1R gene family have been associated with differences in the perception of sweetness and umami (Fushan et al. 2009; Shigemura et al. 2009). For example, variations of TAS1R3 are associated with lower sensitivity to sweetness (Fushan et al. 2009) and to umami taste (Shigemura et al. 2009). On the contrary, variations of TAS1R1 are related to higher sensitivity to umami taste (Shigemura et al. 2009). Variability in responses to salty stimuli has been examined for decades, but a direct genetic link to human salty taste perception has yet to be uncovered. However, very recent studies have suggested that changes in humans genes coding for the channel TRPV1 and the beta sub-unit of the epithelial sodium channels (ENaCs) could change salty taste perception (Dias et al. 2013). Little is known about inter-individual and inter-population variation in sour taste perception, and how such variation may be linked to genetic variation. It is possible that some polymorphisms of the PKD2L1 and PKD1L3 genes may affect sour taste perception (Garcia-Bailo et al. 2009).

Another heritable trait is taste responsiveness to phenylthiocarbamide (PTC) and 6-n-propylthiouracil (PROP) (Fox 1931). The perception threshold of PROP, (now used instead of PTC, because it lacks the sulphurous odour of PTC and may be less toxic), distinguishes subjects into *non taster* (high threshold and low sensitivity) and *taster* (low threshold and high sensitivity) groups (Duffy, Davidson, et al. 2004). Ratings of the intensity of a super concentrated PROP solution (3.2 mM) further divide tasters into *medium tasters*, who perceive the bitter intensity as strong, and *super tasters*, who perceive the bitter intensity as very strong. As a result, PROP responsiveness is typically expressed categorically as PROP taster status (PTS), which consists of three groups: PROP super tasters (STs), PROP medium tasters (MTs), and PROP non tasters (NTs) (Bartoshuk 1993). Insensitivity to PTC or PROP is estimated at 30% in European populations (Guo & Reed 2001).

Genetic studies have generally demonstrated that tasting was a dominant trait and non tasting a recessive trait (Snyder 1931). Indeed, tasters and non tasters differ in the molecular form of the bitter receptor (TAS2R38 gene) (Duffy, Davidson, et al. 2004). There are two common molecular forms [proline-alanine-valine (PAV) and alanine-valine-

isoleucine (AVI)] of this receptor defined by three nucleotide polymorphisms that result in three amino acid substitutions: Pro49Ala, Ala262Val, and Val296Ile. The ancestral human haplotype of these three amino acids is PAV (Kim et al. 2003). This molecular form is common in humans and is associated with tasting; the other common form, the triply substituted form AVI, is associated with non tasting. Genetic variation in TAS2R38 accounts for up to 85% of the phenotypic variance in PTC/PROP perception (Wooding et al. 2004; Bufe et al. 2005).

PTS has been shown to be an influence on food preferences (Keller et al. 2002), acceptability of bitter foods (Drewnowski & Gomez-Carneros 2000; Duffy & Bartoshuk 2000; Kaminski et al. 2000; Turnbull & Matisoo-Smith 2002; Dinehart et al. 2006) as well as total energy intake (Goldstein et al. 2007). A range of bitter foods, including Brussels sprouts, cabbage, broccoli and spinach, coffee (Drewnowski et al. 1999) and grapefruit juice (Drewnowski et al. 1997), have been reported as more bitter and/or less preferred by PROP tasters than by NTs.

Moreover, PTS has long been used as general orosensory responsiveness to a variety of stimuli (Bartoshuk et al. 1992; Bartoshuk et al. 1993; Prescott et al. 2001). PROP tasters rate the intensity of other bitter compounds, including caffeine, quinine, and urea (Leach & Noble 1986; Mela 1989), as more intense than do NTs, sucrose as sweeter (Bartoshuk 1979), sodium chloride as more salty (Bartoshuk et al. 1998), and citric acid as more sour (Prutkin et al. 1999). PTS is also associated with responsiveness to other orosensory stimuli apart from tastes: STs perceive irritation from capsaicin, cinnamaldehyde, ethanol (Prescott & Swain-Campbell 2000) and astringency (Pickering et al. 2004) with greater intensity than NTs.

There have been a number of investigations suggesting that PROP super tasters have more fungiform papillae (FP) than non tasters (Bartoshuk et al. 1994; Tepper & Nurse 1997; Prutkin et al. 2000; Delwiche et al. 2001b; Essick et al. 2003; Hayes & Duffy 2007; Duffy et al. 2010; Nachtsheim & Schlich 2013). In fact, overall taste sensitivity is reflected in the density of lingual fungiform papillae (FP) (Miller & Reedy 1990b). FP contain taste-sensing receptor cells, which are accessible by tastants via pores in taste buds (Correa et al. 2013). Because 99% of papillae in humans contain at least 1 taste bud (Segovia et al. 2002),

papillae density is related to taste sensitivity with subjects who have higher numbers of papillae being more sensitive to taste stimuli (Smith 1971; Stein et al. 1994; Delwiche et al. 2001b). Some studies have also reported relationships between FP density and food consumption/preference such as alcoholic beverages (Duffy, Peterson, et al. 2004), sugar-fat dairy mixtures (Hayes & Duffy 2008), bread (Bakke & Vickers 2011). However, the relationship between PTS and FP number remains unclear. In one study, FP number was found to be related to perceived PROP intensity only in the TAS2R38 homozygote group and not in the TAS2R38 heterozygote group (Hayes et al. 2008). Other works have reported no significant associations between TAS2R38 haplotype or PROP status and FP density (Duffy, Davidson, et al. 2004; Fischer et al. 2013; Garneau et al. 2014).

Individual differences in sensitivity have been demonstrated also for non-taste oral sensations, such as astringency and oral irritation.

Astringency is a tactile sensation, involving dryness of the oral surface and tightening and puckering sensations of the mucosa and muscles around the mouth (Lee & Lawless 1991). Astringency arises from the interaction of dietary tannins with lubricating salivary proteins (Nayak & Carpenter 2008; Dinnella et al. 2010). Individual differences in astringency perceptions have been demonstrated (Dinnella et al. 2009). In particular, individual variation of saliva characteristics modulates the sensitivity to astringency induced by phenolic stimuli (Fischer et al. 1994; Horne et al. 2002; Condelli et al. 2006) and two groups of subjects, Low Responding (LR) and High Responding (HR) to phenolic astringent stimuli have been identified based on their ability to maintain constant salivary characteristics after repeated oral stimulation (Dinnella et al. 2009). This variation significantly affects responsiveness to complex astringent stimuli and influences the overall acceptance for and the consumption of phenol-rich foods (Dinnella et al. 2011). Furthermore, tentative evidence of the genetic effects underlying the perception of this sensation has been found (Törnwall et al. 2011).

Individual differences in responsiveness to oral irritation have been reported for zingerone (Prescott & Stevenson 1996), capsaicin (Cliff & Green 1996; Prescott 1999; Prescott & Swain-Campbell 2000), menthol (Cliff & Green 1996), cinnamaldehyde and ethanol (Prescott & Swain-Campbell 2000). These variations seem to depend on the irritant and, secondly, on the inter-individual variability in responses to a given irritant. After repeated

short-term stimulation irritants can produce sensitization (an increase in irritation intensity) or desensitization (a decrease in sensation intensity) (Stevens & Lawless 1987; Green 1989). However, some individuals can show little or no change in rated intensity over successive samples (Prescott & Swain-Campbell 2000). It has been suggested that frequency consumption of spicy foods could influence responses to irritants. However, this factor has been demonstrated to be important for capsaicin (Prescott & Stevenson 1995) but not for zingerone (Prescott & Stevenson 1996). It could be also possible that pre-existing individual differences in sensitivity to irritation influence consumption frequency of spicy foods. For example, different responses between PROP tasters and non tasters has been reported for capsaicin (Karrer & Bartoshuk 1991), ethanol (Bartoshuk et al. 1993), and cinnamaldehyde (Prescott & Swain-Campbell 2000).

Another marker of individual variation in oral sensation is thermal taste (TT). Thermal taste is the ability to perceive 'phantom' taste sensations during heating or cooling of a small area of tongue (Cruz & Green 2000). In particular, warming the tip of the tongue from 20 to 35 °C can cause a transient sensation of sweetness, and cooling it to  $\leq 20$  °C can induce a sour taste which turns to saltiness at temperatures below 10 °C (Cruz & Green 2000). This phenomenon may be linked to the TRPM5 cation channel, which is a highly temperature-sensitive, heat activated channel, with a key role in the transduction of umami, sweet and bitter tastes (Talavera et al. 2005). It has been further suggested that other tastes, such as salty and sour, perceived by thermal taste subjects (TTs) may be linked to the temperature sensitivity of the channels involved in their chemical transduction (Talavera et al. 2007). TTs have also been reported to perceive taste and some trigeminal stimuli (Green et al. 2005; Bajec & Pickering 2008), vanilla aroma (Green & George 2004) and metallic taste (Bajec et al. 2012; Yang et al. 2014) with greater intensity than thermal non tasters (TnTs).

### **1.1.3. Individual differences in flavour-based learning**

Several individual differences have been reported to impact on sensitivity to both FFL and FCL. FFL depends on the individual hedonic evaluation of training UCS. Differences in changes in liking of sweet-paired odours between sweet-likers and sweet-dislikers have been reported (Yeomans et al. 2006). Furthermore, hunger can modulate hedonic evaluation of sweet stimuli (Yeomans & Mobini 2006). In particular, although all participants

rated the sucrose-paired odour as sweeter post- training, only those who were hungry reported increased liking for the odour. In general, liking for a sweet taste is much greater when hungry than when sated (Cabanac 1971). However, hunger state alone cannot explain the sweet liker–disliker dichotomy. PROP taster status has been suggested to have a role in acquired liking for sweet-paired odours (Yeomans et al. 2009), because PROP non tasters are reportedly more likely to be sweet-likers as determined by their responses to sweet tastes in solution (Looy & Weingarten 1991; Looy & Weingarten 1992). However, PROP taster status did not affect hedonic changes to odours by association with saccharin; but it predicted acquired odour bitterness (Yeomans et al. 2009).

The hunger state plays a role also in the expression of acquired preference induced by associations between a flavour and nutrient ingestion, as reported for a novel yoghurt with added fat (Kern et al. 1993) and for the flavour of a novel drink with added sucrose (Mobini et al. 2007).

Insensitivity to manipulated energy in a study of nutrient-reinforced FCL has been showed by women who scored highly on a measure of dietary restraint, defined as the tendency to restrict food intake as a consequence of concerns about body weight (Brunstrom 2005).

Consumption frequency affects changes in liking for flavours through association with the post-ingestive effects of caffeine. In caffeine-deprived conditions, regular caffeine consumers increase their liking for caffeine-associated flavours (Yeomans et al. 1998; Yeomans et al. 2005), on the contrary individuals who rarely consume caffeine acquire an aversion for caffeine-paired flavours (Rogers et al. 1995; Tinley et al. 2004; Dack & Reed 2009).

## **1.2. The case study: Coffee preference patterns**

Food behaviour is influenced by a mix of variables, among which the main factors are the product, the person and the context (Meiselman et al. 2000). Each choice factor consists of multiple sub-factors and it is related to the other ones (Jaeger et al. 2011). Indeed, contextual variables (such as location, time and social settings) act together with product variables (such as sensory properties and packaging) and consumer variables (such as preferences, attitudes, experiences) to determine consumers' food choices and preferences (Giacalone et al. 2015).

It is increasingly important to study all three main factors - product, person, context - to fully understand food choices and preferences. In particular, phenotypic individual differences should be taken into account, because of their role in perception and thus in determining preference. Similarly, it is important to understand whether the context effect smoothes phenotypic individual variables in food choice or if the variations in underlying physiology affects food behaviour within a cultural context.

It is to be hoped that we will have a better understanding of the interplay between sensory experience, post-ingestive consequences and preference expression. In this way, it would be possible to customize products to consumer needs, deriving from differences both in own physiological / metabolic / psychological characteristics and in situations / frequencies / modalities of consumption.

In this perspective, coffee is an interesting case study because, despite its bitter taste, it is one of the most consumed beverages in the world. Its consumption, in terms of both amount and type, is affected by several environmental factors, such as the culture of individual countries, religious restrictions, the availability of coffee and its cost, and also individual preferences (Luciano et al. 2005). Moreover, genetic studies in twins have shown inheritable influences on habitual coffee consumption (Conterio & Chiarelli 1962; Pedersen 1981; Carmelli et al. 1990). The heritability estimates of coffee consumption range from 0.39 to 0.56 (Silverman et al. 1994; Laitala et al. 2008; Vink et al. 2009). Also the preference for coffee instead of other caffeinated beverages, such as tea, has been reported to be influenced by both genetic and environmental factors. In particular, an inverse relation between tea and coffee consumption was demonstrated in a large community sample of Australian twins (Luciano et al. 2005).

Even in high coffee consuming cultures such as Italy there are clear sensory variations in the coffees that are produced and consumed. In 2013/2014 8.5 million bags of green beans were imported in Italy, mainly from Brazil, Vietnam, India, Uganda and Indonesia (76% of total green bean imports) (AA.VV. 2015). 57% of the coffee sold on the Italian market is *Coffea arabica* (Arabica coffee), while 43% is *Coffea canephora* (Robusta coffee) (<http://www.thecoffeeguide.org/coffee-guide/the-markets-for-coffee/Europe---European->

Union---Summary-data/). Usually, Arabica and Robusta coffees are blended: Arabica is used for its aroma, Robusta for taste and body (Sanz et al. 2002). Robusta is also used in blends to add earthy/phenolic notes to some dark roasted coffees (Parliment & Stahl 1995) and for its low cost. At the same degree of roasting, Arabica coffee is sourer, less bitter and astringent than Robusta coffee. Arabica is characterized by toasted, chocolate, cocoa, caramel and dried fruit aromatic notes. Woody, earthy and cocoa notes are typical of Robusta coffee (Lindinger et al. 2008). Italians prefer medium to dark roasted coffee, mostly from arabica-rich blends, which serves as the basis for most types of coffee preparations consumed in Italy (Giovanazzi 2010).

Apart from sensory differences due to variety and/or processes (Van Der Vossen 2009), coffee can be drunk on its own or mixed with other ingredients (milk, sugar, condensed milk, etc). Sugar and milk are added to reduce the perceived intensity of bitterness (Calviño et al. 1990; Bücking & Steinhart 2002). According to a recent survey on the coffee consumption of Italians (Ghedini 2015) involving thirty-eight million individuals aged from 18 to 65 years old, about 37% of respondents drink black coffee and 63% drink coffee with sweeteners (sugar, milk, cream). Moreover, 76.2% of respondents habitually choose normal coffee and it is the preferred choice of 51.2% of them; 41.2% usually drink *cappuccino* (coffee with hot frothy milk) and 37.4% *macchiato* coffee (coffee with milk); 23.5% drink *ristretto* coffee (short coffee) and 20.3% *long* coffee (with a larger volume of water than normal).

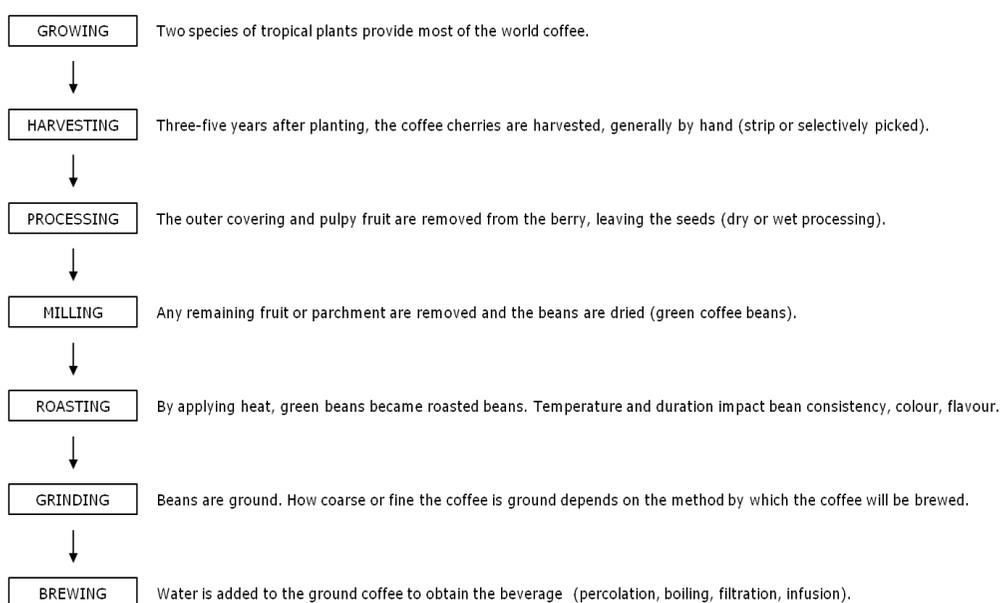
Thus, the preference for coffee can include several aspects, such as the frequency of consumption (coffee instead of other caffeinated beverages such as tea or energy drinks), the choice based on sensory profile (strong vs mild flavour) and the use modality (with or without sweeteners).

For these reasons, coffee is an ideal product to evaluate the effect of physiological, metabolic, contextual and attitudinal factors on preference. This project proposes a general framework to determine consumers' preference patterns. The proposed approach investigates which sensory properties maximize acceptance of consumers and how individual differences due to different psycho-physiological characteristics, such as attitudes, pre-existing preferences, different sensitivity in taste, can affect consumers' preferences.

### 1.2.1. The flavour of coffee

Coffee is a brewed beverage prepared from roasted beans of plants belonging to the *Coffea* genus. Commercial coffee production is based on two species, *Coffea arabica* (Arabica coffee) and *Coffea canephora* (Robusta coffee). Coffee plants are cultivated in over 70 countries, primarily in the subtropical and equatorial regions of the Americas, Southeast Asia, India and Africa.

From plantations to the cup, the production of coffee can be summarized in seven steps (Figure 3):



**Figure 3.** Flow chart of coffee production process: main steps and relevant explanations.

Flavour is one of the major motivations for consumer preferences, and also the most important criterion for coffee quality evaluation (Farah et al. 2006). Coffee sensory properties are affected by several factors: plant varieties, growing region/conditions, processing and brewing methods (Maeztu et al. 2001; Schenker et al. 2002; Andueza et al. 2003; Nebesny & Budryn 2006; Lindinger et al. 2008).

Roasting is the thermal treatment which transforms green coffee into roasted coffee. In the conventional roasting, temperatures ranging from 170°C to 240 °C are applied to coffee

beans for 10 to 15 min to obtain respectively, a light to dark roast (Yeretzian et al. 2002). This step is the most important one in the coffee production, causing chemical, physical, structural and sensory changes (Pittia et al. 2001). Green coffee contains about 300 volatile compounds (Flament 2001). During roasting, the content of volatiles changes and 650 new volatile compounds build-up: about 850 volatiles have been identified in roasted coffee (Czerny & Grosch 2000; Flament 2001). Several compounds are responsible for the sensory profile of roasted coffee (Czerny & Grosch 2000; Mayer & Grosch 2001; Schenker et al. 2002). In particular, some peculiar sensory attributes, such as “coffee”, “roasted”, “burnt”, “brown”, are positively related to roasting degree (Bhumiratana et al. 2011) and influence consumers’ preference (Geel et al. 2005). Vocabulary and definitions to describe the flavour of a coffee brew have been edited by International Coffee Organization (Table 1).

**Table 1.** The vocabulary to describe the flavour of a coffee brew (International Coffee Organization).

Sensory attributes		
<i>Odours</i>	<i>Tastes</i>	<i>Mouthfeel</i>
Animal-like	Acidity	Body
Ashy	Bitterness	Astringency
Burnt/Smokey	Sweetness	
Chemical/Medicinal	Saltiness	
Chocolate-like	Sourness	
Caramel		
Cereal/Malty/Toast-like		
Earthy		
Floral		
Fruity/Citrus		
Grassy/Green/Herbal		
Nutty		
Rancid/Rotten		
Rubber-like		
Spicy		
Tobacco		
Winey		
Woody		

The Maillard and pyrolysis reactions taking place during roasting lead the formation of sensory active molecules, responsible for aroma and colour, as well as compounds having potential harmful effect (Yeretian et al. 2002; Buffo & Cardelli-Freire 2004). Furan is one of the main undesirable compounds formed during thermal treatment of foods and drinks (Moro et al. 2012). Coffee represents a significant source of furan intake given its high level of consumption (Zoller et al. 2007). Acrylamide formation during coffee roasting has also been confirmed (Stadler et al. 2002; Taeymans et al. 2004). The harmful effects of this compound are known even though they are still controversial (Lipworth et al. 2012). The acrylamide level peaks during coffee roasting and only a small amount remains in the fully roasted product (Lantz et al. 2006; Bagdonaite et al. 2008).

Thermal treatments ranging from 140 to 160 °C -defined as under-roasting- have been proposed to reduce the content of potentially harmful compounds in coffee (Guenther et al. 2007; Altaki et al. 2011) and also to preserve the antioxidant activity due to phenolics such as chlorogenic acids (Perrone et al. 2010). In fact, moderate consumption of coffee (3-4 cups for day) is associated with a reduction in the relative risk of several diseases (Nehlig 2012). Chlorogenic acids are thermally labile phenolics contributing bitterness, sourness and astringency to the coffee sensory profile (Farah et al. 2008). They are mainly degraded in the latter stages of roasting (Del Pino-García et al. 2012) even though their degradation products can still contribute to the overall antioxidant activity of roasted coffee (Perrone et al. 2012).

The sensory quality of the coffee brew depends also on the brewing technique (López-Galilea et al. 2006; Gloess et al. 2013). Coffee brewing and extraction methods have been developed according to geographic, cultural and social context as well as personal preferences (Petracco 2001). In Italy the most popular coffee beverage is the *espresso coffee* (Ghedini 2015). *Italian espresso* is "a small cup of concentrated brew prepared on request by extraction of ground roasted coffee beans, with hot water under pressure for a defined short time" (Illy 2004, p.19). Furthermore, espresso is a specific brewing method (Table 2) obtained through "percolation of hot water under pressure through a compacted cake of roasted ground coffee, where the energy of the water pressure is spent within the cake" (Petracco 2004, pp.310-311). The optimal volume in the cup is 25-30 ml. The physical/chemical characteristics of espresso are reported in Table 3 (Petracco 2004,

pp.310–311). The sensory profile of *Italian espresso* has been described as characterised by “a rich body, a full and fine aroma, a balanced bitter-sweet taste with an acidic note and a pleasant lingering after-taste” (Petracco 2004, p.311). Sensory attributes such as “coffee”, “bitter”, “cocoa”, “roasted”, “woody”, “cereal”, “butter toffee”, “acid”, “citrus”, “winey”, “flowery” are usually used to describe *espresso* sensory profile (Navarini et al. 2004; Andueza et al. 2007; Lindinger et al. 2008).

**Table 2.** Technological variables to obtain an espresso coffee (Petracco 2004).

Variable	Range
Ground coffee portion	6.5 ± 1.5 g
Water temperature	90 ± 5 °C
Inlet water pressure	9 ± 2 bar
Percolation time	30 ± 5 s

**Table 3.** Physical and chemical characteristics of espresso coffee (Petracco 2004).

Parameter	Value
Viscosity at 45 °C	> 1.5 mPa.s
Total solids	20-60 g/l
Total lipids	2 mg/ml
Droplet size count (90%)	< 10 µm
Caffeine	< 100 mg/cup

### 1.2.2. The effect of context

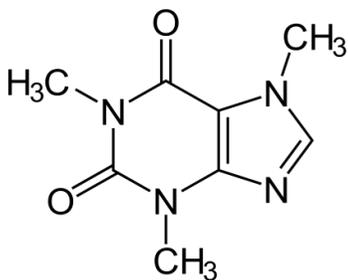
Coffee was first consumed in the West during the 17<sup>th</sup> century and specific local traditions have developed. Coffee has radically reshaped social relations and the tone of both work and leisure within the consuming countries. Historically, coffee houses served as forums for political discussion and learning (Pendergrast 1999), but they have become transformed over the years into "retail theatres" used primarily for socializing (MacLeod 1997). Coffee has become a fixture of Western consumer society and a marker of identity. The last two decades have witnessed sweeping changes in coffee culture across Europe, both in terms of the coffee beverages consumed and the places in which consumption has taken place (Morris 2013). The beverage itself has become integral to working and professional culture: it is simultaneously a pick-me-up that provides greater productivity and a pause (in the form of the coffee break) (Elliott 2001).

Coffee drinking is also an emotional experience depending on time of day, situation, cultures and tradition, or context of consumption (Bhumiratana et al. 2014). Context is often referred to a location/place with physical characteristics, but other associations (e.g., feelings, events, activities, etc.) regarding when the product is consumed can be also considered as context (Stroebele & De Castro 2004). By removing the product from the context when it is normally consumed, a consumer may have less involvement with the product and accurate hedonic ratings may not be obtained. The context in which a product is consumed can be specific to the individual consumer, thus it is important to have an approach that allows different types of consumption contexts to be evoked and allows consumers to personalize the context (Hein et al. 2012). Several studies have demonstrated the efficacy of these approaches in understanding consumers' preference for coffee (Petit & Sieffermann 2007; Hein et al. 2010; Sester et al. 2013; Lipchock et al. 2012; Bangcuyo et al. 2015). The presence of contextual cues relevant to a coffeehouse scenario generated specific expectations of a certain coffee profile and led to different coffee choices (Sester et al. 2013; Bangcuyo et al. 2015). For instance, if a video clip of an iceberg landscape was shown in an experimental bar while people had to choose a drink within a large range of drinks, they more frequently selected warm beverages like coffee, tea or hot chocolate than other drinks (Sester et al. 2013). Furthermore, comparing liking scores for five different coffees, no differences were found when the test was performed in traditional testing environment (individual booths). On the contrary, when tested in a coffeehouse scenario, the two most liked samples were the two coffees obtained from actual coffeehouse purveyors (Bangcuyo et al. 2015).

### **1.2.3. Caffeine: characteristics and properties**

#### **1.2.3.1. The post-ingestive effects of caffeine**

Caffeine (1,3,7-trimethylxanthine; Figure 4) is a xanthine alkaloid naturally found in coffee (*Coffea spp.*), tea (*Camellia spp.*), kola nut (*Cola acuminata*), cacao bean (*Theobroma cacao*), yerba mate (*Ilex paraguariensis*), and guarana berries (*Paullinia cupana*). It is consumed most frequently in beverages such as coffee, soft drinks, and tea (Heckman et al. 2010).



**Figure 4.** The molecular structure of caffeine.

Caffeine is a central nervous system and metabolic stimulant (Nehlig et al. 1992). In particular, caffeine acts as an adenosine-receptor antagonist: it binds to the receptors A1 and A2A and stimulates the central nervous system (CNS) (Fredholm et al. 1999). Thus, caffeine promotes wakefulness, enhances mood and cognition, and produces stimulatory effects (Lieberman et al. 2002; Haskell et al. 2005). At low doses, its psychological effects include mild euphoria, alertness, and enhanced cognitive performance (Lieberman et al. 1987), but at higher doses, it produces nausea, anxiety, trembling, and jitteriness (Daly & Fredholm 1998).

Several studies indicate that the post-ingestive effects of caffeine are sufficient to reinforce changes in flavour preferences (Rogers et al. 1995; Yeomans et al. 1998). Moreover, conditioning is more likely in regular caffeine users (Rogers et al. 1995) and in individuals who are caffeine deprived (Yeomans et al. 1998). The expression of flavour preferences seems to be modulated by the levels of caffeine deprivation (Yeomans, Jackson, Lee, Nesic, et al. 2000; Yeomans et al. 2001).

The mode of action of caffeine in developing flavour preference is not immediate (Yeomans, Jackson, Lee, Steer, et al. 2000). During the development of flavour preferences, the inclusion of a mildly addictive compound such as caffeine to a food will increase the dependence and liking of that food, which will in turn increase consumption (Myers & Sclafani 2007). Caffeine may elicit no perceived flavour or bitterness in the mouth depending on concentration (Keast & Roper 2007). In any case, the positive effects occur post-consumption with increased vigilance and attention, enhanced mood and arousal as well as enhanced motor activity (Keast & Riddell 2007).

### **1.2.3.2. The bitterness of caffeine**

Caffeine is one of the bitter compounds (Wiener et al. 2012) naturally occurring in foods (Drewnowski 2001) and may elicit bitterness depending on the concentration (Keast & Roper 2007).

Differences in the quality and quantity of the multiple cellular mechanisms associated with bitter taste cells cause the large individual variation observed in bitter taste perception (Keast & Breslin 2002). Caffeine can translocate through cellular membranes and interfere with second messenger systems associated with bitter taste (Peri et al. 2000). These multiple, independent putative mechanisms are responsible for multiple perceptual mechanisms for caffeine bitterness (Peri et al. 2000). Genetic differences in bitter taste sensitivity and the perceived intensity of the compound 6-n-propylthiouracil (PROP status) were shown to affect the perception of bitterness of caffeine (Ly & Drewnowski 2001) and the liking for coffee (Pirastu et al. 2014).

Recent work (Dsamou et al. 2012) has suggested that sensitivity to the bitterness of caffeine is related to salivary composition. In particular, hypersensitive subjects contained higher levels of amylase fragments, immunoglobulins, and serum albumin and/or serum albumin fragments in their saliva. However, because these proteins are secreted by different salivary glands and are dependent on a subject's health, it is not clear how they can act to determine sensitivity to caffeine.

Our knowledge of the molecules which induce the bitter taste in coffee is still approximate. Caffeine and trigonelline, which are responsible for the bitter taste of coffee, contribute only 10-30% and 1% respectively (Frank et al. 2006). In fact, decaffeinated coffee is also perceived as bitter, but with less intensity than caffeinated coffee (Macrae 1985). Further research has shown that bitter compounds in coffee are varied and include alkaloids, furfural alcohol, 5-idrossimetil-2-furaldeide, pyrazines and dichetopirazine, 4-vinilcatecol oligomers, quinic acid lactones (Frank et al. 2006; Frank et al. 2007). The formation of bitter compounds also depends on the time and temperature of roasting (Blumberg et al. 2010). Different species and blends of coffee can also contribute to obtain different levels of perceived bitterness in beverages (Lindinger et al. 2008). Moreover, people can use some strategies to suppress coffee bitterness, depending on their own sensitivity to bitterness, such as adding sugar or milk (Keast 2008).

### **1.2.3.3. Individual differences in caffeine metabolism rate**

Caffeine is the major purine alkaloid in green coffee (Horman & Viani 1972). The average content of caffeine is 1.2-1.3% in Arabica and 2.2-2.4% in Robusta (Bee et al. 2004). Coffee contains also paraxanthine, theobromine, theophylline and other trace purines (Weidner & Maier 1999; Kappeler & Baumann 1985). However, caffeine is the major psychoactive substance in coffee and also the most widely consumed in the world (Nehlig 1999). Moreover, bitterness perception of caffeine and sensitivity to its psychoactive effects seem to be related (Fischer & Griffin 1964).

Caffeine is absorbed rapidly and completely from the gastrointestinal tract and is metabolized in the liver (Nawrot et al. 2003). Peak plasma concentrations are reached within 15 (Arnaud 1993) to 60 (Hinds et al. 1996) minutes after intake, but can take as long as 120 minutes after ingestion (Arnaud 1993). In humans, the half-life of caffeine ranges from 2 to 4.5 hours (Daly 1993; Nehlig 2012), but can be as long as 12 hours (Benowitz 1990). Over 95% of caffeine is metabolized in the liver and converted to paraxanthine (81.5%) (Campbell et al. 1987; Gu et al. 1992). The pathway converting caffeine to paraxanthine is exclusively mediated via CYP1A2. However, other enzymes (CYP2E1, CYP2A6 and CYP1A1) contribute to the formation of the other metabolites, theobromine (10.8%) and theophylline (5.4%) (Gu et al. 1992; Tassaneeyakul et al. 1994).

Inter-individual variability in the activity of CYP1A2 is typically between 5- and 15-fold (Schrenk et al. 1998; Tantcheva-Poòr et al. 1999), but may be up to 60-fold (Sesardic et al. 1988). Several factors contribute to the individual differences in sensitivity to the stimulant effects of caffeine, such as genetic, demographic and environmental factors (Yang et al. 2010). Some variability in CYP1A2 is due to genetic polymorphisms in the gene which cause increased or decreased inducibility of the enzyme (Grosso & Bracken 2005). Moreover, several single nucleotide polymorphisms (SNPs) may explain inter-individual variation in caffeine metabolism (Nakajima et al. 1999; Sachse et al. 1999; Chevalier et al. 2001; Han et al. 2001; Signorello et al. 2001; Aklillu et al. 2003; Sachse et al. 2003). Twenty-one alleles, comprising more than 30 SNPs, have been identified in the CYP1A2 upstream sequence and in intron 1 of the CYP1A2 gene (Perera et al. 2012). An A to C substitution at position -163 (rs762551) in the CYP1A2 gene decreases enzyme inducibility as measured by

plasma or urine caffeine metabolites ratio (Sachse et al. 1999). Carriers of the -163C allele (AC and CC genotypes) can be considered slow caffeine metabolizers, on the other hand homozygotes for the -163A allele (AA genotype) are more rapid caffeine metabolizers (Sachse et al. 1999; Cornelis et al. 2007). The A/A variant seems to be associated with high inducibility of the CYP1A2 enzyme in smokers (Sachse et al. 1999).

