TITLE: Effect of Ultra High Temperature (UHT) treatment on coffee brew stability

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ABSTRACT

In this work, the influence of an Ultra High Temperature (UHT) treatment on chemical and sensory composition of Arabica coffee brews for a longer shelf-life has been studied. A temperature of 120 ºC for 2 s allows to obtain a microbiologically safe coffee brew, good valued from the sensory point of view. The behavior of the UHT vs non UHT treated coffee brew was followed throughout 120 days of storage at 4 ºC. The UHT treatment keeps longer the typical acidity of the brews, delaying and softening the pH decrease and the development of sourness, which is one of the main causes for the rejection of stored coffee brews. The UHT treatment hardly affects the concentrations of caffeine and trigonelline, and of some phenolic compounds such as 5-caffeoylquinic (5-CQA), caffeic or ferulic acids. Sixteen key odorants and staling volatiles were analyzed by HS-GC-MS and lower changes were observed in the UHT treated coffee brew throughout storage. Higher DPPH’ scavenging activity was observed in the UHT treated coffee brew from days 60 to 120. In conclusion, the application of an UHT treatment is proposed to extend the shelf-life (up to 60 days) of stored coffee brews.

KEYWORDS: coffee, UHT treatment, volatiles, antioxidant, phenolic compounds, storage
1. INTRODUCTION

Coffee is one of the most consumed drinks in the world (Petracco, 2001). In some countries, such as Japan or Taiwan, canned or packed coffee beverages are very popular (Yamada, Komatsu, & Shirasu, 1997), probably due to the lack of a traditional image of coffee as a freshly brewed beverage. In fact, there is a great variety of packed coffee drinks, such as cappuccino, latte, mocha, regular with milk and sugar, plain black, etc. In western countries, although this type of coffee drinks are not so accepted, they appear as alternative products, able to play functions different from and complementary to the traditional coffee brews. Thus, ready-to-drink coffee beverages have reached a great acceptance among certain populations because they are inexpensive and storable, providing affordable alternatives to freshly brewed coffee. However, ready-to-drink coffee brews present the disadvantage that their sensory properties are often lower than those of the freshly prepared ones. The deterioration of coffee brews during storage is generally accompanied by acidity development, detectable by a pH decrease, even at refrigeration temperatures (Nicoli, Severini, Dalla Rosa, & Lerici, 1991). In fact, pH has been used by some authors to establish the shelf-life of stored coffee beverages (Dalla Rosa, Barbanti, & Nicoli, 1986), even though other studies have shown that this factor is not enough to predict the shelf-life of coffee brews stored for a period of time (Pérez-Martínez, Sopelana, de Peña, & Cid, 2008a). In order to avoid or, at least, minimize, the increase of acidity of coffee brews during storage, some strategies have been used. Thus, in an attempt to stabilize and extend the shelf-life of coffee beverages, coffee industry has applied sterilization and pasteurization techniques traditionally used for other food products such as milk or juices. However, these thermal treatments modify the aromas and flavors of coffee beverages during storage (Yamada et al., 1997; Kumazawa, & Masuda, 2003), what affects negatively their sensory characteristics. Severini, Nicoli, Romani, & Pinnavaia, (1995) employed both high pressures and pasteurization to
extend the shelf-life of coffee brews but, although they achieved the microbiological stability, the treatments applied did not help the chemical stability of the product.

In view of all mentioned before, it would be interesting to obtain a pure coffee brew able to be maintained throughout time and consumed at any occasion, preserving its hygienic and sensory characteristics as much as possible. For this reason, in this work, the influence of an ultra high temperature (UHT) treatment, which is a thermal treatment shorter than pasteurization or sterilization, on certain physico-chemical and sensory parameters of freshly prepared Arabica coffee brews has been studied. Moreover, in order to know the behavior of the chemical and sensory composition of the UHT vs non UHT treated coffee brew during storage, all the changes were monitored. For this purpose, Arabica coffee brews were bottled without headspace and stored at 4 °C. Microbiological, physico-chemical and sensory analyses were performed.

2. MATERIALS AND METHODS

2.1. Coffee. Vacuum-packed Colombian Arabica ground roasted coffee (2.25% water content, L* 19.57±0.09) was provided by a local factory.

