TITLE: Application of multivariate analysis to the effects of additives on chemical and sensory quality of stored coffee brew.

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RUNNING HEAD: Effects of additives on coffee brew quality

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ABSTRACT

The aim of this work was to obtain a black coffee brew to be consumed hot by extension of its shelf-life, by addition of additives. Four pH-regulator agents (sodium and potassium carbonates and bicarbonates), one pH-regulator and antioxidant (sodium citrate), three antioxidants (sodium ascorbate, ethylenediaminetetracetic acid (EDTA), and sodium sulfite), and lactoserum were tested by sensory analysis. Sodium carbonate and bicarbonate were selected for a study of the physico-chemical (soluble and volatile compounds related to the sensory properties) and sensorial quality of coffee brew stored during 90 days at 4ºC. Although both additives extended the shelf-life of the coffee brew up to 60 days, sodium carbonate was the chosen additive because it was the most useful in limiting the pH decrease and perception of sourness, which are some of the main factors involved in the rejection of stored coffee brews, and maintain better the aroma and taste/flavor. Moreover, the application of multivariate analysis facilitated, firstly the description of the global changes of the coffee brews with or without additives throughout the storage using the Principal Component Analysis (PCA), and secondly, to obtain a simple equation only with pH and caffeic acid parameters to discriminate the three types of coffee brews and simplify the analytical process, by means of the Stepwise Discriminant Analysis (SDA).

INTRODUCTION

It is well known that the storage of coffee brews leads to deterioration of their sensory characteristics (1-3). This quality loss is generally accompanied by sourness development, partially detectable by a pH decrease, even at refrigeration temperatures (2, 4). This is of importance when aiming for a storage stable packed coffee brew. Although these types of coffee beverages are very popular in some countries, such as Japan, their sensory quality is lower than that of freshly prepared coffee brews (5). This could probably be one of the reasons for the less success of this type of coffee drinks in Western countries, where the traditional image of coffee as a freshly brewed beverage is still deeply rooted. Even so, ready-to-drink coffee beverages have reached a great acceptation among certain populations because they are inexpensive and storable, providing affordable alternatives to freshly brewed coffee. However, there is still the need to obtain a stable good quality black coffee brew to be consumed hot.

Several patents proposed the addition of acid-neutralizing, such as carbonates, hydroxides, etc., antioxidants and other additives in order to avoid or, at least, reduce, the chemical and sensory evolution of coffee brews during storage, particularly the increase in acidity, (6-8). However, an aroma loss and salty taste were observed (7). Moreover, most of the patents where additives are used in coffee are focused on milk-coffee beverages, cappuccino-type coffee, soluble coffee or to obtain cold coffee beverages.

Taking into account that patents show limited detail, to the best of our knowledge, there are not detailed studies dealing with the effect of additives on the changes of coffee chemical compounds and the sensory quality of stored coffee brews. For these reasons, the main aim of this work was the contribution to the knowledge of the coffee brew changes during storage using additives in order to obtain a black coffee to be consumed hot by extension of the shelf-life of the coffee brew obtained before (4, 9). Both physico-chemical (soluble and volatile

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compounds) and sensorial quality were evaluated. Moreover, multivariate statistical analyses were applied as practical tools to know the global patterns of the coffee brew samples during storage by means of Principal Component Analysis (PCA), and to obtain a simple equation in order to discriminate the coffee brews with or without additives and simplify the analytical process by means of the Stepwise Discriminant Analysis (SDA).

MATERIALS AND METHODS

Coffee. Vacuum-packed Colombian Arabica ground roasted coffee (2.25% water content, L* 19.57±0.09) was provided by a local factory. L* value was analyzed by means of tristimulus colorimeter (Chromameter-2 CR-200, Minolta, Osaka, Japan) using the D65 illuminant. The instrument was standardized against a white tile before sample measurements. Ground roasted coffee was spread out in an l cm Petri plate and the L* value was measured in triplicate and on the CIELab scale.

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, pentoxyfilline, 5-caffeoylquinic acid, caffeic acid, ferulic acid, 4-vinylguaiacol, 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).

Sodium ascorbate, sodium sulfite, sodium citrate and lactoserum were provided by ANVISA (Madrid, Spain). Sodium carbonate, sodium bicarbonate, potassium carbonate, potassium bicarbonate and ethylenediaminetetracetic acid (EDTA) were purchased from Panreac (Barcelona, Spain).

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 a French press coffeemaker. Extraction time was 3 min and water temperature 90±2°C (pH=7.0). Each additive was added immediately after coffee brew extraction in a laminar flow cabin. A reference coffee brew

without any additive was prepared. .Sterilized glass flasks were filled up to the top (330 mL) with freshly coffee brews in a laminar flow cabin, to assure aseptic conditions and avoid the microbiological contamination of the samples. Afterwards, coffee brews were stored at 4ºC until their analysis. This experiment was made in duplicate.

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

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

Caffeine. Extract preparation, clean-up and HPLC analysis were performed following the method described by Maeztu et al. (10). HPLC analysis was achieved with an analytical HPLC unit model 1100 (Agilent Technologies, Palo Alto, CA), 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/milliQ water (15:85) in isocratic conditions at a constant flow rate of 2.0 mL/min at 36 ºC. 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. Conditions of the used gradient solvent system and flow are shown in Table 1. Wavelength of detection was 325 nm.

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. (11). 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/milliQ water adjusted to pH 2.5 with a

phosphoric acid solution (4). The wavelengths of detection were 314 nm for caffeic acid, 325 nm for ferulic acid and 210 nm for 4-vinylguaiacol.

Volatile compound analysis. The profiles of volatile compounds were obtained with the method described by Sanz et al. (12), adapted to coffee brew by Maeztu et al. (13), and using Static Headspace-Gas Chromatography-Mass Spectrometry (SH-GC-MS).

After the flask was opened, six mL of a homogenized coffee brew was introduced into a 10 mL vial, which was immediately sealed with a silicone rubber Teflon cap. Each vial was equilibrated at 40ºC for 60 min in the static headspace sampler (model 7694, Agilent Technologies, Palo Alto, CA). Each vial was pressurized with carrier gas for 12 s, and 3 mL of the coffee headspace sample was 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 180ºC, and carrier gas was Helium (1 mL/min linear speed). The oven temperature was maintained at 40ºC for 6 min and then raised at 3ºC/min to 190ºC. Mass spectrometry analysis was performed with a Hewlett-Packard mass selective detector model 5973 (Agilent Technologies) operating in the electron impact ionization mode (70 eV), with a scan range of 33-300 amu. Ion source temperature was set at 230ºC. Each sample was analyzed in triplicate.

