TITLE: Effects of refrigeration and oxygen on the coffee brew composition

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ABSTRACT

The aim of this work was to monitor the changes both in the composition and in some sensory parameters of Colombian Arabica coffee brews stored at room and refrigeration temperatures, with and without oxygen. Some nonvolatile compounds related to the taste of coffee brews were determined, in an attempt to study possible relationships between chemical and sensory changes. Storage time hardly affects the amounts of chlorogenic, caffeic and ferulic acids, reported to have some beneficial health effects, mainly due to their antioxidant activities. In contrast, pH decreases in all the coffee brews along the time, mainly in that stored at 25ºC with oxygen. The appearance of sourness and other non typical coffee tastes (rancid taste, aftertaste) and an increase in astringency leads to establish a shelf-life of 10 days for coffee brews stored at 25ºC with oxygen, 15 days for coffee brews stored at 4ºC with oxygen and at 25ºC without oxygen, and 20 days for coffee brews stored at 4ºC without oxygen. The behaviour of 5-caffeoylquinic acid, caffeic acid and 4-vinylguaiacol throughout time was different from other studies conducted at higher temperatures to accelerate the staling, what reveals that stability studies of coffee brews should be made in real time and temperature.

KEYWORDS: coffee brew, storage, temperature, oxygen, shelf-life, sensory analysis

INTRODUCTION

Nowadays, coffee is the second most consumed drink in the world after water [1] and more and more consumers demand its availability during the whole day. However, there are some situations when it is not possible to have the required equipment, such as a coffee maker and a heat source, or the time to prepare it. In collective catering, coffee brews are usually kept at high temperatures for a period of time; however, in more reduced fields this option does not seem feasible or practical. Moreover, this is a solution only for a few hours. Another possibility is the storage of coffee brews at refrigeration or at room temperatures, to be reheated, for example in a microwave oven, or to prepare cold coffee Although the shelf-life of coffee brews is very limited because of fungi development, it could maybe be possible to obtain fresh brews for commercial purposes, without any additives, if they are aseptically stored. Nevertheless, it is well known that the storage of coffee beverages causes the deterioration of their sensory characteristics. Concretely, an increase in acidity has been observed in coffee beverages stored for a period of time, being this the most important factor in limiting their acceptability [2-5].

Acidity is an appreciated attribute of Colombian and other light roasted Arabica coffee brews. However, as time passes, this initial acidity is replaced by an excessively sharp, biting and unpleasant flavour (such as vinegar or acetic acid), which is known as sourness [6, 7]. This latter should not be confused with the typical coffee acidity, but most of the papers use both terms without distinction. The increase of sourness in warm stored coffee brews has been attributed to the increase of acids produced by the hydrolysis of the quinic acid lactones and chlorogenic acid lactones formed during roasting, the hydrolysis of low molecular weight esters, and the thermal degradation of chlorogenic acids into their corresponding hydroxycinnamic acids [8, 9]. However, most of these conclusions have been obtained from the results of (a) accelerated ageing studies where coffee brews were

maintained at high temperatures (60 to 95ºC) [3, 4, 10-13], (b) studies of coffee beverages added with sugar and other additives, and pasteurized, stored at room or refrigeration temperatures [5, 14]; or (c) sterilized sugary coffee beverages stored at high temperature [15]. In all of them, an additional thermal process is included after coffee brew preparation, which could be relevant to the degradation of chlorogenic acids and, consequently to the coffee quality. Besides, in the scarce studies where coffee beverages are stored at room or refrigeration temperatures [5, 14], only a few parameters are considered, mainly pH and other properties such as colour, electrical conductivity or optical density. In contrast, as far as we know, no work about the changes undergone by some typical components of coffee brews, such as caffeine, trigonelline or chlorogenic acids, in coffee brews stored at low temperatures has been carried out. These compounds are related to some sensory attributes of coffee brews, such as acidity, bitterness or astringency, whose intensification is associated with a loss of coffee brew quality. Thus, chlorogenic acids in general are known to be responsible for coffee astringency [16] and their thermal degradation into caffeic and quinic acids also contributes significantly to the development of acidity at elevated temperatures, causing the coffee to become acerbic (bitter and sour) [17]. Moreover, some phenolic compounds released during the thermal degradation of chlorogenic acids, such as caffeic or ferulic acids, contribute to bitterness [18]. These latter can, in turn, act as precursors of other compounds which also influence the flavour of coffee brews, such as 4-vinylguaiacol [16].