In general, given the same caffeine intake, slow metabolizers will be more exposed to high internal caffeine levels than fast metabolizers (Bech et al. 2006; Santos et al. 2015) and seem to be more sensitive to caffeine effects (Nehlig 2012). For these reasons, people tend to adapt their coffee consumption to balance perceived negative and reinforcing symptoms of caffeine (Coffee and Caffeine Genetics Consortium & et al. 2014). Moreover, caffeine intake itself increases the rate of caffeine metabolism (Berthou et al. 1995; Tantcheva-Poòr et al. 1999). It has been suggested that the inducing effect of coffee on CYP1A2 activity is due not only to caffeine content but also to other secondary coffee components, such as polycyclic aromatic hydrocarbons (Djordjevic et al. 2008). For this reason, coffee consumption has been reported to significantly affect CYP1A2 activity; on the contrary heavy tea consumption and more than one cola beverage a day have been reported not to have a significant impact on CYP1A2 activity (Perera et al. 2012). The pharmacological activity of caffeine seems to be a major source of coffee consumption and thus preferences. It plausible to hypothesize that the higher coffee consumption associated with a faster caffeine metabolism rate would allow a more rapid development of a preference for stronger coffee flavour, as is found with exposure to other unpalatable tastes (Methven et al. 2012). In turn, this process may be further enhanced by a relatively low sensitivity to bitter taste. Thus, both fast caffeine metabolism rate and low sensitivity to bitter taste would favour the preference for, and consumption of, the more bitter black coffee. On the other hand, the use of sweeteners in coffee to mask its unpleasant bitter taste could result from the interplay between the high sensitivity to bitterness and the relatively low consumption induced by a lower caffeine metabolism rate. However, there has been no exploration of the potential impact of caffeine metabolism rate, the variants in CYP1A2, sensitivity to bitterness and caffeine consumption on coffee preference.

## **2. Aim**

The aim of this research was to study the relationship between the preference patterns for and bitterness perception in coffee. For this purpose, some factors that can determine the preference for coffee, such as the sensory properties of coffee, individual differences in bitterness perception and caffeine metabolism, were considered. In particular, the objectives of this research were the following:

I. To evaluate the effect of roasting degree on coffee sensory profile and preference by describing sensory properties of brews prepared with under-roasted coffees and by evaluating the acceptance of these beverages by coffee regular consumers.

II-III. To investigate the effect of individual physiological and metabolic differences on bitterness perception and coffee preference. This was carried out by evaluating the effect of fungiform papillae number, PROP taster status and caffeine metabolism rate on preference for coffee and bitterness perception both in coffee and standard solutions of bitter compounds.

### **3. Materials and Methods**

This thesis consists of a study describing the sensory properties of brews prepared with under roasted coffees and the acceptance of these beverages by regular coffee consumers (I) and another study investigating the effect of physiological and metabolic factors on consumers' appreciation of coffee (II, III). Together they form an overall picture of coffee consumption as regards both sensory and consumer aspects.

The search for suitable tests for the consumer study (II-III) was completed in several pilot tests. The improvement of measurements was not covered in the published articles (II-III) but it is included in this work to provide a background of the selected methodologies.

#### **3.1. Evaluation principles**

All studies adhered to the tenets of the Declaration of Helsinki. Approval for the research protocol was obtained from the Institutional Review Board of the Agricultural PhD School - Sustainable Management of Agricultural, Forestry and Food Systems - GESAAF, University of Florence.

The summary of the participants, samples, measures and scales is in Table 4.

The following conditions were applicable to all the tests:

- Subjects were recruited in Florence area. The subjects had no history of disorders in smell and oral perception. They were paid for their participation. Written informed consent was obtained from each subject after the description of the experiment.
- Training to recognize the sensations of sweetness, sourness, bitterness, and astringency was performed using the following standard solutions, respectively - sucrose: 8.00, 12.00, 18.00 g/l; citric acid: 0.25, 0.38, 0.50 g/l; quinine monohydrochloride dihydrate 0.025, 0.037, 0.050 g/l; aluminium potassium sulphate: 0.3, 0.6, 0.9 g/l.
- Samples were presented in 80cc plastic cups identified by three digit codes. Sample presentation was balanced across subjects within each session.
- In the adopted experimental conditions, aroma evaluation was performed at 64-67°C and the in-mouth evaluation at 54-57°C for coffee samples.
- After each coffee sample, subjects rinsed their mouth with distilled water for 30-50 sec, ate some plain crackers for 30-50 sec and finally rinsed their mouth with water for

a further 30-50 sec. After each standard solution, subjects rinsed their mouths with distilled water for 90 sec.

Subjects took a 10-15 min break after every two samples.

- Evaluations were performed in individual booths under red lights for sensory evaluations and under white lights for affective evaluations.
- Data were collected with the software Fizz (ver.2.47.B, Biosystemes).

Conditions differing from these were highlighted in the text.

**Table 4.** Participants, number of samples, measures, and scales in the studies I-III and in the pilot tests.

Study	Participants	Samples	Measures	Scales
I	<i>Trained panel</i> n=14 (9F 5M), 22-44 y	12 under and 2 standard roasted coffees	Intensity of 28 attributes	9-point
	<i>Consumer panel</i> n=75 (49F 26M), 19-62 y	6 under roasted coffees	Liking	9-point
		/	Consumption of coffee and herb and vegetable-based brews	5-point
		/	Neophobia	7-point
Pilot tests	<i>Trained panel</i> n=8 (6F 2M), 20-38 y	7 espresso coffees	Intensity of 22 attributes	9-point
	n=18 (13F 5M), 20-48 y	8 coffees with added caffeine	Intensity of sourness, bitterness, astringency	LMS
	n=29 (24F 5M), 20-26 y	6 caffeine and 6 quinine-HCl solutions	Intensity of bitterness	gLMS
	<i>Consumer panel</i> n=10 (6F 4M), 22-27 y	6 saliva samples	Caffeine content in saliva	/
II-III	<i>Consumer panel</i> n=135 (76F 59M), 20-60 y	6 espresso coffees	Liking	9-point
		6 coffees with added caffeine	Intensity of sourness, bitterness, astringency	LMS
		6 caffeine and 6 quinine-HCl solutions	Choice	/
		1 solution of PROP	Intensity of bitterness	gLMS
		2 coffee packs	Intensity of bitterness	gLMS
		3 saliva samples	Choice	/
		/	Caffeine content in saliva	/
		/	Fungiform papillae number	/
		/	Frequency of coffee consumption	5-point
		/	Consumption of coffee: black, with sugar, with milk	3-point
		/	Preference for bitter/caffeine containing foods	9-point
		/	Choice from pairs of caffeine-containing foods	7-point
	/	Familiarity with bitter/caffeine containing foods	5-point	
	/	Consumption of foods affecting CYP1A2 activity	7-point	
	/	Perceived effect of caffeinated coffee intake	7-point	

## 3.2. Study I

### 3.2.1. Descriptive analysis

#### 3.2.1.1. Subjects

Fourteen subjects, nine females and five males, from 22 to 44 years old, participated in the study.

#### 3.2.1.2. Samples

Coffea Arabica from two countries of origin was used (Brazil-B and Guatemala-G). Under-roasting was performed in a temperature range from 140 to 165°C. Standard roasting was performed at 220°C. Six under-roasted (140, 145, 150, 155, 160 and 165°C) and one standard roasted products were obtained for each origin. Total weight loss and colour (Probat, Colorette 3b) were determined on coffee beans after processing in order to check the extent of thermal treatment (Clarke 2003) (Table 5). Products were packed in 250 g sealed cans and used within 3 months.

**Table 5.** Total weight loss (%) and colour index (CI) of coffee beans processed at different temperatures and from different origins.

Temperature (°C)	Total weight loss (%)		Colour index (CI)	
	Brazil	Guatemala	Brazil	Guatemala
140	7.85	8.78	200	200
145	8.60	9.20	200	200
150	11.18	10.14	188	192
155	10.01	11.00	154	169
160	11.19	11.60	142	143
165	12.24	13.12	133	129

Brewed coffees were prepared in a plunger coffee maker by pouring 200 g of deionised water at 100°C over 15 g of coffee powder according to European standard of Brewing control chart (ideal coffee/water ratio 7.5-6.5%). After five min the mixture was filtered and brewed coffee samples were immediately served. Fourteen coffee samples were evaluated in total.

### *3.2.1.3. Training*

Subjects participated in 9 training sessions:

1. Recognition and intensity rating of sensations perceived in standard solutions (2 sessions): subjects were asked to rate the perceived intensity of sweetness, sourness, bitterness and astringency in standard solutions on a 9-point category scale (1="extremely weak"; 5="moderate"; 9="extremely strong").
2. Generation of a list of attributes describing the perceived sensations in coffee samples (4 sessions): assessors developed a vocabulary describing differences among samples according to the Generic Descriptive Analysis (Lawless & Heymann 2010). Assessors were presented with two pairs of coffees in each session and were asked to freely describe similarities and differences between them. A consensus list of 28 attributes was generated (Table 6).
3. Recognition and intensity rating of attributes in coffee samples (2 sessions, 2 samples in each session) on a 9-point category scale labelled at the extremes with "extremely weak" and "extremely strong".
4. Assessing panel calibration and assessor performance (2 sessions). Two individual sessions were performed on three samples (G140, G150, G160) replicated two times.

### *3.2.1.4. Evaluation*

The trained panel participated to seven evaluation sessions: six for under-roasted and one for standard roasted samples. Samples (30 ml) were presented in 100cc closed amber glass bottles. In each session six samples were evaluated. Each sample was evaluated 3 times. For each sample, assessors were asked to rate the intensity of aroma descriptors first. Then they were asked to take a sip of the sample and rate the intensity of flavour attributes. Finally they had to take a second sip and rate taste and mouthfeel attributes. The perceived intensity of each sensation was rated on a 9-point category scale labelled at the extremes with "extremely weak" and "extremely strong".

**Table 6.** Consensus list of attributes describing aroma, flavour and mouthfeel sensations of the samples: definition and standard.

Attribute	Definition	Standard
<i>Aroma and Flavour</i>		
Barley coffee	Odour of barley coffee	1 g of barley coffee in 20 ml of boiling water
Burnt	Odour of over-cooked, almost scorched product	/
Caramel	Odour of the sugar caramelizing without burning	1 g of caramel in 20 ml of water
Cereals cooking water	Odour of water after the cooking of cereals and legumes	1 g of oatmeal in 20 ml of boiling water
Coffee	Odour characteristic of coffee	/
Grains/oats	Odour characteristic of cereal, malt and oats	12 g of oatmeal
Metallic	Odour of metallic utensils, for example clean moka or cutlery	FeSO <sub>4</sub> 0.9 g/l
Nuts	Odour of roasted nuts	3 g of peanuts without shell
Puffed grains	Odour of uncooked or unroasted grain	1 g of rice crackers
	(including unroasted corn, barley or wheat) and malt extract	
Roasted	Odour characteristic of products cooked to a high temperature	/
Vegetables	Odour associated with fresh vegetables, fresh earth, wet soil or humus	Peanuts shell (30 g)
Watered coffee	Odour of coffee prepared by moka with a too low coffee powder amount	1 g of instant coffee in 20 ml of boiling water
Sweet	Taste associated with sucrose	Sucrose 8.0 g/l
Bitter	Taste of chicory, tonic water and caffeine	Quinine-HCl 0.025 g/l
Sour	Taste of lemon juice and vinegar	Citric acid 0.25 g/l
<i>Mouthfeel</i>		
Astringency	Dryness of the oral surface and tightening and puckering sensation of the mucosa and muscles around the mouth	Alum sulphate 0.3 g/l

Standards were presented in a 100ml amber glass bottle. Standards were prepared to induce a moderate intensity corresponding to the central point (5) of the 9-point scale.

### **3.2.2. Consumer test**

#### *3.2.2.1. Subjects*

Seventy-five regular coffee consumers, forty-nine females and twenty-six males, from 19 to 62 years old, participated in the study.

#### *3.2.2.2. Samples*

Six samples were selected based on descriptive analysis results: B140, B150, B160, G140, G150, G160. In particular, samples from coffee processed at temperature lower than 150°C were characterised by vegetable and grain notes, while samples from coffee processed at temperature higher than 150°C were characterised by coffee and toasted notes. Samples from coffee processed at 150°C had an intermediate profile between the others.

#### *3.2.2.3. Evaluation*

Subjects were presented with each sample (30 ml) together with 10 g of sugar (about three coffee spoons) in a plastic cup, both identified with the same random three-digit code. Assessors were asked to smell the sample once and rate liking for aroma, to take a sip and rate liking for flavour (flavour1). Then they were asked to freely add sugar, take a sip and rate again liking for flavour (flavour2). The amount of sugar (g) used for each sample by each subject was measured at the end of evaluation session.

#### *3.2.2.4. Questionnaire*

Subjects were asked to fill a questionnaire including four types of questions: demographic, neophobia, consumption frequency of coffee, herb and vegetable-based brews, consumption of these drinks with or without sweeteners added. The herb and vegetable-based beverages were included in the questionnaire as distracters and the relevant frequency consumption data were not considered.

Ten statements belonging to the food neophobia scale were rated on a 7-point scale from strongly disagree (1) to strongly agree (7) (Pliner & Karen Hobden 1992).

Participants expressed their consumption frequencies using 5-point scales, labelled 1 "less than once a day", 2 "once a day", 3 "twice or three times a day", 4 "four or five times a day", 5 "more than five times a day". They were also asked to answer to the question "Which of these beverages do you usually consume sweetened?"

### 3.3. Pilot tests

#### 3.3.1. Descriptive analysis

##### 3.3.1.1. Subjects

Eight subjects, six females and two males, from 20 to 38 years old, participated in the study.

##### 3.3.1.2. Samples

Although under-roasted coffees were accepted by regular consumers, we decided to move to *espresso coffee* for the main study of this project. First of all, because *espresso* is the most popular coffee beverage in Italy (Ghedini 2015) and so Italian consumers are very familiar with this type of coffee beverage. Moreover, espresso as a brewing technique is easier and more practicable than the French press used for under-roasted coffee samples. The use of an automatic machine also allowed standardization of the procedure.

The aim of this pilot test was to select coffee samples with different level of bitterness intensity. Seven espresso coffees varying in roasting degree (light, medium, dark) and caffeine content (<0.05 – 2%) were evaluated (Table 7).

Coffee samples (25 g) were prepared with an espresso machine using coffee capsules.

**Table 7.** Coffee samples: roasting degree of coffee beans and caffeine content (%) of coffee powder.

Sample	Roasting degree	Caffeine (%)
A	Light	0.8-0.9
B	Dark	0.8-0.9
C	Medium	1.50
D	Dark	1.50
E	Medium	<0.05
F	Medium	0.8-0.9
G	Dark	about 2

##### 3.3.1.3. Training and vocabulary generation

The training consisted of three phases:

1. Recognition and intensity rating of sensations perceived in standard solutions (2 sessions).

Subjects were trained to recognize and rate the perceived intensity of sweetness, sourness, bitterness, and astringency on a 9-point category scale (1="extremely weak"; 5="moderate"; 9="extremely strong").

2. Sensory vocabulary development and reference standards (4 sessions).

Assessors participated in two language sessions according to the Conventional Descriptive Analysis (AA.VV. 1994; Lawless & Heymann 2010). The consensus-building process, managed by the panel leader, ended with the list of attributes reported in Table 8. In order to train subjects to rate the intensity of each attribute, panellists were asked to individually evaluate the intensity of attributes on a score-card, using a 9-point category scale labelled at the extremes with "extremely weak" and "extremely strong".

3. Assessor and panel performance validation (1 session).

Sensory performances were validated by evaluating a subset of samples to be used for the study (three samples for two repetitions). Panel and assessors data were analyzed by using Panel Check software (ver 1.4.0, Nofima, Norway).

**Table 8.** Consensus list of attributes describing aroma, flavour and mouthfeel sensations of samples: definitions and standards.

Attribute	Definition	Standard
Coffee	Odour characteristic of coffee	10 g of coffee powder
Barley coffee	Odour characteristic of barley coffee	1 g of barley coffee in 20 ml of boiling water
Roasted	Odour characteristic of products cooked at high temperature	/
Burnt	Odour of over-cooked, almost scorched product	/
Vegetables	Odour associated with fresh vegetables, fresh earth, wet soil or humus	30 g of peanuts with shell
Caramel	Odour of the sugar caramelizing without burning	20 g of caramel
Dried fruit	Odour of dried fruit, for example roasted nuts and roasted almonds	20 g of grinded roasted nuts
Metallic	Odour of metallic utensils, for example clean moka or cutlery	FeSO <sub>4</sub> 1.75 g/l
Overall intensity	Overall intensity of odour	/
Sour	Taste of lemon juice and vinegar	Citric acid 0.38 g/l
Bitter	Taste of chicory, tonic water and caffeine	Quinine-HCl 0.037 g/l
Astringency	Dryness of the oral surface and tightening and puckering sensation of the mucosa and muscles around the mouth	Alum sulphate 0.6 g/l
Body	It is used to describe the physical properties of the beverage. A strong full mouthfeel characteristic as opposed to being thin	/

Standards were presented in a 100ml amber glass bottle. Standards were prepared to induce a moderate intensity corresponding to the central point (5) of the 9-point scale.

#### *3.3.1.4. Evaluation*

The panel participated in five evaluation sessions. In each session, four or five samples were evaluated. Each sample was evaluated three times. For each sample (25 g), assessors were asked to smell the sample once and rate the intensity of odour descriptors perceived by nose (aroma) first. Then they were asked to wait 3 minutes, take a sip of the sample and rate the intensity of odours perceived retro-nasally, taste and mouthfeel attributes. The intensity of each sensation was rated on a 9-point category scale labelled at the extremes with "extremely weak" and "extremely strong".

#### *3.3.1.5. Sample selection*

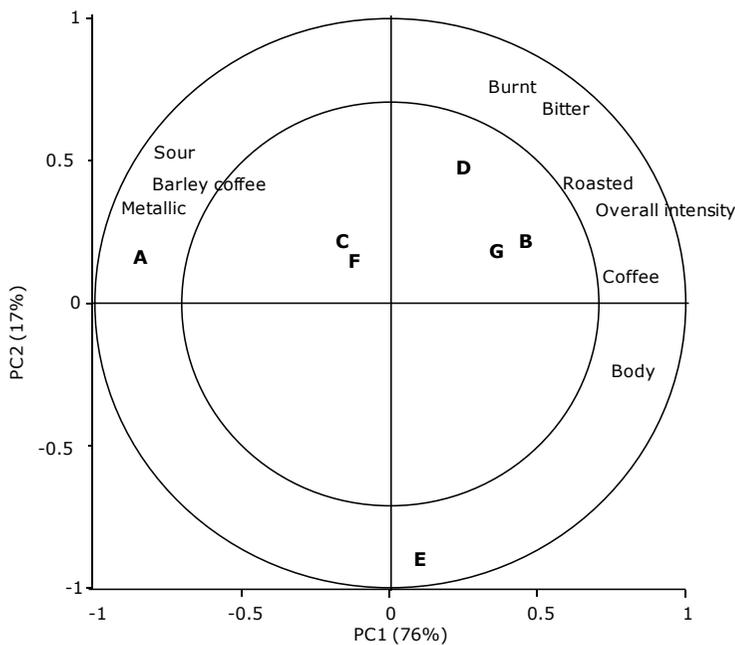
Intensity data from the trained panel were analyzed by multiblock PCA (Tucker-1) and by p/MSE plot (Panel Check software, ver 1.4.0, Nofima, Norway) to assess panel calibration and assessor performance, respectively (Næs et al. 2010). No cases of disagreement among panellists were found for any of the attributes and all subjects were considered reliable and thus included in further data analysis.

Intensity ratings from coffee samples were independently analyzed by a two-way ANOVA mixed model (sample as fixed and assessors as random factors), followed by a Fisher LSD post hoc test (significant for  $p \leq 0.05$ ). Principal Component Analysis (PCA) was computed on panel averages for each significant attribute arising from the ANOVA models. Samples were included as dummy variables (down-weighted in the data matrix) to improve the visual interpretation (Martens & Martens 2001). The full cross validation was computed to validate the interpretation of the first two components.

The results of the PCA computed on descriptive data are summarized in the correlation loading plot reported in Figure 5. The first two significant dimensions of the perceptual map accounted for 93% of the variation (PC1:76% and PC2:17%). PC1 was positively associated with flavour descriptors "coffee", "overall intensity", "roasted", "bitter", "burnt" and mouthfeel descriptor "body", while a negative correlation was found for "metallic", "barley coffee" and "sour" flavour descriptors. Samples were mainly discriminated along the first component according to roasting degree. Roasting degree and time/temperature combinations determine the formations of compounds responsible for the coffee sensory

profile (Clarke 2003; Baggenstoss et al. 2008). PC2 contributed mainly by separating product E from the other coffees. This sample was the only one without caffeine and it was judged differently by the trained panel.

The relative positions of samples on the perceptual map resulting from descriptive data were used for the selection of samples to be tested in the consumer test. Samples A, B, C, D, E, G spanned the relevant variability of the sensory attributes within the whole sample set according to different caffeine content and roasting degree.



**Figure 5.** Correlation loading plot from Principal Component Analysis on panel averages of each significant attribute describing sample sensory properties.

### 3.3.2. Evaluation of coffees with added caffeine

#### 3.3.2.1. Subjects

Eighteen subjects, thirteen females and five males, from 20 to 48 years old, participated in the study.

#### 3.3.2.2. Samples

In the case of attributes where only low levels are desired, such as bitterness in coffee or in chocolate (Harwood et al. 2012), it is crucial to determine the point at which liking is

adversely affected. The consumer rejection threshold is based on using a paired preference test within a threshold methodology (Prescott et al. 2005). This method has been successfully applied to acceptability of cork taint (trichloroanisol, TCA) in white wine (Prescott et al. 2005), eucalyptol in red wine (Saliba et al. 2009), caffeine in coffee, citric acid in orange juice, and salt in beef broth (Lee et al. 2008), and sucrose octaacetate in milk chocolate (Harwood et al. 2012). In the present study, a series of increasing concentrations of caffeine were added to decaffeinated coffee, and consumers were asked to indicate which of the two coffees samples (one with, and one without, added caffeine) they preferred. In this perspective, eight samples differing in caffeine content were evaluated by a trained panel to select 6 samples for the consumer test (Table 9). The concentrations were chosen considering that on average the caffeine content in coffee is 3.2-6 g/l. Samples were prepared by dissolving caffeine (Sigma-Aldrich) in decaffeinated coffee (sample E in the descriptive analysis).

**Table 9.** Concentration of caffeine (g/l) in coffees with added caffeine.

Sample	Caffeine (g/l)
1	0.50
2	0.75
3	1
4	1.50
5	2
6	3
7	4
8	6

### 3.3.2.3. Training

Subjects were trained to recognize sourness, bitterness and astringency, and also in the use of the Labelled Magnitude Scale (LMS) (Green et al. 1993), a quasi-logarithmic scale with the bottom labelled as "barely detectable" (1.4 mm) and the top as "strongest imaginable oral sensation" (100 mm). Intermediate labels include "weak" (6.1 mm), "moderate" (17.2 mm), "strong" (35.4 mm) and "very strong" (53.3 mm) oral sensations. The LMS is particularly suitable for sensations with broadly defined perceptual qualities (Green et al. 1996). Subjects were instructed that their evaluation need not be anchored to the verbal labels but could be located anywhere in the scale. The descriptor at the top end of the scale

- *strongest imaginable* - was defined in the context of all oral sensations, including painful ones.

#### *3.3.2.4. Evaluation*

Subjects were instructed to hold the sample (8 ml) in their mouth for 10 s, then swallow, wait 20 s and evaluate the intensity of sourness, bitterness and astringency using the LMS. The presentation order of attributes was balanced across subjects. Sourness and astringency were included as evaluated attributes to be sure that samples were different only in the perceived intensity of bitterness.

#### *3.3.2.5. Sample selection*

Intensity ratings were independently analyzed by a three-way ANOVA fixed model [samples (8 levels), assessors (18 levels), repetitions (2 levels) as fixed factors], followed by a Fisher LSD post hoc test (significant for  $p \leq 0.05$ ). A significant effect of sample was found only for bitterness. No significant effects of repetition and of interactions were found. To estimate differences between samples for perceived intensity of each attributes, intensity ratings were independently analyzed by a two-way ANOVA mixed model (sample (8 levels) as fixed and assessors (18 levels) as random factors), followed by a Fisher LSD post hoc test (significant for  $p \leq 0.05$ ). The perceived intensity of bitterness significantly increased with the concentration, but not for all levels of concentration. No significant differences between samples were found both for sourness and astringency (Table 10). Based on these results, six samples were selected for final tests: 1, 3, 5, 6, 7, 8.

**Table 10.** Two-way ANOVA on intensity scores of bitterness, sourness, astringency in caffeine reinforced coffee samples: mean intensity, F and p values.

Sample	Bitterness	Sourness	Astringency
1	25.92 <sup>e</sup>	21.28	20.19
2	27.22 <sup>e</sup>	19.19	19.28
3	28.44 <sup>de</sup>	18.69	18.94
4	29.64 <sup>de</sup>	18.47	16.22
5	31.86 <sup>d</sup>	19.06	17.39
6	41.83 <sup>c</sup>	19.97	16.83
7	48.06 <sup>b</sup>	20.75	17.17
8	58.22 <sup>a</sup>	17.08	16.08
$F_{7;287}$	61.18	1.59	1.43
P	≤0.0001	0.145	0.201

Different letters indicate significantly different values ( $p \leq 0.05$ ).

### 3.3.3. Evaluation of standard solutions of caffeine and quinine-HCl

#### 3.3.3.1. Subjects

Twenty-nine subjects, twenty-four females and five males, from 20 to 26 years old, participated in the study.

#### 3.3.3.2. Samples

The bitterness of caffeine has been associated with the bitterness of both quinine-HCl (Delwiche et al. 2001a) and PROP (Hansen et al. 2006). However, recent advances in knowledge of the peripheral organization of the taste system strongly indicate that taste receptor cells are quality specific (Mueller et al. 2005; Huang et al. 2006). For this reason, both caffeine and quinine-HCl were selected to study bitterness sensitivity. The concentrations were chosen considering that on average the caffeine content in coffee is 3.2-6 g/l and also based on the reported results by Keast and Roper (Keast & Roper 2007) comparing responses to equi-intense solutions of caffeine and quinine-HCl.

Twelve solutions, six of caffeine and six of quinine-HCl, were evaluated (Table 11).

Standard solutions were prepared by dissolving caffeine (Sigma-Aldrich) and quinine-HCl (Sigma-Aldrich) in deionised water, were stored in glass bottles and were brought to room temperature prior to testing.

**Table 11.** Concentration (g/l) of caffeine and quinine-HCl in standard solutions.

Caffeine (g/l)	Quinine-HCl (g/l)
0	0
0.58	0.016
1.16	0.032
2.33	0.048
4.66	0.064
9.32	0.081

#### 3.3.3.3. Training

Subjects were trained to recognize the bitter taste. Subjects were also trained to use the General Labelled Magnitude Scale (gLMS). In this case, the descriptor at the top end of the gLMS - *strongest imaginable of any kind* - was defined in the context of all sensations, including taste, sight, touch, smell, hearing (Bartoshuk 2000; Prutkin et al. 2000). This change has been introduced to establish the domain of the scale as the entire range of perceived intensities for making comparisons across subjects with different sensitivity to bitterness (Bartoshuk et al. 2002).

#### 3.3.3.4. Evaluation

Subjects were instructed to hold the sample (10 ml) in their mouth for 10 s, then expectorate, wait 20 s and evaluate the perceived intensity of bitterness, using the gLMS.

#### 3.3.3.5. Sample selection

Intensity ratings from standard solutions of caffeine and quinine-HCl were independently analyzed by a three-way ANOVA fixed model [samples (6 levels), assessors (29 levels), repetitions (2 levels) as fixed factors], followed by a Fisher LSD post hoc test (significant for  $p \leq 0.05$ ). A significant effect of sample was found both for caffeine and quinine-HCl. No significant effects of repetition and no interactions were found. To estimate differences between samples for bitterness, intensity ratings from standard solutions were independently analyzed by a two-way ANOVA mixed model [sample (6 levels) as fixed and assessors (29 levels) as random factors], followed by a Fisher LSD post hoc test (significant at  $p \leq 0.05$ ). Bitterness significantly increased with the concentration both for caffeine and quinine-HCl (Table 12). Moreover, to verify that bitterness was perceived at the same

intensity both in caffeine and quinine-HCl, intensity ratings from standard solutions of both caffeine and quinine-HCl were analyzed by a two-way ANOVA mixed model [samples (12 levels) as fixed factor and assessors (29 levels) as random factor], followed by a Fisher LSD post hoc test (significant at  $p \leq 0.05$ ). No significant differences between caffeine and quinine-HCl were found comparing the perceived intensities at the same level of concentration (Table 13). Based on these results, all concentrations were selected for final tests.

**Table 12.** Two-way ANOVA on intensity scores of bitterness in standard solutions of caffeine and quinine-HCl: mean, F and p values.

Caffeine (g/l)	Mean Intensity	Quinine-HCl (g/l)	Mean Intensity
0	1.60 <sup>f</sup>	0	1.59 <sup>f</sup>
0.58	6.00 <sup>e</sup>	0.016	7.55 <sup>o</sup>
1.16	12.29 <sup>d</sup>	0.032	15.17 <sup>d</sup>
2.33	21.90 <sup>c</sup>	0.048	24.81 <sup>c</sup>
4.66	31.26 <sup>b</sup>	0.064	34.24 <sup>b</sup>
9.32	46.98 <sup>a</sup>	0.081	44.60 <sup>a</sup>
F <sub>5,347</sub>	139.16	F <sub>5,347</sub>	319.26
P	$\leq 0.0001$	P	$\leq 0.0001$

Different letters indicate significantly different values ( $p \leq 0.05$ ).

**Table 13.** Two-way ANOVA on intensity scores of bitterness in standard solutions of caffeine and quinine-HCl (in italic): mean, F and p values.

Concentrations (g/l)	Mean Intensity
0	1.60 <sup>f</sup>
<i>0</i>	<i>1.59<sup>f</sup></i>
0.58	6.00 <sup>e</sup>
<i>0.016</i>	<i>7.55<sup>e</sup></i>
1.16	12.29 <sup>d</sup>
<i>0.032</i>	<i>15.17<sup>d</sup></i>
2.33	21.90 <sup>c</sup>
<i>0.048</i>	<i>24.81<sup>c</sup></i>
4.66	31.26 <sup>b</sup>
<i>0.064</i>	<i>34.24<sup>b</sup></i>
9.32	46.98 <sup>a</sup>
<i>0.081</i>	<i>44.60<sup>a</sup></i>
F <sub>5,347</sub>	129.78
p	≤0.0001

Different letters indicate significantly different values (p≤0.05).

### 3.3.4. Evaluation of caffeine metabolism rate

#### 3.3.4.1. Subjects

Ten subjects, six females and four males, from 22 to 27 years old, participated in the study.

#### 3.3.4.2. General indication

External factors influencing CYP1A2 activity have been investigated in some studies. CYP1A2 activity is induced by polycyclic aromatic hydrocarbons (e.g. cigarette smoking), caffeine, Cruciferous vegetables, heavy exercise, grilled meat and certain drugs such as omeprazole and carbamazepine (Vistisen et al. 1991; Nousbaum et al. 1994; Kall & Clausen 1995; Le Marchand et al. 1997; Parker et al. 1998). On the contrary, CYP1A2 activity is inhibited by Apiaceous vegetables, fluvoxamine, quinolone antibiotics, pregnancy and oral contraceptives (Gardner et al. 1983; Fuhr et al. 1992; Cook et al. 1996; Christensen et al. 2002). Before phenotyping, a methylxanthine abstinent period of 12 to 36 hours is suggested (Faber et al. 2005). Subjects were instructed to avoid foods and beverages containing caffeine for at least 12 hours before the session started; foods affecting CYP1A2 activity for at least 24 hours before the session started; medicines interfering with CYP1A2

activity for at least 48 hours before the session started. A list of such products was provided. Subjects were also instructed to refrain from smoking, eating, and drinking for 2 hours before the session.