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 also by comparison of their Kovats indices with those of standard compounds. The Kovats indices were calculated according to the method of Tranchant (14). Peak areas were measured by calculation of each volatile total area based on integration of a single ion. The Quantification Ion of each volatile compound is given in the Table 5.

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. (10, 13) to have a 10-member panel. Retraining and sensory standards were described by Pérez-Martínez, et al. (4, 9). A scorecard with the most frequently perceived sensory attributes was developed during training. Two lines for "other" aromas and flavors were added. All the descriptors 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±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 coffee brew was evaluated first, as a reference and 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 (15). Rinse water was provided between samples. After the individual evaluation of each sample, results were discussed in order to find new other sensory attributes that could be developed in the coffee brew during the study and to establish the shelf-life by consensus.

Statistical analysis. Each parameter was analyzed in triplicate. Results are shown as means ± standard deviations. A two-way analysis of variance (ANOVA) was performed to establish the impact of both the additive addition (sodium carbonate and bicarbonate) and the storage time on several physico-chemical and aroma parameters of coffee brew samples (Table 4). When interactions are significant, a one-way ANOVA was applied. T-Tukey test was applied as a test *a posteriori* with a level of significance of 95%.

Correlations among variables were assessed by means of the Pearson correlation test. Principal Component Analysis (PCA), based on the Pearson correlation matrix, was applied to the data. Principal Components (PC) with eigenvalues higher than 1 were selected. Stepwise Discriminant Analysis (DA) was applied to obtain a simple equation by which the coffee brew samples could be classified. Wilk's Lambda stepwise method was used. The criteria

were 0.05 for maximum significance of F to enter and 0.10 minimum significance of F to remove. All statistical analyses were performed using the SPSS v.15.0 software package.

RESULTS AND DISCUSSION

Selection of the additives

Previous studies on the Colombian Arabica coffee brews showed that staling is mainly due to the development of sourness and other non typical coffee taste/flavors (rancidity, aftertaste) and loss of aroma, and it is faster in the presence of oxygen (4, 9). For these reasons, pH-regulator and antioxidant agents were previously selected in order to extend the shelf-life of coffee brew. A preliminary study on the sensory effects of additives to coffee brew was made taking into account that sensory properties are crucial for the coffee quality. Four pH-regulator agents (sodium and potassium carbonates and bicarbonates), one pH-regulator and antioxidant (sodium citrate), three antioxidants (sodium ascorbate, ethylenediaminetetracetic acid (EDTA), and sodium sulfite), and lactoserum were tested by sensory analysis and the results are shown in Table 2.

Carbonates and bicarbonates are used to reduce acidity in beverages, included coffee-type. Also, because these chemical compounds together with polymers are foam making agents very useful for cappuccino-type or milk-coffee beverages. Seventy-five ppm of sodium or potassium carbonates or bicarbonates was added to coffee. Sodium carbonate or bicarbonate showed no influence on aroma and taste/flavor of coffee when they were compared with a reference coffee brew (without additives). However, the addition of potassium salts increased not only bitterness, effect which is very well-known, but also astringency and aftertaste, and slightly decreased freshness aroma, diminishing Colombian coffee quality. Consequently, potassium carbonate and bicarbonate were rejected.

Citric acid is a pH-regulator and reducing agent very used in beverages and many other foods because its safety. In this study, to avoid the increase of protons, citric acid was added as sodium salt. However, sourness, and also bitterness and aftertaste, were the highest, and

freshness aroma and the typical acidity of Colombian coffee were the lowest in comparison to the reference coffee. For this reason, sodium citrate was also rejected.

Ascorbic acid acts as a potent water-soluble antioxidant by scavenging free radicals such as hydroxyl, peroxyl and hydroperoxyl radicals (16). Ascorbic acid was added to coffee brew as sodium salt, and a sensory profile similar to the reference coffee was observed.

EDTA is a transition metal chelator. Therefore, it inhibits the formation of hydroxyl radicals from hydrogen peroxide (17). The addition of EDTA to coffee increased the bitterness of the Colombian coffee brew, and consequently was rejected.

Sodium sulfite is a potent oxygen scavenger under neutral and basic conditions. Although it is broadly applied for shelf life extension in food industry, its application is progressively limited because its allergenicity. The addition of sodium sulfite to coffee decreased the aroma and increased the undesirable taste/flavors (bitterness, sourness, astringency and aftertaste). Therefore, this additive was also rejected.

Taking into account that the addition of milk to coffee and tea is very common to reduce bitterness and astringency and that milk has an almost neutral pH, lactoserum was also used in the preliminary study. This milk component was preselected because the aim of this study is focused on black coffee. The addition of lactoserum to coffee only decreased the bitterness, but did not modify any other sensory attributes of the Colombian coffee brew. For this reason, this additive was selected for the next step.

The effect of a higher concentration (100 ppm) of the preselected additives (sodium carbonate, bicarbonate and ascorbate, and lactoserum) was also studied. However, the results of the sensory analysis (data not shown) revealed that the use of a higher dose had a negative influence on the coffee brews. Therefore, the concentration of 75 ppm was used in the subsequent studies.

The next step for the selection of the additives was to carry out a short-term study (8 days) with coffee brews prepared with sodium carbonate, sodium bicarbonate, sodium ascorbate and lactoserum at a concentration of 75 ppm. As in the previous experiments, sensory analysis was the selection test. A recent coffee brew without additives was used as reference coffee, namely fresh coffee, because the final goal of this study was to obtain a stored coffee brew similar to fresh coffee. The results of the sensory analysis are shown in Table 3. Both aroma intensity and freshness kept quite stable throughout the study, even though a tendency to decrease with time was observed. The relative aroma stability might be due to the absence of oxygen. As Charles-Bernard and co-workers (2005) observed, this absence had a higher stabilizing effect on the volatile thiols, some of them related to coffee freshness aroma, than antioxidants such as sodium ascorbate (18). Rancid burnt and/or spicy aromas and flavors were not perceived in any of the studied coffee brews along the storage time. Bitterness and astringency maintained low scores, with small variations, throughout the time. In contrast, typical coffee acidity and persistence showed a tendency to decrease whereas sourness and aftertaste tended to increase. These changes were more intense in coffee brews with lactoserum. Moreover, sourness was perceived in the 4th day in coffee brews with sodium ascorbate. Therefore, sodium carbonate and bicarbonate were selected for the long-term study.