On the other hand, the influence of oxygen on the changes of coffee brews has only been studied focused on pH, colour and turbidity during hot storage [19-21]. Therefore, the aim of this study was to monitor the changes of some sensory and chemical parameters related to the taste of coffee brews stored at room (25ºC) and refrigeration (4ºC) temperatures. The advantages of the package in absence of oxygen were studied too. Some microbiological

parameters were also measured in order to guarantee the safety of the stored coffee brews. These results constitute a first point for the development of coffee brews for commercial purposes, aseptically stored and with a longer shelf-life, destined for being consumed as hot coffee brews.

MATERIALS AND METHODS

Materials

Colombian Arabica ground roasted coffee was provided by a local factory.

Pure reference standards of caffeine, trigonelline, pentoxyfilline, 5-caffeoylquinic acid, caffeic acid, ferulic acid and 4-vinylguaiacol were purchased from Sigma-Aldrich (Steinheim, Germany).

Coffee brew preparation

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±2ºC (pH=7.0). The freshly prepared coffee brews were immediately poured into 330 mL sterilized glass flasks, either up to a volume of 135 mL (with air headspace, this is, with oxygen) or up to the top (without air headspace, this is, without oxygen), and hermetically closed. The filling of the flasks was carried out aseptically in a laminar flow cabin to avoid the microbiological contamination of the samples. Afterwards, both coffee brews bottled with and without oxygen were stored at 4ºC and 25ºC for 30 and 60 days, respectively.

Microbiological analysis. Aerobic mesophilic flora was analyzed by colony count technique at 30ºC (ISO 4833:2003). Enumeration of yeasts and molds was made by colony count technique at 25ºC (ISO 7954:1987).

Caffeine and Trigonelline. Extract preparation, clean-up and HPLC analysis were performed following the method described by Maeztu et al. [22]. HPLC analysis was achieved with an analytical HPLC unit (Agilent Technologies 1100), 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) in isocratic conditions at a constant flow rate of 2.0 mL/min at 36ºC during 15 min.

The injection volume was 20 μL. Detection was accomplished with a diode-array detector, and chromatograms were recorded at 280 nm.

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. Acetonitrile and water adjusted to pH 3.0 with a phosphoric acid solution were the solvents. The flow conditions and the proportion of the solvents are shown in Table 1. The temperature of analysis was 25ºC and the injection volume 100 μL. Wavelength of detection was 325 nm. (Figure 1).

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 et al. [23]. The HPLC analysis was carried out with the same equipment described above. The chromatographic separation was achieved at 25ºC by using a complex gradient solvent system with acetonitrile / water adjusted to pH 2.5 with a phosphoric acid solution (Table 2) [23]. Injection volume was 100 μL. The wavelengths of detection were 314 nm for caffeic acid, 325 nm for ferulic acid and 210 nm for 4-vinylguaiacol. (Figure 2).

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

Sensory Descriptive Analysis. Twenty judges were recruited among members of the Food Science and Technology Department at the University of Navarra. Selection and training were carried out as described by Maeztu et al. [22] to have a 10-member panel. Although judges had experience on sensory evaluation of coffee brews, they were retrained during four sessions to adapt their evaluation to the detection and quantification of parameters related to staling. Attention was focused in rancidity and the distinction of acidity, typical from a Colombian coffee, and sourness, typical from an old coffee brew. Reference coffee brews were prepared with a Colombian Arabica ground roasted coffee stored for 1-2 years

for rancidity, other stored for less than 1 month for acidity and the positive flavours, and a coffee brew prepared with the same coffee but stored for 1 month for sourness. A scorecard with the most frequently perceived sensory attributes was developed during training. Two lines for "other" flavours were added. Acidity, sourness, bitterness, astringency, rancidity and aftertaste were rated on 11-point scales from "none" (0) to "very high" (10).

Each coffee brew sample was heated in a microwave oven at 90±2ºC immediately before tasting and served monadically in a white porcelain coffee cup. The order of presentation was randomized among sessions. A freshly prepared coffee brew was evaluated at first as a reference and in order to avoid first impressions. All evaluations were conducted in isolated sensory booths illuminated with white light in the sensory laboratory under standardized conditions by UNE 87-004-79 [24]. Rinse water was provided between samples. After the individual evaluation of each sample, results were discussed and established by panel consensus.

Statistical analysis. Each parameter was analyzed in triplicate. Analysis of Variance (ANOVA) was applied for each storage temperature. The source of variation was the time. T-Tukey was applied as the test *a posteriori* with a level of significance of 95%. t-Student analysis was applied to the results obtained for both storage temperatures. 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.

RESULTS AND DISCUSSION

Neither aerobic mesophilic flora nor molds and yeasts growth (< 1 cfu) were observed in the coffee brews stored at 25ºC and at 4ºC, both with and without oxygen, throughout storage time. Consequently, both coffee brews could be considered as stable products from the microbiological point of view.