2.2. Chemicals and reagents. The methanol used was of spectrophotometric grade from Panreac (Barcelona, Spain). Acetonitrile, supragradient HPLC grade, was provided by Scharlau (Barcelona, Spain). Pure reference standards of caffeine, trigonelline, pentoxyfilline, 5-caffeoylquinic acid, caffeic acid, ferulic acid, 4-vinylguaiacol, 2,2-diphenyl-1-picrylhydrazyl (DPPH·), propanal, hexanal, 2-ethyl-6-methylpyrazine and acetic acid were obtained from Sigma-Aldrich (Steinheim, Germany); acetaldehyde, 2-methylpropanal, 2-methylbutanal, 3-methylbutanal, 2-propanone, 2-butanone, 2,3-butanedione, 2,3-pentanedione, 2-ethylpyrazine, 2-ethyl-3,5-dimethylpyrazine and guaiacol (2-methoxyphenol) were purchased from Acros Organics (Springfield, New Jersey, USA).
2.3. **Coffee brew samples.** The ground coffee packages were opened immediately before the preparation of the coffee brew to avoid aroma losses. Coffee brews were prepared from 90 g of ground roasted coffee for a water volume of 1 L, using French press coffeemakers. Extraction time was 3 min and water temperature 90±2 ºC (pH=7.0). The freshly prepared coffee brews were immediately filtered, poured into sterilized glass flasks and hermetically closed without headspace; these were considered as reference coffee brews. The UHT treated coffee brews were subjected to the UHT treatment before being bottled. For both types of sample, the flasks were filled up to the top (330 mL) in a laminar flow cabin, to assure aseptic conditions and avoid the microbiological contamination of the samples. Afterwards, coffee brews were stored at 4 ºC until their analysis.

2.4. **UHT treatment.** This was carried out with an FT74X UHT/HTST system, provided with a plate heat exchanger and a recirculating glycol chiller FT63 (Armfield Ltd, Ringwood, England). The temperatures tested were 90, 100, 105, 110 and 120 ºC for 2 s.

2.5. **Microbiological analysis.** Aerobic mesophilic flora was analyzed by colony count technique at 30 ºC in Plate Count Agar (Biolife, Milano, Italy) (ISO 4833:2003). Enumeration of molds and yeasts was made by colony count technique at 25 ºC in oxytetracycline-Glucose Yeast extract Agar (Oxoid, Basingstoke, England) (ISO 7954:1987). Mesophilic aerobic sporulates were also analyzed by colony count technique at 37 ºC after heating at 80 ºC for 10 min. These analyses were performed monthly.

2.6. **pH.** The measure was obtained with a Crison Basic 20 pH-meter.

2.7. **Caffeine and Trigonelline.** Extract preparation, clean-up and HPLC analysis were performed following the method described by Maeztu, Sanz, Andueza, de Peña, Bello, & Cid, (2001a). HPLC analysis was achieved with an analytical HPLC unit model 1100 (Agilent Technologies, Palo Alto, CA, USA), equipped with a binary pump and an automated sample injector. A reversed-phase Hypersil-ODS (5 μm particle size, 250 x 4.6 mm) column was
used. The mobile phase was acetonitrile/water (15:85, v/v) in isocratic conditions at a constant flow rate of 2.0 mL/min at 36 °C. Detection was accomplished with a diode-array detector, and chromatograms were recorded at 280 nm.

**2.8. 5-Caffeoylquinic acid (5-CQA).** 500 μL of the coffee brew were diluted up to 50 mL with milliQ water. 5-CQA HPLC analysis was carried out with the same equipment described above. The chromatographic separation was performed using a gradient of acetonitrile and Milli-Q water starting with 12% acetonitrile at 1 mL/min flow for 5 min, then 7.5% acetonitrile at 1.6 mL/min for 5 min, then 8.0% acetonitrile at 1.6 mL/min for 5 min, then 25% acetonitrile at 1.6 mL/min for 5 min more and, finally, to return to initial conditions (12% acetonitrile) at 1.1 mL/min in 5 min. Wavelength of detection was 325 nm.

**2.9. Caffeic acid, Ferulic acid and 4-Vinylguaiacol.** The extraction, clean-up and HPLC analysis of these three compounds were performed simultaneously, according to the method developed by Álvarez-Vidaurre, Pérez-Martínez, de Peña, & Cid, (2005). The HPLC analysis was carried out with the same equipment described above. The chromatographic separation was achieved at 25 °C by using a complex gradient solvent system with acetonitrile/water adjusted to pH 2.5 with a phosphoric acid solution. The wavelengths of detection were 314±4 nm (reference wavelength 260±4 nm) for caffeic acid, 325±10 nm (reference wavelength 450±10 nm) for ferulic acid and 210±8 nm for 4-vinylguaiacol. The absorbance of the rest of the chromatogram was not registered in order not to interfere visually.

**2.10. Volatile compound analysis.** The profiles of volatile compounds were obtained with the method described by Sanz, Ansorena, Bello, & Cid (2001), adapted to coffee brew by Maeztu et al. (2001a), and using Static Headspace-Gas Chromatography-Mass Spectrometry (SH-GC-MS).