Influence of sodium carbonate and bicarbonate on the coffee brew stability

Coffee brews with sodium carbonate or sodium bicarbonate (75ppm) and a reference coffee brew (without additives), aseptically bottled without headspace and stored at 4ºC for 90 days, were analyzed.

The microbiological analysis of the coffee brews during the long-term study showed a colony count number lower than 1 cfu/mL both for mesophilic flora and for moulds and

yeasts. Therefore, the aseptic handling of the sample preparation and the bottling storage were effective to avoid the microbiological contamination of the coffee brews.

pH and soluble and volatile compounds related to the sensory properties of coffee brews were studied. A two-way ANOVA was performed to establish the impact of the additive and the storage time on the pH and the chemical compounds of coffee brews (Table 4). In most cases, significant interaction between the additive and the storage time has been observed. Those compounds that have no significant interaction effect were significantly affected by both factors, except caffeine, ferulic and acetic acids which were not significantly affected by the additives. Moreover, F values corresponding to the storage time were higher than the F values of the additives for all soluble compounds and most of the volatiles, showing greater importance of the storage time effect which was in detail described in previous works (4, 9).

The effect of the sodium carbonate and bicarbonate on the pH of coffee brews throughout storage at 4ºC is shown in Figure 1. As pH-regulator agents, both additives significantly suppressed the reduction in pH over storage time increased the pH of coffee brews. At initial time, there were significant higher pH of the sodium carbonate (5.04) and bicarbonate (5.02) coffee brews than the reference (coffee brew without additives, 4.97). Sodium carbonate coffee brew pH decreased the least. Although both additive coffee brews did not reach pH lower than 4.8 considered as the limit of the acceptance by some authors (19, 20), sodium bicarbonate coffee brew pH decreased faster than the carbonate brew. In fact, at 7 days pH was not significantly different than reference coffee one. But, after 20 days, pH decrease of bicarbonate coffee brew was progressively slower in comparison to the reference and similar to carbonate coffee brew. This different behavior may be attributed to the hydrogen cation of the bicarbonate that partially contributed to the pH decrease.

Figure 2 shows the effect of the sodium carbonate and bicarbonate on the soluble compounds of coffee brews throughout storage at 4ºC. The addition of these pH-regulator

agents did not influence the changes induced by the storage time (4). Only a higher, but not statistically significant, decrease of 5-CQA in sodium carbonate coffee brew could be observed. This slightly lower amount of 5-CQA could be due to the influence of a higher pH on a lower hydrolysis of chlorogenic acid lactones formed during coffee roasting (21), a higher isomerization to 3-CQA and 4-CQA or a lower release of chlorogenic acids from non covalently linked polymeric skeletons, such as melanoidins (22), but not to decomposition to caffeic and quinic acids because the former was also lower, but not significantly, in the carbonate coffee brew.

Coffee aroma is one of the most appreciated characteristics of coffee brews and its loss is one of the consequences of staling. For this reason, the influence of the sodium carbonate and bicarbonate on the most frequently reported coffee aroma impact compounds (13, 23-29). One sulfur compound, 6 aldehydes, 4 ketones, 3 pyrazines, 1 acid and 1 phenolic compound were analyzed and the results are shown in Table 5.

Neither methanethiol, a sulphur compound responsible for freshness aroma in ground roasted coffee (30) and in espresso coffee (13), nor guaiacol (2-methoxyphenol), responsible for phenolic and spicy aromas (24) and phenolic and burnt flavors (26), were present at detectable levels in any coffee brew throughout storage. Two ethylpyrazine, 2-ethyl-6-methylpyrazine and 2-ethyl-3,5-dimethylpyrazine, associated with roasty and earthy/musty flavors in ground roasted and brewed coffees (23, 24), and with flowery and fruity aromas of coffee brews in the case of 2-ethyl-6-methylpyrazine (29) were not detected. Similar results were also observed in coffee brews stored in the presence of air (9).

The most abundant volatile compounds, 3-methylbutanal, 2-methylbutanal and 2-methylpropanal (Strecker aldehydes) did not show significant interaction in the two-way ANOVA (Table 4), but they were significantly affected by both additive and storage time. The addition of sodium carbonate and bicarbonate to coffee brew induced to a lower amount

of these volatiles, but also for most of the other aldehydes and ketones. Moreover, the absence of oxygen induced less change over time in comparison with results previously reported for coffee brews stored with air headspace, which should lead to better maintenance of coffee aroma (9).

Acetic acid, which has no significant interaction effect in the two-way ANOVA (table 4), was significantly affected only by storage time. The absence/presence of oxygen seems not to have influence in the increase of this volatile because similar results were observed in coffee brews stored with air at the same temperature (4ºC) (9). However, the storage temperature was critical.

Finally, the influence of the sodium carbonate and bicarbonate on the sensory quality of coffee brews throughout storage at 4ºC is shown in Figure 3. Although the additives decreased the original acidity of the Colombian coffee brews, the acidity score was adequately high and the other sensory attributes were hardly affected. With time, the typical acidity of the Colombian coffee brews was maintained up to 30 days in sodium carbonate coffee brew whereas it decreased in the others because the increase of sourness unbalanced the global acidity. Even though the perception of sourness and other non typical coffee taste and flavors, such as aftertaste and astringency, were established at 20 days shelf-life for reference coffee brew (confirming the shelf-life established in Pérez-Martínez et al. 4, 9) and 60 days for both coffee brews with sodium carbonate or bicarbonate, the addition of carbonate maintained better the aroma and taste/flavor of coffee brews.

Principal Component Analysis.