As it is shown in Figure 3, caffeine and trigonelline did not suffer significant changes along the time, neither due to the storage temperature nor due to the presence of oxygen.

In Figure 4, it is shown that the initial pH of the coffee brews was between 5.1 and 5.2. An increase of chemical acidity, measured by pH, was observed in all coffee brews along the time, being faster at 25ºC vs 4ºC and in oxygen packaged coffee brews. Although both temperature and the presence of oxygen influenced the pH decrease, the former seemed to have higher incidence. These results partly agree with those reported by Nicoli et al. [19] who found that the temperature had a high influence on the pH decrease of coffee brews but the use of nitrogen hardly influenced the pH value.

Figure 5 shows the changes of 5-CQA, caffeic acid, ferulic acid and 4-vinylguaiacol in coffee brews stored at 4 and 25ºC, with and without oxygen. A significant increase ($p<0.05$) of 5-CQA during the first 3 days of storage was observed for all coffee brews, except for that stored at 4ºC without oxygen. In the latter, there was an initial decrease followed by an increase up to concentrations similar to the other coffee brews at 10 days. This increase could maybe be due to the hydrolysis of chlorogenic acid lactones formed during the roasting of coffee [8], or to the release of CQAs from non covalently linked polymeric skeletons, such as melanoidins [25]. After this increase, 5-CQA was maintained up to 20 and 30 days when a decrease was observed in the coffee brews stored without oxygen at 25ºC and 4ºC, respectively. It should be noticed that 5-CQA was significantly ($p<0.05$) higher in coffee brews stored at 25ºC vs 4ºC. In the coffee brews stored without

oxygen, the concentration of caffeic acid increased significantly ($p<0.05$) only during the first day and smoothly decreased with time. In those stored with oxygen, a maximum concentration of caffeic acid was observed at 25ºC before than at 4ºC. These increases of caffeic acid might be originated by the hydrolysis of caffeoylquinic acids, but not only 5-CQA, because there was no correlation between 5-CQA and caffeic acid changes. On the other hand, the presence of oxygen seems to have a role in the formation, but also degradation, of caffeic acid which needs further investigation. The changes of these acids were in contrast with those observed in heat-maintained coffee brews [11] or in heat sterilized canned coffee beverages [15] where a decrease of 5-CQA was observed. This decrease was attributed to isomerisation of 5-CQA to 3-CQA and 4-CQA and to decomposition to caffeic and quinic acids. This different behaviour could be due to the mild storage temperatures used in our study which might be not high enough to induce the isomerisation and degradation of 5-CQA. Severini et al. [26] also found that the concentration of chlorogenic acid hardly changed during the conservation of a coffee brew at 40ºC.

In Figure 5 more changes in the concentration of ferulic acid in oxygen packaged coffee brews, and a less pronounced decrease in the coffee brews packaged without oxygen can be observed. This smooth evolution, at both storage temperatures, suggests a slight degradation of its precursors, feruloylquinic acids, which seem to be more stable than caffeoylquinic acids [27].

4-vinylguaiacol is the product originated by the thermal decarboxylation of ferulic acid. In aged canned beverages, such as coffee [15] and orange juices [28] it has been reported as one of the most detrimental off-flavour compounds. However, in our study, the initial amount of 4-vinylguaiacol (3.92-3.95 μg/mL) showed a significant decrease during storage. This decrease was higher in coffee brews stored with oxygen, maybe because 4-

vinylguaiacol could be oxidized to vanillin and vanillic acid [29], and also it was higher at 25ºC vs 4ºC. Therefore, this compound seems not to be the main responsible for off-flavours in coffee brews stored at mild temperatures (room and refrigeration). On the other hand, the different behaviour of this off-flavour compound at mild and high (60-80ºC) temperatures would question the conclusions derived of some kinetic studies [15] based on accelerated coffee aging experiments, because they might not be good predictors for normal coffee beverages storage conditions (room or refrigeration temperatures).

In Figure 6, the changes in the taste sensory attributes of the studied coffee brew are shown. Acidity scores were high at the initial time with a tendency to decrease throughout storage. This decrease in perceived acidity apparently contrasts with the findings of other authors**,** who have reported an increase of acidity in coffee brews stored at room temperature over a period of just 5 hours [2, 30]. However, the fall observed in this study must be interpreted as a disappearance of the typical acidity initially perceived in Arabica coffee brews, being substituted by a sour taste [6]. The members of the panel described sourness as "bad acidity" or "acidity non characteristic of coffee", and it was perceived for the first time at 15 days for coffee stored at 25ºC with oxygen, at 20 days for both coffee brews stored at 4ºC with oxygen and at 25ºC without oxygen, and at 30 days for coffee stored at 4ºC without oxygen. From these days onwards, the judges not only perceived that sourness increased with storage time, but also that the coffee brew became unacceptable from a sensory point of view. If data concerning pH are considered (see also Figure 4), it can be checked that, in the coffee brew stored at 25ºC with oxygen, this perception coincides with the moment when pH reaches a value of 4.8 (day 15). As early as 1972, Sivetz [31] proposed that the pH of beverages prepared from mild roasted coffee should be 4.9-5.2 and at pH $\leq$4.8 the coffee tastes strongly sour and below 4.7 milk might be curdled. Moreover, the pH value of 4.8 has been considered as the limit of acceptability for stored