After the flask was opened, 6 mL of a homogenized coffee brew were introduced into a 10 mL vial, which was immediately sealed with a silicone rubber Teflon cap. Each vial was
equilibrated at 40 °C for 60 min in the static headspace sampler (model 7694, Agilent Technologies). Each vial was pressurized with carrier gas for 12 s, and 3 mL of the coffee headspace sample were injected into an HP-Wax glass capillary column (60 m x 0.25 mm x 0.5 μm film thickness) in an HP 6890 gas chromatograph (Agilent Technologies). Injector temperature was 180 °C, and carrier gas was Helium (1 mL/min linear speed). The oven temperature was maintained at 40 °C for 6 min and then raised at 3 °C/min up to 190 °C. Mass spectrometry analysis was performed with a mass selective detector model 5973 (Agilent Technologies) operating in the electron impact ionization mode (70 eV), with a scan range of 33-300 amu. The ion source temperature was set at 230 °C.

Identification and quantification of the volatile compounds. The volatile compounds were identified by comparing their mass spectra with those of the pure reference compounds and the Wiley library and, in addition, by comparison of their Kovats indices and retention times with those of standard compounds. The Kovats indices were calculated according to the method of Tranchant (1982). Peak areas were measured by calculation of each volatile total area based on integration of a single ion. The quantification ion (QI) of each volatile compound is given in Table 1.

2.11. Antioxidant Capacity. The antioxidant capacity was measured by using the DPPH\(^-\) (2,2-diphenyl-1-picrylhydrazyl) decolorization assay (Brand-Williams, Cuvelier, & Berset, 1995). A 6.1 x10^{-5} M DPPH\(^-\) methanol solution was prepared immediately before use. The DPPH\(^-\) solution was adjusted with methanol to an absorbance of 0.7 (± 0.02) at 515 nm in a 3 mL capacity cuvette (1 cm length) with a spectrophotometer Lambda 25 UV-VIS (Perkin-Elmer Instruments, Madrid, Spain) connected to a thermostatically controlled chamber (25 °C) and equipped with UV WinLab software (Perkin Elmer). Coffee brews were diluted 1:50 in water prior to analysis. Samples (20 μL) were added to the DPPH\(^-\) solution (1.98 mL). After mixing, the absorbance was measured at 515 nm after exactly 1 min, and then every
minute for 18 min. Reaction rates were calculated using the equation proposed by Manzocco, Anese, & Nicoli (1998).

\[
1/Abs^3 - 1/Abs_o^3 = -3kt
\]

where \( k \) is the DPPH· bleaching rate, \( Abs_o^3 \) is the initial absorbance value, and \( Abs^3 \) is the absorbance at increasing time, \( t \). The antioxidant capacity was expressed as the slope obtained from the equation \((-Abs^{-3} \text{ min}^{-1})\) per mL of sample.

2.12. Redox Potential. The redox potential measurements of the coffee brews were made with a platinum-indicating electrode connected to a voltmeter (mod. 5261, Crison, Spain). Calibration was performed against 220 and 468 mV redox standard solutions at room temperature (Crison, Spain). Electrodes were placed in a 50 mL 3-neck flask containing a volume of 16 mL of coffee brew together with 20 mL of milliQ water. Prior to analysis, oxygen was removed from the system by continuous nitrogen flushing for a period of 15 min. Millivolt values were recorded for at least 10 min at room temperature, until a stable potential was reached. A stable redox potential was arbitrarily defined as a change of less than 1 mV in a 3 min period.

2.13. Browned Compounds (Abs 420 nm). 50 μL of coffee brew were diluted up to 2 mL with milliQ water. Browned compounds were measured by measuring the absorbance of the sample at 420 nm after exactly 1 min, in a 3 mL capacity glass cuvette (1 cm length) at 25 ºC (Lambda 25 UV-VIS spectrophotometer, Perkin-Elmer Instruments, Madrid, Spain). This measurement is employed as a convenient index of development of caramelization and Maillard reactions.

2.14. Color Analysis. Color analysis was carried out using a tristimulus colorimeter (Chromameter-2 CR-200, Minolta, Osaka, Japan). The instrument was standardized against a white tile before sample measurements. Color was expressed in \( L^* \), \( a^* \) and \( b^* \) CIELab scale parameters.
2.15. Sensory Descriptive Analysis. Twenty judges were recruited among members of the Nutrition, Food Science, Physiology, and Toxicology Department at the University of Navarra. Selection and training were carried out as described by Maeztu et al. (2001a); Maeztu, Sanz, Andueza, de Peña, Bello, & Cid (2001b); and Pérez-Martínez, Sopelana, de Peña, & Cid (2008b) to have a 10-member panel. Both aroma and taste/flavor attributes were evaluated. The aroma parameters selected were intensity, freshness, rancid/old, burnt and spicy. Bitterness, acidity, sourness, astringency, persistence, aftertaste, spiciness, rancidity and burnt flavor were included in the group of taste/flavor attributes. All of them were rated on 11-point scales from “none” (0) to “very high” (10).