Principal Component Analysis (PCA) is a method that aims to recognize patterns in multivariate data sets or to reduce the dimensionality of a data set obtaining linear combinatios of original variables called Principal Components (PCs). In this paper, taking into account the high number of physico-chemical and sensorial parameters and points of

analysis, this method appeared to be very useful in order to describe the global changes of the coffee brews with or without additives throughout the storage at 4ºC. Five PCs with eigenvalues higher than 1 were selected by PCA. PC1 and PC2 explained 70.2% of the total variance. Figure 4 shows bidimensional plots of PC1 and PC2 parameter loadings and sample scores. PC1, which explained 53.2% of the total variance, is mainly characterized by sensory attributes, pH and most of the coffee aroma compounds. PC2, which explained 17.0% of the total variance, is mainly characterized by soluble compounds (caffeic acid, 5-CQA and caffeine) and the rest of the volatiles.

As can be seen, when the storage time was increased, coffee brews were moved on the left half-graphic from the top to the bottom due to coffee aroma decrease, however, the typical attributes of Colombian coffee were maintained. On the loss of coffee quality, the products moved to the right half-graphic because the decrease of pH, the increase of acetic acid and the presence of sourness and other non typical coffee taste and flavors, such as rancidity. Moreover, although all the coffee brews shows a global pattern very similar, the reference coffee brew (without additives) is placed on the right of the coffee brews with additives mainly because of the lower pH and good sensory attributes even during the first days, and sodium carbonate coffee brew is on left because this coffee brew maintain longer the coffee quality.

Discriminant analysis.

Discriminant Analysis (DA) is the best-known and most often used supervised classification method in which knowledge of the grouping structure is used to develop rules which predict the group that a new object belongs to. Stepwise Discriminant Analysis (DA) was applied to obtain a simple equation by which the coffee brew samples could be classified. When Stepwise Discriminant Analysis (SDA) was applied to all physico-chemical

parameters, two discriminant functions (DF) were obtained. The DF1 which explained 96% of the total variance is shown:

y = 32.250*pH – 2.830*caffeic acid + 0.003*2-propanone– 0.001*2,3-butanodione + 0.003*acetic acid -158.306

DF1 allowed the classification of the coffee samples into their respective group with a success rate of 75.3%. However, very different parameters participated in the obtained functions. Some of them, such as pH, were measured by simple methods, but others were measured by HPLC (soluble compounds) or HS-GC-MS (volatiles). Consequently, from the analytical point of view, this equation only partially contributed to simplify the analyses in order to differentiate the three types of coffee brews. For this reason, and because the changes in acidity and taste/flavor related compounds are the most relevant in coffee brews, a new SDA was applied only to pH and soluble compounds. Two discriminant functions, using only pH and caffeic acid, were obtained. DF1 which explained 100.0% of the total variance was:

y = 18.985*pH - 3.427*caffeic acid - 87.204

Figure 5 shows the sample results for DF1 and DF2, and the centroids scores. DF1 allowed the classification of the coffee samples into their respective group with a success rate of 81.5%. The sodium carbonate and bicarbonate coffee samples stored during 90 days were misclassified and included in the group of reference coffee brews, maybe because they overpassed the shelf-life and consequently they lost the coffee quality.

In summary, among all the tested additives both sodium carbonate and sodium bicarbonate were the most effective to keep the coffee brew quality longer. In fact, a shelf-life of 60 days was proposed for these coffee brews, in comparison with the 20 days shelf-life established for a coffee brew without additives (4). However, sodium carbonate was the chosen additive because is the most useful to reduce the pH decrease and the appearance of sourness, which

are some of the main factors involved in the rejection of stored coffee brews and maintain better the aroma and taste/flavor. Moreover, the application of multivariate analysis facilitated, firstly the description of the global changes of the coffee brews with or without additives throughout the storage at 4ºC using the PCA, and secondly, to obtain a simple equation with pH and caffeic acid parameters to discriminate the three types of coffee brews and simplify the analytical process, by means of the SDA.

ACKNOWLEDGEMENTS

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FIGURE CAPTIONS

Figure 1. Effect of the sodium carbonate and bicarbonate on the pH of coffee brews throughout storage at 4ºC.

Figure 2. Effect of the sodium carbonate and bicarbonate on the soluble compounds.

Figure 3. Effect of the sodium carbonate and bicarbonate on the sensory profile of coffee brews at 0, 30, 60 and 90 days.

Figure 4 Principal Component Analysis (PCA) of the coffee brews throughout storage at 4ºC, a) parameter loadings, b) sample scores.

Figure 5. Discriminant scores and centroid values of the coffee brew samples.

Table 1. Gradient solvent system and flow used in the method for the determination of 5-caffeloilquinic acid.

Time (min)	Dilution (acetonitrile:water)	Flow (mL/min)
0	12.0: 88.0	1.000
5	7.5: 92.5	1.600
10	8.0: 92.0	1.600
15	25.0: 75.0	1.600
20	12.0: 88.0	1.100

Table 2. Sensory analysis of the coffee brews with additives (75 ppm).

Parameter	Reference	Sodium carbonate	Potassium carbonate	Sodium bicarbonate	Potassium bicarbonate	Sodium citrate	Sodium ascorbate	EDTA	Sodium sulfite	Lactoserum
AROMA										
Intensity	8±1	7±1	7±1	8±1	6±1	7±0	7±1	7±1	6±1	7±1
Freshness	8±1	7±1	6±1	8±0	6±1	6±1	7±1	7±0	6±0	7±2
TASTE/FLAVOR										
Bitterness	3±1	3±1	5±1	3±1	4±1	6±1	3±1	4±2	5±1	0±1
Acidity	8±1	7±1	7±1	7±1	5±1	5±0	7±1	7±1	7±1	7±0
Sourness	0±0	1±1	1±0	1±1	1±1	4±1	0±1	1±1	3±0	1±1
Astringency	1±1	2±1	4±1	2±0	4±1	3±0	2±1	2±1	5±1	2±1
Persistence	5±1	4±1	4±0	5±1	5±0	4±1	5±1	5±0	1±0	4±1
Aftertaste	0±0	1±1	3±1	1±0	4±1	5±1	0±0	1±1	4±1	1±1
Spicy	0±0	1±0	0±1	0±0	0±0	0±0	0±0	0±0	1±0	0±1
Burnt	0±0	1±1	0±1	0±0	0±0	0±0	0±1	0±0	0±0	0±0

All values are shown as means ± standard deviations.