coffee brews by some authors [3, 32]. It should be noticed that, in other studies [5], this pH was not reached after 50 days of storage at 25ºC. This could be due to differences in the raw coffee used (a mixture of Arabica and Robusta 50:50), since coffees of different origins provide extracts with different pHs, and Colombian is one of the most acidic [31]. The brewing procedure or the storage conditions could also affect the pH decrease.

As it can be observed in Figure 6, bitterness hardly changed during the first days but slightly tended to decrease with time. This could be due to the little variations observed in nonvolatile compounds related to bitterness, such as caffeine, trigonelline, 5-CQA, caffeic and ferulic acids, even though a possible hydrolysis of chlorogenic acid lactones to chlorogenic acids might contribute to the decreasing tendency. Related to this, Frank et al. [33] established the importance of the caffeoyl- and feruloyl-quinides as well as more complex esters between quinides and cinnamoyl derivates as the most intense bitter taste compounds in coffee brews.

Another cause of the quality loss of coffee brews is the appearance of unpleasant tastes such as astringency and aftertaste which increased mainly during the first days. The caffeic acid content was very high significantly ($p<0.001$) correlated with astringency (0.659) and 5-CQA content very high significantly ($p<0.001$) correlated with aftertaste (0.734). On the other hand, sourness was very high significantly ($p<0.001$) correlated with pH values (-0.841 and -0.855 in coffee brews packed with and without oxygen, respectively). These results agree with those obtained by Maeztu et al. (2001). In contrast, the concentration of 5-CQA content was poorly correlated ($p<0.05$) with sourness (0.554).

Taking the sensory results into account, shelf-life was tentatively established at 10 days for coffee stored at 25ºC with oxygen, 15 days for coffee brews stored at 4ºC with oxygen and at 25ºC without oxygen, and 20 days for coffee stored at 4ºC without oxygen. At these times, sourness and other non desirable coffee taste attributes such as rancid taste,

aftertaste and astringency were perceived by the panel. It must be noticed that only for the coffee brew stored at 25ºC with oxygen, the shelf-life established by sensory analysis and the criterion of the pH value of 4.8 as the limit of acceptance was coincident. However, in the other coffee brews, the shelf-life was established before pH reached this value. Consequently, the shelf-life calculation based on the pH decrease kinetic and the pH value of 4.8 proposed as the "limit of acceptance" for stored coffee beverages by Dalla Rosa et al. [5], are not suitable to be applied for Colombian Arabica coffee brew. This might be because the pH established as the limit of acceptance should be different for Arabica and Robusta coffee, or because pH should not be considered as the unique criterion for coffee brew acceptance.

CONCLUSIONS

The storage temperature and the packaging in presence or absence of oxygen influence the changes not only of some typical coffee components, but also of certain sensory characteristics. Thus, the maintenance of coffee brew at 25ºC packaged in the presence of oxygen, results in general, in more accused and quicker changes in most of the parameters studied than the maintenance of coffee brew at 4ºC packaged in the presence of oxygen and at both temperatures packaged in the absence of oxygen. Even so, some of the compounds studied keep quite stable at the temperatures tested, mainly at 4ºC, which reveals that storage temperature is a key factor to reduce some of the changes which contribute to the deterioration of coffee brews throughout time. Besides, it could be added that a low storage temperature contributes to preserve the beneficial effects of coffee brews for human health, since some of the studied compounds, such as 5-CQA or caffeic acid, have been reported to have antioxidant activity [34, 35].

Nevertheless, neither storage at refrigeration temperature nor the absence of oxygen are effective to avoid completely the pH decrease and the development of sourness and other non desirable tastes in stored coffee brews.

The results of this study also reveal that the deterioration of the coffee brews cannot be exclusively attributed either to the evolution of pH or to the studied compounds, and also that pH is not the only factor which determines the limit of acceptance of coffee brews. Consequently, further investigations should be carried out in order to know more deeply which are the factors involved in the staling of this type of product stored at room or refrigeration temperatures and with or without oxygen.