Each stored coffee brew sample was heated in a microwave oven at 90±2 °C immediately before tasting and served monadically in a white porcelain coffee cup. The order of presentation was randomized among sessions. A freshly prepared filtered coffee brew was evaluated at first place, as a reference and in order to avoid first impression. All evaluations were conducted in isolated sensory booths illuminated with white light in the sensory laboratory under standardized conditions by UNE 87-004-79 (AENOR, 1997). Rinse water was provided between samples. After the individual evaluation of each sample, results were discussed and established by panel consensus.

2.16. Statistical analysis. All the analyses were performed in triplicate. Results are shown as means ± standard deviations. t-Student test was used to know whether there were differences between the values obtained for each parameter from the reference and the UHT treated coffee brews in each point of the storage time. One-way analysis of variance (ANOVA) was applied both to the results obtained from the coffee brews subjected to different UHT treatments and to those obtained from the analysis of the reference and UHT treated coffee brews throughout time, being time the source of variation. T-Tukey-b was applied as the test a posteriori with a level of significance of 95%. Correlations among
variables were assessed by means of the Pearson’s correlation test. All statistical analyses were performed using the SPSS v.11.0 software package.

3. RESULTS AND DISCUSSION

3.1. Selection of the optimal UHT temperature in coffee brews.

Five temperatures (90, 100, 105, 110 and 120 ºC) during 2 s were applied to coffee brews in an UHT equipment. The selection of the optimal UHT temperature was based on sensory and microbiological analyses in order to obtain coffee brews with similar sensory quality than the reference coffee brew (without UHT treatment) and safe from the microbiological point of view. Sensory analysis (data not shown) revealed lower aroma intensity and freshness in most of the UHT treated coffee brews (scores of 6-7) in comparison with the reference (score of 8), being less pronounced in those subjected to 110 and 120 ºC (score of 7). Neither rancid nor burnt and spicy aroma notes were perceived in the studied coffee brews. In relation to taste/flavor attributes, slight astringency and aftertaste flavors (scores of 1-2) have been detected in most of the UHT treated coffee brews, but not in the reference coffee brew. Moreover, lower taste/flavor persistence (score of 4 vs 6 in reference) has been observed in all UHT treated coffee brews, except at 120ºC which showed a similar score to that of the coffee brew without UHT treatment (reference). Sour, rancid, spicy and burnt flavors were not perceived in any of the tested coffee brews. In conclusion, those coffee brews UHT-treated at 110 and 120 ºC were the best sensorially valued because maintained similar characteristics to those of the reference coffee brew (without UHT treatment).

The microbiological analysis of the reference coffee brew revealed a colony count number of \(8.5 \times 10^2\) cfu/mL for mesophilic flora. So that, the effectiveness of the UHT treatment as a sterilization technique to assure the safety of UHT-treated coffee brews for the subsequent long-term storage has been checked by microbiological analysis. The lowest temperature
applied (110°C), among the best sensory valued ones (110 and 120 °C), was high enough to remove microorganisms initially present in coffee brew, because there was no growth of microorganisms. Consequently, UHT treatment applied to coffee brew at least 110°C during 2s assures that bottled coffee brews were safe to be consumed.

According to the results of several studies (Yamada et al., 1997; Schrader, Kiehne, Engelhardt, & Maier, 1996), the use of high temperatures can cause chemical reactions in coffee brews, such as decomposition of chlorogenic acids, with the release of quinic, ferulic and caffeic acids. This can lead to an increase in acid, bitter and/or astringent tastes (Clifford, 1985; Lingle, 1996), and to a quicker deterioration of coffee brews during further long-term storage. For this reason, the influence of the UHT treatment on some chemical parameters (caffeine, trigonelline, 5-CQA, caffeic acid, ferulic acid and 4-vinylguaiacol) in the coffee brews subjected to 110°C and 120°C was also studied. It was observed that, for most of the compounds studied, there were no significantly differences between the reference coffee brew (without UHT treatment) and those subjected to UHT treatment. Therefore, although neither of the two UHT treatments applied seems to affect the most relevant chemical compounds of coffee brews, the coffee brew subjected to 120 °C was selected for the subsequent long-term storage study because it achieved better scores in some sensory attributes like taste/flavor persistence.

3.2. Study of the influence of the UHT treatment on the coffee brews during storage.

This study was performed with coffee brews subjected to an UHT treatment (120°C-2 s), aseptically bottled without headspace and stored at 4°C for 120 days. Taking into account that the acidity development seems to be one of the main factors involved in the quality loss of stored coffee brews (Nicoli et al., 1991; Dalla Rosa et al., 1986), the pH of the coffee brews was measured throughout all the long-term study (Figure 1). It must be noticed that the
application of the UHT treatment did not affect the initial pH of the coffee brew. pH decreased significantly in both the reference and the UHT treated coffee brews until day 90, but this fall was less pronounced in the latter. Moreover, stronger differences between the reference and the UHT treated coffee brews pH were found between days 30 and 90.