Table 3. Sensory analysis of the coffee brews with additives during 8 days.

	Fresh coffee (Control)	Storage time (days)				
		0	**1**	**4**	**6**	**8**
AROMA						
AROMA INTENSITY	9-10					
Sodium carbonate		7±1	7±1	7±1	6±1	6±1
Sodium bicarbonate		7±1	6±0	7±1	6±1	7±1
Sodium ascorbate		6±1	7±1	6±1	5±0	6±1
Lactoserum		8±1	7±1	7±2	7±1	6±0
AROMA FRESHNESS	9-10					
Sodium carbonate		7±1	7±1	7±1	6±1	6±1
Sodium bicarbonate		7±1	6±1	6±1	5±0	7±1
Sodium ascorbate		7±1	7±1	6±0	5±1	6±1
Lactoserum		7±1	7±0	7±1	7±1	5±0
TASTE/FLAVOR						
BITTERNESS	0-1					
Sodium carbonate		2±0	2±0	2±1	3±1	2±0
Sodium bicarbonate		3±1	3±1	2±0	3±0	2±0
Sodium ascorbate		2±1	1±0	1±0	2±0	2±0
Lactoserum		2±1	3±1	1±0	1±0	3±1
ACIDITY	8-10					
Sodium carbonate		7±1	8±1	6±1	6±0	6±1
Sodium bicarbonate		6±0	7±1	7±1	6±1	7±1
Sodium ascorbate		7±1	8±1	6±2	6±1	6±1
Lactoserum		8±1	6±2	6±1	7±1	4±1
SOURNESS	0					
Sodium carbonate		0±0	0±0	0±0	0±0	1±0
Sodium bicarbonate		0±0	0±0	0±0	1±1	0±0
Sodium ascorbate		0±1	0±1	2±1	1±0	1±1
Lactoserum		0±0	1±0	1±0	2±1	3±1
ASTRINGENCY	0-1					
Sodium carbonate		1±0	0±1	1±1	1±1	0±0
Sodium bicarbonate		1±1	1±0	1±1	0±1	0±1
Sodium ascorbate		2±1	0±1	1±0	1±0	0±1
Lactoserum		1±0	1±0	1±1	1±0	1±1
PERSISTENCE	9-10					
Sodium carbonate		6±1	6±2	6±1	4±0	5±1
Sodium bicarbonate		4±1	5±1	5±1	5±1	5±1
Sodium ascorbate		5±1	4±1	6±1	5±0	4±1
Lactoserum		5±1	3±1	5±0	5±1	3±1
AFTERTASTE	0					
Sodium carbonate		0±1	1±0	1±1	2±1	3±1
Sodium bicarbonate		1±1	2±1	1±0	1±0	0±1
Sodium ascorbate		1±0	2±1	1±0	2±1	1±1
Lactoserum		2±1	4±1	2±1	1±1	4±1

All values are shown as means ± standard deviations.

Table 4. Two-Way ANOVA results of coffee pH and chemical compounds.

	Additive effect		Storage time effect		(Additive x storage time)	
	F	*p*	*F*	*p*	*F*	*p*
pH	399.48	***	295.34	***	15.68	***
Caffeine	0.97	ns	6.18	***	1.17	ns
5-CQA	18.29	***	67.60	***	4.83	***
Caffeic acid	14.29	***	77.32	***	2.79	**
Ferulic acid	1.50	ns	23.52	***	1.77	ns
4-vinylguaiacol	4.64	*	17.31	***	1.42	ns
Acetaldehyde	72.70	***	437.04	***	2.68	**
Propanal	26.08	***	22.99	***	2.87	**
2-Methylpropanal	30.85	***	28.00	***	0.64	ns
2-Propanone	13.26	***	34.57	***	2.10	*
2-Butanone	20.76	***	23.00	***	2.22	*
2-Methylbutanal	51.57	***	33.98	***	1.45	ns
3-Methylbutanal	17.34	***	2.77	*	1.05	ns
2,3-Butanedione	5.42	**	10.39	***	5.27	***
2,3-Pentanedione	6.48	**	28.21	***	4.07	***
Hexanal	5.34	**	10.05	***	4.16	***
Acetic acid	0.14	ns	27.83	***	0.51	ns

p: ns non-significant ($p > 0.05$); * significant ($p < 0.05$); ** very significant ($p < 0.01$); *** highly significant ($p < 0.001$).

Table 5. Effect of the sodium carbonate and bicarbonate on the aroma impact compounds (Area x 10^{-3}) of coffee brews throughout storage at 4ºC.[a]