Finally, the use of high temperatures to simulate an accelerated ageing of coffee brews does not seem to be a proper way to study the evolution of this product during storage, since these temperatures favor reactions which do not occur at lower temperatures. It

makes it necessary to develop stability studies of coffee brews in real time and temperatures.

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Table 1. Flow and gradient solvent conditions for 5-caffeoylquinic acid HPLC analysis.

Time (min)	**Dilution** (% acetonitrile:% water)	**Flow** ($mL.min^{-1}$)
0	12.0 : 88.0	1.0
5	7.5 : 92.5	1.6
10	8.0 : 92.0	1.6
15	25.0 : 75.0	1.6
20	12.0 : 88.0	1.1

Table 2. Flow and gradient solvent conditions for caffeic acid, ferulic acid and 4-vinylguaiacol HPLC analysis.

Time (min)	**Dilution** (% acetonitrile:% water)	**Flow** ($mL.min^{-1}$)
0-20	91 : 9	1.0
20-25	14 : 86	1.1
25-35	12 : 88	1.1
35-42	14 : 86	1.1
42-45	18 : 82	1.3
45-48	27 : 73	1.3
48-55	25 : 75	1.7
55-58	19 : 81	1.6
58-68	20 : 80	1.8
68-70	10 : 90	1.6
70-75	9 : 91	1.0

FIGURE CAPTIONS

Figure 1. HPLC chromatogram of 5-CQA.

Figure 2. HPLC chromatogram of caffeic acid, ferulic acid and 4-vinylguaiacol.

Figure 3. Changes in caffeine and trigonelline of coffee brews stored at 4 and 25ºC with and without oxygen.

Figure 4. Changes in pH of coffee brews stored at 4 and 25ºC with and without oxygen.

Figure 5. Changes in 5-CQA, caffeic acid, ferulic acid and 4-vinylguaiacol of coffee brews stored at 4 and 25ºC with and without oxygen.

Figure 6. Changes in taste sensory attributes of coffee brews stored at 4 and 25ºC with and without oxygen.

Figure 1

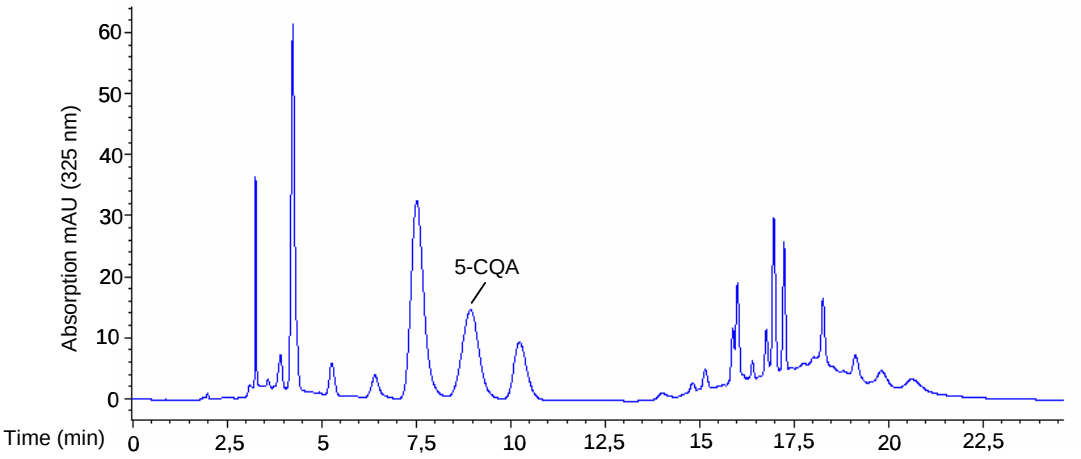

Absorption mAU (325 nm)
60
50
40
30
20
10
0
5-CQA
Time (min)
0
2,5
5
7,5
10
12,5
15
17,5
20
22,5

Figure 2

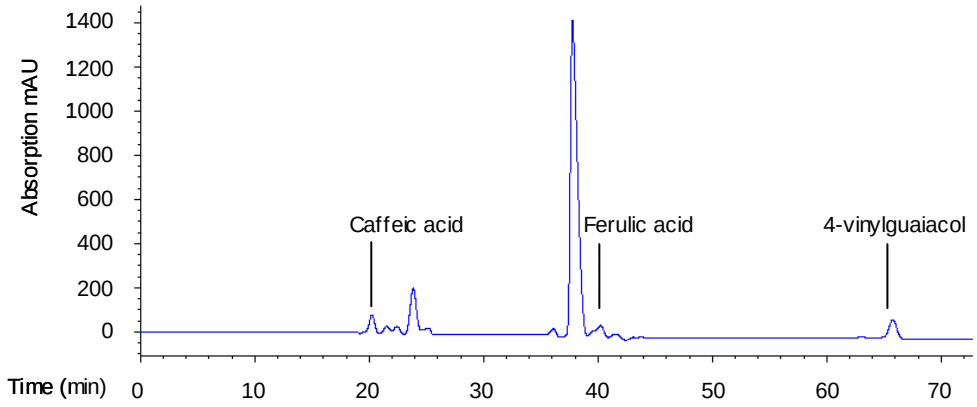

Absorption mAU
1400
1200
1000
800
600
400
200
0
Caffeic acid
Ferulic acid
4-vinylguaiacol
Time (min)
0
10
20
30
40
50
60
70