#### *3.3.4.3. Saliva collection*

The 'gold standard' measurement of CYP1A2 phenotype is the apparent clearance of caffeine (Kalow & Tang 1993), both in plasma and saliva (Newton et al. 1981; Perera et al. 2011). Saliva is an efficient matrix because it is easily obtainable, economical and non-invasive.

The saliva was collected as described by Dinnella et al. 2009 (Dinnella et al. 2009). Subjects received tap water to rinse their mouths, then they were instructed to mechanically evoke saliva by chewing Parafilm (3 cm \* 3 cm) for a total collection time of 3 min (first saliva collection, T0). Subjects then received a dose of caffeine (100 mg in 15 ml of water). After 30 min, they rinsed their mouths with water and evoked saliva by chewing Parafilm for a further 3 min (second saliva collection, T30). The procedure for saliva collection was repeated after 90, 150, 210, 270 min (four saliva collections, T90, T150, T210, T270). Saliva samples were immediately frozen.

#### *3.3.4.4. Determination of caffeine content in saliva samples*

Enzymatic immunoassays have been proposed to assess CYP1A2 activity using both saliva and plasma (Miceli et al. 1984; Zysset et al. 1984; McDonagh et al. 1991; Brice & Smith 2001; Lieberman et al. 2002; Haskell et al. 2005). These tests are sensitive, simple, harmless, inexpensive and easily repeated (McDonagh et al. 1991).

Salivary caffeine was measured using the caffeine ELISA assay (Abraxis LLC, 54 Steamwhistle Drive Warminster, PA 18974). The absorbance at 450 nm (Abs) was read using a microplate ELISA photometer (1420 Victor 3™ Multilabel Counter, Wallak, PerkinElmer) within 15 minutes after stopping the reaction. The caffeine concentration is inversely proportioned to the absorbance at 450 nm. Saliva samples were analyzed in duplicate.

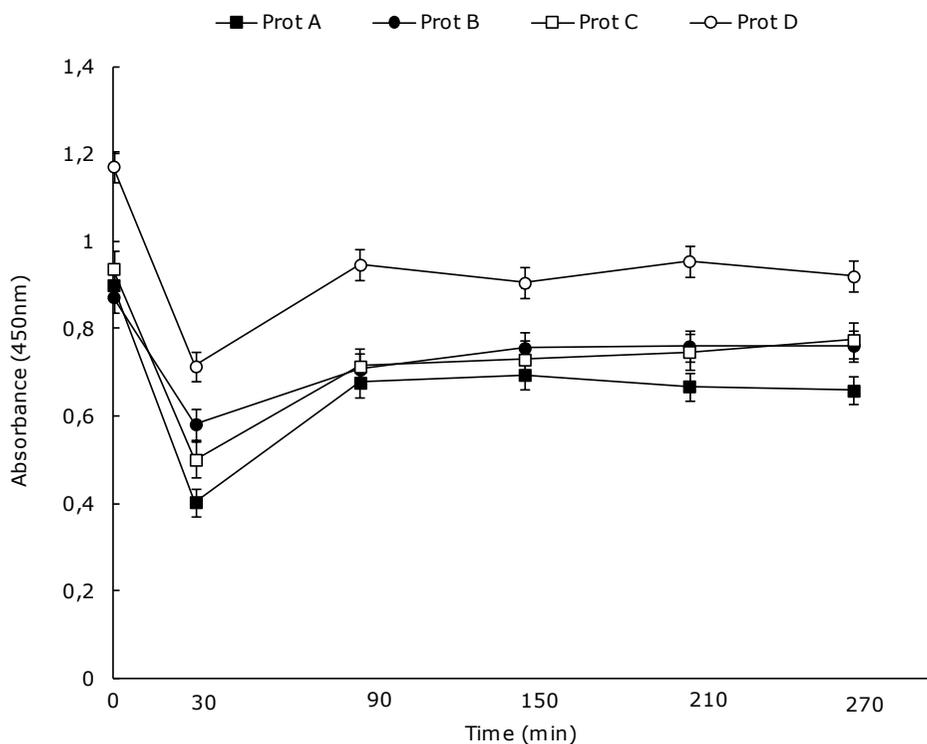
Four different protocols were applied to optimize ELISA assay:

- Protocol A: the same reported in the kit instructions, saliva samples were diluted 1:500 and 30 min were spent for the reaction enzyme-substrate;
- Protocol B: saliva samples were diluted 1:500 and 60 min were spent for the reaction enzyme-substrate;
- Protocol C: saliva samples were diluted 1:1000 and 30 min were spent for the reaction enzyme-substrate;
- Protocol D: saliva samples were diluted 1:1000 and 60 min were spent for the reaction enzyme-substrate.

The protocol A was the one suggested by the ELISA assay producer. In the user's guide of the assay, a further dilution is suggested to obtain accurate results (Protocols C and D). The results may also be improved by spending more time for the enzyme-substrate reaction (Protocols B and D).

#### *3.3.4.5. Caffeine metabolism index*

Caffeine content in saliva was monitored for 270 min during the pilot test (Figure 6). The minimum of absorbance value was reached at 30 min after intake and corresponded to the maximum detectable concentration of caffeine in the adopted experimental conditions. Then, caffeine salivary concentration decreased with metabolism and a sharp absorbance increase was observed after 90 min from the intake. No further absorbance variations were observed between 90 and 270 min possibly because the changes in caffeine concentration were lower than the immune assay sensitivity (75 ppb ( $\mu\text{g/L}$ ) in saliva). Protocol D, combining the 1:1000 sample dilution and 60 min for the reaction enzyme-substrate, was the most effective because it allowed higher levels of registered absorbance and also it was the most sensitive to the variations in caffeine concentration.



**Figure 6.** Absorbance values at 450 nm detected in saliva samples collected at 0, 30, 90, 150, 210, 270 min after caffeine ingestion using for different protocols (A, B, C, D): mean absorbance values for one subject.

Based on this evidence, three sampling times – 0 min (T<sub>0</sub>), 30 min (T<sub>30</sub>) and 90 min (T<sub>90</sub>) – were selected to evaluate caffeine metabolism rate in the final tests. Metabolism rate was expressed as a caffeine metabolism index (CmI). Three absorbance values (Abs) were determined: AbsT<sub>0</sub>, the baseline assay response; AbsT<sub>30</sub>, corresponding to the highest amount of caffeine detected in saliva samples after intake and assumed as the peak of adsorption under the adopted experimental conditions; and AbsT<sub>90</sub>, corresponding to the amount of caffeine metabolized after the adsorption peak at the shortest time when the absorbance variation was detectable under the adopted experimental conditions. The CmI for each subjects was calculated using the following formula:  $(AbsT_{90} - AbsT_{30}) / (AbsT_0 - AbsT_{30}) * 100$ . The higher the CmI value, the higher the amount of metabolized caffeine in one hour after the adsorption peak.

### **3.4. Study II-III**

#### *3.4.1. Subjects*

One-hundred thirty-five regular coffee consumers, seventy-six females and fifty-nine males, from 20 to 60 years old, participated in the study.

#### *3.4.2. Data collection*

Subjects participated in five sessions over five consecutive days, as follows:

##### 1. Assessment of liking for coffee samples

Written scenarios have successfully been used to evoke or create different contexts. Unlike the use of physical means, written scenarios do not require major modifications to the physical environment and allow consumers to personalize the context being evoked (Hein et al. 2010). The written scenario instructs a consumer to imagine an occasion when a food or beverage could be consumed and requires them to think of that occasion in their own mind. Several studies have demonstrated the efficacy of these approaches in understanding consumers' preference for coffee (Petit & Sieffermann 2007; Hein et al. 2010; Sester et al. 2013; Lipchock et al. 2012; Bangcuyo et al. 2015). Just before starting the test, subjects were asked to describe the most preferred situation for consuming coffee (time of the day, place, company, ecc.) and to imagine that they were doing coffee tasting in that particular situation.

Subjects were presented with each sample (25 g) along with 10 g of sugar (about three coffee spoons) in a plastic cup. For each sample, subjects were asked to smell the sample once and rate their liking for the aroma first. Then they were asked to take a sip and rate their liking for the flavour (flavour1). Finally they were asked to freely add sugar, if they thought it was necessary, independent of their habits, to take a sip and rate their liking for the flavour again (flavour2). Hedonic ratings were collected using a 9-point hedonic scale (Peryam & Pilgrim 1957), from 1 ("dislike extremely") to 9 ("like extremely") with a neutral point at 5 ("neither like nor dislike"). The amount of sugar used for each sample by each subject was measured by weighting the sugar cup after each evaluated sample.

## 2. Paired comparison between coffee samples: rejection threshold for caffeine

Subjects were presented with six pairs of coffee samples. Each pair consisted of the less concentrated sample (0.5 g/l caffeine) and one of the other samples (0.5, 1, 2, 3, 4, 6 g/l caffeine). The presentation order of the pairs and of the samples in the pair was balanced across subjects. Subjects were instructed to taste the first sample, rinse their mouths with water, taste the other sample and finally choose the most preferred sample in the pair (Prescott et al. 2005). No sugar was provided to subjects.

## 3. Evaluation of sourness, bitterness and astringency in coffee samples

First, subjects were trained to recognize sourness, bitterness, and astringency and to use the Labelled Magnitude Scale as described above. Subjects were then instructed to hold each sample (8 ml) in their mouth for 10 s, then expectorate, wait 20 s and evaluate the intensity of sourness, bitterness and astringency using the LMS. The evaluation order of attributes was balanced across subjects.

## 4. (a) Coffee choice and (b) evaluation of bitter standard solutions (caffeine, quinine-HCl, PROP)

(a) At the beginning of the session, subjects were presented with two coffee packs available on the market and were asked to choose which one they wanted to drink. One coffee was dark roasted and labelled with "strong flavour" (in Italian "*flavour intenso*"); the other one was medium roasted and labelled with "balanced flavour" (in Italian "*flavour equilibrato*"). These terms were chosen to indicate two different degrees of overall flavour, in particular bitterness.

(b) Then, subjects were trained to use the gLMS and were presented with two sets of samples (one for caffeine and one for quinine-HCl), each consisting of six samples. The presentation order of the sets and of the samples in the set was balanced across subjects. Subjects were instructed to hold the sample (10 ml) in their mouth for 10 s, then expectorate, wait 20 s and evaluate the intensity of bitterness, using the gLMS.

At the end of the session, PROP taster status was evaluated. The one solution test was conducted (Prescott et al. 2004). Subjects rated the intensity of a suprathreshold PROP

solution (3.2 mM), using the gLMS (Bartoshuk et al. 2002). This method has been found to provide group separation equivalent to methods using multiple PROP solutions or PROP/NaCl ratio scores (Tepper et al. 2001).

The evaluation of caffeine and quinine-HCl solutions was performed once, the evaluation of PROP solution twice.

#### 5. (a) CYP1A2 phenotyping and genotyping and (b) fungiform papillae counting

To participate in this session, subjects had to follow the general indications reported in 3.3.4.2. At the beginning of the session, subjects repeated the choice test between the two coffee packs.

(a) For CYP1A2 genotyping, saliva was collected by DNA swabs (DNA Swab - 20 ISO SK-2S – 100 x 1 swab with 2 ml tube and special release cap, individually wrapped and gamma treated, Teltec srl) and DNA was then extracted using the QIAAsymphony (www.qiagen.com) automatic extractor. Finally, genotypes for rs762551 were obtained through direct Sanger sequencing.

After this, three saliva samples for each subject were collected as described above (3.3.4.3.).

During breaks in saliva collection, subjects filled in a questionnaire that included information on:

(1) demographics – gender, age –, smoking habits and contraceptive use;

(2) mode and frequency of coffee consumption: subjects expressed their coffee consumption frequency using a 5-point scale, labelled 1 "less than once a day", 2 "once a day", 3 "twice or three times a day", 4 "four or five times a day", 5 "more than five times a day". They were also asked to indicate if they usually consume coffee with added sweetener (1 "never", 2 "occasionally", 3 "regularly");

(3) liking for 41 bitter or caffeine-containing drinks/foods (Drewnowski & Gomez-Carneros 2000; Dinnella et al. 2011; Hayes et al. 2011) (Table 14) using a 9-point hedonic scale (Peryam & Pilgrim 1957);

(4) choice from 8 pairs of caffeine-containing foods: the food pairs were formed based on different level of perceived bitterness or on added ingredients that counteract the perceived bitterness. Participants expressed their choice for one of the products within a pair using a 7-point scale where "1" indicated that the choice was definitively for the product on the left side and "7" that the choice was definitively for the product on the right (Roininen et al. 2001). The number 4 was considered as a neutral point. The points 2, 3, 5 and 6 were used to indicate any other intermediate judgements. The position of products at left vs right was counterbalanced across the 8 pairs;

(5) familiarity with 16 bitter or caffeine-containing drinks/foods (Table 15) using the familiarity scale consisted of five options, labelled 1 "I do not recognize the product", 2 "I recognize the product, but I have not tasted it", 3 "I have tasted, but I do not use the product", 4 "I occasionally eat the product" and 5 "I regularly eat the product" (Bäckström et al. 2004);

(6) consumption of foods affecting CYP1A2 activity (cabbage/broccoli, radishes, valerian, grilled meat, carrots, celery, celeriac, spices, parsley, parsnip, grapefruit juice) using a 7-point scale, labelled 1 "once a month or less", 2 "less than once a week", 3 "once a week", 4 "twice or three times a week", 5 "four or five times a week", 6 "once a day", 7 "more than once a day";

(7) perceived effect of caffeinated coffee intake (Table 16) (Huntley & Juliano 2012) were rated on a 7-point scale from strongly disagree (1) to strongly agree (7).

**Table 14.** 41-item list of bitter or caffeine-containing drinks/foods used in the questionnaire.

Checklist of bitter/caffeine containing foods	
Apple juice	<i>Tea with milk*</i>
Blueberry juice	<i>Tea with sugar*</i>
Carrot juice	<i>Tea with sugar and with milk*</i>
Grapefruit juice	<i>Tea without sugar and without milk*</i>
Lemon juice	<i>Cocoa</i>
Orange juice	<i>Dark chocolate*</i>
<i>Burn</i>	<i>Dark chocolate 70%*</i>
<i>Chinotto</i>	<i>Dark chocolate 99%*</i>
<i>Coke</i>	<i>Milk chocolate*</i>
<i>Pepsi</i>	Asparagus
<i>Red bull</i>	Aubergine
<i>Lemon soda</i>	Broccoli
<i>Orange soda</i>	Cabbages
<i>San bitters</i>	Endive
<i>Tonic water</i>	Rape
Beer	Soy products
Coffee liqueur	Spinach
<i>Guarana</i>	
<i>Maté</i>	
Cappuccino	
<i>Coffee with milk*</i>	
<i>Coffee with sugar*</i>	
<i>Coffee with sugar and milk*</i>	
<i>Coffee without sugar and without milk*</i>	

\* Indicates that product was selected for choice from 8 pairs of caffeine-containing foods

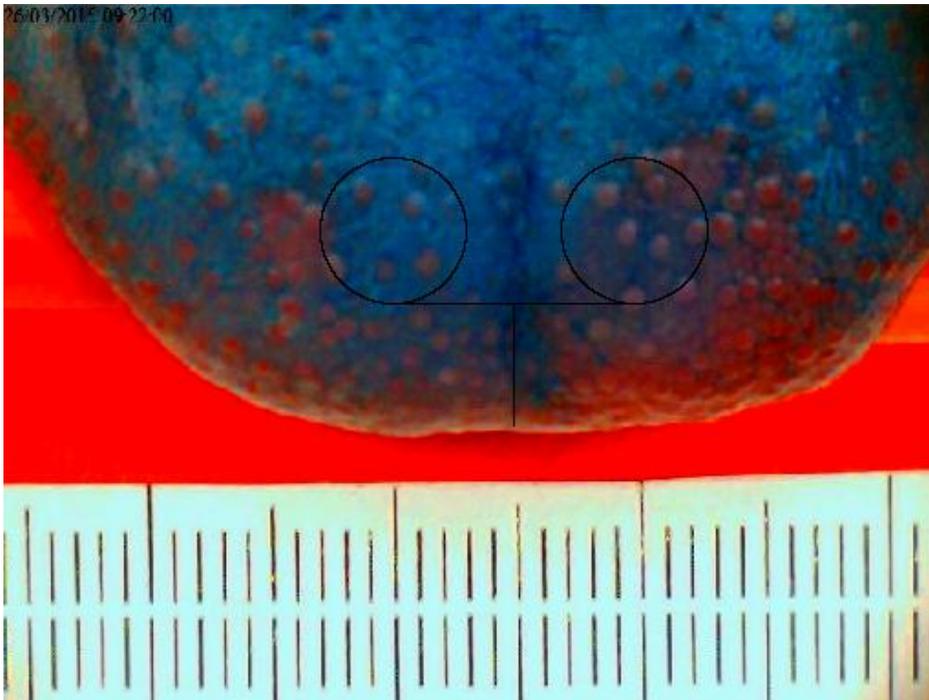
*Italic* indicates that product was selected for familiarity assessment

**Table 15.** Statements to measure perceive effect of caffeinated coffee consumption.

Statements
1. Coffee picks me up when I am feeling tired
2. Coffee improves my athletic performance
3. Coffee increases my motivation to work
4. Coffee improves my concentration
5. Coffee makes me jittery
6. Coffee at any time of day throws off my sleep
7. Coffee makes my heart beat irregularly
8. Consuming coffee late in the day disrupts my sleep
9. The number of coffees that I consume during the day depends on taken benefit
10. Coffee makes me feel good

(b) At the end of the session, a photograph of the tongue of each subject was collected. To facilitate visualization of the FP, the anterior portion of the dorsal surface of the tongue was swabbed with household blue food colouring (F.lli Rebecchi), using a cotton-tipped applicator. This made the FP easily visible as red structures against the blue background of the stained tongue (Miller & Reedy 1990a). Images of the tongue were recorded using a digital microscope (MicroCapture, version 2.0 for 20x-400x).

For each participant, the clearest image was selected, and the number of FP was counted in two 0.6 cm diameter circles, one on the right side and one on the left side of the tongue, 0.5 cm from the tip and 0.5 cm from the tongue midline (Figure 7) (Shahbake et al. 2005). The number of FP was counted by two researchers independently, blind to the performance of participants in the sensory evaluation tests, and the average of these values was used for each subject. The Denver Papillae Protocol (or simply DPP), was applied to reduce variance and increase accuracy in FP counting (Garneau et al. 2014). DPP is a dichotomous key with clear and distinct characteristics of FP that were selected based on previous literature, but for the first time pooled and prioritized for a more complete and objective method.



**Figure 7.** Example of the procedure used to count fungiform papillae.

### **3.5. Data analysis**

#### **3.5.1. Descriptive data**

Intensity data from the trained panel were analyzed by multiblock PCA (Tucker-1) and by p/MSE plot (Panel Check software, ver 1.4.0, Nofima) to assess panel calibration and assessor performance, respectively. Intensity ratings from coffee samples were independently analyzed by a two-way ANOVA mixed model (sample as fixed and assessors as random factors), followed by a Fisher's LSD post hoc test (significant for  $p \leq 0.05$ ). Principal Component Analysis (PCA) was computed on panel averages of each significant attribute arising from the ANOVA models. Samples were included as dummy variables (down-weighted in the data matrix) to improve the visual interpretation. The full cross validation was computed to validate the interpretation of the first two components.

#### **3.5.2. Liking data**

Liking ratings expressed for aroma, flavour1 and flavour2 were independently submitted to a two-way ANOVA model (assessors and sample as factors) with Fisher's LSD post hoc test

(significant for  $p \leq 0.05$ ). Liking ratings expressed for flavour1 and flavour2 were analyzed by a two-way ANOVA model (sample -6 levels- and condition -2 levels: with or without sugar added- as factors), with Fisher LSD post hoc test (significant for  $p \leq 0.05$ ).

Individual ratings expressed for aroma, flavour1 and flavour2 were analyzed by an internal preference map (IMP). Samples were included as dummy variables (down-weighted in the data matrix) to improve the visual interpretation. The full cross validation was computed to validate the interpretation of the first 2 components.

In study I a visually oriented approach, based on the inspection of IMP loading plot, was used for subject clustering and Y-axis was set as the limit between consumer segments. The individual neophobia values were computed as the sum of ratings given to 10 statements, after the negative items had been reversed; the neophobia scores thus ranged from 10 to 70 (10 maximum value to 70 minimum value). Neophobia values of clusters were compared by means of an independent t-test. The associations of clusters with gender, coffee consumption frequency and consumption of sweetened/ unsweetened coffee (categorical variables) were estimated by a homogeneity chi-square test.

### **3.5.3. Individual variables**

In studies II and III the relationships between FP number, PROP status and caffeine metabolism index were independently estimated by Pearson's correlation coefficient. A two-way ANOVA (group and sample as factors), with Fisher's LSD test ( $p \leq 0.05$ ) were used to independently test the effect of FP number, PROP status and caffeine metabolism rate on liking and intensity ratings in both coffee samples and standard solutions. The associations of clusters with gender, coffee consumption frequency and consumption of sweetened/unsweetened coffee (categorical variables) were estimated by a homogeneity chi-square test.

In study II, subjects were divided in two groups based on median value of FP distribution (12.12; range 4 - 22): Low FP (LFP; n=60) and High FP (HFP; n=60). PROP taster status was based on the average rating of the two replicates, and groupings were based on percentile distribution: PROP non tasters (NTs; n=30)  $\leq 21.75$ ; PROP medium tasters (MTs; n=60), 22– 65; and PROP super- tasters (STs; n=30)  $\geq 65.125$ .

In study III, subjects were divided in two groups based on the median value of the CmI z-score distribution (0.0056; range: from -1.7109 to 2.8752): Low CmI (LCmI; n=45) and High CmI (HCmI; n=45). The subject with the CmI corresponding to the median value of the CmI z-score distribution (0.0056) was excluded from the further analysis. A linear regression between CmI and coffee consumption was computed, subjects were divided in four groups based on CmI percentile distribution and the coffee consumption of each group was calculated as sum of frequency scores by each subject. The effect of cluster on perceived effect of caffeinated coffee consumption was estimated by a two-way ANOVA. The effect of genotype group on CmI was estimated by a one-way ANOVA.

#### **3.5.4. Text analysis**

Text analysis was conducted combining the statistical tools provided by the software for text analysis T-LAB 9.1 (Italy) and semiotic analysis, applied on 10% of the whole sample to guide the pre-processing of the data.

A Thematic Analysis was conducted using the software T-LAB 9.1, indicating as unit of analysis the different texts produced by the consumers (context units). Themes were identified by an inductive approach (bottom-up), which allows themes to “emerge” from textual data. An unsupervised clustering was performed, including a type of co-occurrence analysis and, subsequently, a type of comparative analysis. In particular, comparative analysis uses the categories of the new variable (categories of the new variable = thematic clusters) derived from the co-occurrence analysis to form the contingency table columns. Analysis results can be considered as an isotopy (iso = same; topoi = places) map where each theme is characterized by the co-occurrences of semantic traits (Lancia 2014). The label of each theme was chosen throughout the inspection of the context units assigned to each clusters considering their score (weight) and the characteristic lemma of each cluster. Four clusters (or themes, groups of words semantically associated) focusing on different aspects of the coffee experience were identified.

Global Chi-square was used for testing the independence between rows – physiological variables (FP number, PROP taster status, CmI) - and columns – clusters - of the contingency table. Chi-square per cell was applied to analyse if the observed values of each cell of the contingency table were significantly higher, lower or equal to the theoretical values, applying Fisher Exact Probability Test with  $\alpha=0.05$  (Symoneaux et al. 2012).

## 4. Results

### 4.1. Study I: the effect of roasting degree on coffee sensory profile and liking

Increasing the bean processing temperature affected the intensity of specific sensory attributes. In particular, coffees processed at temperature higher than 150°C were described by terms generally used to describe samples from standard roasted coffees, such as “coffee”, “roasted”, “burnt” (International Coffee Organization n.d.). Moreover, samples processed at temperatures higher than 155-160°C were not significantly different from the coffees roasted at the standard temperature of 220°C. This was affected also by origin ( $F_{1,50}=18$ ;  $p\leq 0.001$ ), with coffees from Guatemala being more intense than coffees from Brazil.

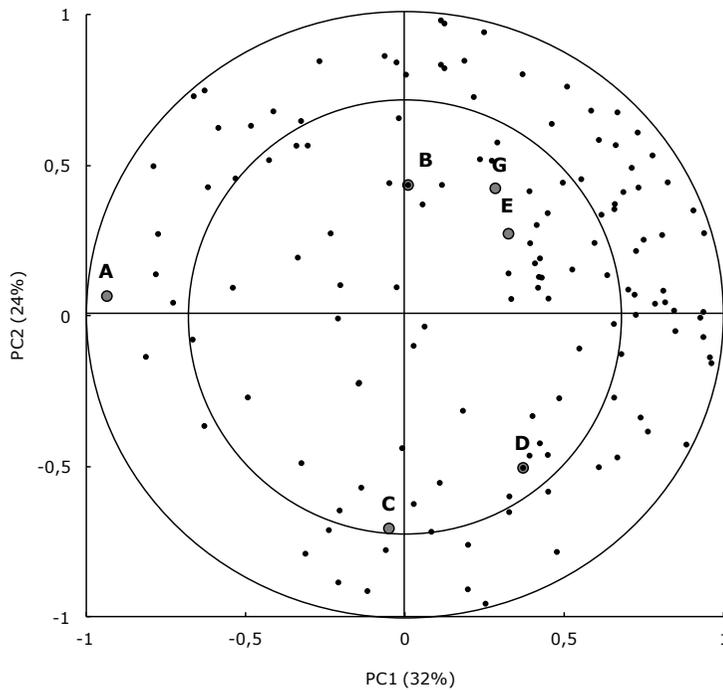
Mean liking scores for flavour were significantly affected by the conditions adopted for data acquisition (with or without sugar) ( $F_{1,888}=120.25$ ;  $p\leq 0.001$ ). In particular, mean liking data for flavour1 (without sugar) resulted significantly lower than those for flavour2 (with sugar). Furthermore, 80% of subjects added sugar after the first tasting. Consumers’ liking was also positively related to roasting temperature ( $F_{5,888}=2.5$ ;  $p=0.03$ ). The liking in general was higher for samples processed at temperature higher than 150°C. However, two cluster were evident: CL1 (n=29), who preferred the sample from beans processed at 140°C, and CL2 (n=46), who preferred the sample from beans processed at 160°C. These two groups differed only for neophobia score, with CL1 subjects were less neophobic than CL2 subjects ( $t_{73; 1.99}=-2.08$ ;  $p=0.04$ ).

### 4.2. Study II-III: the effect of individual characteristics on coffee liking and consumption

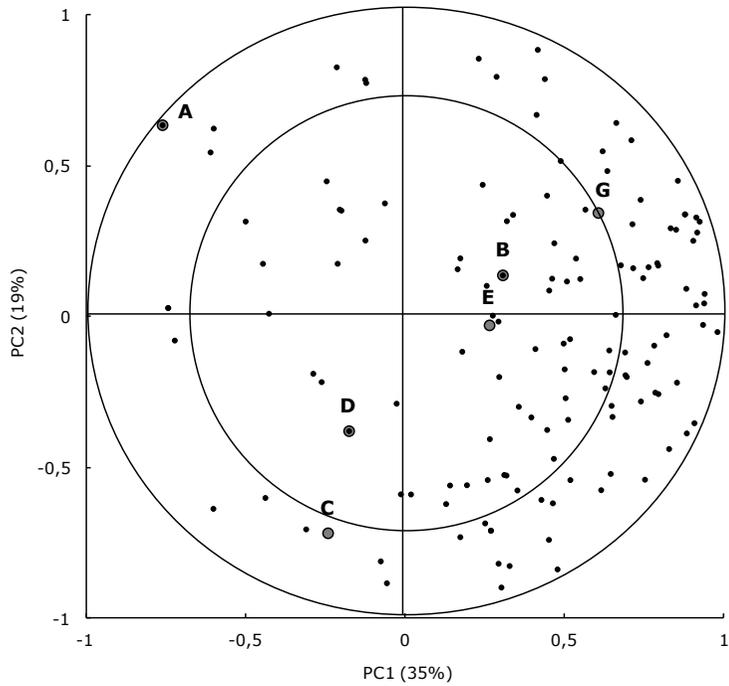
The internal preference map related to aroma is shown in Figure 8. The variance explained by the model using the first 2 significant dimensions was 56%. A relatively even spread of consumer loadings was observed. However, the first dimension of the correlation loading plot indicated that, in general, subjects’ preferences were oriented toward samples on the right side of map consisting of samples G, E, D. Coffee samples A and C were positioned of the left side and were preferred by a very limited number of subjects. The internal preference map related to flavour1 (without sugar) and flavour2 (with sugar) are shown in Figure 9-10. The variance explained by the model using the first 2 significant dimensions

was 54% and 58%, respectively. The first dimension of the correlation plot indicated that, in general, consumers' liking was oriented toward samples B, G, E. Samples B and G were the dark roasted samples, while sample E was the decaffeinated coffee.

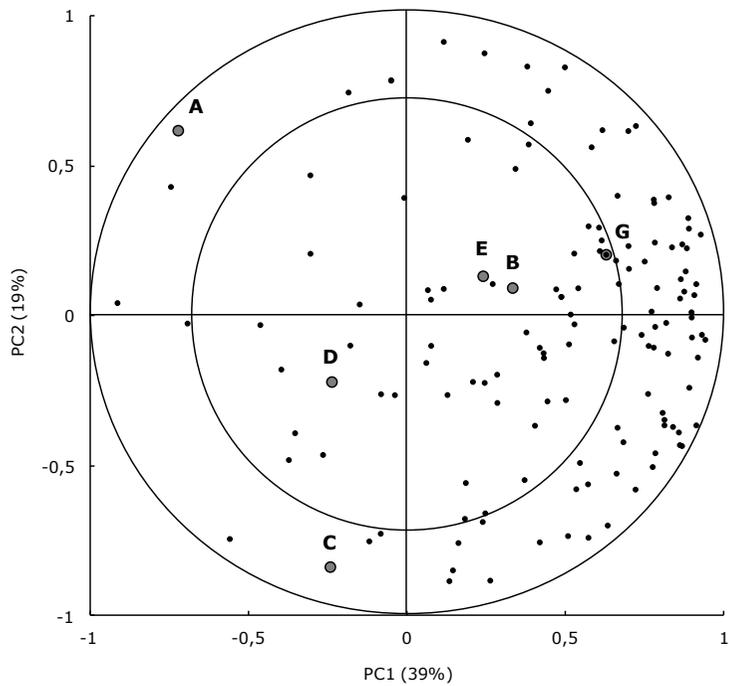
Mean liking scores for flavour were significantly affected by the conditions adopted for data acquisition (with or without sugar) ( $F_{1;1618}=60.14$ ;  $p\leq 0.001$ ). In particular, mean liking data for flavour1 was significantly lower than those for flavour2. Furthermore, 84% of subjects added sugar after the first tasting.



**Figure 8.** Internal preference map on individual liking expressed for aroma.



**Figure 9.** Internal preference map on individual liking expressed for flavour 1 (without sugar).



**Figure 10.** Internal preference map on individual liking expressed for flavour 2 (with sugar).

No significant relationships were found between the FP number, the perceived intensity of PROP and the caffeine metabolism index (FP&PROP:  $r^2=0.002$ ,  $p=0.592$ ; PROP&CmI:  $r^2=0.0004$ ,  $p=0.858$ ; FP&CmI:  $r^2=0.027$ ,  $p=0.121$ ). So the effect of these factors on coffee liking and bitterness perception has been analyzed independently.

#### *4.2.1. The effect of fungiform papillae number on coffee liking and perception*

Subjects were divided in two groups based on median value of FP distribution (12.12; range 4 - 22): Low FP (LFP) and High FP (HFP). Subjects with a higher FP number (HFP) added more sugar to the samples when asked to freely do it ( $F_{1,708}=9.26$ ,  $p=0.002$ ) and gave higher liking ratings for coffees than subjects with a lower FP number (LFP) ( $F_{1,708}=6.79$ ,  $p=0.009$ ). No significant effect of FP group on liking for aroma and flavour without sugar were found ( $F_{1,708}=0.59$ ,  $p=0.443$ ;  $F_{1,708}=2.17$ ,  $p=0.141$ , respectively).

HFP subjects rated the bitterness of both caffeine and quinine-HCl solutions as stronger ( $F_{1,708}=4.21$ ,  $p=0.041$ ;  $F_{1,708}=10.42$ ,  $p=0.001$ , respectively), and also sourness in coffee, than did LFP subjects ( $F_{1,708}=3.83$ ,  $p=0.050$ ). No significant differences between FP groups were found for coffee bitterness and astringency ( $F_{1,708}=0.001$ ,  $p=0.980$ ;  $F_{1,708}=1.40$ ,  $p=0.286$ , respectively).