QI[b]	KI[c]		Storage time (days)								
			0	**3**	**7**	**10**	**15**	**20**	**30**	**60**	**90**
SULFUR COMPOUNDS											
45	635	Metanethiol									
		Reference	nd	nd	nd	nd	nd	nd	nd	nd	nd
		NaHCO$_3$	nd	nd	nd	nd	nd	nd	nd	nd	nd
		Na$_2$CO$_3$	nd	nd	nd	nd	nd	nd	nd	nd	nd
ALDEHYDES											
43	645	Acetaldehyde[d]									
		Reference	1670±1[j]	981±18[c-f]	1061±27[e-h]	932±26[abc]	995±45[c-f]	965±62[cde]	990±49[c-f]	1128±69[gh]	1233±20[i]
		NaHCO$_3$	1645±47[j]	852±27[ab]	924±16[abc]	940±43[bcd]	934±43[bc]	923±16[abc]	944±38[bcd]	1054±21[efg]	1153±32[hi]
		Na$_2$CO$_3$	1660±5[j]	828±5[a]	897±21[abc]	829±19[a]	914±5[abc]	830±42[a]	849±26[ab]	1036±3[d-g]	1071±72[fgh]
58	712	Propanal									
		Reference	1120±1[abc]	1434±51[f-i]	1390±26[d-h]	1278±90[a-f]	1240±22[a-f]	1236±36[a-f]	1294±49[b-g]	1484±36[ghi]	1619±94[i]
		NaHCO$_3$	1228±107[a-f]	1178±188[a-d]	1292±98[b-g]	1208±38[a-e]	1230±56[a-f]	1182±75[a-d]	1214±31[a-e]	1312±72[c-g]	1533±49[hi]
		Na$_2$CO$_3$	1276±96[a-f]	1198±63[a-e]	1216±12[a-e]	1080±40[ab]	1172±54[a-d]	1072±30[a]	1120±42[abc]	1306±69[c-g]	1410±94[e-h]
41	747	2-Methylpropanal									
		Reference[3]	4915±1[a]	4717±296[a]	4941±336[a]	4472±269[a]	4573±434[a]	4610±631[a]	4715±365[a]	5222±309[ab]	6035±286[b]
		NaHCO$_3$[2]	4630±505[ab]	4139±152[a]	4521±60[ab]	4420±143[ab]	4484±92[ab]	4280±45[a]	4454±84[a]	4956±24[b]	5544±31[c]
		Na$_2$CO$_3$[1]	4330±9[a]	4153±143[a]	4096±154[a]	4145±187[a]	4266±70[a]	4017±206[a]	4100±259[a]	4888±122[b]	5388±71[c]
39	880	2-Methylbutanal									
		Reference3	5216±2[ab]	4868±89[a]	5277±241[ab]	4519±304[a]	4710±178[a]	4611±343[a]	5086±352[ab]	5607±329[b]	6304±368[c]
		NaHCO$_3$[2]	5026±661[bc]	4243±206[a]	4779±110[ab]	4351±70[ab]	4508±153[ab]	4400±110[ab]	4550±61[ab]	5026±109[bc]	5641±185[c]
		Na$_2$CO$_3$[1]	4257±178[a]	4328±196[a]	4481±34[a]	4017±115[a]	4454±69[a]	4063±194[a]	4167±29[a]	5067±80[b]	5373±415[b]
44	884	3-Methylbutanal									
		Reference[2]	7739±1[a]	8636±909[a]	7774±1104[a]	7898±1313[a]	7179±1225[a]	7789±1887[a]	7284±264[a]	7214±808[a]	8889±952[a]
		NaHCO$_3$[1]	6760±793[a]	6162±555[a]	6911±1340[a]	6497±1624[a]	6672±1066[a]	6989±1440[a]	6927±216[a]	7255±344[a]	7681±479[a]
		Na$_2$CO$_3$[1]	5309±17[ab]	6253±624[abc]	6538±746[abc]	5163±641[a]	5532±41[ab]	6286±1505[abc]	6500±798[abc]	7453±29[bc]	7968±1232[c]
56	1084	Hexanal									
		Reference	425±5[b-e]	443±34[b-e]	507±82[de]	369±86[b-e]	318±93[a-d]	318±48[a-d]	263±57[abc]	145±4[a]	144±63[a]
		NaHCO$_3$	399±4[b-e]	370±63[b-e]	415±51[b-e]	302±34[a-d]	445±28[b-e]	345±99[a-e]	223±29[ab]	328±84[a-e]	261±67[abc]
		Na$_2$CO$_3$	461±37[cde]	430±110[b-e]	473±4[cde]	343±52[a-e]	413±34[b-e]	253±70[abc]	228±10[ab]	346±56[a-e]	544±7[e]
KETONES											
58	753	2-Propanone									
		Reference	1841±1[ab]	2111±166[b-e]	2101±141[cde]	1916±78[abc]	1916±79[abc]	1872±109[abc]	1966±135[a-d]	2301±71[ef]	2574±127[g]
		NaHCO$_3$	1914±73[abc]	1867±95[abc]	1969±50[a-d]	2015±118[a-e]	1895±53[abc]	1844±30[ab]	1917±106[abc]	2219±126[def]	2452±77[fg]
		Na$_2$CO$_3$	2017±149[a-e]	1858±43[abc]	1779±25[a]	1826±97[ab]	1856±119[abc]	1779±42[a]	1754±101[a]	2159±66[cde]	2295±190[ef]
43	866	2-Butanone									
		Reference	493±1[a-d]	513±31[a-g]	572±39[c-g]	518±30[a-g]	502±46[a-f]	519±19[a-g]	542±16[b-g]	592±46[e-h]	672±19[h]
		NaHCO$_3$	574±20[d-g]	501±24[a-e]	520±27[a-g]	497±6[a-e]	489±8[a-e]	473±21[ab]	474±26[abc]	604±8[gh]	607±75[gh]
		Na$_2$CO$_3$	476±24[abc]	482±25[a-d]	451±22[ab]	446±25[ab]	481±22[a-d]	438±63[a]	474±12[abc]	577±18[d-g]	596±56[fgh]
43	962	2,3-Butanedione									
		Reference	795±6[b-e]	774±128[b-e]	798±236[b-e]	813±12[cde]	760±32[b-e]	722±12[b-e]	707±28[b-d]	703±25[a]	849±55[de]
		NaHCO$_3$	834±39[de]	815±26[cde]	829±28[cde]	822±2[cde]	721±6[b-e]	697±34[b-e]	662±61[bcd]	802±46[b-e]	866±61[e]
		Na$_2$CO$_3$	854±14[de]	771±7[b-e]	779±16[b-e]	709±15[b-e]	705±12[b-e]	615±36[b]	634±26[bc]	769±13[b-e]	754±121[b-e]
43	1058	2,3-Pentanedione									
		Reference	1321±2[h]	1315±49[gh]	1196±262[fgh]	1046±203[d-h]	1057±88[d-h]	684±17[a-d]	521±90[ab]	544±42[a]	589±12[abc]
		NaHCO$_3$	1298±33[gh]	1164±88[fgh]	1234±69[fgh]	1127±180[e-h]	873±26[b-f]	1101±98[d-h]	958±148[c-h]	823±189[b-f]	720±63[a-e]
		Na$_2$CO$_3$	1326±85[h]	1182±63[fgh]	1187±32[fgh]	1069±196[d-h]	979±137[c-h]	547±86[ab]	740±429[b-e]	893±120[b-g]	827±17[b-f]
PYRAZINES											
107	1359	2-Ethylpyrazine									
		Reference	nd	nd	nd	nd	nd	nd	nd	nd	nd
		NaHCO$_3$	nd	nd	nd	nd	nd	nd	nd	nd	nd
		Na$_2$CO$_3$	nd	nd	nd	nd	nd	nd	nd	nd	nd
121	1395	2-Ethyl-6-methylpyrazine									
		Reference	nd	nd	nd	nd	nd	nd	nd	nd	nd
		NaHCO$_3$	nd	nd	nd	nd	nd	nd	nd	nd	nd
		Na$_2$CO$_3$	nd	nd	nd	nd	nd	nd	nd	nd	nd