Figure 3

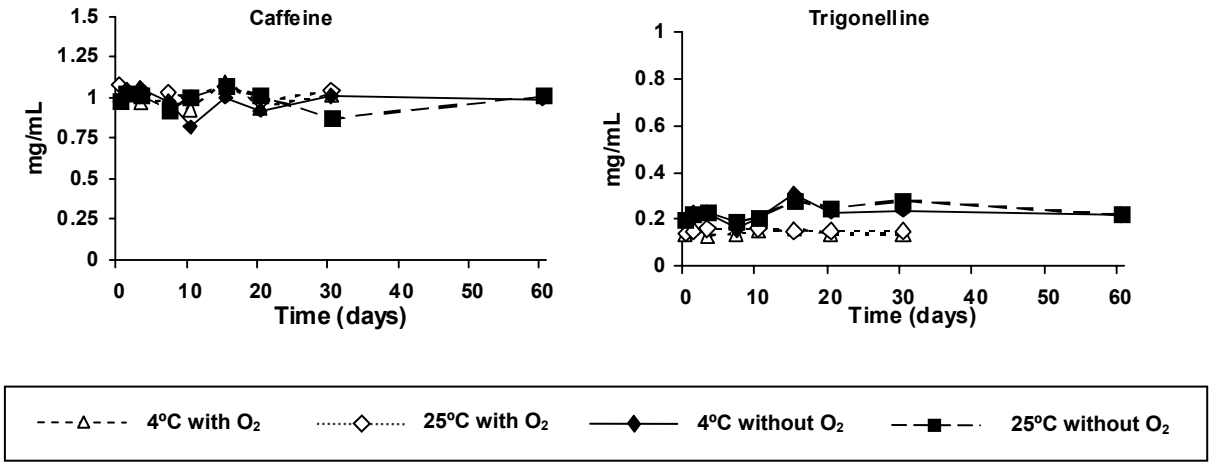

Caffeine
mg/mL
1.5
1.25
1
0.75
0.5
0.25
0
0
10
20
30
40
50
60
Time (days)
Trigonelline
mg/mL
1
0.8
0.6
0.4
0.2
0
0
10
20
30
40
50
60
Time (days)
4ºC with O2
25ºC with O2
4ºC without O2
25ºC without O2