Caffeine and trigonelline that are compounds typically related to the bitterness of coffee (Macrae, 1985), exhibited only minor changes throughout the storage time in the two studied coffee brews (Figure 2). In fact, significant differences were hardly observed either throughout time or between the reference and the UHT treated coffee brews. These results are in agreement with those obtained in a previous work (Pérez-Martínez et al., 2008a).

The changes in 5-CQA, caffeic acid, ferulic acid and 4-vinylguaiacol throughout the storage of coffee brews can be observed in Figure 3. These compounds are related to sensory attributes of coffee brews, such as acidity, bitterness or astringency, whose intensification is associated with a loss of quality. The concentrations of these compounds were very similar in the reference and in the UHT treated coffee brews showing small, and non significant in most of them, changes throughout the storage time. However, a significant increase in the concentration of 5-CQA was observed from days 90 to 120. This could be due to the hydrolysis of chlorogenic acid lactones formed during the roasting of coffee (Maier, Engelhardt, & Scholze, 1984), or to the release of CQAs from non-covalently linked polymeric skeletons, such as melanoidins (Delgado-Andrade, & Morales, 2005; Bekedam, Schols, Van Boekel, & Smit, 2008). Moreover, chlorogenic acids were stable during storage at 4°C because no increases in their degradation products, caffeic and ferulic acids, were observed. Although the amounts of ferulic acid was maintained with little changes and after 60 days decreased, its main degradation product, 4-vinylguaiacol, which influences unfavorably coffee flavor (Yamada et al., 1997), also decreased significantly at day 15, to increase afterwards and significantly decreased again. These changes in 4-vinylguaiacol could
be due to the balance between its formation by ferulic acid degradation, the release of ferulic acid linked to melanoidins, and its oxidation into vanillin and vanillic acid. All these results are in agreement with those obtained in a previous study (Pérez-Martínez et al., 2008a).

Coffee aroma, due to the volatile compounds released during the roasting and brewing processes, is one of the most appreciated characteristics of coffee. However, not all the volatile compounds present in coffee contribute to its aromatic quality. Compounds considered as coffee aroma impact compounds by different authors (Holscher, Vitzthum, & Steinhart, 1990; Blank, Sen, & Grosch, 1991; Blank, Sen, & Grosch, 1992; Semmelroch, & Grosch, 1995; Semmelroch, & Grosch, 1996; Sanz, Czerny, Cid, & Schieberle, 2002; López-Galilea, Fournier, Cid, & Guichard, 2006) were studied. The changes of these compounds in reference and UHT treated coffee brews throughout storage is shown in Table 1. Six aldehydes, 4 ketones and 1 acid were identified and quantified in the headspace of the coffee brews. Methanethiol, a sulphur compound related to aroma freshness in ground roasted coffee (Holscher, & Steinhart, 1992) and espresso coffee (Maeztu et al., 2001a) was found. Nor guaiacol (2-methoxyphenol), responsible for phenolic, spicy and burnt aromas and flavors (Blank et al., 1991; Semmelroch, & Grosch, 1995), was present at detectable levels in the studied coffee brews. Pyrazines associated with roasty and earthy/musty flavors in ground roasted and coffee brews (Holscher et al., 1990; Blank et al., 1991), and with flowery and fruity notes of coffee brews for 2-ethyl-6-methylpyrazine (López-Galilea et al., 2006), were not detected either.

Some authors (Kumazawa, & Masuda, 2003) have reported that heat processing affects the aroma of coffee drinks. However, in this work, at the beginning of the study significant lower areas in UHT treated coffee brews were only found for 2-butanone and 2,3-butanedione. For the other identified aromatic compounds, no significant differences between the reference and the UHT treated coffee brews were observed. This could be because UHT treatment (120°C
for 2 s) in this study was much shorter than the sterilization process applied by the mentioned authors (121°C for 10 min).

With regard to the changes of the identified aromatic compounds throughout the coffee brews storage, Table 1 shows that, except for hexanal, the area of the aldehydes decreased significantly up to 15 days in both the reference and the UHT-treated coffee brews. Afterwards, they increased during the next 15 days to remain quite constant in the UHT-treated coffee brew up to the end of the storage where the aldehydes areas were significantly lower than the initial ones in most of them. However, in the reference coffee brew, at 90 days a significant decrease in aldehydes areas was observed, to increase again at the end of the study, reaching slightly higher values than the initial ones for acetaldehyde and propanal, and very similar for Strecker aldehydes (2-methylpropanal, 2-methylbutanal and 3-methylbutanal).