135	1455	2-Ethyl-3,5-dimethylpyrazine									
		Reference	nd	nd	nd	nd	nd	nd	nd	nd	nd
		NaHCO$_3$	nd	nd	nd	nd	nd	nd	nd	nd	nd
		Na$_2$CO$_3$	nd	nd	nd	nd	nd	nd	nd	nd	nd

ACIDS

45	1480	Acetic acid									
		Reference[1]	338±8^a	512±12^{ab}	620±68^{ab}	596±40^{ab}	514±82^{ab}	675±33^b	908±156^c	1090±507^c	1472±473^d
		NaHCO$_3$[1]	343±67^a	494±98^{ab}	542±36^{ab}	558±20^{ab}	573±142^{ab}	750±118^b	1003±162^c	990±412^c	1394±32^d
		Na$_2$CO$_3$[1]	343±40^a	592±40^{ab}	663±30^{ab}	587±31^{ab}	567±23^{ab}	672±40^b	1032±27^c	1003±298^c	1161±18^d

PHENOLIC COMPOUNDS

109	1864	Guaiacol									
		Reference	nd	nd	nd	nd	nd	nd	nd	nd	nd
		NaHCO$_3$	nd	nd	nd	nd	nd	nd	nd	nd	nd
		Na$_2$CO$_3$	nd	nd	nd	nd	nd	nd	nd	nd	nd

[a] All values are shown as means ± standard deviations. In each row and column, different letters indicate statistically significant differences (p<0.05) throughout the time. nd, not detected. The number in the name in the sample indicates that there was no interaction with the storage time and the additive addition. Different number in the name in the sample indicate statistically significant differences (p<0.05) throughout the time due to the additive addition.

[b] QI: Ion used for the Quantification of the compound.

[c] KI: Kovats Index calculated for the HP-Wax capillary column.