Figure 4

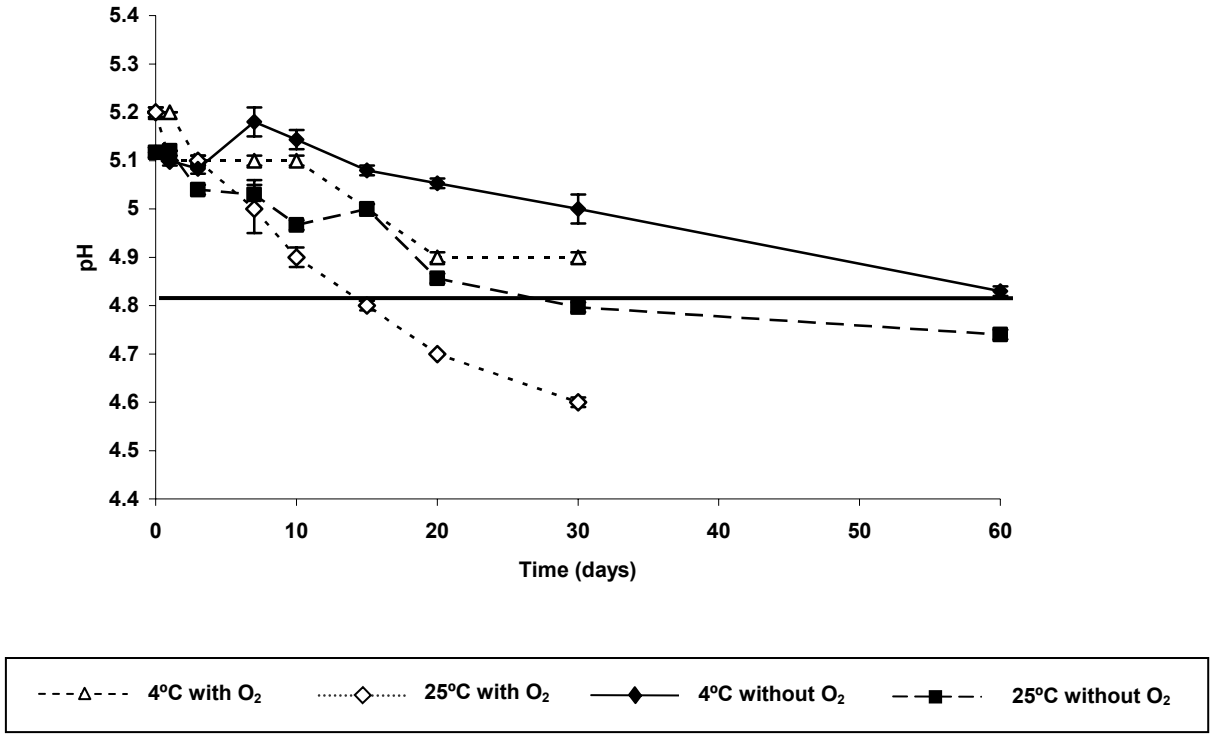

5.4
5.3
5.2
5.1
5
4.9
4.8
4.7
4.6
4.5
4.4
pH
0
10
20
30
40
50
60
Time (days)
4ºC with O2
25ºC with O2
4ºC without O2
25ºC without O2