#### *4.2.2. The effect of PROP taster status on coffee liking and perception*

While coffee liking was unrelated to PROP status (aroma:  $F_{2,702}=0.03$ ,  $p=0.968$ ; flavour1:  $F_{2,702}=1.25$ ,  $p=0.288$ ; flavour2:  $F_{2,702}=0.06$ ,  $p=0.944$ ), non-tasters (NTs) added more sugar to the coffees than did either medium tasters (MTs) or supertasters (STs) ( $F_{2,702}=8.34$ ,  $p\leq 0.0001$ ). However, STs rated coffee sourness ( $F_{2,702}=6.73$ ,  $p\leq 0.0001$ ), bitterness ( $F_{2,702}=16.68$ ,  $p\leq 0.0001$ ) and astringency ( $F_{2,702}=15.09$ ,  $p\leq 0.0001$ ) as stronger than NTs and MTs. Moreover, STs rated the bitterness of both caffeine and quinine-HCl solutions as stronger than did PROP NTs and MTs ( $F_{2,702}=16.33$ ,  $p\leq 0.0001$ ;  $F_{2,702}=15.83$ ,  $p\leq 0.0001$ , respectively).

#### *4.2.3. The effect of caffeine metabolism index on coffee liking and perception*

No significant effect of genotype class on CmI was found ( $F_{2,48}=0.571$ ,  $p=0.569$ ).

Subjects with a lower caffeine metabolism index (LCmI) added more sugar to the coffee samples than did subjects with a higher caffeine metabolism index (HCmI) ( $F_{1,528}=23.87$ ,

$p \leq 0.0001$ ). However, no significant effect of CmI group on liking for aroma and flavour without and with sugar were found (aroma:  $F_{1,528}=2.51$ ,  $p=0.143$ ; flavour1:  $F_{1,528}=1.74$ ,  $p=0.187$ ; flavour2:  $F_{1,528}=0.25$ ,  $p=0.615$ ). Bitterness was rated as stronger by LCmI subjects than by HCmI subjects ( $F_{1,528}=5.88$ ,  $p=0.016$ ) but there were no significant effects of group on sourness ( $F_{1,528}=2.65$ ,  $p=0.104$ ) and astringency ( $F_{1,528}=2.45$ ,  $p=0.118$ ) perception in coffee. Moreover, LCmI subjects rated the bitterness of caffeine solutions as stronger than did HCmI subjects ( $F_{1,528}=5.21$ ,  $p=0.023$ ), but there were no group differences for quinine-HCl solutions ( $F_{1,528}=2.36$ ,  $p=0.125$ ).

A significant positive relationship between CmI and the amount of coffee consumed daily was found ( $r^2=0.93$ ,  $p=0.033$ ), with HCmI subjects consuming more coffees daily than LCmI subjects. In choosing a coffee based on information ("strong" or "balanced" flavour), after 12 h abstention from caffeine the majority of subjects, independent of CmI group, chose the "strong" product most often ( $\chi^2=0.185$ ,  $df=1$ ,  $p=0.667$ ). On the other hand, after no restrictions on caffeine intake before the test, LCmI subjects chose the "balanced" product most often (57.78%), while HCmI subjects chose the "strong" product ( $\chi^2=1.601$ ,  $df=1$ ,  $p=0.206$ ). The major impact of caffeine abstention was to produce a tendency for LCmI subjects to invert their choice ( $\chi^2 = 2.18$ ,  $df=1$ ,  $p=0.140$ ).

No significant differences were found between CmI groups in response to questions on the perceived effects of caffeinated coffee consumption.

#### *4.2.4. The link between individual characteristics and the preferred context for having coffee*

The one-hundred thirty-five texts describing the most preferred situation for consuming coffee were considered in the analysis, for a total of 5567 words (on average 41,23 per subject). The texts differed in length, degree of details and focus, constituting an heterogeneous corpus of analysis.

A Thematic Analysis was performed to identify the main themes in the texts and to study the relationship between individual characteristics of the subjects and the preferred context for having coffee (Figure 11).

The variance explained by the model using the first two significant dimensions was 72.57%, representing 44.59% and 27.98% of the variance respectively. Four clusters (or themes,

groups of words semantically associated) were identified, focusing on different aspects of the coffee experience. The clusters were indicated with a label chosen to sum up the focus of the preferred context:

Cluster 1 - Savouring: focus on an experience of relaxation, comfort (even brief);

Cluster 2 - Social: focus on the social dimension (company, friends, chatting); preference for a coffee in the afternoon with friends to recover from work.

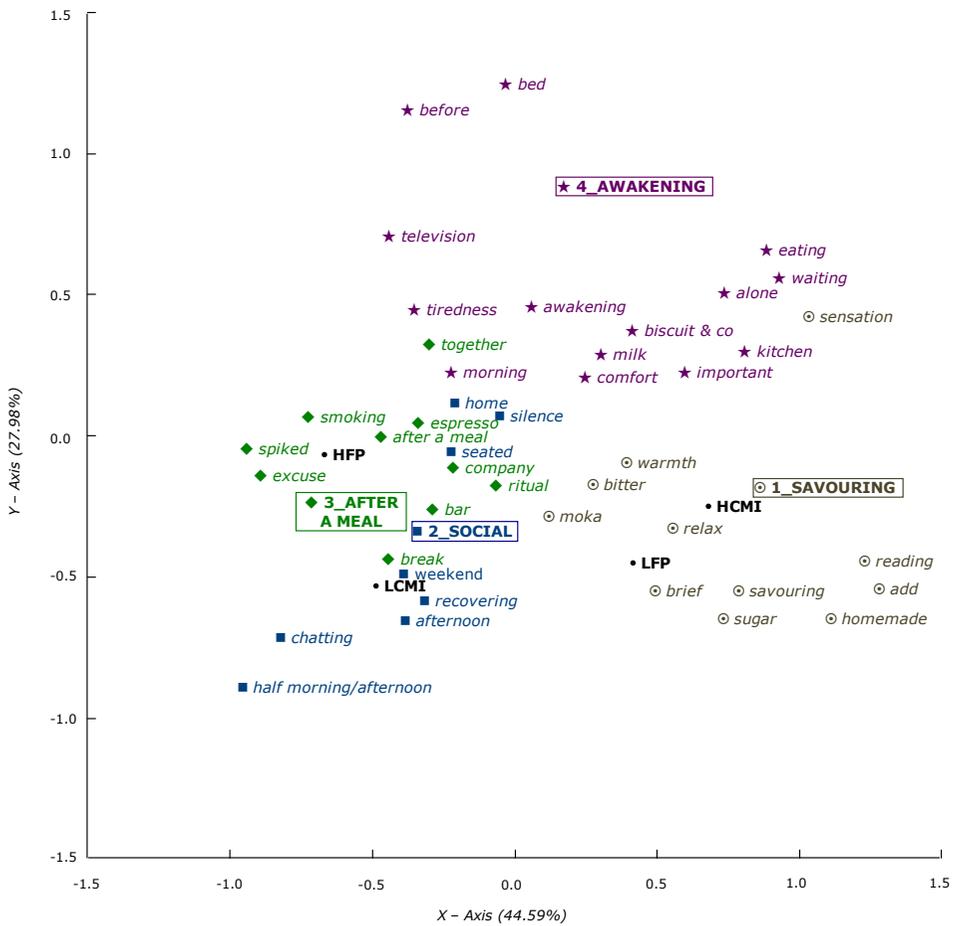
Cluster 3 - After a meal: focus on the situation; preference for a coffee after meal or during a break at work, associated with another ritual (e.g. smoking).

Cluster 4 - Awakening: focus on the waking-up function of coffee and the tasting experience.

According to the thematic analysis, the descriptions were clearly separated along the first axis based on the focus: cluster "Savouring" focused on the product and the experience of tasting was on the right of the map, while cluster "After a meal" and "Social", more focused on the situation, were on the left. Along the second axis the main differences were found between the cluster "Awakening", focused on the functionality of coffee, and the other clusters, particularly "Social" and "Savouring".

Projecting the categorical variables (physiological indices) on the map it was possible to notice that individual factors were related to different preferred contexts.

The main differences were registered on the first dimension (44.59%): Low FP (LFP) subjects and High CMI (HCmI) were more represented in cluster 1 (Savouring), compared to High FP (HFP) and Low CMI (LCmI) that resulted more focussed on the situation (Cluster 2 and 3: Social and After a meal).



**Figure 11.** Map obtained with Thematic Analysis.

Results from global Chi-Square and Chi-square per cell on the contingency tables for each set of variables allowed exploring the results more in depth. Comparing LCmI and HCmI subjects, our findings showed that the social context (cluster 2) was significantly less preferred by HCmI subjects, while the savouring context (cluster 1) was significantly less preferred by LCmI subjects (Table 16). Comparing LFP and HFP subjects, there was a tendency for HFP subjects to prefer less the savour context (cluster 1), while LFP tended to prefer less the awakening context (cluster 4) (Table 17).

**Table 16.** Contingency table: frequency of LCmI and HCmI subjects in each context cluster (1-Savouring; 2-Social; 3-After a meal; 4-Awakening); (homogeneity chi-square test:  $\chi^2=7.497$ ,  $df=3$ ,  $p=0.058$ ).

	LCmI	HCmI	Total
CL1 Savouring	<b>8</b> <	15>	23
CL2 Social	17>	<b>8</b> <	25
CL3 After a meal	14>	10<	24
CL4 Awakening	6<	11>	17
Total	45	44	89

< and > indicate that the observed value is lower or higher than the expected theoretical value (Chi-square per cell).

In bold significant differences based on Fisher Exact Probability Test with  $\alpha=0.05$ .

**Table 17.** Contingency table: frequency of LFP and HFP subjects in each context cluster (1-Savouring; 2-Social; 3-After a meal; 4-Awakening); (homogeneity chi-square test:  $\chi^2=5.892$ ,  $df=3$ ,  $p=0.117$ ).

	LFP	HFP	Total
CL1 Savour	19>	<b>11</b> <	30
CL2 Social	16>	14<	30
CL3 After a meal	17<	19>	36
CL4 Awakening	<b>7</b> <	16>	23
Total	59	60	119

< and > indicate that the observed value is lower or higher than the expected theoretical value Chi-square per cell).

In bold significant differences based on Fisher Exact Probability Test with  $\alpha=0.05$ .

No differences in the preferred context were found considering gender (M/F), smoke (Yes/No), use of sugar in coffee (Yes/No), PROP taster status (Super-/Medium-/Non-taster) as variables.

## **5. Discussion**

These studies show that coffee consumption and preference, in terms of frequency and modality, are influenced by both the sensory properties of coffee and the psychological, physiological and metabolic characteristics of consumers. The roasting degree is a step in the production process crucial for the development of coffee sensory properties affecting consumer preference. Furthermore, both the physiological indices – FP number and PROP taster status – and the caffeine metabolism rate play a significant role in taste sensitivity for bitter compounds in general and also in the preference for coffee with or without sugar.

### **5.1. The effect of roasting degree on the sensory profile and consumers' acceptance of coffee**

The most significant finding of study I is that the sensory profiles of coffee from beans processed at temperatures higher than 150°C were not significantly different from the sensory profiles of coffee from beans roasted at the standard temperature of 220°C. Under-roasting processes (from 140 to 160 °C) have been successfully proposed to reduce the content of potentially harmful compounds in coffee. However, no information was available on the sensory properties of and consumer liking for brews prepared with under-roasted coffee. This study reports the description of the sensory properties of coffee beverages obtained by thermal processing means which are milder than the standard roasting. Moreover, this study demonstrates that these products were accepted by consumers, thereby suggesting a new possibility for the market of coffee-based beverages.

In any case, roasting degree is one of the main factors contributing to the coffee sensory profile (Lindinger et al. 2008). In general, the perceived intensity of bitterness and odours increases from light to dark roasts while the perceived intensity of sourness decreases (Bhumiratana et al. 2011). This pattern of findings is confirmed by the data from study I on under-roasted coffees. Increasing the bean processing temperature increased the frequency of panellists' descriptors referring to "coffee" and "burnt" and decreased the frequency of terms referring to "grain/oats" and "vegetables". In general, brews from coffee processed at higher temperature (in this case  $\geq 150^{\circ}\text{C}$ ) were described in terms that explicitly referred to coffee; on the other hand, samples from beans processed at lower temperature (in this case  $< 150^{\circ}\text{C}$ ) were mainly described in terms that were unusual for standard roasted coffee.

These findings were also confirmed by the descriptive analysis run in the pilot tests for study II and III, showing that roasting temperature was positively associated with the perceived intensity of coffee flavours. Moreover, as previously reported by other authors (Frank et al. 2006; Blumberg et al. 2010), the results demonstrated that caffeine content and the degree of roasting contribute to the perceived intensity of bitterness in coffee.

In general, consumers' preference followed the same patterns as well, with the most preferred products being the samples roasted at higher temperatures. These results were not surprising considering that coffee from under-roasted beans is not common in the Italian market and so it is a quite unconventional product. These products were liked by subjects with a lower neophobia index, that is, they were more inclined to try and consume unfamiliar foods. These results confirmed the fact that both attitudes and psychological factors influence liking for a product, as well as for widely accepted products such as coffee. In studies II and III the most preferred samples were the dark roasted samples B and G, with the exception of the decaffeinated coffee E (medium roasted) and the sample D that was dark roasted but not one of those most liked by consumers. Based on the descriptive analysis, the dark roasted samples B, D, G had very similar sensory profiles; on the contrary, the decaffeinated coffee was very different from the all other coffee with different caffeine contents. It seems that in this case, the liking was based on the lack/presence of sour taste.

## **5.2. The effect of individual variables on coffee perception and preference**

Another important general finding of consumers' studies, both on under roasted and standard roasted coffees, was that tasting coffee in standard conditions is not appropriate. In particular, it seems to be better if subjects were free to add sugar to the samples. The lack of a significant sample effect on liking for unsweetened coffees was probably due to the general disliking for unsweetened samples, considering that the majority of subjects usually consumed coffee with sugar and also added sugar when they were asked to do so freely. The results of studies II and III on liking for standard roasted coffees showed that the use of sugar in coffee is influenced by both physiological and metabolic factors, such as the density of FP, sensitivity to the bitter compound PROP, and the caffeine metabolism rate.

In particular, subjects with higher FP numbers added more sugar when asked to do so for the study samples, and gave higher liking ratings than did low FP subjects for the sweetened coffee samples. High FP subjects were also more sensitive to sourness in coffee. This suggests that these subjects were trying to modify an undesirable sensory characteristic that they perceived in the coffees using sugar. The use of sugar also caused a general increase in liking for the coffee samples. Sugar is generally added to enhance flavour and to mask undesired tastes. In fact, sweetness is the only innate preference and the addition of sweetness to a wide variety of foods and drinks increases their immediate acceptability (Yeomans 2007). This practice helps the development of preference for bitter and strong flavoured foods like coffee or alcohol beverages.

Sugar use was related also to PROP taste status. In particular, PROP NTs, who rated coffee sourness, bitterness and astringency as less intense than did PROP STs, added more sugar to the coffee than did either MTs and STs. It could be suggested that NTs might be attempting to modify the sensory properties of the coffee, enhancing its overall flavour by adding sugar. Moreover, PROP NTs are reportedly more likely to be sweet-likers as determined by their responses to sweet tastes in solution (Yeomans, Tepper, et al. 2007), suggesting that they are more inclined to consume sweetened products.

Also caffeine metabolism rate was associated with the use of sugar in coffee. In particular, subjects with a lower caffeine metabolism rate, who were more sensitive to bitterness of coffee, added more sugar to coffee. In this case, sugar was used to mask the bitterness in coffee, because no differences were found between slow and fast metabolizers in the perceived intensity of both sourness and astringency.

### **5.3. The effect of individual variables on bitterness perception**

Both subjects with higher FP numbers and STs rated the intensity of caffeine and quinine-HCl bitterness in solutions as stronger than did subjects with lower FP numbers and NTs, confirming that both FP density and PROP taster status are a general index of taste sensitivity. However, PROP ratings and FP counts were not positively correlated as they were in some previous studies (Bartoshuk et al. 1994; Tepper & Nurse 1997; Prutkin et al. 2000; Delwiche et al. 2001b; Essick et al. 2003; Hayes & Duffy 2007; Duffy et al. 2010; Nachtsheim & Schlich 2013). There were also other works which had reported no significant

association between TAS2R38 haplotype or PROP status and FP density (Duffy, Davidson, et al. 2004; Fischer et al. 2013; Garneau et al. 2014).

The lack of a PROP/FP association found in the present study may depend on several factors. First, different methods can be applied to both FP identification and counting and taste intensity measurements. Some papillae are flat and short with little elevation, others are double papillae. Thus counting FP by hand is cumbersome and a somewhat subjective task, because the identification of FP is not always easy. Furthermore, the FP remain intact even when the chorda tympani nerve is severed. Thus damage to taste structures alter taste experience without altering the number of FP (Prutkin et al. 2000). The number of FP can be measured, but not the number of taste pores within each papilla (Fischer et al. 2013). Thus, the number of FP is only an estimate of gustatory functionality, based on the fact that 99% of papillae in humans contain at least 1 taste bud (Segovia et al. 2002). Moreover, PTS can be assessed in different ways and the type of method used can affect the results (Tepper et al. 2001; Bartoshuk et al. 2002). For this reason, the results may not be comparable across studies (Fischer et al. 2013). Later, the majority of studies on the relationship between the FP number and PTS has been performed almost exclusively in small study cohorts or convenience samples. And so, those results may not be representative when these relationships are compared with other populations (Fischer et al. 2013).

Apart from methodologies, this lack of an association between PROP taster status and FP number can also depend on the fact that PROP taster status is due to both genetic variations in TAS2R38 and general taste sensitivity, while FP number is related only to taste sensitivity. Indeed, as previously reported by Hayes and co-authors (Hayes et al. 2008), PROP taster status and FP number are associated only in the TAS2R38 homozygote group and not in the TAS2R38 heterozygote group.

For the first time a relationship between caffeine metabolism rate and sensitivity to bitterness of caffeine has been highlighted. In particular, subjects with a lower caffeine metabolism rate perceived caffeine as more bitter than subjects with higher caffeine metabolism rate. On the contrary, the bitterness of quinine-HCl and PROP was perceived at the same level in both slow and fast metabolizers. As far as it is known, neither quinine-HCl

nor PROP have any post-ingestive effects; on the contrary caffeine has a stimulatory effect. Thus, since the relationship between caffeine metabolism and bitterness sensitivity is exclusive to caffeine and independent from the sensitivity to other bitter compounds, it seems to be mediated exclusively by the psychoactive effect.

#### **5.4. The effect of caffeine metabolism rate on coffee-consumption behaviour**

Caffeine metabolism rate was related to different coffee-consumption behaviour. Relationships were found between caffeine metabolism rate and both the frequency of coffee consumption and the choice of the coffee to consume. In particular, fast metabolizers favoured a coffee associated with greater stimulatory effects (here labelled as "strong"). In contrast, subjects with the lower caffeine metabolism index chose the "strong" coffee only after 12 hours abstinence from caffeine, probably because this label conveys the expectation that caffeine effects will last longer. In fact, subjects who slowly metabolize caffeine are affected by caffeine stimulatory effects for longer (Bech et al. 2006; Santos et al. 2015). On the other hand, fast metabolizers may be motivated to consume more coffee than slow metabolizers. It is a reasonable assumption that those who quickly metabolize caffeine are affected by caffeine's stimulatory effects for less time. This implies that quick metabolizers will need coffee more frequently to obtain its pharmacological effects, e.g., maintaining alertness, since these have been shown to have positive reinforcing effects (Tinley et al. 2003). It seems that subjects adapt their habits based on their metabolic characteristics (Coffee and Caffeine Genetics Consortium & et al. 2014). This is probably the reason why we did not find any differences in perceived effects, either alerting or anxiogenic, of caffeine consumption. It is plausible that, in regular coffee consumers, different caffeine metabolism rates are reflected in different amounts of consumed coffee to achieve the same desired stimulatory effect.

#### **5.5. Caffeine metabolism rate and CYP1A2 genotype**

While a significant association between the caffeine genotypes and different metabolism rates might be expected, the failure to find it in the present study is not surprising since genotyping is effective when the individual variability in enzyme activity is mostly due to known DNA polymorphisms. In contrast, phenotyping is more effective when other major factors of influence (e.g. enzyme induction/inhibition) are prevalent. Indeed, phenotypes

are a reflection of the combined effects of genetic, environmental and endogenous factors on enzyme activity (Streetman et al. 2000). In the case of CYP1A2, apart from genetic factors, a great deal of variability remains unexplained. Twin studies have suggested that genetic factors play an important role in determining habitual caffeine consumption and sensitivity to caffeine stimulatory effects (Yang et al. 2010), but the specific genes involved have not yet been identified. Indeed, CYP1A2 activity depends on several environmental factors, such as the induction of CYP1A2 activity by smoking, or its inhibition by oral contraceptive use, as confirmed in this study. Furthermore, no SNP or haplotype in the CYP1A2 gene has yet been identified that might unequivocally be used to predict the metabolic phenotype in any individual. Moreover, in this study, a small group of subjects was selected to estimate the effect of genotype group on caffeine metabolism index.

#### **5.6. The effect of individual variables on preferred context for having coffee**

The liking test was run using the immersive technique of written scenario, that is, subjects were asked to describe the most preferred situation for having coffee and to imagine that they were tasting coffee in that particular situation. The aim of this approach was to explore the possibility that people with different physiological characteristics preferred different situations for consuming coffee. By the Thematic Analysis of the texts it was possible to identify four clusters (or themes, groups of words semantically associated), focused on different aspects of the coffee experience: the cluster "Savouring" focused on the product and the experience of tasting; the clusters "After a meal" and "Social", focused on the situation; the cluster "Awakening", focused on the functionality of coffee. The preferred context for coffee consumption seemed to be influenced by the physiological characteristics of the subjects, such as the caffeine metabolism rate and the number of FP.

Subjects with a higher CmI (HCmI) seemed not to prefer a social context, while on the contrary, subjects with a lower CmI (LCmI) tended not to prefer a savouring context. We can hypothesise that HCmI subjects were focused more on the product itself than on the experience of drinking coffee. In addition High CMI also tended to prefer the context of "awakening", probably because they associated coffee to the alerting effect of caffeine. On the contrary, LCmI subjects were focused on the coffee-drinking situation. In this case, drinking coffee was associated with other "rituals", such as smoking, having a break during work, staying with friends, or after a meal. These results are probably related to the fact

that, as discussed above, people with different caffeine metabolism rates are differently affected by the psychoactive effects of caffeine for different times. Likely, subjects with a lower caffeine metabolism rate seem to need an excuse to consume coffee, considering that they are affected by the psychoactive effects of caffeine for a longer time. On the contrary, subjects with a higher caffeine metabolism rate consume more frequently coffee and thus they have learned to savour and appreciate coffee for both its sensory profile and the alerting effects of caffeine.

Subjects with higher FP (HFP) tended to prefer the context focused on the product (Savouring context) less, while subjects with lower FP (LFP) were less inclined to prefer the context focused on the function (Awakening context). This result could be related to the different taste sensitivity of the two groups. Subjects with a higher FP number, who were more sensitive to bitterness and sourness, were less inclined to link coffee consumption to a sensory experience. It is probable that they did not appreciate the sensory profile of coffee and thus they were less focused on the experience of savouring coffee in itself, and more on the situation of drinking coffee. On the other hand, subjects with a lower FP number, who were less sensitive to undesired tastes/flavours, were probably more focused on the product and neglected the psychoactive functionality of coffee.

### **5.7. Limitations**

This work aimed to understand the factors affecting bitterness perception and coffee preference. For this reason only regular coffee consumers were selected. However, in some way, this has limited the research on bitterness perception. For example the range of FP (4 – 22) was similar to (Shahbake et al. 2005), but also lower than has been reported (Hayes & Duffy 2007; Nachtsheim & Schlich 2013). It could be that our sampling excluded high FP individuals who either did not regularly consume coffee, perhaps because of its high sourness and bitterness, or those who were less inclined to volunteer for research on this topic, possibly for the same reasons. Moreover, the lack of significant effects of physiological factors on preference for bitter and/or caffeine-containing foods assessed by questionnaire could depend on the considered sample. Probably, more subjects are needed to better explore the preference patterns of bitter foods other than coffee. Several other factors are involved in the preference for vegetables, alcoholic beverages, energy drinks and so on, such as familiarity, availability, health care.

Other results may also depend on sampling. Because of the effect of smoking and of the somewhat lower mean for oral contraceptive users on the caffeine metabolism index, non-smokers and non-contraceptive users (n=91) were selected for investigating the role of metabolism rate in coffee preference. The results found should be confirmed with a larger sample. In particular no significant association was found between the caffeine genotypes and different metabolism rates. However, genotype was determined from only fifty-one individuals, a population too small to reliably estimate the effect of genotype on caffeine metabolism index.

Paired comparison between coffees with added caffeine was unsuccessful. No differences were found in the results for all considered groups (subjects with different FP numbers, PROP taster status, caffeine metabolism rate). Probably, the experimental plan was not effective. In fact samples were presented without sugar and adding sugar was not permitted. The majority of subjects added sugar to coffee samples, thus it could be that coffees without sugar were not liked independently of their caffeine content and perceived intensity of bitterness. On the other hand, it may also be possible that subjects were not able to discriminate between samples in the pair. Both of these factors could cause the choice to be made randomly.

## **6. Conclusions and future prospects**

This study describes the several factors affecting the sensitivity to bitterness and the preference for coffee. The results not only confirm that FP density and PROP status play a significant role in taste sensitivity for bitter compounds, but that a food-related behaviour – sugar use – is partly a function of fundamental individual differences in physiology. In addition, for the first time, a relationship between caffeine metabolism rate and bitterness sensitivity to caffeine has been demonstrated. Moreover, the effects of this link on coffee perception and consumption have been highlighted. Considering that caffeine is the most commonly ingested psychoactive substance in the world, this research could be the starting point to a deeper investigation of the responses to the post-ingestive effects of caffeine and the possible relation between individual differences in acquired preferences and variations in caffeine metabolism rate.

The proposed approach may be considered as a general framework for investigating the effect of physiological and metabolic factors on food habits with health-related consequences, such as the use of sweeteners. Based on their physiological and metabolic characteristics, subjects could adjust their food choices by avoiding unhealthy food behaviour. For example, subjects who are more sensitive to tastes which might be unpalatable in a food can compensate by altering its overall flavour or by choosing products with a lower perceived intensity of the unwanted tastes. In the case of bitterness, more sensitive subjects may consume bitter foods or beverages with the addition of some bitter-masking agents or may choose preparation methods which reduce bitterness, instead of having to abstain from these foods altogether.

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## **Appendix A. Original papers I-III**

**Paper I**

# Sensory Properties of Under-Roasted Coffee Beverages

Camilla Masi, Caterina Dinnella, Massimo Barnabà, Luciano Navarini, and Erminio Monteleone

**Abstract:** Conditions milder than standard roasting (under-roasting: 140 to 170 °C for 20 to 12 min) have been proposed to reduce the formation of potentially harmful compounds developing during standard coffee roasting. In the present study, sensory properties of brews prepared with under-roasted coffees (140 to 165 °C for 20 min) were described by descriptive analysis. Two meta-attributes were defined: “coffee” consisting of attributes positively related to the increasing of process temperature and “no-coffee” consisting of those negatively related to temperature. The progressive lowering of “no-coffee” and the corresponding increasing of “coffee” mean values were induced by temperature increasing in under-roasting conditions. The processing temperature of 150 °C seems to represent the changing point between the 2 meta-attributes with “no-coffee” prevailing at lower and “coffee” at higher temperatures. Consumer responses indicate the positive effect of both product processing temperature and “coffee” attribute intensity on liking. Brews from coffee treated at temperature lower than 150 °C showed a sensory profile characterized by “no-coffee” attributes and resulted the less preferred by regular coffee consumers.

**Keywords:** coffee origin, consumer preference, descriptive analysis, roasting temperature

**Practical Application:** Results from this study represent a contribution to the description of the sensory properties of coffee beverages obtained by means thermal processes milder than standard roasting. A new set of sensory descriptors, and relative reference standards, was defined to describe the specific sensory properties of beverages from under-roasted coffee. Furthermore, thermal treatment conditions leading to under-roasted coffee beverages accepted by coffee regular consumers were determined.

## Introduction

Coffee is one of the most traded food products and of the beverages consumed most widely throughout the world. According to data reported by Intl. Coffee Organization (ICO), consumption has continuously increased in the last 20 y. Coffee is consumed for its pleasant flavor and aroma as well as for the stimulatory properties arising from its caffeine content.

Coffee sensory properties are affected by several factors: plant varieties, growing region/conditions, processing, and brewing methods (Illy and Viani 2005). Roasting degree and time/temperature combinations determine the formation of compounds responsible for the desirable coffee flavors and aromas (Clarke 2003; Baggenstoss and others 2008). Temperatures ranging from 170 to 240 °C for 15 to 10 min are applied to obtain from light to dark roasted coffee beans (Yeretzian and others 2002; Illy and Viani 2005).

Several chemical reactions take place simultaneously during roasting (Farah and others 2006). The main constituents of coffee beans, such as polysaccharides, proteins, and sucrose, undergo Maillard and pyrolysis reactions thus leading the formation of sensory active molecules, responsible for aroma and color as well as traces of compounds having potential harmful effect, that is, furan and acrylamide (Stadler and others 2002; Yeretzian and others 2002; Buffò and Cardelli-Freire 2004; Taeymans and others

2004; Illy and Viani 2005; Ribeiro and others 2009; Farah 2011; Moro and others 2012). The positive relationships between roasting process and both sensory properties and aroma profile have been widely studied (Czerny and Grosch 2000; Lyman and others 2003; Dofner and others 2004; López-Galilea and others 2006; Baggenstoss and others 2008). Thermal treatments performed in a range from about 140 to 170 °C for 20 to 12 min, corresponding to the beginning of Maillard reaction in standard roasting conditions (Illy and Viani 2005), can be defined as under-roasting. Under-roasting has been proposed as effective strategy to reduce the content of potentially harmful compounds in coffee (Guenther and others 2007; Altaki and others 2011) and to preserve its endogenous antioxidant activity (Perrone and others 2010). However, under-roasting process, affecting the complex reaction network taking place during standard thermal treatment is likely to widely influence the evolution of sensory active molecules responsible for typical coffee aroma and flavor. At the moment, no information is available on sensory properties of and consumer liking for brews prepared with under-roasted coffee. On the other hand, this represents an important point since coffee is a highly familiar food with well-defined sensory expectations by consumers. Flavor is the most important criterion for coffee quality evaluation by experts, and also one of the major drivers for consumer preferences (Farah and others 2006). Coffee brew characteristic attributes, such as “coffee,” “roasted,” “burnt,” and “brown,” were positively related to both roasting temperature (Bhumiratana and others 2011) and consumer preference (Geel and others 2005).

The main objective of the present study was to describe sensory properties of brews prepared with under-roasted coffees in order to explore the development of typical coffee flavor and aroma in

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**Table 1—Coffee samples: processing temperature (°C) and origin of coffee beans.**

Temperature (°C)	Origin	
	Brazil	Guatemala
140	B140	G140
145	B145	G145
150	B150	G150
155	B155	G155
160	B160	G160
165	B165	G165
220	B220	G220

process conditions milder than standard roasting. For the purpose, coffees from 2 origins were processed in a temperature range from 140 to 165 °C for 20 min. Furthermore, the relationship between sensory properties and consumers' liking for the brews was investigated in order to identify optimal process conditions.

## Materials and Methods

### Samples

Coffea Arabica from 2 countries of origin was used (Brazil-B and Guatemala-G). Green coffee beans were roasted in a pilot plant for 20 min at the assigned temperature. Under-roasting was performed in a temperature range from 140 to 165 °C. Standard roasting was performed at 220 °C. Six under-roasted (140, 145, 150, 155, 160, and 165 °C) and 1 standard roasted products were obtained for each origin. Products were packed in 250 g sealed cans and used within 3 mo.

Coffee beans were ground in a Mazzer Super Jolly Man coffee bean grinder, 30 min before the sensory evaluations. Brewed coffees were prepared in a plunger coffee maker by pouring 200 g of deionized water at 100 °C over 15 g of coffee powder according to European standard of Brewing control chart (ideal coffee/water ratio 7.5% to 6.5%). After 5 min, the mixture was filtered and brewed coffee samples immediately served.

Fourteen coffee samples were evaluated in total (Table 1).

### Physical and chemical analysis

Total weight loss and color (Probat, Colorette 3b) were determined on coffee beans after processing in order to check the extent of thermal treatment (Clarke 2003).

### Descriptive analysis

**Panel.** Subjects were recruited in Florence area (Tuscan, Italy). They had seen or received an invitation to fill in an online questionnaire about familiarity with vegetable-based infusions and volunteered based on their interest and availability. Questionnaire consisted of 16 items on familiarity with coffee and 15 herb- and vegetable-based infusions (barley coffee, berries, black tea, chamomile, fennel, ginseng coffee, green tea, hawthorn, karkadè, laurel, lavender, lemon balm, linden, mallow, and wild rose). Herb- and vegetable-based infusions were included as distracters. The order of questionnaire items was balanced across subjects.