Hexanal, an odor impact compound related to rancid flavor development in ground roasted coffee (Vila, de Peña, & Cid, 2005) due to lipid oxidation, showed only little changes, remaining at low levels, in both the reference and the UHT-treated coffee brews throughout the overall storage time. Also low areas in this volatile compound during coffee brew storage have been reported in a previous work (Pérez-Martínez et al., 2008b) maybe because the low amount of fat in coffee brews.

The changes in the area of ketones were very similar to those of the aldehydes, showing a quick and significant decrease during the first 15 days in the two studied coffee brews followed by a significant increase from days 15 to 30. Afterwards, the area of 2-propanone and 2-butanone tended to increase until the end of the storage time, reaching values significantly higher than the initial ones. In contrast, 2,3-butanedione and 2,3-pentanedione, key odorants responsible for the buttery flavors in espresso and other coffee brews (Maeztu et al., 2001a; Blank et al., 1991; Semmelroch, & Grosch, 1996), showed a decrease with storage.
time. This fact could be related to the aroma freshness loss in coffee brews after the first 8 days of storage. In fact, a very significant ($p<0.01$) positive correlation (0.612) was found between aroma freshness and the amount of 2,3-pentanedione. Degradation of these two diketones has also been observed during the storage of roasted coffee (Holscher, & Steinhart, 1992; Kallio, Leino, Koullias, Kallio, & Kaitaranta, 1990; Czerny, & Schieberle, 2001) and Arabica coffee brews (Pérez-Martínez et al., 2008b).

The initial levels of acetic acid in the UHT treated and the reference coffee brews (see day 0 in Table 1) were not significantly different. This maybe due to the short time (2 s) of the UHT heat treatment because this result disagrees with the acetic acid increase in coffee beverages after heat treatment at 121°C for 10 min observed by Kumazawa and Masuda (2003). During the 120 days of storage, a significant increase in acetic acid was observed, mainly in the reference coffee brew. This increase was very highly significant ($p<0.001$) positive correlated (0.826) with the appearance of sourness, a non typical unpleasant acidity flavor of Arabica coffee brews (Pérez-Martínez et al., 2008a). Also acetic acid was negatively correlated (-0.815, $p<0.001$) with the acidity of Colombian coffee brews.

During last few years, coffee brews have been proposed as an important source of antioxidants in the diet. Thus, to study the effect of the UHT treatment and storage on the antioxidant capacity of coffee brews, both radical scavenging activity (DPPH· assay) and redox potential were analyzed. The results are shown in Figure 4. At the beginning of the study (day 0), the DPPH· antioxidant capacity was slightly higher, and redox potential lower, in the reference coffee brew than in the UHT treated one. In relation to the changes of these parameters with storage time, DPPH· values increased until day 10 in the UHT treated coffee brew and until day 15 in the reference brew. Then, they decreased until days 15 and 30 in the UHT treated and in the reference coffee brews, respectively, however, the reduction was significantly higher in the UHT treated coffee brew. After that, this parameter remained
almost constant until day 60, to drop sharply until the end of the study in the reference coffee brew. In contrast, in the UHT treated coffee brew, a slight increase was observed from days 60 to 120. Furthermore, it could be observed that DPPH· scavenging activity in the UHT treated coffee brew during storage was always higher than the initial value. An initial increase and a further decrease in the chain breaking activity of dark-roasted coffee brews stored at 30 ºC, in the absence of oxygen, was also observed by Anese and Nicoli (2003). These changes could be due to non-oxidative polymerization reactions of melanoidins or melanoidins precursors. The same authors (Nicoli, Anese, & Calligaris, 2001) also suggested that the coffee extraction procedures and/or the storage conditions could favor a further development of the Maillard reaction with the formation of phenol-type intermediates, which can increase the overall radical scavenging properties of the coffee brew.

Redox potential showed opposite behaviors in the UHT treated coffee brew and in the reference up to days 15. This could be explained because the thermal treatment could favor the formation of reactive oxygen species (ROS) (Stadler, 2001), inducing to a loss of reducing power in UHT treated coffee brew. Then, the redox potential decreased until days 60 and 90 in the UHT treated and in the reference coffee brew, respectively, to finally increase until the end of the study, showing a loss in the overall antioxidant efficiency of both coffee brews. This increase in redox potential apparently disagrees with the increase in DPPH· scavenging activity for UHT-treated coffee brew. However, it should be taking into account that redox potential evaluates the oxidation/reduction efficiency of all the antioxidants and pro-oxidants in coffee. Thus, although DPPH· scavengers were increased during last two months of the storage in UHT-treated coffee brews, other antioxidants with different action mechanisms (Anese, & Nicoli, 2001) might be lost or pro-oxidants could be generated.