Figure 5

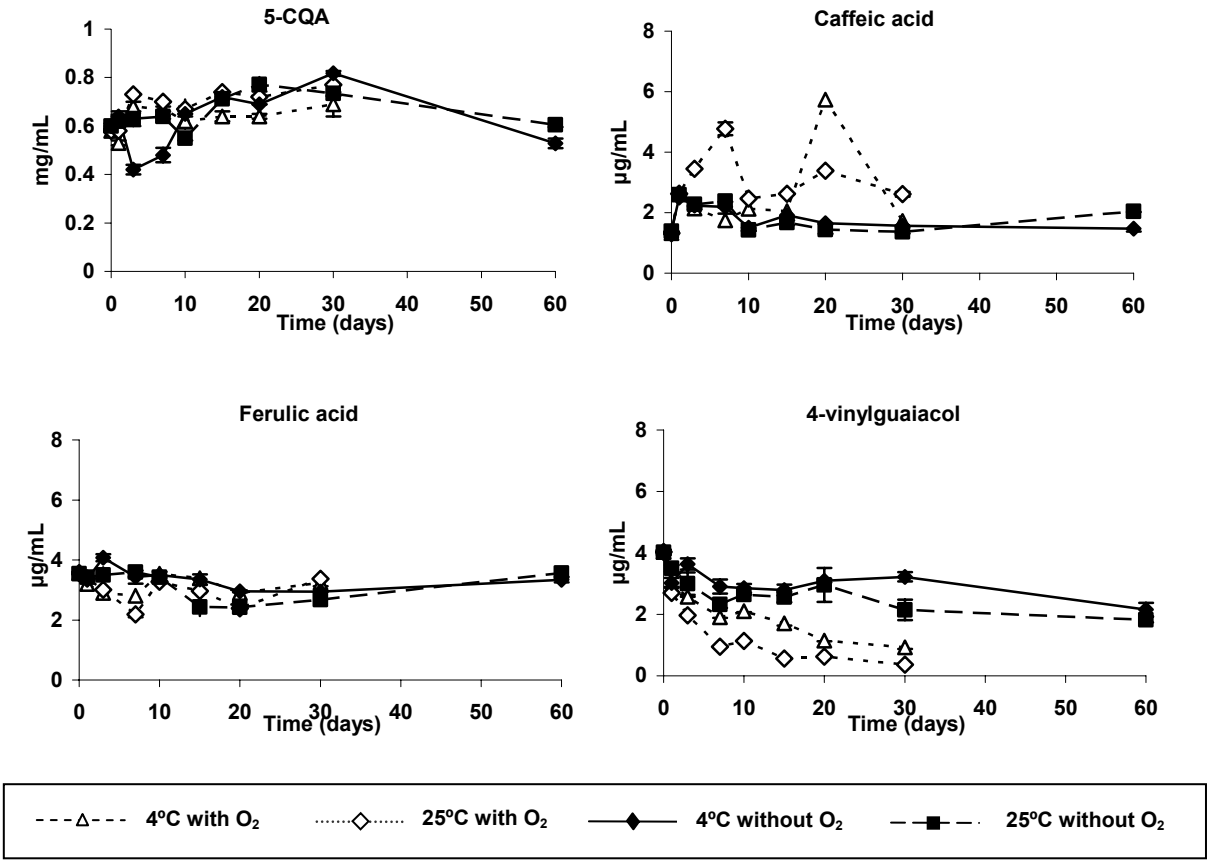
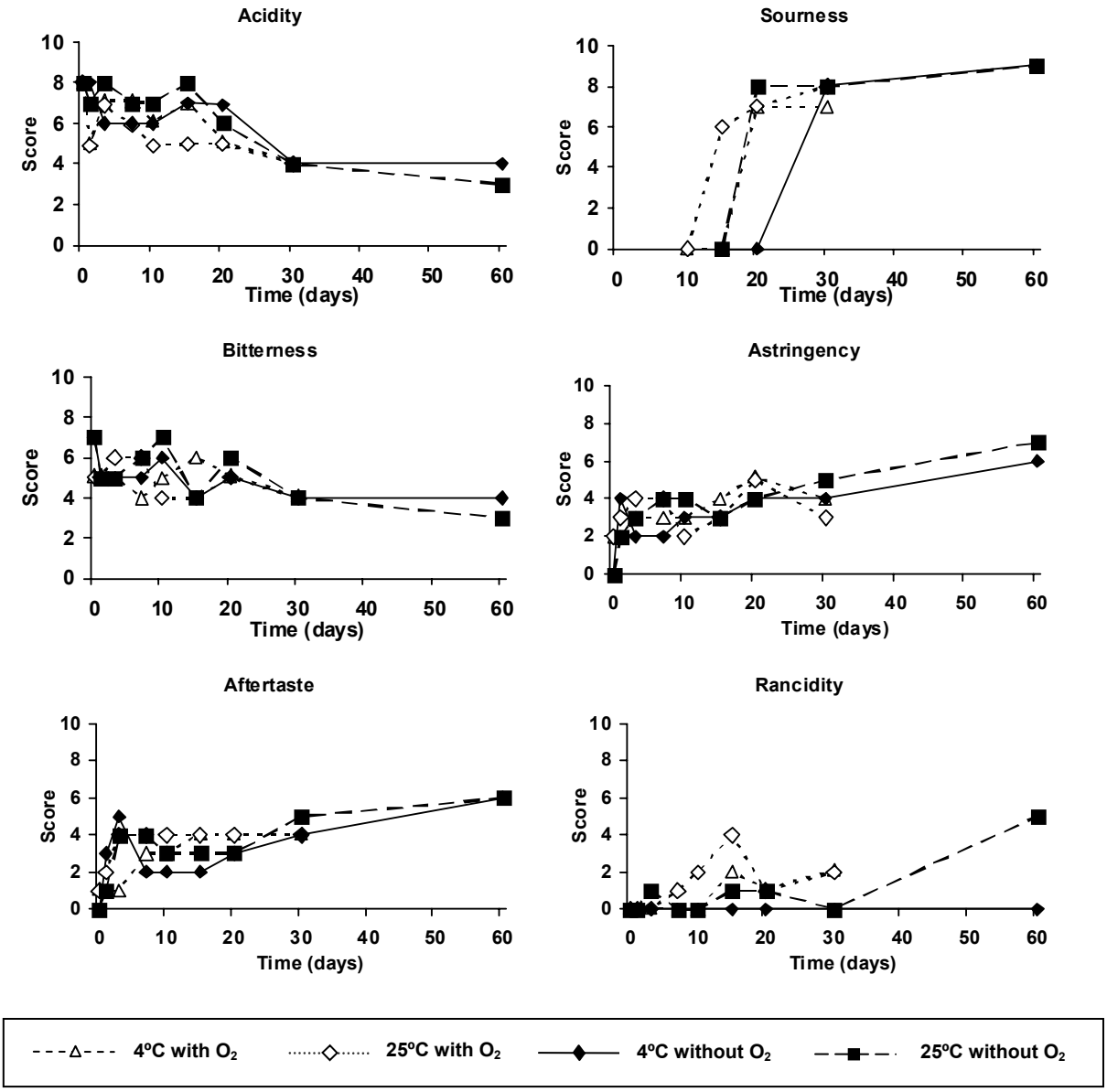

5-CQA
mg/mL
Time (days)
Caffeic acid
µg/mL
Time (days)
Ferulic acid
µg/mL
Time (days)
4-vinylguaiacol
µg/mL
Time (days)
4ºC with O2
25ºC with O2
4ºC without O2
25ºC without O2

Figure 6


Acidity
Sourness
Bitterness
Astringency
Aftertaste
Rancidity
Score
Time (days)
4ºC with O2
25ºC with O2
4ºC without O2
25ºC without O2