The familiarity scale consisted of 5 options, labeled 1 "I do not recognize the product," 2 "I recognize the product, but I have not tasted it," 3 "I have tasted, but I do not use the product," 4 "I occasionally eat the product," and 5 "I regularly eat the product" (Bäckström and others 2002).

**Table 2—Classes of descriptors: terms and occurrences.**

Terms	Occurrences	Classes
Barley coffee	38	Barley coffee
Barley coffee odor	4	
Relevant to barley coffee	5	
Burnt	35	Burnt
Burnt coffee	18	
Caramel	11	Caramel
Cereals cooking water	27	Cereals cooking water
Cooked cereals	3	
Starch	1	
Wet cereals	5	
Bitter coffee	1	Coffee
Coffee	121	
Coffee beans	1	
Coffee odor	5	
Coffee powder	2	
Strong black coffee	2	
Sweetened coffee	8	
Barley	22	Grains/oats
Cereals	38	
Oats	9	
Sesame	2	
Starchy foods	3	
Wheat	2	
Ferrous	3	Metallic
Metallic	15	
Odor of moka	6	
Hazelnut	3	Nuts (roasted nuts)
Nut	4	
Peanut	14	
Puffed grains	6	Puffed grains
Bakery	2	Roasted
Highly roasted coffee	2	
Roasted	76	
Smoked	3	
Woody	2	
Artichoke	1	Vegetables
Chicory	1	
Earthy	6	
Grassy	3	
Nut shell	2	
Radish	1	
Roots	2	
Vegetables	39	
Coffee grounds	14	Watered coffee
Coffee-stained water	3	
Drip coffee	2	
Fresh-made coffee with moka	2	
Instant coffee	8	
Relevant to coffee	1	
Watered coffee	101	
Weak black coffee	2	

Fourteen subjects, 5 males and 9 females, aged from 22 to 44 y, regular coffee consumers, were selected. Subjects were informed that the aim of the evaluation was the description of herb- and vegetable-based infusions containing natural substances with stimulatory properties analogous to caffeine. The subjects had no history of disorders in oral perception. They were paid for their participation in the study. Written informed consent was obtained from each subject after the description of the experiment.

### Sensory vocabulary development, subject training, and subjects reproducibility.

1. Recognition and intensity rating of sensations perceived in standard solutions (2 sessions): Subjects were trained to recognize and rate the perceived intensity of the following

**Table 3—Consensus list of attributes describing aroma, flavor, and mouthfeel sensations of samples: definitions and standards.**

Attribute	Definition	Standard
<i>Aroma and Flavor</i>		
Barley coffee	Odor of barley coffee	1 g of barley coffee (Nestlè Orzoro moka) in 20 mL of boiling water
Burnt	Odor of overcooked, almost scorched product	
Caramel	Odor of the sugar caramelizing without burning	1 g of caramel in 20 mL of water
Cereals cooking water	Odor of water after the cooking of cereals and legumes	1 g of oatmeal (Germinal, Bio) in 20 mL of boiling water
Coffee	Odor characteristic of coffee	
Grains/oats	Odor characteristic of cereal, malt and oats	12 g of oatmeal (Germinal, Bio)
Metallic	Odor of metallic utensils, for example, clean moka or cutlery	FeSO <sub>4</sub> 0.9 g/L
Nuts	Odor of roasted nuts	3 g of peanuts without shell
Puffed grains	Odor characteristic of puffed rice and puffed grains	1 g of rice crackers (Sunny Nature, Bio)
Roasted	Odor characteristic of products cooked at high temperature	
Vegetables	Odor associated with fresh vegetables, fresh earth, wet soil, or humus	Peanuts shell (30 g)
Watered coffee	Odor of coffee prepared by moka with a too low coffee powder amount	1 g of instant coffee (Nescafé Classic) in 20 mL of boiling water
Sweet	Taste associated with sucrose	Sucrose 12.0 g/L
Bitter	Taste of chicory, tonic water, and caffeine	Quinine dichloride 0.037 g/L
Sour	Taste of lemon juice and vinegar	Citric acid 0.38 g/L
<i>Mouthfeel</i>		
Astringency	Dryness of the oral surface and tightening and puckering sensation of the mucosa and muscles around the mouth	Alum sulphate 0.6 g/L

All standards were presented in a 100 mL amber glass bottle. Standards were prepared to induce a moderate intensity, corresponding to the central point (5) of the 9-point scale.

**Table 4—Food neophobia scale.**

Items
1. I am constantly sampling new and different foods
2. I don't trust new foods <sup>®</sup>
3. If I don't know what is in a food, I won't try it <sup>®</sup>
4. I like foods from different countries
5. Ethnic foods look too weird to eat <sup>®</sup>
6. At dinner parties I will try a new food
7. I am afraid to eat things I have never had before <sup>®</sup>
8. I am very particular about the foods I will eat <sup>®</sup>
9. I will eat almost anything
10. I like to try new ethnic restaurants

Items negative to food neophobia, marked with <sup>®</sup>, were reversed prior to analysis.

different sensations: sweetness, sourness, bitterness, and astringency using the following standard solutions—sucrose: 8.00, 12.00, and 18.00 g/L; citric acid: 0.25, 0.38, and 0.50 g/L; quinine monohydrochloride dihydrate 0.025, 0.037, and 0.050 g/L; aluminium potassium sulphate: 0.3, 0.6, and 0.9 g/L. During training sessions, subjects were asked to rate the perceived intensity in standard solutions on a 9-point category scale (1 = “extremely weak”; 5 = “moderate”; 9 = “extremely strong”).

- Descriptive term elicitation: A simplified version of the repertory grid method (Piggott and Watson 1992) was applied to allow assessors to individually elicit sensory descriptors of the under-roasted coffees. In total 3 sessions were run in 3 d. In each session, 2 pairs (diads) of samples were presented. All subjects received the same diads and in particular in the 1st session, they received the samples B140 and B145 first and, after a break of 10 min, samples B150 and B155.

Similarly, in the 2nd session, they received the diads B145 compared with B155 and B160 compared with B165. Finally, in the 3rd session, they received the diads G140 compared with G150

**Table 5—Total weight loss (%) and CI of coffee beans processed at different temperature.**

Temperature (°C)	Total weight loss (%)		CI	
	Brazil	Guatemala	Brazil	Guatemala
140	7.85	8.78	200	200
145	8.60	9.20	200	200
150	11.18	10.14	188	192
155	10.01	11.00	154	169
160	11.19	11.60	142	143
165	12.24	13.12	133	129

and G155 compared with G165. These sessions were run in a room of the sensory lab and each subject performed the task individually. In order to prevent the effect of expectation for coffee sensory properties on the generation of attributes describing samples, subjects were given general information about samples (herb- and vegetable-based infusions) and any explicit reference to coffee was avoided. They were asked to compare the aroma and the flavor of the samples of each diad and freely describe similarities and differences between them on a sheet. Panelists were encouraged to use associative and cognitive terms rather than quantitative or affective ones (such as good, bad, intense, and so on). At the end of each session, the panel leader listed all the elicited terms across subjects taking note of the occurrences of each term. All the elicited terms across subjects and the relative occurrences are reported in Table 2. Terms were classified in 12 classes according to the consensual decision of the panel.

- Sensory vocabulary development and reference standards: Before assessment of products, assessors participate in a series of language sessions (4) according to the Generic Descriptive Analysis (ISO 11035 1994; Lawless and Heymann 2010). The initial list of attributes was reduced to achieve a list that comprehensively and accurately described the product space: redundant and/or less-cited terms were

grouped on a semantic basis and/or eliminated according to the subjects' consensual decisions. The consensus-building process, managed by the panel leader, ended with the list of attributes reported in Table 3. To facilitate the consensus and to calibrate the subjects, reference standards were presented to the panel, discussed and modified until the panelists reached a consensus. Standards were prepared to induce a moderate intensity, corresponding to the central point (5) of the 9-point scale. In order to train subjects to rate the intensity of each attribute, 2 sessions were run. Panelists were presented with 2 samples in each session (1st session: B145 and B160; 2nd session: B140 and B155), and were asked to individually evaluate the intensity of attributes on a score card. A 9-point category scale labeled at the extremes with "extremely weak" and "extremely strong" was used. At the end of each session, individual evaluation results were collectively discussed and panel agreed on attributes and relevant intensities describing each sample. Thus, an evaluation sheet consisting of 28 ratings was defined. They consisted in 12 aroma descriptors ("odor" sensations evaluated by nose, orthonasal perception), 15 flavor descriptors (12 "odor" sensations evaluated by mouth, retronasal perception, and 3 "taste" descriptors), and 1 descriptor of "tactile" sensations.

- Assessor and panel performance validation: sensory performances were validated by evaluating a subset of samples to be used for the study. Two sessions were performed in individual booths on 3 samples (G140, G150, and G160) replicated 2 times. Panel and assessors data were analyzed using Panel Check software (ver 1.4.0, Nofima, Tromsø, Norway).

**Evaluations.** The panel participated to 7 evaluation sessions: 3 for under-roasted sample B, 3 for under-roasted sample G, and 1 for standard roasted samples B and G. In each session, 6 samples were evaluated. Each sample was evaluated 3 times. Samples (30 mL) were presented in 100cc closed amber glass bottles identified by a 3-digit code. Sample presentation was balanced across subjects within each session. For each sample, assessors were asked to rate

the intensity of odor descriptors perceived by nose (aroma) first. Then, they were asked to take a sip of the sample and rate the intensity of odors perceived retronasally. Finally, they had to take a 2nd sip and rate taste and mouthfeel attributes. The perceived intensity of each sensation was rated on a 9-point category scale labeled at the extremes with "extremely weak" and "extremely strong."

After each sample, subjects rinsed their mouths with distilled water for 30 s had some plain crackers for 30 s and finally rinsed their mouths with water for a further 30 s. Subjects took a 15 min break every 2 samples. In the adopted experimental conditions, aroma evaluation was performed at 64 to 66 °C and the in-mouth evaluation at 54 to 56 °C.

Evaluations were performed in individual booths under red lights. Data were collected with the software Fizz (ver.2.47.B, Biosystemes, Couternon, France).

### Consumer Test

**Subjects.** Seventy-five regular coffee consumers, 26 males and 49 females, aged from 19 to 62 y, were recruited using the same modality described for the descriptive analysis.

**Samples.** Six samples representing the whole sensory variation within the 12 experimental samples were tested by consumers (B140, B150, and B160; G140, G150, and G160). A perceptible map obtained by submitting descriptive data to a principal component analysis (PCA) was used to select the samples.

**Evaluations.** Subjects were presented with each sample (30 mL) in a closed 80cc plastic cup along with 10 g of sugar (about 3 coffee spoons) in a plastic cup both identified with the same random 3-digit code. Samples were presented 1 by 1 and the presentation order was balanced across subjects. For each sample, consumers were asked to smell and rate their liking for the aroma first. Then, they were asked to take a sip and rate their liking for the flavor (flavor1). Finally, they were asked to freely add sugar, take a sip, and rate again their liking for the flavor (flavor2).

The amount of sugar used for each sample by each subject was measured by weighting the sugar cup after each evaluated sample.

Evaluations were performed in individual booths adopting the same general evaluation conditions reported for descriptive analysis.

**Questionnaire.** After sample evaluation, subjects were asked to fill in a questionnaire including: demographics (age, gender), neophobia scale, consumption frequency of coffee, and consumption of coffee with or without sweeteners added.

Ten statements belonging to the food neophobia scale were rated on a 7-point scale from strongly disagree (1) to strongly agree (7) (Pliner and Hobden 1992) (Table 4).

Participants expressed their consumption frequency using a 5-point scale, labeled 1 "less than once a day," 2 "once a day," 3 "twice or 3 times a day," 4 "4 or 5 times a day," 5 "more than 5 times a day." They were also asked to indicate if they usually consume coffee with sweetener added.

### Data Analysis

**Descriptive data.** Intensity data from the trained panel were analyzed by multiblock PCA (Tucker-1) and by p/MSE plot (Panel Check software, ver 1.4.0, Nofima) to assess panel calibration and assessor performance, respectively (Næs and others 2010). In particular, the occurrence of assessor-attribute combinations deviating from the rest of the panel was checked, for each attribute, by means the analysis of a correlation plot generated by the multiblock PCA. For an assessor interpreting an attribute differently

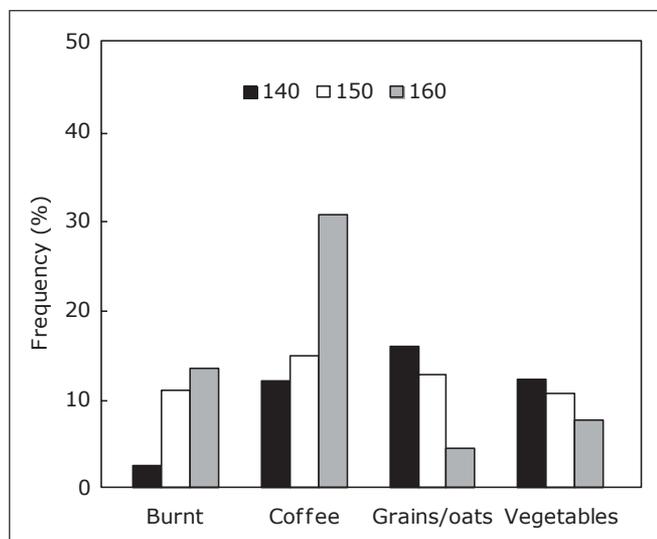


Figure 1—Frequency of elicitation of descriptors referring to "coffee," "burnt," "grains/oats," and "vegetables" classes in brews from coffees processed at 140, 150, and 160 °C.

from the rest of the panel, the corresponding correlation loadings are located closer to the center than others, making it possible to detect assessor–attribute combinations with weak relation to the general underlying data structure. No cases of disagreement among panelists were found for all the attributes. Furthermore, differences among assessors in product differentiation ability and in consistency over replicates were analyzed by  $p$ /MSE plots. These plots are derived from the computation of a one-way ANOVA for each assessor and each attribute combination. For each attribute, individual ability of discriminating among samples, expressed as  $P$ -value, is plotted along the vertical axis, while the consistency over replicates, expressed as ratio of sums of squares of the differences between average values of products and the variance of replicates (MSE), is plotted along the horizontal axis. The lower are both  $P$  and MSE values, the better is the assessor performance and thus the presence of weak performers can be detected (see Næs and others 2010 for details). On the basis of the  $p$ /MSE plots, 3 out of 14 subjects were considered unreliable and were taken out from further data analysis.

Intensity ratings from Brazil and Guatemala under-roasted samples and from standard roasted samples were independently analyzed by a two-way ANOVA mixed model (sample as fixed and assessors as random factors), followed by a Fisher LSD posthoc test (significant for  $P \leq 0.05$ ).

PCA was computed on panel averages of each significant attribute arising from the ANOVA models. Samples were included as dummy variables (downweighted in the data matrix) to improve the visual interpretation (Martens and Martens 2001). The full cross validation was computed to validate the interpretation of the first 2 components.

Two meta-attributes were defined based on the correlation loading values of attributes resulting from the PCA (Johansen and others 2010). The correlation between attributes in each meta-attribute was checked by Chronbach  $\alpha$  test. Mean intensity scores of each attribute within the same meta-attribute for each sample were summarized. Meta-attribute values were expressed as the percentage of the maximum value. The maximum value is given by the number of attributes belonging to the meta-attribute\*9 (maximum of intensity scale).

A paired  $t$ -test was used to compare meta-attribute values of under-roasted and reference samples.

Meta-attribute values were analyzed by a three-way ANOVA mixed model (sample and origin as fixed and assessors as random factors), with Fisher LSD posthoc test (significant for  $P \leq 0.05$ ).

A paired  $t$ -test was used to further investigate the effect of origin on meta-attribute values.

**Consumer data.** Liking ratings expressed for aroma, flavor1 and flavor2 were independently submitted to a two-way ANOVA

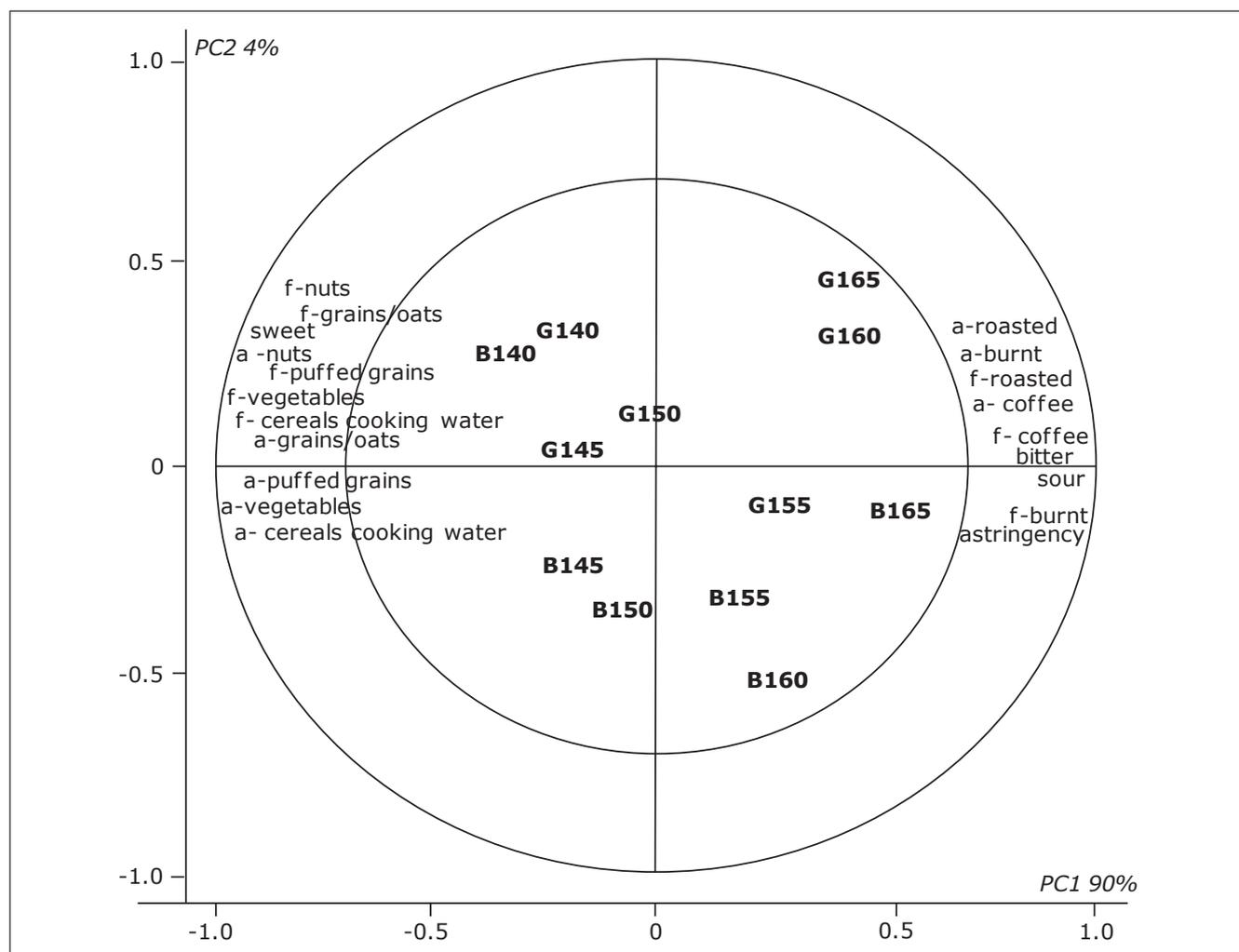


Figure 2—Correlation loading plot from PCA on panel averages of each significant attribute describing sample sensory properties.

model (assessors and sample as factors) with Fisher LSD posthoc test (significant for  $P \leq 0.05$ ).

Liking ratings expressed for flavor1 and flavor2 were analyzed by a two-way ANOVA model (sample-6 levels and condition-2 levels: with or without sugar added as factors), with Fisher LSD posthoc test (significant for  $P \leq 0.05$ ).

Individual ratings expressed for aroma and flavor2 were analyzed by an internal preference map (IMP). Samples were included as dummy variables (downweighted in the data matrix) to improve

the visual interpretation. The full cross validation was computed to validate the interpretation of the first 2 components.

A visually oriented approach, based on the inspection of loading plot, was used for subject clustering and Y-axis was set as limit between consumer segments (Næs and others 2010).

The individual neophobia values were computed as the sum of ratings given to 10 statements, after the negative items had

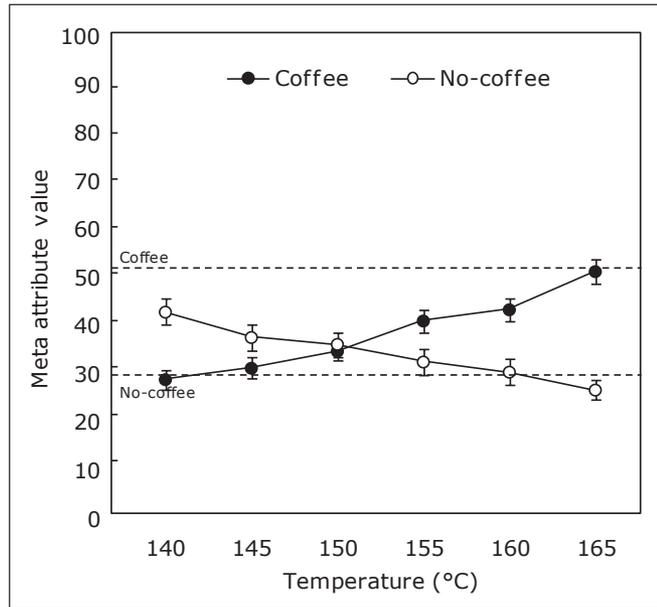


Figure 3—Effect of coffee processing temperature on mean values of “coffee” and “no-coffee” meta-attributes in samples from Brazil. Dotted lines represent the mean meta-attribute values in reference sample roasted at 220 °C. Bars represent the standard error of the mean (SEM).

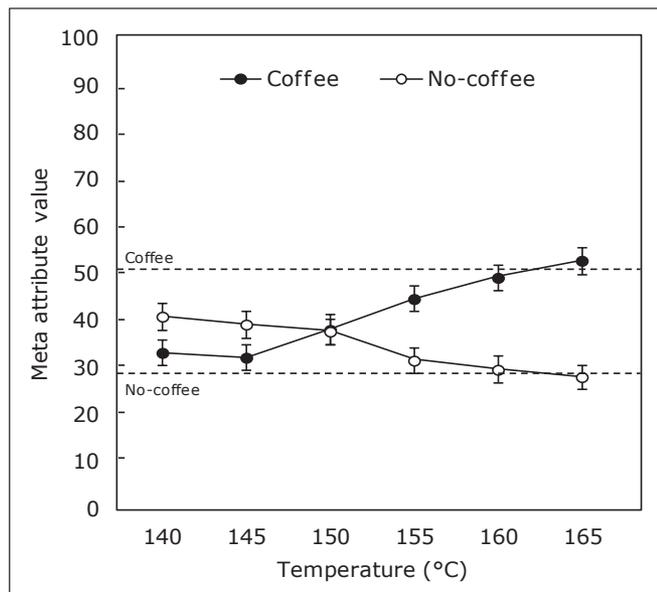


Figure 4—Effect of coffee processing temperature on mean values of “coffee” and “no-coffee” meta-attributes in samples from Guatemala. Dotted lines represent the mean meta-attribute values in reference sample roasted at 220 °C. Bars represent the standard error of the mean (SEM).

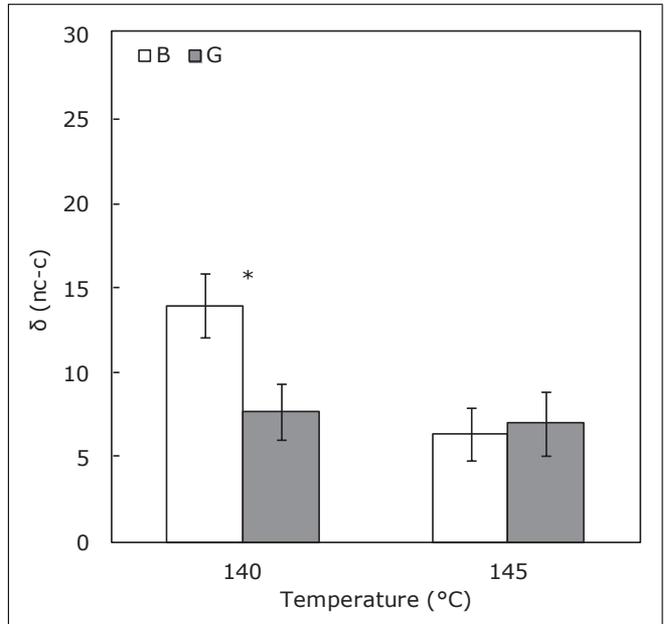


Figure 5—Effect of origin on  $\delta$  values (“no-coffee”–“coffee”) of samples from Brazil (B) and Guatemala (G) processed at temperature < 150 °C. Bars represent the standard error of the mean (SEM). \*indicates significantly different values ( $P \leq 0.05$ ).

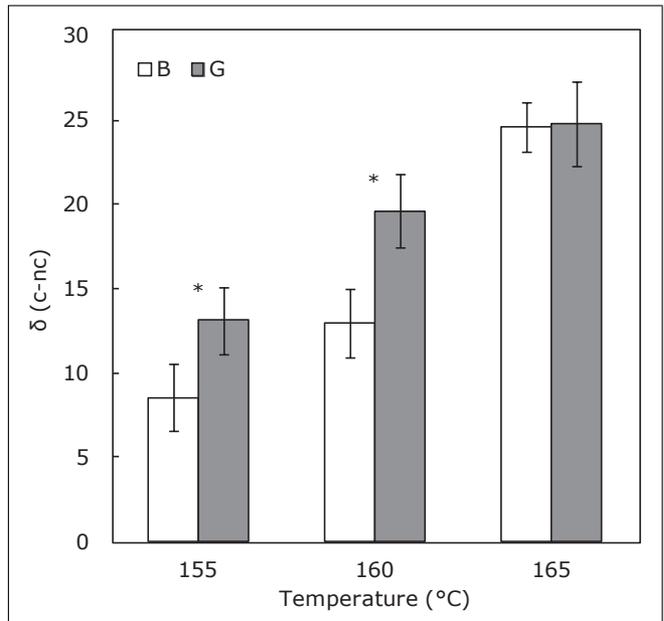


Figure 6—Effect of origin on  $\delta$  values (“coffee”–“no-coffee”) of samples from Brazil (B) and Guatemala (G) processed at temperature > 150 °C. Bars represent the standard error of the mean (SEM). \*indicates significantly different values ( $P \leq 0.05$ ).

**Table 6—Effect of coffee processing temperature on liking for aroma and flavor2: mean ratings, *F* and *P* values.**

Sample	Aroma	Flavor2
B140	5.23 <sup>c</sup>	5.64 <sup>bc</sup>
B150	5.24 <sup>c</sup>	5.49 <sup>c</sup>
B160	6.07 <sup>a</sup>	5.95 <sup>ab</sup>
G140	5.19 <sup>c</sup>	5.48 <sup>c</sup>
G150	5.69 <sup>b</sup>	5.52 <sup>c</sup>
G160	6.25 <sup>a</sup>	6.12 <sup>a</sup>
<i>F</i> <sub>5,449</sub>	12.88	3.12
<i>P</i>	≤0.001	0.009

Different letters indicate significantly different values ( $P \leq 0.05$ ).

been reversed; the neophobia scores thus ranged from 10 to 70 (10 maximum value to 70 minimum value). Neophobia values of clusters were compared by means an independent *t*-test.

The associations of clusters with gender, education level, coffee consumption frequency, and consumption of sweetened/unsweetened coffee (categorical variables) were estimated by a homogeneity chi-square test.

## Results and Discussion

### Physical index of coffee samples

Total weight loss and color for or each coffee product were determined in order to check the degree of thermal treatment (Table 5). Total weight loss ranged from 7.85% to 13.20% according to thermal treatment lower than 170 °C (Perrone and others 2010). The color index (CI) values indicate thermal treatment lower than light-medium roasting conditions. Thus, as expected, physical index values indicate thermal treatment milder than standard roasting conditions.

### Descriptive analysis

**Effect of under-roasting temperature on elicitation of sample descriptors.** The increasing of bean processing temperature induces greater frequency of descriptors referring to “coffee” and “burnt” classes, while the lowering of frequency of terms referring to “grain/oats” and “vegetables” classes is observed (Figure 1). In general, data show that samples from coffee processed at temperature higher than 150 °C are described by terms explicitly referred to coffee and often used to describe sensory properties of standard roasted samples (ICO 2002; Illy and Viani 2005). On the other hand, samples from beans processed at temperature lower than 150 °C are mainly described by terms unusual for standard roasted coffee. Thus, it seems that even in blind evaluation conditions, sensory properties of under-roasted samples processed at temperature higher than 150 °C match with coffee product schemata stored in the memory of regular consumers (Tourila and others 1998), thus inducing the elicitation of terms describing aroma and flavor of standard roasted coffee.

**Effect of under-roasting temperature on coffee sensory profile.** Results from two-way ANOVA models showed a significant sample effect on 19 out of 28 attributes for Brazil samples and on 19 out of 28 attributes for Guatemala samples. No significant sample effect on aroma and flavor descriptors “watered coffee,” “barley coffee,” “caramel,” and “metallic” was found for both B and G coffees. Thus, these 8 attributes were not included in the subsequent PCA.

The PCA correlation loading plot (Figure 2) shows that samples are mainly discriminated along the 1st component (PC1: 90% explained variance) according to bean process temperature. Coffee samples from beans processed at ≤150 °C are positioned on the left side of the map, while those from beans processed at ≥155 °C are located on the right. Visual inspection of the correlation loading plot allows the observer to easily identify 2 attribute groups along PC1. A 1st group is composed of 11 attributes (aroma and flavor of nuts, vegetables, grains/oats, puffed grains, cereals cooking water, and sweet taste) positioned on the left of the plot and characterizing samples obtained at lower processing temperatures. A 2nd group of attributes, strongly correlated each other and positioned on the right of the plot, is composed of 9 attributes (coffee, roasted and burnt aroma and flavor descriptors, astringent, bitter, and sour) that characterize samples obtained at higher processing temperatures. The correlation between attributes within the 2 groups was tested and resulted very high in terms of Cronbach  $\alpha$  value (0.97 for the attribute group on the left and 0.98 for those on the right). Thus, 2 sensory dimensions, each related to several attributes, can be identified and defined as meta-attribute (Johansen and others 2010). In particular, meta-attribute allocated on the right of the map, consisting of attributes describing brews from standard roasted beans (ICO 2002; Narain and others 2003; Geel and others 2005; Bhumiratana and others 2011), was named “coffee.” On the contrary, the group of variables on the left of the map was named “no-coffee” and included: nuts, vegetables, grains/oats, puffed grains, cereals cooking water aroma and flavor descriptors, and sweet. “No coffee” meta-attribute consists of terms generally not included in standard coffee descriptive vocabulary. This meta-attribute groups sensations perceived in brewed coffees from beans in which processing temperature did not permit the formation of sensory active molecules responsible for the distinctive coffee profile.

**Sensory difference between standard and “under-roasted” coffee samples.** The effect of under-roasting temperature on coffee profiles was further investigated by comparison with coffee from beans processed at standard temperature (B220 and G220). No significant differences were found between attribute intensities of B220 and G220 samples. Mean values of “no-coffee” and “coffee” meta-attributes from both B220 and G220 were computed and used as reference values (REF coffee and REF no-coffee). These mean values were compared to “no-coffee” and “coffee” meta-attributes mean values of under-roasted samples from Brazil (Figure 3) and Guatemala (Figure 4).

“No-coffee” mean values of under-roasted samples were significantly higher than the standard one for bean processing temperature ≤150 °C ( $P \leq 0.0001$ ), and no significant differences were found for processing temperature ≥155 °C ( $P \geq 0.35$ ). “Coffee” mean values of under-roasted samples are significantly lower than the standard one for processing temperature ≤160 °C in sample from Brazil ( $P \leq 0.0001$ ) and ≤155 °C in sample from Guatemala ( $P \leq 0.0001$ ). No significant differences were found for higher processing temperatures ( $P \geq 0.50$ ).

In general, sensory data indicate the progressive lowering of “no-coffee” and the corresponding increasing of “coffee” mean values induced by temperature increasing in under-roasting conditions. The processing temperature of 150 °C represents the changing point between the 2 meta-attributes with “no-coffee” prevailing at lower and “coffee” at higher temperatures.