Browned compounds formed as a result of caramelization and Maillard reactions during roasting process, and particularly melanoidins, are also related to the antioxidant capacity of
coffee brews (Delgado-Andrade, & Morales, 2005). Table 2, shows the changes in browned compounds expressed as Absorbance at 420nm throughout the storage of the reference and the UHT-treated coffee brews. At the beginning of the study, the amounts of browned compounds were significantly similar in both coffee brews, suggesting that the UHT treatment does not induce their formation. During storage, the changes in this parameter were very slight. Therefore, although some authors (Anese, & Nicoli, 2003) have suggested that the increase in both radical-scavenging and the reducing activity of coffee brews during storage could be attributable to the formation of Maillard reaction products with antioxidant properties from radical precursors, in this study, browned compounds do not seem to be involved in the changes observed in antioxidant capacity.

The results of color analysis are also shown in Table 2. The statistical analysis revealed very significant differences between the values of lightness (L*) in the reference and the UHT treated coffee brews throughout the overall storage time. This parameter, which takes values between 0 (black) and 100 (white), was always lower in the UHT treated coffee brew. This was in agreement with the darker color perceived by visual examination in the UHT treated coffee brew in comparison to the reference one. The changes in L* parameter with time were very small. Also Anese and Nicoli (2003) observed that lightness does not change in coffee brews stored in the absence of oxygen. On the other hand, the values of the chromaticity coordinates (a* and b*), which ranges from +60 (red for a* and yellow for b*) to -60 (green for a* and blue for b*), were also statistically different in the reference and in the UHT treated coffee brews in most of the analysis times, being, in general, higher in the reference. However, it must be noticed that these parameters varied between very close values, both in the reference (a*: 0.71-1.97; b*:0.82-2.67) and in the UHT treated coffee brew (a*: 0.29-1.39; b*: 0.32-1.68).
Figure 5 shows the changes in the sensory attributes of the studied coffee brews mostly affected by storage. The sensory evolution of the studied coffee brews was mainly characterized by a decrease in aroma freshness and in the acidity of a Colombian Arabica coffee brew. This acidity was progressively replaced by a non desirable sour taste, responsible to a great extent for the rejection of the coffee brews by the sensory panel, in a quicker way in the reference coffee brew. The descriptor sourness refers to an excessively sharp, biting and unpleasant flavor (such as vinegar or acetic acid), and it should not be confused with acidity, considered a pleasant and desirable taste in Colombian Arabica coffee brews. A highly significant ($p<0.001$) negative correlation (-0.897) was observed between sourness and pH. Taste/flavor persistence, a good characteristic of freshly prepared coffee brews, also decreased throughout the study. All these changes, which were observed mainly from day 7 onwards, were slower and less pronounced in the UHT treated coffee brew. Moreover, other taste and flavor notes non typical of coffee brews, related to their quality loss, began to be detected with storage time. This is the case of aftertaste, which was firstly perceived by the judges at day 8.

From the observation of Figure 5, three stages in the sensory evolution of the studied coffee brews may be distinguished: a decrease of the typical coffee attributes together with an increase in some non desirable notes until days 10-15; a second stage until day 60 when changes were less pronounced; and a third stage from day 60 onwards, when the loss of the typical coffee attributes and the appearance of non desirable notes became more pronounced. For example, slight astringency was perceived from days 60 to 120. It is worth mentioning that both aftertaste and astringency were found to be very high and significant ($p<0.001$) correlated with 5-CQA content (0.872 and 0.846, respectively). These results partly agree with those obtained in a previous work (Pérez-Martínez et al., 2008a). For the other sensory attributes studied (data not shown), very small differences between the reference and the UHT
treated coffee brews were observed. Aroma intensity hardly changed throughout the storage time for any of the studied coffee brews (scores of 6-8), and low bitterness (0-2) was maintained during the study. Burnt and spicy notes were not perceived either in aroma or in flavor of the studied coffee brews throughout time.

All these results have led us to propose a shelf-life of 60 days for the UHT treated coffee brew, in contrast with the 20 days established for Arabica coffee brews bottled and stored in the same conditions but without UHT treatment (Pérez-Martínez et al., 2008a).

In summary, it could be said that the UHT treatment of coffee brews at 120 ºC for 2 s allows us to obtain a microbiologically safe and good sensory valued product, with hardly changes either its volatiles or non-volatiles compounds. However, the UHT treatment applied in this study seems to be useful to preserve somehow the sensory quality of stored coffee brews. Thus, although the UHT treated coffee brew is considered more plain in terms of sensory quality, this treatment keeps longer the typical acidity of the brews, delaying and softening the pH decrease and the development of sourness, which is one of the main causes for the rejection of stored coffee brews (Pérez-Martínez et al., 2008a). In view of all the results, it could be concluded that the application of an UHT treatment is effective to extend the shelf-life (up to 60 days) of stored coffee brews while assuring their microbiological stability and keeping its chemical characteristics.

ACKNOWLEDGEMENTS

The authors thank the “Ministerio de Educación y Ciencia” for the financial support (AGL 2003-04045), for the grant given to M. Pérez-Martínez and for the “Juan de la Cierva” contract given to P. Sopelana. The authors thank the Unión Tostadora, S.A., for providing the coffee. The authors also thank the members of the sensory panel and the Department of Microbiology at the University of Navarra for its technical assistance.
REFERENCES


### Table 1. Changes in the aroma impact compounds of the coffee brews throughout storage at 4 °C.

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<th>Sulfur Compounds</th>
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<th>8</th>
<th>10</th>
<th>15</th>
<th>30</th>
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<td>nd</td>
<td>nd</td>
<td>nd</td>
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<td>93.7 ± 10.6</td>
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<td>1005 ± 10</td>
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<td>989 ± 32</td>
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<td>1030 ± 4</td>
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</tbody>
</table>
All values are shown as means ± standard deviations (n=3). In each row, different letters indicate statistically significant differences (p<0.05) throughout the time. SL, significance level between the two types of coffee brews (reference and UHT treated) in the same day: ns, non-significant (p >0.05); * significant (p <0.05); ** very significant (p <0.01); highly significant *** (p <0.001). nd, not detected.

### PHENOLIC COMPOUNDS

|     | 107 | 1359 | 2-Ethylpyrazine                  | Reference | nd | nd | nd | nd | nd | nd | nd | nd | UHT | nd | nd | nd | nd | nd | nd | nd | nd | nd |
|-----|-----|------|---------------------------------|-----------|----|----|----|----|----|----|----|----|-----|----|----|----|----|----|----|----|----|----|----|
|     | 121 | 1395 | 2-Ethyl-6-methylpyrazine        | Reference | nd | nd | nd | nd | nd | nd | nd | nd | UHT | nd | nd | nd | nd | nd | nd | nd | nd |
|     | 135 | 1455 | 2-Ethyl-3,5-dimethylpyrazine    | Reference | nd | nd | nd | nd | nd | nd | nd | nd | UHT | nd | nd | nd | nd | nd | nd | nd | nd |

**Guaiacol (2-methoxyphenol)**

|     | 109 | 1864 | Guaiacol (2-methoxyphenol)      | Reference | nd | nd | nd | nd | nd | nd | nd | nd | UHT | nd | nd | nd | nd | nd | nd | nd |

---

^a All values are shown as means ± standard deviations (n=3). In each row, different letters indicate statistically significant differences (p<0.05) throughout the time. SL, significance level between the two types of coffee brews (reference and UHT treated) in the same day: ns, non-significant (p >0.05); * significant (p <0.05); ** very significant (p <0.01); highly significant *** (p <0.001). nd, not detected.

^b QI: Ion used for the quantification of the compound.

^c KI: Kovats Index calculated for the HP-Wax capillary column.
Table 2. Changes in browned compounds and color parameters in coffee brews throughout storage at 4 ºC. Values are given as the mean value ± standard deviation.

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<th>90</th>
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<tr>
<td><strong>Browned compounds (Abs 420 nm)</strong></td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Reference</td>
<td>0.390±0.010</td>
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<td>0.396±0.001</td>
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<td>0.408±0.005</td>
<td>0.391±0.001</td>
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<td>ns</td>
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<td><strong>Color</strong></td>
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<tr>
<td>L*</td>
<td>Reference</td>
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</tr>
<tr>
<td>a*</td>
<td>Reference</td>
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<td>UHT</td>
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<tr>
<td>b*</td>
<td>Reference</td>
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</table>

Different letters in a row indicate significant differences (p<0.05) along the time. SL Signification level between the two storage temperatures in the same day; ns: no significant (p>0.05); *: significant (p<0.05);**: very significant (p<0.01); *** high

significant (p<0.001).
FIGURE CAPTIONS

Figure 1. Changes in pH in the reference and the UHT treated coffee brews throughout storage at 4ºC.

Figure 2. Changes in caffeine and trigonelline in the reference and the UHT treated coffee brews throughout storage at 4ºC.

Figure 3. Changes in 5-CQA, caffeic acid, ferulic acid and 4-vinylguaiacol in the reference and the UHT treated coffee brews throughout storage at 4ºC.

Figure 4. Changes in redox potential and DPPH· antioxidant capacity in the reference and the UHT treated coffee brews throughout storage at 4ºC.

Figure 5. Changes in selected sensory attributes in the reference and the UHT treated coffee brews throughout storage at 4ºC. Results are expressed as the panel scores.
FIGURES

Figure 1. Changes in pH in the reference and the UHT treated coffee brews throughout storage at 4°C.
Figure 2. Changes in caffeine and trigonelline in the reference and the UHT treated coffee brews throughout storage at 4°C.
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