**Effect of the origin on the sensory properties of “under-roasted” coffee samples.** A significant effect of origin on “coffee” meta-attribute value was found ( $F_{1;50} = 18; P \leq 0.001$ ); in particular, it was higher in samples G than in samples B. No significant effect of origin on “no-coffee” meta-attribute value was found. No significant effect of sample\*origin interaction was found thus indicating the same relationship between meta-attribute values and processing temperature in samples from both origins.

The effect of origin on meta-attributes values was further investigated. The arithmetic difference between the mean values of the 2 meta-attributes was computed in each sample. In particular, at temperature lower than 150 °C, the difference between the value of “no-coffee” and of “coffee” was computed ( $\delta_{nc-c}$ ). On the contrary, at temperature higher than 150 °C, the difference between the value of “coffee” and of “no-coffee” was computed ( $\delta_{c-nc}$ ).  $\delta$  values of samples from B and G products processed at the same temperature were compared by means a paired *t*-test (Figure 5 to 6).  $\delta$  value of B140 was significantly higher than the G140 one ( $t_{32;2.04} = 3.26, P = 0.002$ ), and  $\delta$  values of G155 and G160 were significantly higher than those of B155 and B160 ( $t_{32;2.04} = -2.01, P = 0.05; t_{32;2.04} = -2.62, P = 0.01$ , respectively).  $\delta$  values comparison indicates that in cof-

fees from beans processed at 140 °C, “no-coffee” attributes prevail on the “coffee” ones more in B than in G samples; whereas in coffee from beans processed at 155 and 160 °C, “coffee” attributes prevail on the “no-coffee” ones more in G than in B samples.

Although the aroma and flavor are mainly characterized by the roast degrees and conditions, the origin of green coffee can also affect the development of the volatile compounds responsible for different aroma (Mayer and others 2000; Bhumiratana and others 2008).

The obtained results indicate that the general distinctive characteristics of coffees from different countries of origin are retained in under-roasted conditions. In fact, coffees from Guatemala, and, in general, all Arabica coffees from Eastern Africa as well from Central and South America, are characterized by a more intense flavor, while those from Brazil are less acid and have a less marked aroma than coffee from other origins (Illy and Viani 2005).

**Consumer test**

**Selection of samples.** Based on descriptive data analysis results, 6 samples were selected for consumer test. Three samples from products treated at 140, 150, and 160 °C were selected for both origins (B140, B150, and B160; G140, G150, and G160).

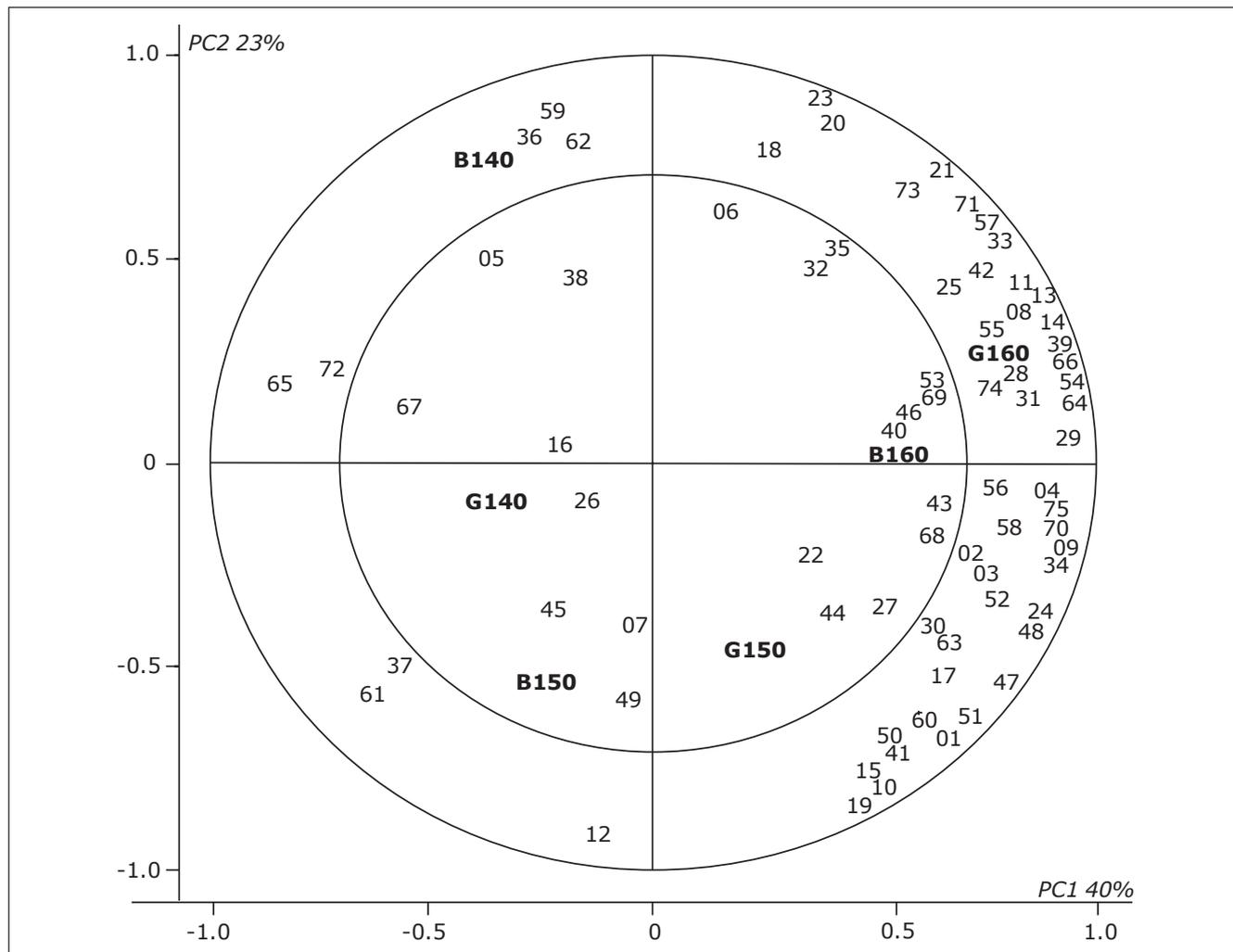


Figure 7–IPM on individual liking expressed for aroma.

B140 and G140 were considered as representative of coffees characterized by “no-coffee” attributes, and samples B160 and G160 were considered as representative of coffees characterized by “coffee” attributes. B150 and G150 were selected as representative of an intermediate profile where intensities of “no-coffee” and “coffee” attributes are almost the same.

**Effect of sensory profile on liking.** The coffee processing temperature significantly affects the liking for sample aroma ( $F_{5,449} = 12.88$ ;  $P \leq 0.001$ ). Mean liking scores for flavor resulted significantly affected by the conditions adopted for data acquisition (with or without sugar) ( $F_{1,888} = 120.25$ ;  $P \leq 0.001$ ). In particular, mean liking data for flavor1 resulted significantly lower than those for flavor2 (4.4 and 5.7, respectively). This result was expected since 68% of consumers declared to usually sweeten coffee. Furthermore, 80% of subjects added sugar after the first tasting. The product processing temperature significantly affects liking score for flavor ( $F_{5,888} = 2.5$ ;  $P = 0.03$ ). No significant effect sample\*condition interaction was found. In order to further explore the effect of conditions for data acquisition on liking for flavor, scores for flavor1 and flavor2 were independently analyzed considering sample and assessor as main factors. A significant sample effect was found on liking for flavor2 ( $F_{5,449} = 3.12$ ;  $P = 0.009$ ), while no significant effect was found on liking for flavor1. It seems that the general disliking for

unsweetened samples resulted in the lack of a significant sample effect. Thus, tasting without sugar added does not seem appropriate for evaluation of liking of under-roasted coffee samples. Based on these evidences, ratings for flavor1 were no further considered.

Data reported in Table 6 show the positive effect of product processing temperature on mean liking for aroma and flavor2. In fact, in both conditions, the most preferred samples were those obtained at the highest temperatures, while the least preferred were those from the lowest temperatures. The correlation between the 2 series of liking mean scores was quite strong ( $r = 0.89$ ,  $P = 0.017$ ). Based on this evidence, it is possible to assume that the main “information” from mean linking data collected in both conditions tends to be the same. The main sensory differences between samples from relatively low and high temperatures are related to “coffee” and “no-coffee” meta-attributes mean values.

To better understand the structure of preference data, liking ratings for aroma and flavor2 were independently analyzed by IMPs.

IMP referred to aroma is shown in Figure 7. The variance explained by the model after the first 2 significant dimensions was 63%. The 1st dimension of the correlation loading plot indicated that, in general, subjects’ preference was oriented toward samples on the right side of map consisting of samples from products treated

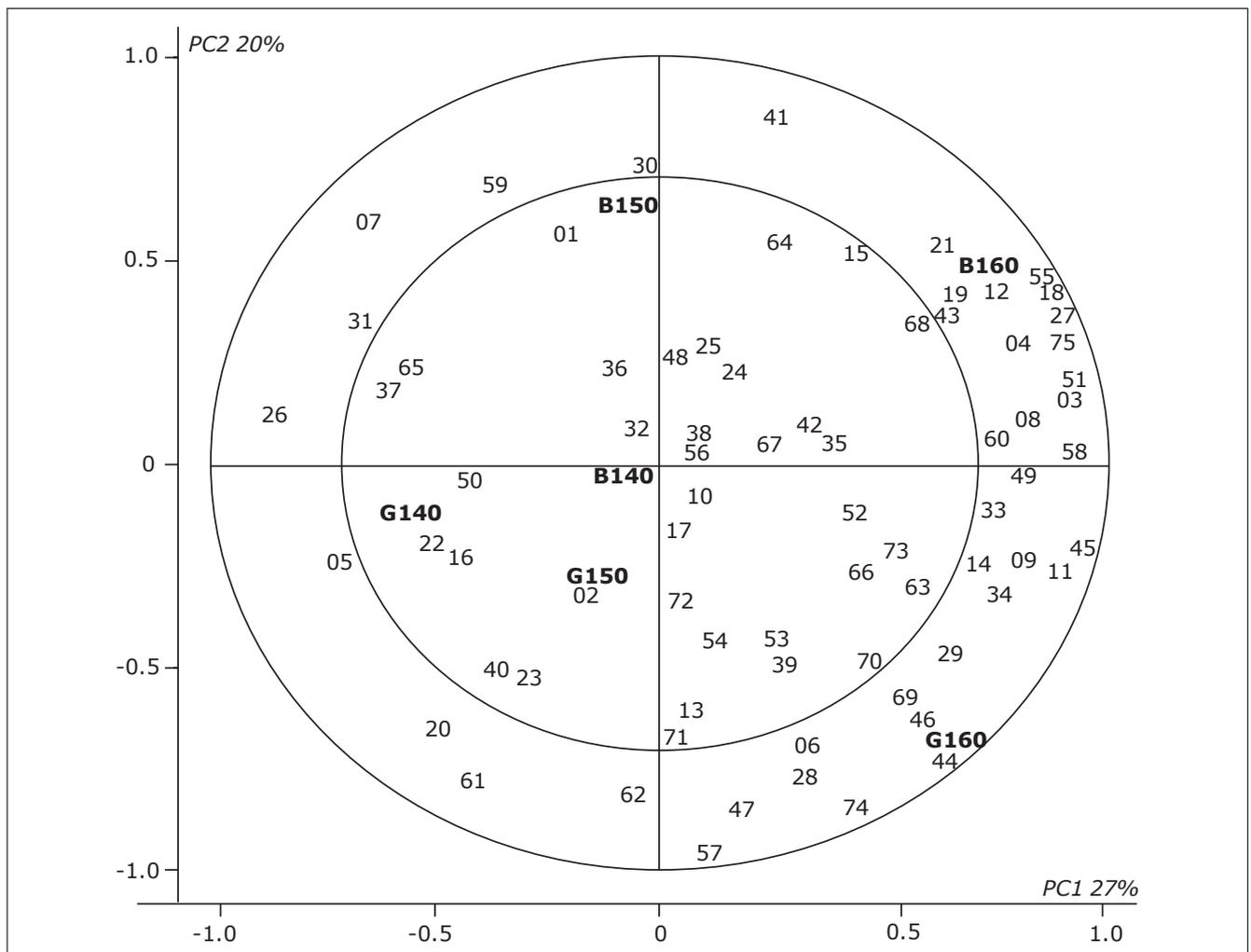


Figure 8—IMP on individual liking expressed for flavor2 (with sugar added).

**Table 7—Sociodemographic background, neophobia, frequency of coffee consumption, and consumption of sweetened coffee of all subjects and of subject clusters.**

Items	All subjects ( <i>n</i> = 75)	CL1 ( <i>n</i> = 29)	CL2 ( <i>n</i> = 46)
Age			
Average	32.9	36.4	31.0
Min	19	21	18
Max	62	62	57
Gender			
Men%	34.7	31.0	37.0
Women%	65.3	69.0	63.0
Instruction level%			
Middle school diploma	0	0	0
High school diploma	46.7	48.3	45.7
Degree	48.0	41.4	52.2
Post degree	5.3	10.3	2.1
Neophobia			
Median	57	57*	55*
25th percentile	48	43	48.5
75th percentile	64	64	64
Coffee consumption			
Frequency%			
< 1 a day	14.7	10.3	17.4
1 a day	22.7	27.7	19.6
2–3s a day	52.0	44.8	54.3
4–5s a day	9.3	13.8	8.7
> 5s a day	1.3	3.4	0
Sweetened %	68	69	67

\*indicates a significant difference for  $P \leq 0.05$ .

at the highest temperatures (B160 and G160). Coffee samples from B140, G140, and B150 were positioned of the left side and were preferred by a very limited number of subjects.

IMP referred to flavor2 is shown in Figure 8. The variance explained by the model after the first 2 significant dimensions was 47%. The 1st dimension of the correlation plot confirms that, in general, consumers' liking was oriented toward samples from products treated at the highest temperatures (B160 and G160). The difference in explained variance after the first 2 components between the 2 IPMs is not surprising since the data were collected in 2 different sensory modalities. In particular, in flavor2, condition taste and mouthfeel sensations got into the play empathizing the individual differences among subjects. However, the 1st dimension of the map from flavor2 confirms that, in general, consumers' liking was oriented toward samples from products treated at the highest temperature (B160 and G160). Furthermore, a relatively even spread of consumer loadings was observed.

In order to further study individual differences in liking, 2 consumer segments were identified setting Y-axis as limit. Segmentation was performed according to whether consumer loadings lie on the left or right side of the axis. As reported by Næs and others 2010, this procedure can be useful when no clear grouping tendency is observed.

Two groups were obtained, one consisting of 29 subjects (CL1) positioned on the left side, in the direction of samples G140 and B140 and the other ( $n = 46$ ; CL2) on the right side of the map oriented toward samples B160 and G160.

CL2 subjects are spread along the 2nd dimension of the correlation plot. One group is positioned on the upper side of the 2nd dimension and tends to prefer sample B160, and another group is positioned on the bottom of the 2nd dimension and tends to prefer sample G160. These different preference patterns could be related to sensory differences between the 2 samples, with "coffee"

attributes prevailing on the "no-coffee" ones more in G than in B samples.

To better understand preference patterns, questionnaire data were considered (Table 7). The neophobia median value was 57, indicating that 50% of subjects was inclined to consume unfamiliar products. Data from all the subjects participating in the study indicate that the 75% has coffee at least once a day and according to data on Italian consumers they can be considered regular coffee consumers (AA.VV. 2012). Sweetened coffee appears the most common modality of consumption (68%). The effect of the questionnaire variables on liking value was investigated by comparing data from CL1 to CL2. Subject groups significantly differed only for neophobia level ( $t_{73; 1.99} = -2.08$ ;  $P = 0.04$ ). In particular, subjects preferring B140 and G140 (CL1) were less neophobic than the subjects preferring B160 and G160 (CL2).

The positive effect of roasting-related attributes on regular consumer preference for coffee has been found (Geel and others 2005). In this study, consumers were not aware they were evaluating brewed coffee. However, most part of subjects tends to prefer samples characterized by familiar and well-known coffee sensory attributes. On the other hand, "no-coffee" attribute intensity negatively affects liking and only a few subjects orient their preference toward samples from products treated at temperature equal or lower than 150 °C.

## Conclusions

Sensory profiles of brews from under-roasted coffees are described by 2 attribute groups, one defined as "coffee" consisting of descriptors relevant to standard roasted coffee and the other one, described as "no-coffee" including terms generally not included in standard coffee descriptive vocabulary. The increasing of temperature in under-roasting conditions induced a progressive lowering of "no-coffee" and the corresponding increasing of "coffee" mean values. This indicates the gradual formation of sensory active molecules responsible for typical coffee flavor and aroma in the adopted under-roasting conditions. Descriptive data indicate that processing temperature equal or higher than 160 °C is needed to obtain brews in which "coffee" prevail on "no-coffee" descriptors. Furthermore, the positive effect of standard-coffee-related attribute intensity on consumer preference was found. In fact, brews from products treated at 160 °C resulted the most preferred.

In conclusion, the results of this study suggest that optimization of coffee beans thermal treatments for reducing the formation of potentially harmful compounds should always consider the risk of disappointing consumer sensory expectations for coffee brews.

The characterization of under-roasted samples, in terms of potentially harmful compound content, is under investigation in order to further optimize processing conditions for both sensory and healthy properties of coffee beverages from under-roasted beans.

## Acknowledgments

Illycaffè s.p.a research activity has been carried out in the framework of "Nutrigenomica e consumo di caffè: effetti fisiologici, genetica del gusto e genetica della pianta" Project partially supported by the POR-FESR 2007–2013 Regione Autonoma Friuli Venezia Giulia—Italy.

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**Paper II**



# The impact of individual variations in taste sensitivity on coffee perceptions and preferences



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## HIGHLIGHTS

- Physiological measures underlying taste perception were investigated
- The impact of physiological indices on coffee liking and consumption was studied
- Fungiform papillae density affects both taste perception and coffee preference
- PROP taste status affects taste perception both in coffee and in standard solutions
- Sugar use depends both on fungiform papillae density and PROP taste status

## ARTICLE INFO

### Article history:

Received 15 July 2014

Received in revised form 28 October 2014

Accepted 30 October 2014

Available online 6 November 2014

### Keywords:

Fungiform papillae

6-n-propylthiouracil

Bitterness

Sugar use

## ABSTRACT

Despite a few relationships between fungiform papillae (FP) density and 6-n-propylthiouracil (PROP) taster status have been reported for sensory qualities within foods, the impact on preferences remains relatively unclear. The present study investigated responses of FP number and PROP taster groups to different bitter compounds and how these affect coffee perception, consumption and liking. Subjects (Ss) with higher FP numbers (HFP) gave higher liking ratings to coffee samples than those with lower FP numbers (LFP), but only for sweetened coffee. Moreover, HFP Ss added more sugar to the samples than LFP Ss. Significant differences between FP groups were also found for the sourness of the coffee samples, but not for bitterness and astringency. However, HFP Ss rated bitter taste stimuli as stronger than did LFP Ss. While coffee liking was unrelated to PROP status, PROP non-tasters (NTs) added more sugar to the coffee samples than did super-tasters (STs). In addition, STs rated sourness, bitterness and astringency as stronger than NTs, both in coffee and standard solutions. These results confirm that FP density and PROP status play a significant role in taste sensitivity for bitter compounds in general and also demonstrate that sugar use is partly a function of fundamental individual differences in physiology.

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## 1. Introduction

Individual sensitivity to taste and other oral sensations shows considerable variability between individuals, and there is increasing evidence these variations are a significant influence on food preference and consumption [1–6]. Overall taste sensitivity is reflected in two commonly studied physiological measures. The first of these, the density of lingual fungiform papillae (FP) is positively associated with taste intensity [7] because the tongue's taste buds are contained primarily within the FP. Thus, those who have higher numbers of FP are more sensitive to tastes [8–12].

The second measure, the intensity of the compounds phenylthiocarbamide (PTC) and 6-n-propylthiouracil (PROP), is a genetically mediated

index of individual variation in oral sensations [13–16]. PROP responsiveness is typically expressed categorically as PROP taster status (PTS), which consists of three groups: PROP super tasters (STs), PROP medium tasters (MTs), and PROP nontasters (NTs) [17]. PROP responsiveness has long been used as general orosensory responsiveness to a variety of stimuli (e.g., [13,18]). PROP tasters rate the intensity of other bitter compounds, including caffeine, quinine, and urea [19–22], as more intense than do NTs, sucrose as sweeter [23,24], sodium chloride as more salty [13], and citric acid as more sour [25]. PTS is also associated with responsiveness to other orosensory stimuli apart from tastes: STs perceive irritation from capsaicin [26,27], cinnamaldehyde [27], ethanol [27–29], and astringency [30–32] with greater intensity than NTs.

PROP intensity and the density of FP are often found to be positively correlated. The most plausible explanation for this is that, while the ability to taste PTC [33] or PROP [29,34] results from the presence of a functional bitterness receptor (TAS2R38), the intensity of all tastes results

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from the spatial summation of number of taste buds stimulated, itself a function of FP density.

Bitterness *per se* is instinctively rejected [35–37] and this is thought to have been crucial to survival via its impact on food choice, specifically the avoidance of bitter toxins [38].

However, sensitivity to bitterness in foods and beverages varies widely among individuals, and some foods are consumed despite the presence of potentially bitter compounds. Both FP density and PROP intensity appear to clearly reflect this variation [39]. A range of bitter foods, including Brussels sprouts, cabbage, broccoli and spinach [40–42], caffeinated coffee [40] and grapefruit juice [43,44], have been reported as more bitter and/or less preferred by PROP tasters than by NTs. Differences between PROP/PTC tasters and NTs have also been found with foods that are sour such as lemon juices, vinegar, and sauerkraut [43]. Some studies have reported relationships between FP density, PROP status and food consumption/preference: Ss who rated the least bitterness intensity of PROP or had lowest numbers of FP reported less burn and disliking of ethanol as well as more frequent consumption of alcoholic beverages [45]; perceived less creamy/sweet sensations of sugar-fat mixtures and their liking was not affected by concentration at high sugar/high fat levels [46]; tasted less bitterness from some vegetables and consumed vegetables most frequently [39]; had significantly higher liking ratings for bread [47].

The universality of some bitter foods/beverages suggests however that their consumption is not limited to bitter insensitive individuals. Coffee, for example, that is one of the world's most consumed beverages despite the presence of caffeine and other bitter compounds. Such preferences are almost certainly the results of flavor-flavor and/or flavor-consequence conditioning via the stimulatory impact of caffeine and the addition of sugar or milk [48–50].

Nevertheless, how these preferences might also be shaped by individual physiological differences remains poorly understood. Moreover, some physiological indices can be determinants of learned preferences. For example, Yeomans et al. [51] demonstrated that PROP taster status, together with the intrinsic pleasantness of the taste of saccharin (sweet liker status), influenced the pleasantness of an odor paired with the saccharin in solution.

Even in high coffee consuming cultures such as Italy there are clear sensory variations in the coffees that are produced and consumed, and there is also potential for modification of bitterness using the addition of sweeteners. The aim of this study was to investigate physiological

measures underlying taste perception and how these influence perception, consumption of, and liking for coffee.

## 2. Methods

### 2.1. Product selection

#### 2.1.1. Subjects

As part of a pilot experiment to select suitable coffee samples for use in the main study, eight subjects (Ss), six females and two males, aged from 20 to 38 years, and regular coffee consumers, were recruited in the Florence area. The Ss had no history of disorders in oral perception. They were paid for their participation in the study. Written informed consent was obtained from each subject after the description of the experiment.

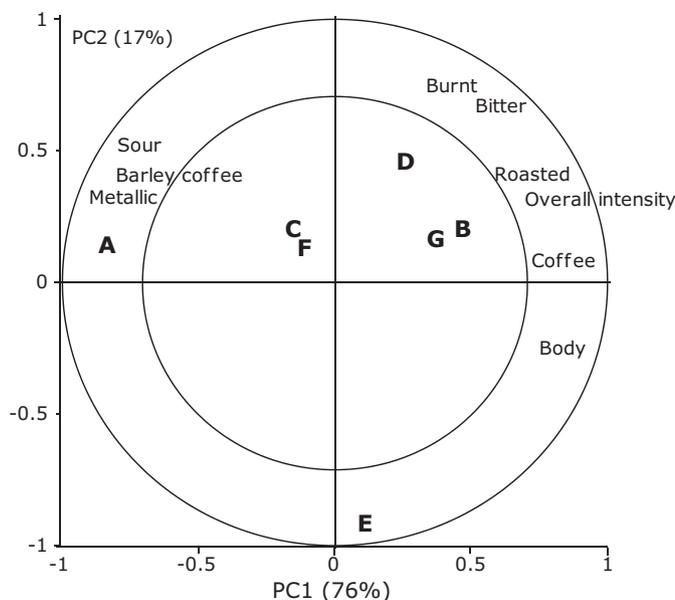
#### 2.1.2. Samples

Seven espresso coffees varying in roasting degree (light, medium, dark) and caffeine content (<0.05–2%) were evaluated (Fig. 1). Coffee samples (25 g) were prepared with an espresso machine using coffee capsules.

#### 2.1.3. Descriptive analysis (DA)

Ss participated in five sessions for training and term generation. Specifically, they were trained to recognize and rate the perceived intensity of the following qualities: sweetness, sourness, bitterness, and astringency using the following standard solutions - sucrose: 8.00, 12.00, 18.00 g/l; citric acid: 0.25, 0.38, 0.50 g/l; quinine monohydrochloride dihydrate 0.025, 0.037, 0.050 g/l; aluminium potassium sulphate: 0.3, 0.6, 0.9 g/l. During training sessions, Ss were asked to rate the intensity of the standard solutions on a 9-point category scale (1="extremely weak"; 5="moderate"; 9="extremely strong").

An evaluation sheet consisting of 22 ratings was defined. In each of the five sessions, four or five samples were evaluated. Each sample was evaluated 3 times. Samples (25 g) were presented in a closed 80 cc plastic cup identified by a three digit code. Sample presentation was balanced across subjects within each session. For each sample, assessors were asked to rate the intensity of odor descriptors perceived by nose (aroma) first. Then they were asked to wait 3 minutes, take a sip of the sample and rate the intensity of odors perceived retro-orally, taste and mouthfeel attributes. After each sample, subjects



Sample	Roasting degree	Caffeine (%)
A	Light	0.8-0.9
B	Dark	0.8-0.9
C	Medium	1.50
D	Dark	1.50
E	Medium	<0.05
F	Medium	0.8-0.9
G	Dark	about 2

**Fig. 1.** Correlation loading plot from Principal Component Analysis on panel averages of each significant attribute describing sample sensory properties. For each sample roasting degree of coffee beans and caffeine content (%) of coffee powder are reported in the table.

rinsed their mouths with distilled water for 50 sec, had some plain crackers for 50 sec and finally rinsed their mouths with water for a further 50 sec. Ss took a 15 min break every two samples. In the adopted experimental conditions aroma evaluation was performed at 65–67 °C and the in-mouth evaluation at 55–57 °C. Evaluations were performed in individual booths under red lights. Data were collected with the software Fizz (ver.2.47.B, Biosystemes, Couternon, France).

Intensity ratings from coffee samples were independently analyzed by a two-way ANOVA mixed model (sample as fixed and assessors as random factors), followed by a Fisher's LSD post hoc test (significant for  $p \leq 0.05$ ). Principal Component Analysis (PCA) was computed on panel averages of each significant attribute arising from the ANOVA models. Samples were included as dummy variables (down-weighted in the data matrix) to improve the visual interpretation [52]. The full cross validation was computed to validate the interpretation of the first two components.

Results of the PCA computed on descriptive data are summarized in the correlation loading plot reported in Fig. 1. The first two significant dimensions of the perceptual map accounted for 93% of the variation (PC1:76% and PC2:17%). PC1 was positively associated with flavor descriptors “coffee”, “overall intensity”, “roasted”, “bitter”, “brunt” and mouthfeel descriptor “body”, while a negative correlation was found for “metallic”, “barley coffee” and “sour” flavor descriptors. Samples were mainly discriminated along the first component according to roasting degree. PC2 contributed to separate product E from other coffees. Coffee sensory properties are affected by several factors: plant varieties, growing region/conditions, processing and brewing methods [53]. Roasting degree and time/temperature combinations determine the formations of compounds responsible for the coffee sensory profile [54,55].

The relative positions of samples on the perceptual map resulting from descriptive data were used for the selection of samples to be tested for consumer test. Samples A, B, C, D, E, G spanned the relevant variability of the sensory attributes within the whole sample set according to different caffeine content and roasting degree.

## 2.2. Study of coffee consumers

### 2.2.1. Subjects

One hundred and twenty Ss (51 males; 69 females; aged 20–60 years, regular coffee consumers), were recruited in the Florence area to participate in 3 evaluation sessions in 3 consecutive days. Data on mode of coffee consumption (black; with sugar; with milk; 1 “never”, 2 “occasionally”, 3 “regularly”) and frequency (1 “less than once a day”, 2 “once a day”, 3 “twice or three times a day”, 4 “four or five times a day”, 5 “more than five times a day”) were collected. The Ss had no history of disorders in oral perception. They were paid for their participation in the study. Written informed consent was obtained from each subject after the description of the experiment.

### 2.2.2. Coffee samples

Six espresso coffees (labeled A, B, C, D, E, G) were evaluated.

### 2.2.3. Taste stimuli

Psychophysical curves were constructed for caffeine and quinine-HCl over 6 concentrations (caffeine: 0, 3, 6, 12, 24, 48 mM; quinine-HCl: 0, 0.05, 0.10, 0.15, 0.20, 0.25 mM). A single solution of PROP (3.2 mM) was rated using the general Labeled Magnitude Scale (gLMS) [56] to determine PROP taster classification [57,58]. All solutions were prepared with deionized water and were stored in glass bottles and were brought to room temperature prior to testing.

### 2.2.4. Procedure

#### 2.2.4.1. First session: liking for coffee samples.

Ss were presented with each sample (25 g) in a closed 80 cc plastic

cup along with 10 g of sugar (about three coffee spoons) in a plastic cup both identified with the same random three digit code. Samples were presented one by one and the presentation order was balanced across Ss. For each sample, Ss were asked to smell and rate their liking for the aroma first. Then they were asked to take a sip and rate their liking for the flavor (flavor1). Finally they were asked to freely add sugar, if they thought it was necessary independently of their habit, take a sip and rate again their liking for the flavor (flavor2). Hedonic ratings were collected using a 9-point hedonic scale [59], from 1 (“dislike extremely”) to 9 (“like extremely”) with a neutral point at 5 (“neither like nor dislike”). The amount of sugar used for each sample by each S was measured by weighting the sugar cup after each evaluated sample.

After each sample, Ss rinsed their mouths with distilled water for 50 s, had some plain crackers for 50 s, and finally rinsed their mouths with water for a further 50 s. Ss took a 10 min break after every 2 samples. In the adopted experimental conditions, coffee temperature was 65 to 67 °C for aroma evaluation and 55 to 57 °C for in-mouth evaluation. Evaluations were performed in individual booths under white lights. Data were collected with the software Fizz (ver.2.47.B, Biosystemes, Couternon, France).

#### 2.2.4.2. Second session: sourness, bitterness and astringency in coffee samples.

Ss were trained to recognize the following qualities and the respective intensities: sourness, bitterness, and astringency using the following standard solutions - citric acid: 0.25, 0.38, 0.50 g/l; quinine monohydrochloride dihydrate 0.025, 0.037, 0.050 g/l; aluminium potassium sulphate: 0.3, 0.6, 0.9 g/l.

In particular, Ss were presented with 9 samples identified with the name of the taste/sensation (sourness, bitterness, astringency) and the respective intensity (weak, moderate, strong). Ss were instructed to hold the sample in their mouth for 10 s, then expectorate, wait 20 s and memorize the perceived taste/sensation and also its intensity. Ss tasted first sour solutions, then bitter solutions and last astringency solutions in increasing intensity order. After each sample, Ss rinsed their mouths with distilled water for 90 s. Ss took a 10 min break after every taste/sensation.

30 min after training, Ss were presented with the six unsweetened coffee samples (8 ml) in a closed 80cc plastic cup identified with a random three digit code. Samples were presented one by one and the presentation order was balanced across subjects.

Ss were instructed to hold the sample in their mouth for 10 s, then expectorate, wait 20 s and evaluate the intensity of sourness, bitterness and astringency using the Labeled Magnitude Scale (LMS) [60]. The evaluation order of attributes was balanced across Ss. After each sample, Ss rinsed their mouths with distilled water for 50 s had some plain crackers for 50 s and finally rinsed their mouths with water for a further 50 s. They took a 10 min break after every 2 samples. The evaluation was performed at 55 to 57 °C. Evaluations were performed in individual booths under red lights. Data were collected with the software Fizz (ver.2.47.B, Biosystemes, Couternon, France).

#### 2.2.4.3. Third session: bitterness of caffeine, quinine and PROP solutions and measure of fungiform papillae number.

Caffeine, quinine-HCl and PROP solutions were presented in 3 different blocks and the evaluation order of the blocks was balanced across Ss. Ss took a 30 min break after each block. Ss were presented with each sample (10 ml) in a 80cc plastic cup identified with a random three digit code. The presentation order of the samples in each block was balanced across Ss. Ss were instructed to hold the sample in their mouth for 10 s, then expectorate, wait 20 s and evaluate the intensity of bitterness, using the gLMS.

After each sample, Ss rinsed their mouths with distilled water for 90 s. Ss took a 10 min break after every 2 samples. Evaluations of caffeine and quinine-HCl were performed once, of PROP twice. Evaluations were performed in individual booths under white lights. Data were

collected with the software Fizz (ver.2.47.B, Biosystemes, Couternon, France).

The anterior portion of the dorsal surface of the tongue was swabbed with household blue food coloring (F.lli Rebecchi), using a cotton-tipped applicator. This made the FP easily visible as red structures against the blue background of the stained tongue [7]. Images of the tongue were recorded using a digital microscope (MicroCapture, version 2.0 for 20x–400x). For each participant, the clearest image was selected, and the number of FP was counted in two 0.6 cm diameter circles, one on right side and one on left side of tongue, 0.5 cm from the tip and 0.5 cm from the tongue midline. The number of FP was counted by two researchers independently, blind to the performance of participants in the sensory evaluation tests. The average of these values was used for each S.

### 2.3. Data analysis

Liking ratings expressed for aroma, flavor1 and flavor2 were independently submitted to a two-way ANOVA model (assessors and sample as factors) with Fisher's LSD post hoc test (significant for  $p \leq 0.05$ ).

Liking ratings expressed for flavor1 and flavor2 were analyzed by a two-way ANOVA model (sample – 6 levels – and condition – 2 levels: with or without sugar added- as factors), with Fisher LSD post hoc test (significant for  $p \leq 0.05$ ).

Ss were divided into groups based on median value of FP distribution (12.12; range 4–22): Low FP (LFP) and High FP (HFP). PROP taster status was based on the average rating of the two replicates, and groupings were based on percentile distribution: PROP nontasters (NTs: 30 Ss)  $\leq 21.75$ ; PROP medium tasters (MTs: 60 Ss), 22–65; and PROP super-tasters (STs: 30 Ss)  $\geq 65.125$ . The relationship between FP number and PROP status was estimated by Pearson's correlation coefficient.

A two-way ANOVA (group and sample as factors), with Fisher's LSD test ( $p \leq 0.05$ ) were used to independently test both the effect of FP number and PROP status on liking and intensity ratings in both coffee samples and standard solutions. The associations of clusters with gender, coffee consumption frequency and consumption of sweetened/unsweetened coffee (categorical variables) were estimated by a homogeneity chi-square test.

## 3. Results

### 3.2.1. General overview

A significant sample effect on liking for aroma was found ( $F_{5;719} = 11.22$ ,  $p \leq 0.0001$ ). Mean liking scores for flavor resulted significantly affected by the conditions adopted for data acquisition (w/o or with sugar) ( $F_{1;1439} = 64.30$ ,  $p \leq 0.0001$ ). In particular, mean liking score for flavor1 resulted significantly lower than those for flavor2 (4.68 and 5.49, respectively). A significant sample effect on liking was found ( $F_{1;1439} = 37.74$ ,  $p \leq 0.0001$ ). No significant effect sample\*condition interaction was found.

A significant sample effect was found on liking for both flavor1 ( $F_{5;719} = 22.36$ ,  $p \leq 0.0001$ ) and flavor2 ( $F_{5;719} = 33.18$ ,  $p \leq 0.0001$ ). However, when sugar was added to the samples (flavor2), different samples were rated as more different than when coffees were evaluated without sugar (flavor1), and in particular a higher difference between the liking rates of the most preferred coffee and of the least one was found. So, flavor2 better discriminated between samples ( $F_{719;1.13} = 1.15$ ,  $p = 0.035$ ).

Significant differences between samples were found for perceived intensity of sourness, bitterness and astringency ( $F_{5;719} = 10.13$ ,  $p \leq 0.0001$ ;  $F_{5;719} = 11.75$ ,  $p \leq 0.0001$ ;  $F_{5;719} = 2.40$ ,  $p = 0.036$ ; respectively). Samples A and C were the most intense for sourness; bitterness significantly differed among samples based on roasting degree; samples C and B were the most and the least astringent respectively.

Psychophysical curves for caffeine and quinine-HCl are shown in Fig. 2. Bitterness significantly increased with concentration

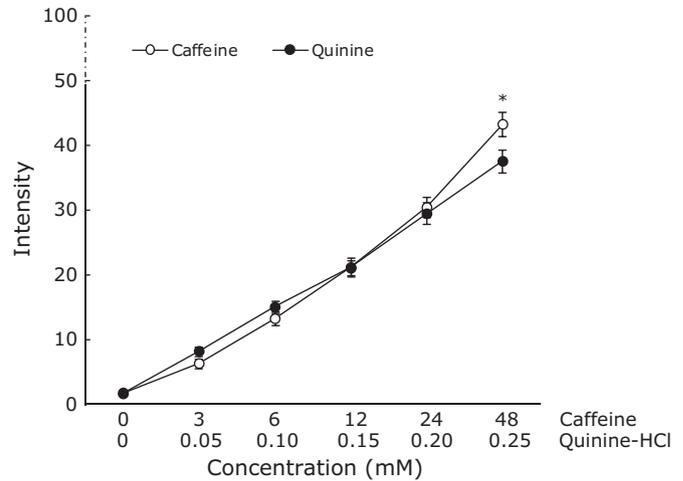


Fig. 2. Mean bitterness ratings as a function of variations in caffeine and quinine-HCl concentration. \* indicates a significant difference ( $p \leq 0.05$ ).

( $F_{5;719} = 348.07$ ,  $p \leq 0.0001$ ;  $F_{5;719} = 279.31$ ,  $p \leq 0.0001$ , respectively). No significant differences in ratings of bitterness between caffeine and quinine-HCl were found, excepted for the most concentrated solutions ( $F_{1;1439} = 242.50$ ,  $p \leq 0.0001$ ).

### 3.2.2. Fungiform papillae number

The distribution of FP papillae is shown in Fig. 3. No significant differences were found for FP counts conducted by the two different researchers ( $t_{238;1.97} = 1.41$ ,  $p = 0.160$ ), or on the two tongue sides ( $t_{238;1.97} = 0.14$ ,  $p = 0.888$ ). No significant difference was found between males and females for FP number ( $t_{118;1.98} = -0.86$ ,  $p = 0.391$ ). No significant difference between the FP groups in terms of either gender ( $\chi^2 = 0.85$ ,  $df = 1$ ,  $p = 0.356$ ) or age ( $t_{118;1.98} = 0.27$ ,  $p = 0.784$ ) was found. No significant difference between the FP groups in terms of coffee consumption frequency was found ( $\chi^2 = 0.04$ ,  $df = 1$ ,  $p = 0.838$ ).

3.2.2.1. Effect of FP number on liking. The effect of FP number on coffee preferences is shown in Fig. 4. No significant effect of FP group on liking for aroma and flavor1 were found. HFP Ss gave higher liking ratings than LFP Ss for coffees overall when asked to add sugar to taste ( $F_{1;708} = 6.79$ ,  $p = 0.009$ ). Moreover, HFP Ss added more sugar to the samples than LFP Ss (2.04 g vs 1.56 g,  $F_{1;708} = 9.26$ ,  $p = 0.002$ ). Consistent with this, greater sugar use by HFP Ss was also evident in the

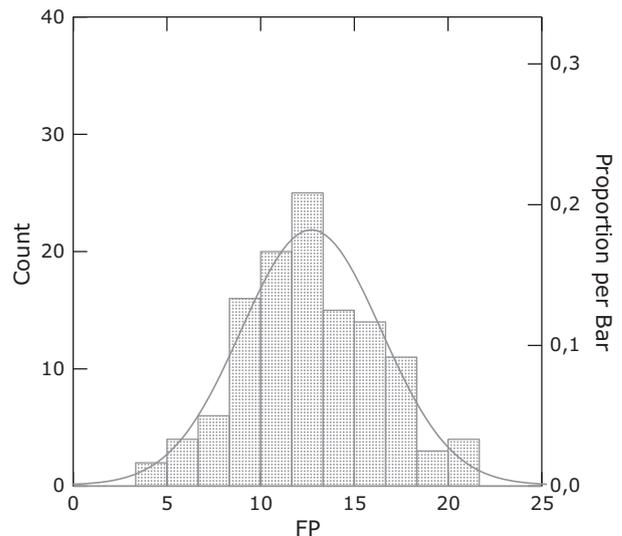
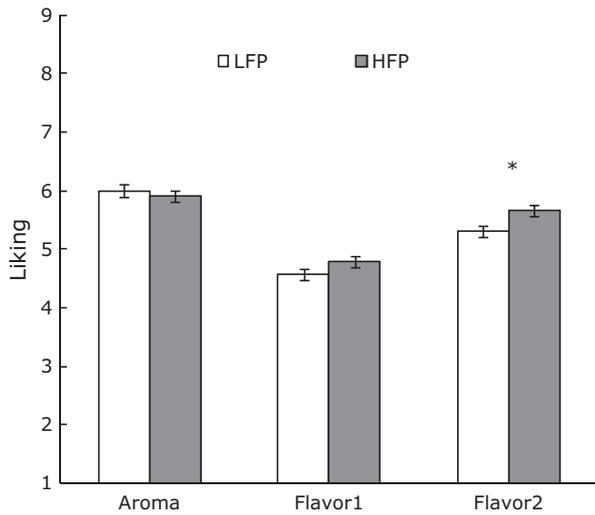
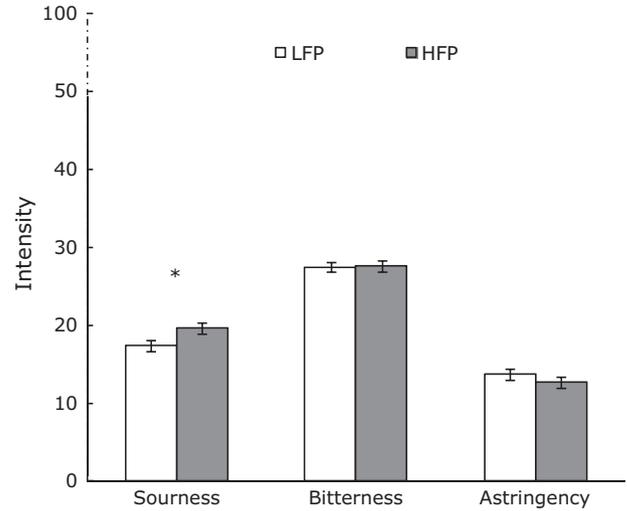


Fig. 3. Fungiform papillae number distribution.



**Fig. 4.** Effect of FP cluster (LFP and HFP) on liking for aroma, flavor1 and flavor2: mean ratings and standard error values. \* indicates a significant difference ( $p \leq 0.05$ ).



**Fig. 6.** Effect of FP cluster (LFP and HFP) on sourness, bitterness and astringency perception in coffee: mean ratings and standard error values. \* indicates a significant difference ( $p \leq 0.05$ ).

consumption data (52% of HFP Ss vs 33% of LFP Ss who consume regularly coffee with sugar;  $\chi^2 = 4.13$ ,  $df = 1$ ,  $p = 0.042$ ). No significant group\*sample interaction was found.

**3.2.2.2. Effect of FP number on taste perception.** HFP Ss rated the bitterness of both caffeine and quinine solutions as stronger than did LFP Ss ( $F_{1;708} = 4.21$ ,  $p = 0.041$ ;  $F_{1;708} = 10.42$ ,  $p = 0.001$ , respectively) (Fig. 5a and b). No significant effect of group\*sample interaction was found. The effect of FP number on taste perception in coffee is shown in Fig. 6. HFP Ss rated coffee sourness as stronger than LFP Ss ( $F_{1;708} = 3.83$ ,  $p = 0.050$ ). No significant differences between FP groups were found for coffee bitterness or astringency. No significant group\*sample interaction was found.

**3.2.3. PROP status**

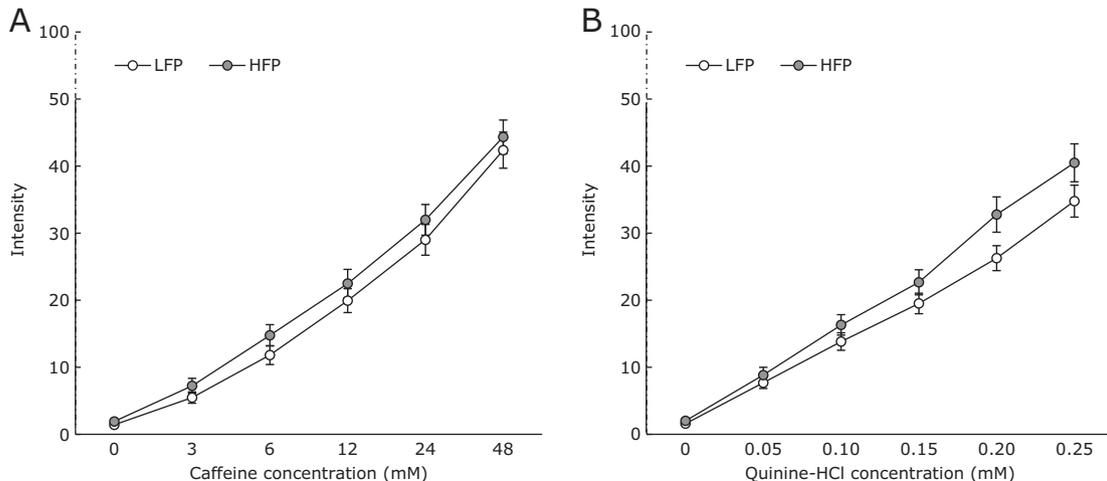
The distribution of PROP intensity ratings is shown in Fig. 7 and is similar to those in other published studies of PROP sensitivity. No significant difference between the two replicates was found ( $t_{119;1.98} = -0.07$ ,  $p = 0.942$ ). No significant difference was found between males and females for PROP status ( $t_{118;1.98} = -0.50$ ,  $p = 0.613$ ). No significant difference between the PROP groups in terms of either gender ( $\chi^2 = 0.85$ ,  $df = 2$ ,  $p = 0.653$ ) or age ( $F_{2;117} = 0.23$ ,  $p = 0.796$ ) was found. No significant difference between the PROP groups in terms of coffee consumption frequency was found ( $\chi^2 = 1.38$ ,  $df = 2$ ,  $p = 0.502$ ).

**3.2.3.1. Effect of PROP status on liking.** No significant effect of PROP status on coffee liking was found (Fig. 8). While coffee liking was unrelated to PROP status, NTs added more sugar to the coffee than did MTs and STs ( $F_{2;702} = 8.34$ ,  $p \leq 0.0001$ ). However, no significantly different habits of sugar use were reported in the consumption data ( $\chi^2 = 0.58$ ,  $df = 2$ ,  $p = 0.748$ ). No significant effect of group\*sample interaction was found.

**3.2.3.2. Effect of PROP status on taste perception.** STs rated the bitterness of both caffeine and quinine solutions as stronger than did PROP NTs and MTs ( $F_{2;702} = 16.33$ ,  $p \leq 0.0001$ ;  $F_{2;702} = 15.83$ ,  $p \leq 0.0001$ , respectively) (Fig. 9a and b). No significant differences between NTs and MTs were found. No significant group\*sample interaction was found. The effect of PROP status on taste perception in coffee is shown in Fig. 10. PROP STs rated coffee sourness ( $F_{2;702} = 6.73$ ), bitterness ( $F_{2;702} = 16.68$ ,  $p \leq 0.0001$ ) and astringency ( $F_{2;702} = 15.09$ ,  $p \leq 0.0001$ ) as stronger than NTs and MTs. Significant difference between NTs and MTs was found only on astringency perceived intensity. No significant effect of group\*sample interaction was found.

**3.2.4. Relationship between FP number and PROP status**

No significant relationship was found between FP number and PROP intensity ( $r = 0.05$ ,  $p = 0.592$ ). No significant effect of FP group



**Fig. 5.** Mean bitterness ratings for each FP group (LFP and HFP) as a function of variations in caffeine (A) and quinine-HCl (B) concentration.

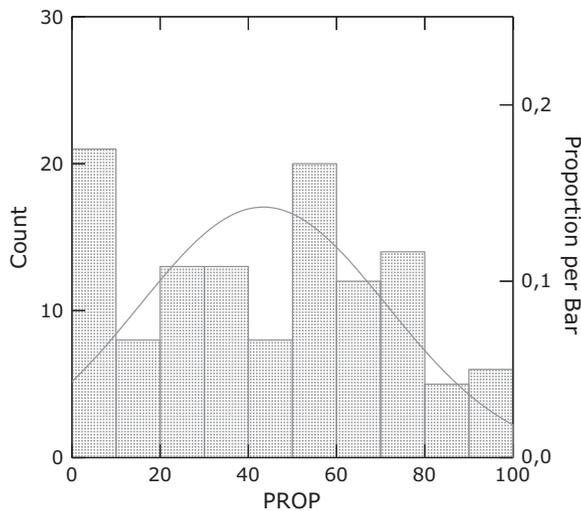


Fig. 7. PROP taster status distribution.

on the bitterness rating of PROP solution was found ( $t_{118;1.98} = 0.88$ ,  $p = 0.382$ ) and no significant differences in PROP groups for FP number were found ( $F_{2;117} = 0.53$ ,  $p = 0.590$ ).

#### 4. Discussion

A number of recent studies have examined the influence of underlying physiology or genetics on food acceptability, preferences or intake [36,41,42,45,57,61]. However, the question of how these underlying influences might shape food behaviors has received little attention. Given that culture is probably the most pervasive influence on food choices, the impact of variations in underlying physiology/genetics will take place within a cultural context. For example, while there are large variations in the perception of hot (spicy) food ingredients [26], in countries such as Mexico or Korea, the consumption of chilli is universal and frequent. However, it might be expected that individuals tailor the degree of spiciness in their foods to a comfortable level. The bite of alcohol is similarly influenced by variations in physiology [45] and it is commonplace in some cultures for alcohol to be diluted when it is first consumed.

The present data on coffee appear to show a similar pattern. The consumption of espresso coffee is near ubiquitous in Italy and it represents

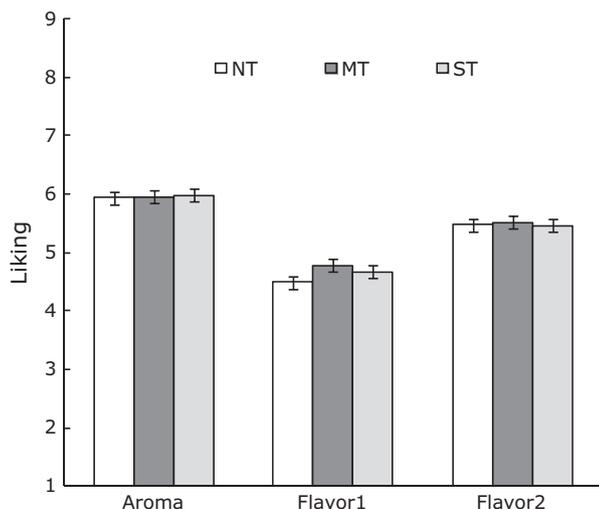


Fig. 8. Effect of PROP taster status (NT, MT, and ST) on liking for aroma, flavor1 and flavor2: mean ratings and standard error values.

a part of the common food culture for Italians. Against this background, and in common with other coffee users worldwide, some Italians modify the sensory characteristics of their coffee using sweeteners or sometimes milk. In this study, 68.33% of consumers declared that they usually sweeten coffee and 82.50% of subjects added sugar after the first tasting. The general disliking for unsweetened samples resulted in a minor significant sample effect. Thus, tasting without sugar added does not seem appropriate for evaluation of liking of coffee samples.

The most significant finding of the current study is the fact that a food-related behavior – sugar use – is influenced by both sensitivity to the bitter compound PROP as well as the density of FP. Subjects with higher FP numbers routinely added more sugar to their coffee, added more sugar when asked to do so for the study samples, and gave higher liking ratings to the coffees than low FP Ss for the sweetened coffee samples. In the context of the finding that HFP Ss rated coffee sourness as stronger than did LFP Ss, this suggests that their addition of sweetness was an attempt to modify an undesirable sensory characteristic that they perceived in the coffees. It is quite plausible that this difference in sugar use underlies the fact that the HFP shows higher liking for the coffees. Sweetness is innately liked, and the addition of sweetness to a wide variety of foods and drinks increases their immediate acceptability [62].

In an apparently paradoxical finding, PROP NTs, who rated coffee sourness, bitterness and astringency as less intense than PROP STs, added more sugar to the coffee than did either MTs and STs. Tentatively, it could be suggested that NTs might be attempting to modify the sensory properties of the coffee, enhancing its overall flavor by adding sweetness. One other factor that might be influential is the fact that PROP NTs are reportedly more likely to be sweet-likers as determined by their responses to sweet tastes in solution [50,63,64].

Unexpectedly, PROP ratings and FP counts were not positively correlated as they have been in some previous studies [11,58,65–73]. This may be explained by sampling effects. Indeed, the distribution of PROP tasters was similar to that found in many other studies, with approximately 25% of the sample NTs and another 25%, STs. The range of FP was, however, lower than has been reported: 4–22, as compared to 11.75–40.25 [58] and 12–51 [73] (see [72] for a review). This raises the prospect that our sampling excluded high FP individuals who either do not regularly consume coffee perhaps because of its high sourness and bitterness, or were less inclined to volunteer for research on this topic, possibly for the same reasons. It is also possible that papillae number measurements were an issue. Papillae counts depend on several factors, such as considered area, resolution of images, as well as the criteria used to decide on the presence of individual papillae. Some papillae are flat and short with little elevation, others are double papillae, thus making consistent identification an issue.

Even without high FP individuals, strong relationships between FP density and the bitterness of quinine and caffeine in solution, sourness in coffee samples, and, importantly, the propensity to add sweetener were found. Together, these effects reinforce the idea of FP density as a general index of taste intensity. It is somewhat puzzling that bitterness in coffee was not indexed by FP density, but to date there are no data on FP density and taste discrimination, as there are for PROP (e.g. [18,30,57]), and it may be that the bitterness of the coffee samples was sufficiently alike not to be discriminated as a function of FP density in this study. Moreover, coffee flavor is very complex. Indeed, coffee solids contain tastants that are sour (such as 4-vinylcatechol oligomers and quinic acid lactones) generated during roasting of the beans, as well as many bitter compounds other than caffeine [74,75]. Hence, the taste of a low concentration of caffeine is liable to be masked by both the bitter taste of the roasted coffee and the sour taste that may be confused with it [76]. Moreover, in coffee, a certain degree of bitterness is expected and liked [77]. On the contrary, sourness is not associated with coffee for consumers and subjects frequently confuse sour and bitter taste qualities [78,79]. The lack of a significant FP group effect on astringency is expected, because astringency is a mouthfeel sensation and its perception is not directly related to FP number [32,80,81].

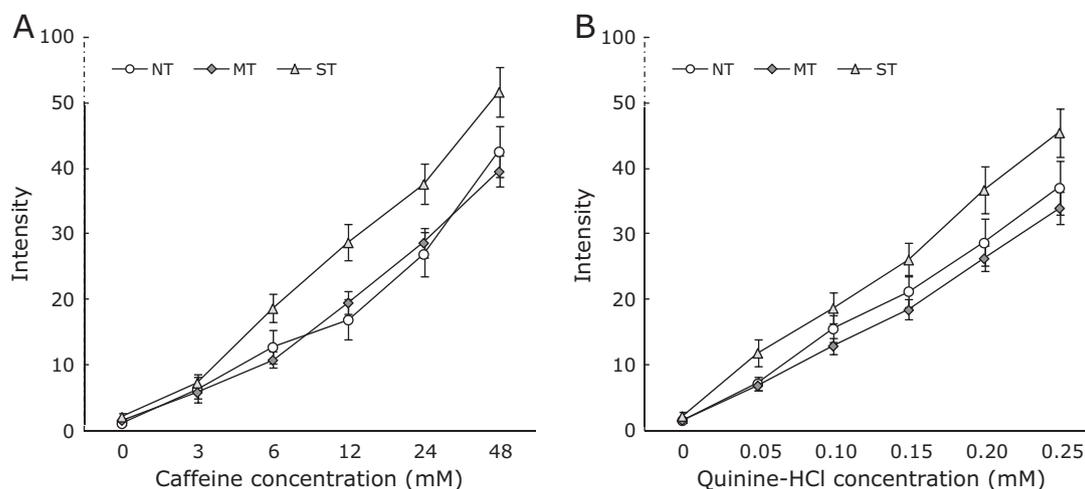


Fig. 9. Mean bitterness ratings for each PROP taster group (NT, MT, and ST) as a function of variations in caffeine (A) and quinine-HCl (B) concentration.

The effect of PROP taste status on sourness and bitterness evaluation in coffee samples also confirms that PROP status is related to general orosensory responsiveness to a variety of stimuli in both standard solutions and foods [18,57]. The results concerning astringency perception are less clear, in particular why MTs rated astringency as weaker than NTs and STs. However, an evident relationship between PROP status and astringency perception is not consistent. Indeed, STs and MTs are reported to perceive astringency with greater intensity than NTs [30]; on the other hand also NTs rate the overall astringency higher than STs [31]. Furthermore, individual differences in astringency perceptions have been shown [81].

Bitterness intensity ratings in standard solutions seem to confirm that bitterness perception is very complex, even though there is large variation in bitter taste perception, there is some commonality to bitter taste elicited by different compounds [82–86]. Furthermore, these results are consistent with previous studies that showed strong relationships between FP density and the intensity of taste compounds [7–9, 11,87] and also that PROP status is linked with heightened sensitivity to bitter compounds as caffeine and quinine hydrochloride [77].

These results not only confirm that FP density and PROP status play a significant role in taste sensitivity for bitter compounds in general but also that sugar use is partly a function of fundamental individual differences in physiology. Thus, it appears that those more sensitive to tastes

that might be unpalatable in coffee compensate by altering its overall flavor. Clearly, measures of food/beverage intake are likely to be only partly related to physiological measures such as FP density or PROP status unless such food behaviors are taken into account.

#### Acknowledgements

This work was completed as part of CM's PhD requirements at University of Florence. The PhD project was funded by illycaffè s.p.a. The authors wish to thank Luciano Navarini and Massimo Barnabà (illycaffè s.p.a.) for cooperation.

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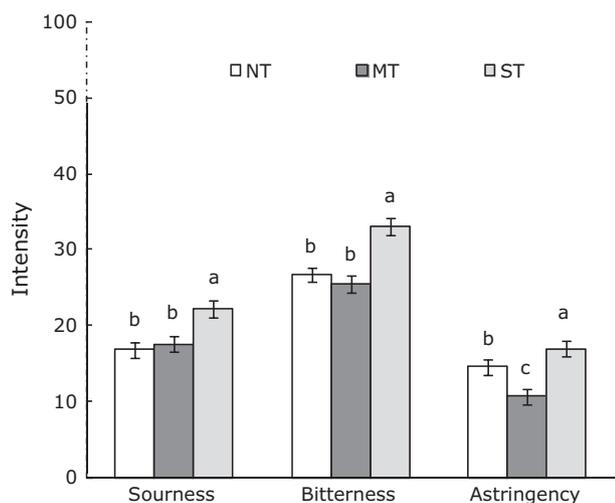


Fig. 10. Effect of PROP taster status (NT, MT, and ST) on sourness, bitterness and astringency perception in coffee: mean ratings and standard error values. Different letters indicate significantly different values ( $p \leq 0.05$ ).

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**Paper III**

# **Caffeine Metabolism Influences Coffee Perception, Preferences and Intake.**

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## Abstract

Inter-individual variability in caffeine metabolism rate influences the pharmacokinetics effect of caffeine. This study explores the possible relationship between caffeine metabolism rate and coffee preferences and consumption habits. In addition, the extent to which caffeine metabolism interacted with variations in bitterness perception was investigated. Caffeine metabolism rate was assayed by competitive immuno-enzymatic assay in one-hundred thirty-five coffee consumers who provided saliva samples after 12 hours caffeine abstinence and at 30 and 90 min after ingestion of caffeine (100mg). A caffeine metabolism index (CmI) was computed as the ratio between the amount of residual caffeine in saliva 60 min after the adsorption peak and the amount of caffeine at the adsorption peak corrected with the baseline. Ninety-one subjects were selected to investigate the relationships between inter-individual variation in caffeine metabolism, bitterness perception and coffee preference. Subjects rated liking for, and sourness, bitterness and astringency of, six unsweetened and freely sweetened coffee samples varying in roasting degree, caffeine content and bitterness. They also rated the bitterness of six caffeine and six quinine (equi-intense) solutions. Finally, subjects choose coffee to drink on the basis of a label (strong vs balanced flavor) both after caffeine abstinence and after no restrictions on caffeine intake. The CmI was strongly associated with the frequency of daily coffee consumption. Subjects with lower CmI gave higher bitterness ratings than other subjects for both coffee and caffeine solutions, but not for quinine solutions. They also added more sugar to the coffee samples. Following caffeine abstinence, all subjects chose the "strong flavor" coffee, while without caffeine restrictions, subjects with lower CmI preferentially tended to choose the "balanced flavor" coffee. These results provide the first link between caffeine metabolism and bitterness perception, and to the use of sugar to modify coffee bitterness.

## Introduction

Understanding the influences on preferences for bitter foods and beverages is a challenge because bitterness *per se* is innately disliked [1]. Despite this, bitter beverages such as coffee and beer are amongst the most consumed beverages worldwide. Preferences for bitter food or beverage flavors are thought to be developed via associations between the flavor and the post-ingestive consequences of the consumed nutrients and pharmacologically active ingredients [2]. Such flavor consequence learning (FCL; [3]), can be produced by ingestion of valued nutrients such as glutamate [4], or energy in the form of sugars or fats [5]. In addition, the physiological and behavioral effects associated with stimulants such as alcohol, caffeine, theophylline, theobromine are also linked to the development of flavor preferences [2, 6].

Caffeine, in particular, has been found to promote flavor preferences [6]. Caffeine is a central nervous system and metabolic stimulant [7] and it promotes wakefulness, enhances mood and cognition, and produces stimulatory effects [8, 9]. At low doses (50-250 mg), caffeine psychological effects include mild euphoria, alertness, and enhanced cognitive performance [10], but at higher doses (400-800 mg), it can produce nausea, anxiety, trembling, and jitteriness [11].

In addition to any direct pharmacological effects, preferences for coffee or other initially unpalatable foods/beverages can be facilitated by the addition of sweeteners that produce FCL due to the delivery of energy [5] and also suppress disliked bitter, sour or irritant qualities [12].

Independently, choosing particular coffee styles that may vary in species, origins, processing factors, and brewing methods [13-17] allows consumers to choose coffees based on their own preference for particular sensory properties.

Coffee preferences, like those of other bitter foods and beverages [18-21], appear to be partially dependent on genetically-determined variations in taste sensitivity [22]. Hence, differences in the

intensity of the compound 6-n-propylthiouracil (PROP) were shown to affect the perception of bitterness of caffeine [23] and the liking for coffee [24]. Moreover, the density of lingual fungiform papillae (FP), the PROP status [25] and the individual responsiveness to astringent stimuli [26] influence the perception of bitter taste and the use modality in coffee (with or without sweeteners).

Another potentially important factor influencing preference for coffee is the individual variability in caffeine metabolism rate. Caffeine is absorbed rapidly and completely from the gastrointestinal tract and is metabolized in the liver [27-28] with peak plasma concentrations of caffeine are reached within 15 [27] to 60 [29] minutes after intake. In humans, the half-life of caffeine typically ranges from 2 to 4.5 hours [30-31]. Caffeine is converted mostly to paraxanthine (81.5%) [32-34], by cytochrome P-450 enzymes, in particular the P-450 1A2, which is coded by the gene CYP1A2 [35-36]. Inter-individual variability in activity of CYP1A2 ranges normally between 5- and 15-fold [37-38], but differences may be up to 60-fold [39], and it represents a major source of the variability in pharmacokinetics of caffeine [40]. Different single nucleotide polymorphisms (SNPs) may explain inter-individual variation in caffeine metabolism rate [41-48]. An A to C substitution at position -163 (rs762551) in the CYP1A2 gene decreases enzyme inducibility [41, 49]. Carriers of the -163C allele can be considered slow caffeine metabolizers, whereas homozygous for the -163A allele are more rapid caffeine metabolizers [41, 49]. The A/A variant seems to be associated with high inducibility of the CYP1A2 enzyme in smokers [41].

In general, given the same caffeine intake, slow metabolizers will be more exposed to high internal caffeine levels than fast metabolizers [50-51]. People tend to adapt their coffee consumption to balance perceived negative and reinforcing symptoms that are affected by genetic variation [52]. However, while caffeine intake itself increases the rate of caffeine metabolism [38, 53], there has been no exploration of the potential impact of caffeine metabolism rate, the variants in CYP1A2 and caffeine consumption on coffee preference.

The study reported here aimed to examine for the first time the relationship between caffeine metabolism rate, coffee preferences and consumption habits. In addition, we examined the extent to which caffeine metabolism interacted with variations in bitterness perception. We predicted that both fast caffeine metabolism rate and low sensitivity to bitter taste favor the preference for, and consumption of, the more bitter black coffee. The logic behind this is that the higher coffee consumption associated with a faster caffeine metabolism rate would allow a more rapid development of a preference for stronger coffee flavor, as is found with exposure to other unpalatable tastes [54]. In turn, this process may be further enhanced by a relatively low sensitivity to bitter taste. On the other hand, the use of sweeteners in coffee to mask its unpleasant bitter taste could result from the interplay between the high sensitivity to bitterness and the relatively low consumption induced by a lower caffeine metabolism rate. Thus, the aim of this study was to investigate the possible relationship between caffeine metabolism rate and bitterness sensitivity and how these can influence intake of and preference for coffee, in terms of both sensory properties and use modality (with or without sweeteners).

## **Materials and Methods**

### **Subjects**

One hundred and thirty-five subjects (Ss) (59 males and 76 females; aged 20-60 years; regular coffee consumers; 108 no smokers and 27 smokers; 19 oral contraceptive users) were recruited in the Florence area. The Ss had no history of disorders of oral perception. They were paid for their participation in the study. All studies adhered to the tenets of the Declaration of Helsinki. Approval for the research protocol was obtained from the Institutional Review Board of the Agricultural PhD School/Sustainable Management of Agricultural, Forestry and Food Systems - GESAAF, University of Florence. Written informed consent was obtained from each subject after the description of the experiment.

## **Samples**

### *Coffee samples*

Six espresso coffee samples (labeled A, B, C, D, E, G) were evaluated. Products were selected based on variations in their caffeine content, the roasting degree, the intensity of bitterness and typical descriptors of coffee flavor, as previously described [25]. Coffee samples (25 g) were prepared with an espresso machine using coffee capsules. In the adopted experimental conditions, coffee temperature was 65 to 67 °C for aroma evaluation and 55 to 57 °C for in-mouth evaluation.

### *Taste stimuli*

Six concentrations of caffeine (0, 3, 6, 12, 24, 48 mM) and quinine-HCl (0, 0.05, 0.10, 0.15, 0.20, 0.25 mM) were selected to obtain equi-intense solutions for bitterness. A single solution (3.2 mM) of 6-n-propylthiouracil (PROP) was selected to determine PROP taster classification [22, 55]. All solutions were prepared with deionized water and were stored in glass bottles and were brought to room temperature prior to testing. Ss were instructed to hold each sample (10 ml) in their mouth for 10 s, then expectorate, wait 20 s and evaluate the intensity of bitterness using the general Labeled Magnitude Scale (gLMS) [56].

## **Procedure**

Ss participated in four separate evaluation sessions: in the first session, Ss were asked to smell and rate their liking for the aroma of the coffee samples first. Then they were asked to take a sip and rate their liking for the flavor (flavor1). Finally, they were asked to freely add sugar, if they thought it was necessary independently of their habit, take a sip and rate again their liking for the flavor (flavor2). In the second session, Ss rated the intensity of sourness, bitterness and astringency in the coffee samples using the gLMS [57]. In the third session, Ss rated the intensity of bitterness in standard solutions of caffeine, quinine, PROP using the gLMS. In the final session, activity of

CYP1A2 was assessed and Ss completed a questionnaire, as described below. Evaluations were performed in individual booths under white (1<sup>st</sup> and 3<sup>rd</sup> sessions) or red (2<sup>nd</sup> session) lights. Data were collected with the software Fizz (ver.2.47.B, Biosystemes, Coutermon, France).

## **CYP1A2 activity**

### *Caffeine metabolism phenotyping*

Caffeine is the most commonly used substance for CYP1A2 phenotyping and several methods and matrices have been employed for this purpose (see [58] for a review). Enzymatic immunoassays have been successfully proposed to assess CYP1A2 activity using both saliva and plasma [e.g. 8-9, 59-62]. These tests are sensitive, simple, harmless, not expensive and easily repeatable [61]. Moreover, most authors suggest that before phenotyping, a methylxanthine abstinent period of 12 to 36 h has to be respected (review [58]) and participants who show high caffeine concentrations before the administration of the caffeine test dose has to be excluded [38, 63]. Other external factors have been reported to influence CYP1A2 activity. CYP1A2 activity is associated with exposure to polycyclic aromatic hydrocarbons (e.g., through cigarette smoking), consumption of cruciferous vegetables and grilled meat, heavy exercise, and certain drugs such as omeprazole and carbamazepine [e.g. 64-68]. Conversely, CYP1A2 activity is inhibited by Apiaceous vegetables, fluvoxamine, quinolone antibiotics, pregnancy and oral contraceptives [e.g. 69-72].

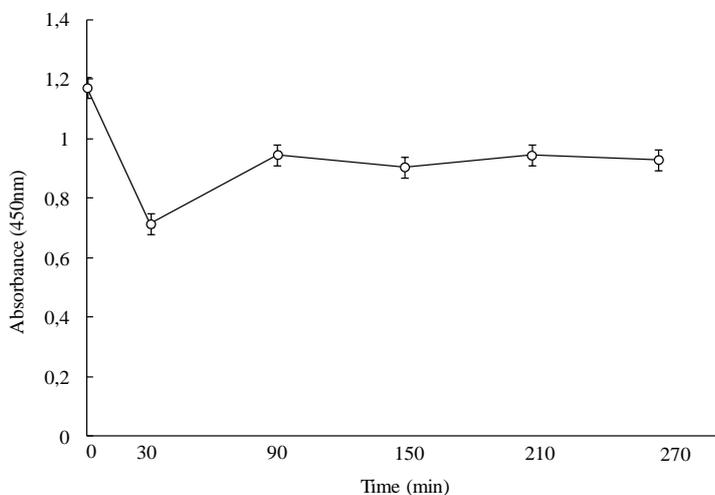
A pilot test was run to determine the times for saliva collection and the protocol for saliva analysis. Ten Ss were instructed to avoid foods and beverages containing caffeine for at least 12 h before the session started, foods affecting CYP1A2 activity for at least 24 h before the session started, and medicines interfering with CYP1A2 activity for at least 48 h before the session started. A list of such products was provided. Ss were also instructed to refrain from smoking, eating, and drinking for 2 h before the session.

The saliva was collected as previously described [73]. Ss received tap water to rinse their mouth, and they were then instructed to mechanically evoke saliva by chewing Parafilm (3 cm \* 3 cm) for a total collection time of 3 min (first saliva collection, T0). Then Ss received a dose of caffeine (100 mg in 15 ml of water). After 30 min, they rinsed their mouth with water and evoked saliva by chewing Parafilm for a further 3 min (second saliva collection, T30). After 90, 150, 210, 270 min they repeated the procedure for saliva collection (four saliva collections, T90, T150, T210, T270). Saliva samples were immediately frozen. Salivary caffeine was measured using the caffeine ELISA competitive immune-assay (Abraxis LLC, 54 Steamwhistle Drive Warminster, PA 18974) using a sample dilution factor of 1:1000 and a reaction time enzyme-substrate of 60 min. The absorbance at 450 nm (Abs) was read using a microplate ELISA photometer (1420 Victor 3<sup>TM</sup>Multilabel Counter, Wallak, PerkinElmer) within 15 minutes after stopping the reaction. The absorbance at 450 nm decreases as the amount of caffeine in saliva increases. The level of caffeine in each sample was calculated by interpolation using the standard curve constructed in a concentration interval from 0 to 2.5 µg/ml ( $y = -0,2545x + 1,1895$ ). Saliva samples were analyzed in duplicate.

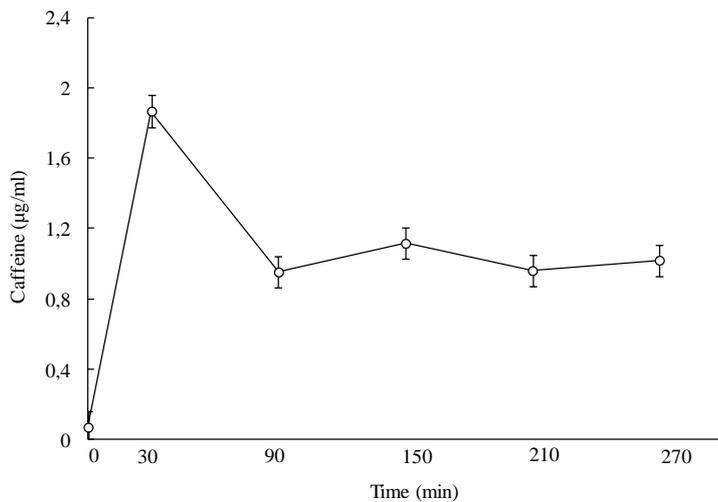
Caffeine content in saliva was monitored for 270 min during the pilot test. The mean absorbance values and the relative caffeine concentrations for one subject are reported as example in Figs 1.a and 1.b. The minimum of absorbance value was reached at 30 min after intake, as previously reported [74], and corresponded to the maximum detectable concentration of caffeine in the adopted experimental conditions. Then, caffeine salivary concentration decreased with metabolism and a sharp absorbance increase was observed after 90 min from the intake. No further absorbance variations were observed between 90 and 270 min possibly because the changes in caffeine concentration were lower than the immune assay sensitivity (0.075 µg/ml) in saliva). Based on these data, saliva was collected in the main study at three sampling times T0, T30 and T90 according to the procedure above and assayed for caffeine content. Three absorbance values were determined:

AbsT0 relevant to the baseline assay response, AbsT30 corresponding to the highest amount of caffeine detected in saliva samples after intake and assumed as the peak of adsorption in the adopted experimental conditions, AbsT90 corresponding to the amount of caffeine metabolized after the adsorption peak at the shortest time to detect the absorbance variation in the adopted experimental conditions.

CPYA12 phenotype was expressed as caffeine metabolism index (CmI). The CmI for each S was calculated as the percentage ratio between the amount of residual caffeine 60 min after the adsorption peak (AbsT90-AbsT30) and the amount of caffeine at the adsorption peak corrected with the base line (AbsT0-AbsT30), as follows:  $(AbsT90 - AbsT30)/(AbsT0 - AbsT30) * 100$ . The higher the CmI value, the higher is the amount of metabolized caffeine in the hour after the adsorption peak.



**Fig 1.a. Absorbance values at 450 nm detected in saliva samples collected at 0, 30, 90, 150, 210, 270 min after caffeine ingestion: mean absorbance values for one subject.**



**Fig 1.b. Caffeine concentrations in saliva samples collected at 0, 30, 90, 150, 210, 270 min after caffeine ingestion: mean values for one subject.** The level of caffeine in each sample was calculated by interpolation using the standard curve constructed in a concentration interval from 0 to 2.5 µg/ml ( $y = -0,2545x + 1,1895$ ).

### CYP1A2 genotyping

Ss' DNA was collected before saliva collection by DNA swabs (DNA Swab - 20 ISO SK-2S – 100 x 1 swab with 2 ml tube and special release cap, individually wrapped and gamma treated, Teltec srl). DNA was then extracted using the QIA *Symphony* (www.qiagen.com) automatic extractor. Finally genotypes for rs762551 were obtained through direct Sanger sequencing. Genotypes for fifty-one Ss were determined.

### Choice between different coffees

Ss were presented with two coffee packs available on the market and were asked to choose which one they wanted to drink. One coffee was dark roasted and labeled with “strong flavor” (in Italian “*flavor intenso*”); the other one was medium roasted and labeled with “balanced flavor” (“*flavor equilibrato*”). These terms were chosen to indicate two different degrees of overall flavor,

in particular bitterness intensity. Ss undertook this test twice: once after 12 h coffee abstinence and once after no restrictions on caffeine intake.

### **Coffee consumption questionnaire**

Data on the Ss' usual mode of coffee consumption (black; with sugar; with milk; 1 "never", 2 "occasionally", 3 "regularly") and frequency (1 "less than once a day", 2 "once a day", 3 "twice or three times a day", 4 "four or five times a day", 5 "more than five times a day") were collected. Intake of other caffeinated beverages was not considered since coffee represents the 91.4% of Italian population daily caffeine intake [75]. Ten statements about perceived effects of caffeinated coffee consumption [76] were rated on 7-point scales with endpoints *strongly disagree* (1) and *strongly agree* (7): 1. Coffee picks me up when I am feeling tired; 2. Coffee improves my athletic performance; 3. Coffee increases my motivation to work; 4. Coffee improves my concentration; 5. Coffee makes me jittery; 6. Coffee at any time of day throws off my sleep; 7. Coffee makes my heart beat irregularly; 8. Consuming coffee late in the day disrupts my sleep; 9. The number of coffees that I consume during the day depends on taken benefit; 10. Coffee makes me feel good.

### **Data analysis**

The effects of gender, smoking and contraceptive use on CmI were independently analyzed by unpaired t-tests; in particular for each factor the others were excluded: gender effect was assessed considering only non-smokers and non-contraceptive users (n=91); smoking effect considering only non-contraceptive users (n=116); contraceptive use consider only women non smokers (n=65) . A linear regression between CmI and coffee consumption was computed. Ss were divided in four groups based on CmI percentile distribution [77] and the coffee consumption of each group was calculated as sum of frequency scores by each S.

Two-way ANOVAs (group and sample as factors), followed by Fisher's LSD test ( $p \leq 0.05$ ) as appropriate, were used to independently test the effect of CPYA12 phenotype (CmI) on liking and intensity ratings for both coffee samples and bitter solutions. The associations of clusters with gender, coffee consumption frequency, consumption of sweetened/unsweetened coffee and choice of different coffees (categorical variables) were estimated by a homogeneity chi-square test. The effect of cluster on perceived effect of caffeinated coffee consumption was estimated by a two-way ANOVA. The effect of genotype group on CmI was estimated by a one-way ANOVA.

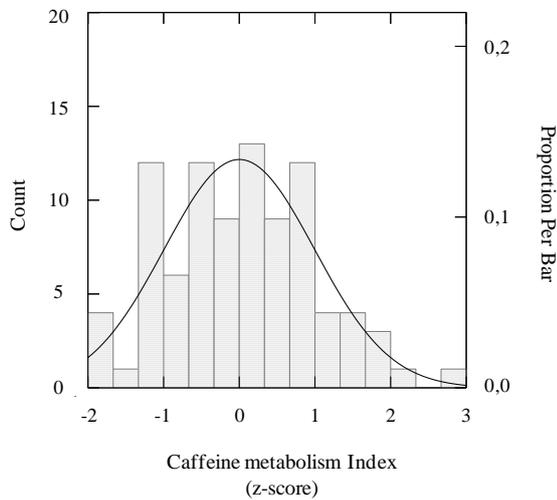
## Results

### *Caffeine metabolism Index*

A significant effect of gender on CmI was found ( $t_{89;1.98}=2.60$ ,  $p=0.011$ ) reflecting the higher CmI of men than of women (40.21 vs 28.10). Smoking significantly increased the mean value of CmI (46.92 vs 33.83;  $t_{114;1.98}=-2.59$ ,  $p=0.011$ ), while there was no significant effect of oral contraceptive use (20.52 vs 28.10;  $t_{63;2.00}=1.39$ ,  $p=0.170$ ). Because of the effect of smoking and the somewhat lower mean for oral contraceptive users, non-smokers and non-contraceptive users ( $n=91$ ) were selected for further analysis.

Because of the gender effect, z-scores were calculated for both male and female distributions. Ss were divided into groups based on the median value of the CmI z-score distribution (0.0056; range: from -1.7109 to 2.8752) (Fig 2): Low CmI (LCmI;  $n=45$ ; 20 males and 25 females) and High CmI (HCmI;  $n=45$ ; 22 males and 23 females) (-0.8137 vs 0.8136;  $t_{88;1.99}=-13.35$ ,  $p \leq 0.0001$ ). The subject with the CmI corresponding to the median value of the CmI z-score distribution (0.0056) was excluded from the further analysis. Thus the following results are based on data from ninety Ss. LCmI Ss were considered as slow metabolizers; on the other hand, HCmI were considered as fast metabolizers. No significant differences between these groups were found for gender ( $\chi^2=0.18$ ,  $df=1$ ,  $p=0.673$ ), age (28.50 vs 31.50;  $t_{88;1.99}=-1.40$ ,  $p=0.167$ ), FP numbers (12.99 vs 12.29;  $t_{88;1.99}=-$

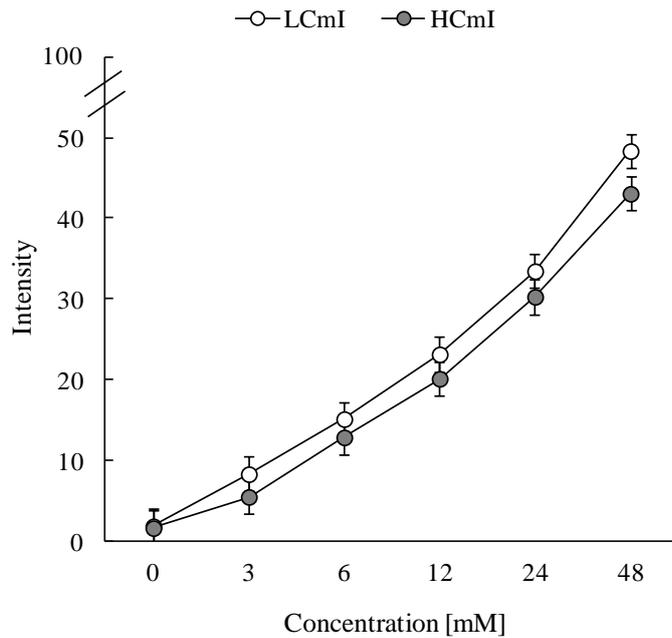
0.96,  $p=0.340$ ), PROP intensity scores (47.75 vs 43.98;  $t_{88;1.99}=-0.67$ ,  $p=0.505$ ) and Body Mass Index (22.82 vs 23.43;  $t_{88;1.99}=-0.77$ ,  $p=0.444$ ).



**Fig 2. Distribution of caffeine metabolism index z-scores.**

#### *Caffeine metabolism and bitterness perception*

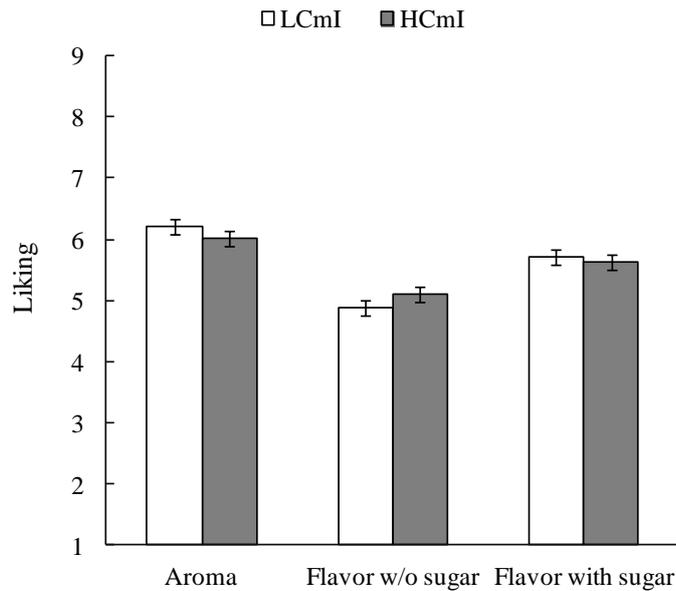
LCmI Ss rated the bitterness of caffeine solutions as stronger than did HCmI Ss ( $F_{1;528}=5.21$ ,  $p=0.023$ ) (Fig 3), but there were no group differences for quinine-HCl solutions. No significant group\*sample interactions were found. Coffee bitterness was rated as stronger by LCmI Ss than by HCmI Ss (28.94 vs 25.67;  $F_{1;528}=5.88$ ,  $p=0.016$ ). No significant group\*(coffee) sample interaction was found, and there were no significant effects of group on ratings of sourness and astringency in coffee.



**Fig 3. Mean bitterness ratings for each CmI group (LCmI and HCmI) as a function of variations in caffeine concentration.**

#### *Caffeine metabolism on liking*

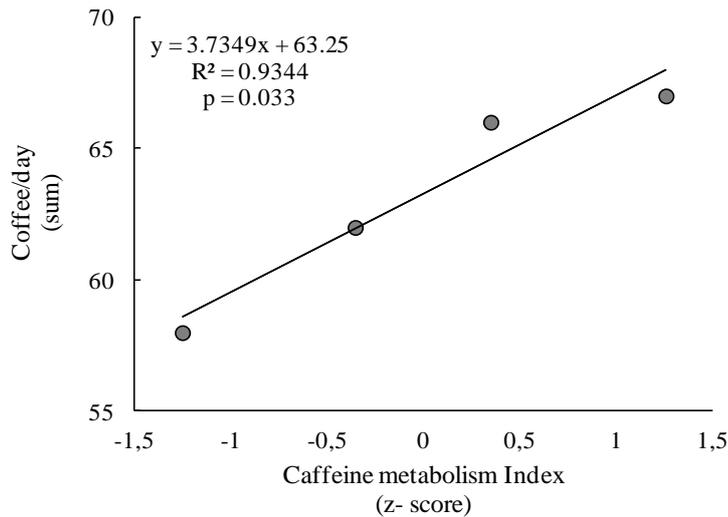
While coffee liking was unrelated to CmI group (Fig 4), LCmI Ss nevertheless added more sugar to the coffee than did HCmI Ss (1.90 g vs 1.10 g;  $F_{1,528}=23.87$ ,  $p \leq 0.0001$ ). Consistent with this, different sugar use by LCmI and HCmI Ss was also evident in the consumption data. Thus, only 11% of LCmI Ss, as compared to 21% of HCmI Ss, consumed coffee regularly without sugar ( $\chi^2=4.85$ ,  $df=1$ ,  $p=0.028$ ).



**Fig 4. Effect of CmI cluster (LCmI and HCmI) on liking for aroma, flavor without sugar and flavor with sugar: mean ratings and standard error values.**

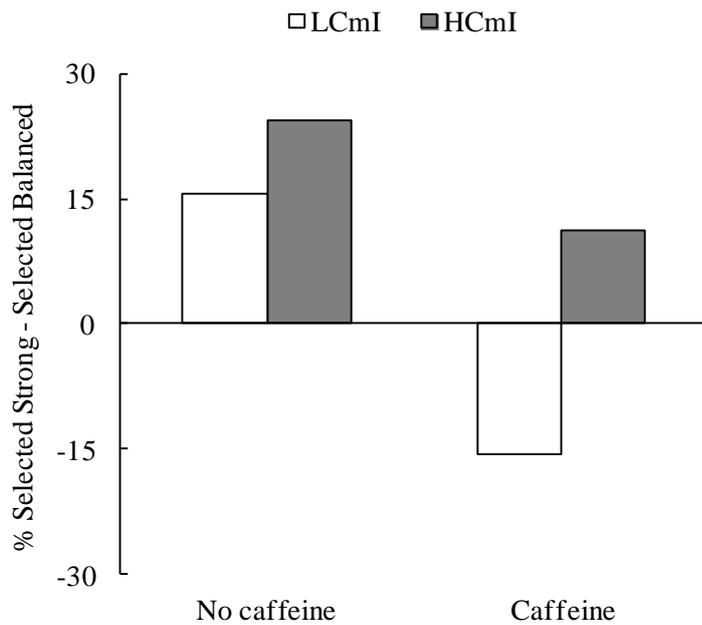
*Caffeine metabolism, coffee consumption and choice, and perceived effects of caffeine*

A significant positive relationship between CmI and the frequency of daily coffee consumption was found ( $r^2=0.93$ ,  $p=0.033$ ) (Fig 5), with HCmI Ss consuming more coffees daily than LCmI Ss.



**Fig 5. Relationship between CmI and coffee consumption.** Ss were divided in four groups based on percentile distribution of CmI z-scores. The coffee consumption was calculated as sum of frequency scores by each S.

In choosing a coffee based on information (“strong” or “balanced” flavor) , no significant differences were found between HCmI and LCmI either after 12 h abstinence from caffeine ( $\chi^2=0.185$ ,  $df=1$ ,  $p=0.667$ ) and after no restrictions on caffeine intake before the test ( $\chi^2=1.601$ ,  $df=1$ ,  $p=0.206$ ). In both groups, after 12 h abstinence from caffeine, the majority of Ss chose the “strong” product most often (57.78% of LCmI Ss; 62.22% of HCmI Ss). The comparison within each Ss group showed that the HCmI Ss chose the “strong” coffee both after 12 h abstinence from caffeine and after no restrictions on caffeine intake ( $\chi^2 =0.413$ ,  $df=1$ ,  $p=0.520$ ). On the other hand, the percentage of LCmI Ss choosing the “strong” coffee after 12 h abstinence tended to be higher than the percentage after no restrictions ( $\chi^2 =2.18$ ,  $df=1$ ,  $p=0.140$ ) (Fig 6).

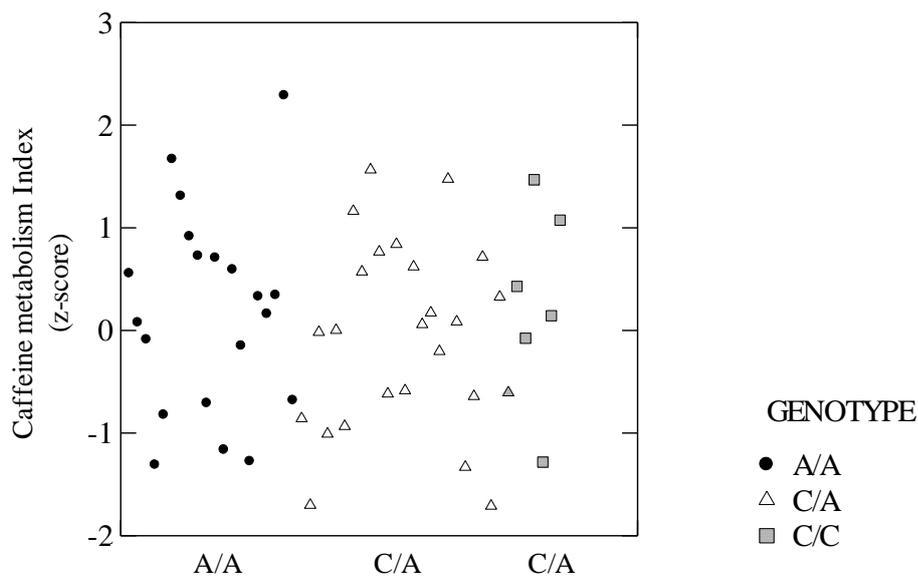


**Fig 6. Effect of caffeine abstinence (no caffeine)/free consumption (caffeine) condition on coffee choice.** For each group on each occasion the difference between the percentage of Ss who chose “strong” coffee and the percentage of Ss who chose “balanced” coffee was computed.

No significant differences between CmI groups in response to questions on the perceived effects of caffeinated coffee consumption were found.

#### *Relationship between caffeine metabolism phenotype and genotype*

The Ss were distributed in the three genotype classes as follows: 20 Ss A/A, 25 Ss C/A, 6 Ss C/C. The CmI of each S in each genotype class is reported in Fig 7. No significant effect of genotype class on CmI was found ( $F_{2,48}=0.571$ ,  $p=0.569$ ).



**Fig 7. Caffeine metabolism Index distribution in each genotype class.**

The z-score of CmI is reported for each subject of each genotype class (A/A, C/A, C/C).

## Discussion

Consistent with the reported positive relation between caffeine consumption and metabolism rate, the present study found a strong linear relationship between the caffeine metabolism index and the frequency of daily coffee consumption. Coffee represents more than the 90% of daily caffeine intake [75] for Italian population and a stimulatory effect on CYP1A2 from other secondary coffee components, such as polycyclic aromatic hydrocarbons, was hypothesized [81-82]. Possible confounding factors influencing CYP1A2 activity were taken into account by instructing Ss participating in the study to avoid sources of possible enhancers or inhibitors of enzyme activity before and during phenotyping procedure [58]. Furthermore, the relation between frequency of coffee consumption and CmI value was computed excluding smokers and oral contraceptive users and the gender confounding effect on CYP1A2 activity considered by data standardization. Thus, in the adopted experimental conditions, it seems possible to consider coffee as the major factor affecting CP1A2 activity.

We assessed individual variability in caffeine metabolism using a rapid determination of current CYP1A2 activity that required only three saliva samples. The observed trend for caffeine adsorption and metabolism is consistent with previous data showing the pharmacokinetics of caffeine both in plasma and saliva [27, 29, 74, 78]. Moreover, the derived index appears to be a valid measure of individual variability in caffeine metabolism, in that it was affected by factors already known to modify CYP1A2 activity such as gender, smoking, and oral contraceptive use [58, for a review]. As previously reported, no effects of age and BMI on caffeine metabolism rate were found [79-80].

Different coffee consumption behaviors appear related to caffeine metabolism rate. Fast and slow metabolizers tended to differ in their choice of coffee types based on information, although the significance level of the difference was lower than 95%. In particular, fast metabolizers favor a coffee – here labeled as “strong” – associated with greater stimulatory effects. In contrast, those Ss with the lower caffeine metabolism index chose the “strong” coffee only after 12 hours abstinence from caffeine. This inversion of the choice suggests that the Ss used the information related to flavor intensity as a predictor of its likely effect on the body and is consistent with findings that the effects of caffeine are only clearly reinforcing following such a period of abstinence [83]. However, no differences between the two groups in perceived effects of caffeine consumption, either alerting or anxiogenic, were found. It is plausible that, in regular coffee consumers, different caffeine metabolism rates are reflected in different amounts of consumed coffee to achieve the same desired stimulatory effect, but confirming this requires further research.

The relationship found between caffeine bitterness and metabolism is important because of the evidence that it influences how coffee was consumed by these Ss. Thus, Ss with lower caffeine metabolism rate were more sensitive to bitterness of both caffeine and coffee and also added more

sugar to coffee. Crucially, slow and fast metabolizers only differed in their responses to caffeine bitterness, and not to the equi-intense bitterness of quinine-HCl, or to other indices of general taste intensity, PROP status and FP number. Caffeine has a post-ingestive stimulatory effect, while, as far as is known, neither quinine-HCl nor PROP have any noticeable post-ingestive effects. Thus, since the relationship between caffeine metabolism and bitterness sensitivity is exclusive to caffeine and independent from the sensitivity to other bitter compounds, it seems to be mediated exclusively by the psychoactive effect. We argue, therefore, that slow metabolizers added more sugar to coffee to overcome aversion to bitterness and to benefit from the stimulatory effects of caffeine. Thus, the use of sweeteners in coffee seems to be related to bitterness sensitivity to caffeine and coffee frequency consumption, which is in turn associated to the caffeine metabolism rate.

No significant association was found between the caffeine genotypes and metabolism rates. This failure might be related to several factors. First, this study used a relatively small group of subjects who were selected to estimate the effect of genotype group on caffeine metabolism index. Furthermore, no SNP or haplotype in the CYP1A2 gene has yet been identified that might unequivocally be used to predict the metabolic phenotype in any individual. Finally, in the case of CYP1A2, apart from genetic factors, a great deal of individual variability relate to several environmental and endogenous factors.

These data underline the interplay between caffeine metabolism rate, caffeine bitterness perception and coffee-consumption behaviors. For the first time, a relationship between caffeine metabolism rate and bitterness sensitivity to caffeine was demonstrated. Moreover, the effects of this link on coffee perception and consumption were highlighted. The importance of this study lies in the finding that responses to bitterness in an ingested beverage – and hence potentially other taste qualities – rely not only on taste receptor genes, but also on the psychoactive or post-ingestive properties of the taste compound. While this has been evident in relation to food and beverage

preference development, the current study shows that this factor remains important in determining ongoing preferences and intake. Moreover, this study suggests further important investigations on responses to the post-ingestive effects of caffeine and on the possible relation between individual differences in acquired preferences and variations in caffeine metabolism rate.

## **Acknowledgements**

This work was completed as part of CM's PhD requirements at University of Florence. The authors wish to thank Luciano Navarini and Massimo Barnabà (illycaffè.p.a.) for cooperation. The analysis of saliva samples was conducted at the Department of Health Sciences (University of Florence), and the authors wish to thank the team, and in particular Roberta Felici, for their assistance and access to the equipment. The authors thank Martina La Bianca for the genetic analysis (Maternal and Child, Health - IRCCS "Burlo Garofolo", Trieste, Italy).

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