Figure 1. Effect of the sodium carbonate and bicarbonate on the pH of coffee brews throughout storage at 4ºC.
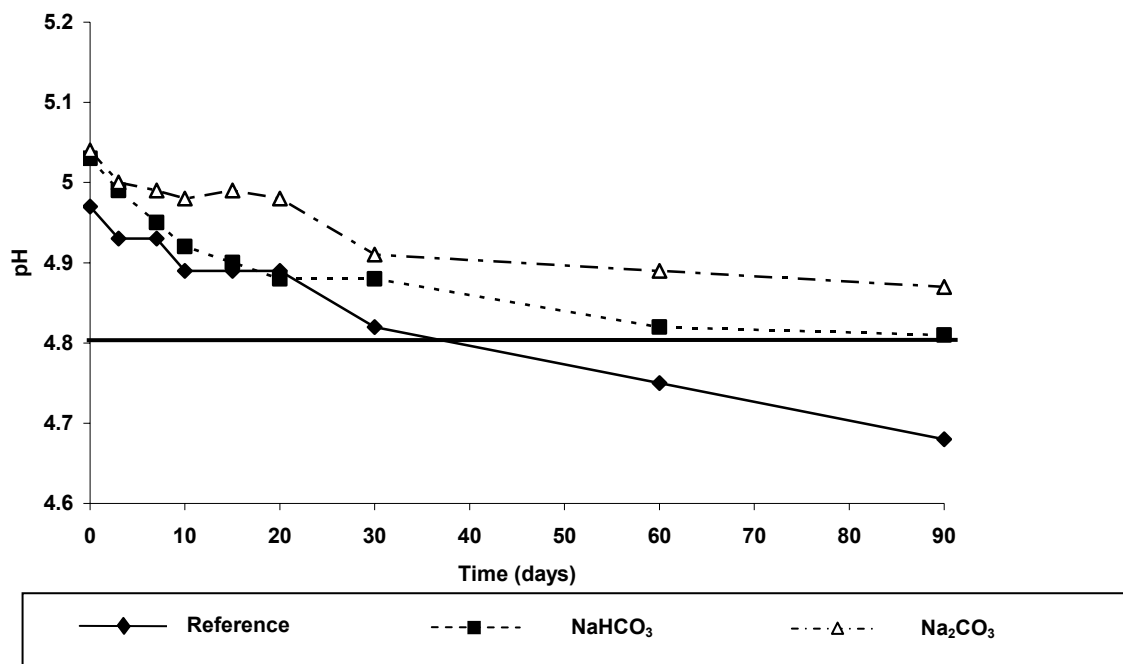
Figure 2. Effect of the sodium carbonate and bicarbonate on the soluble compounds.
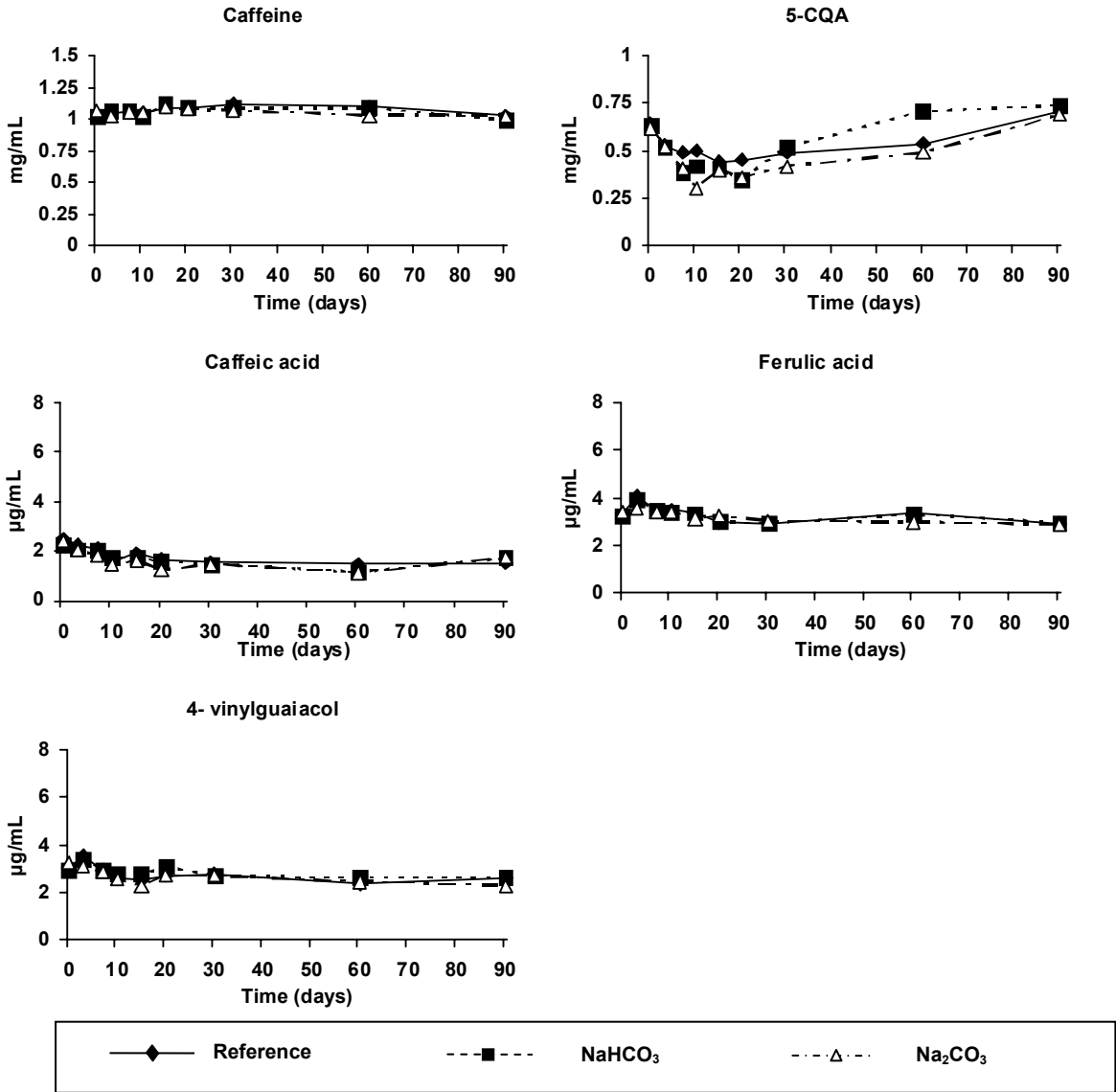
Figure 3. Effect of the sodium carbonate and bicarbonate on the sensory profile of coffee brews at 0, 30, 60 and 90 days.
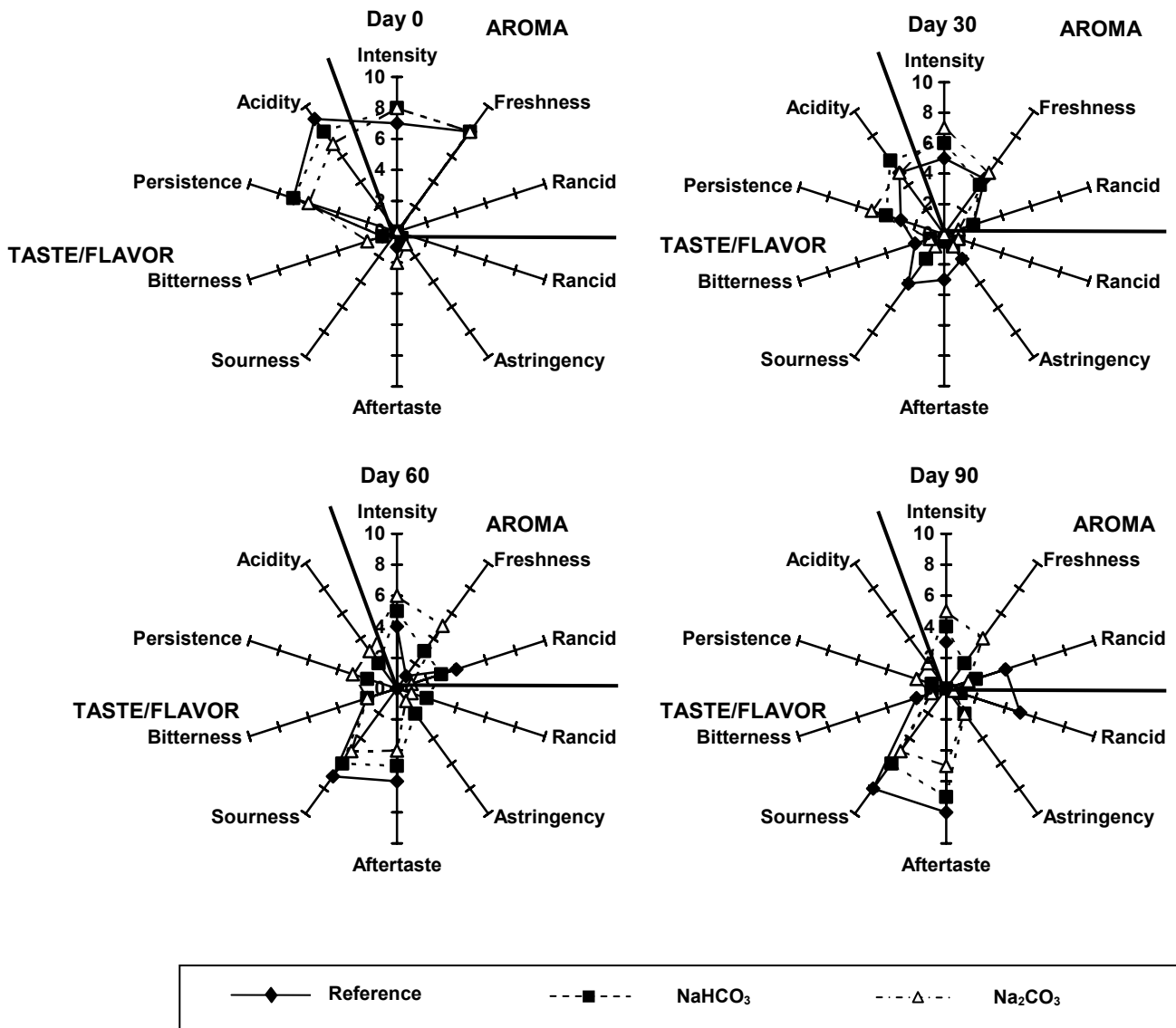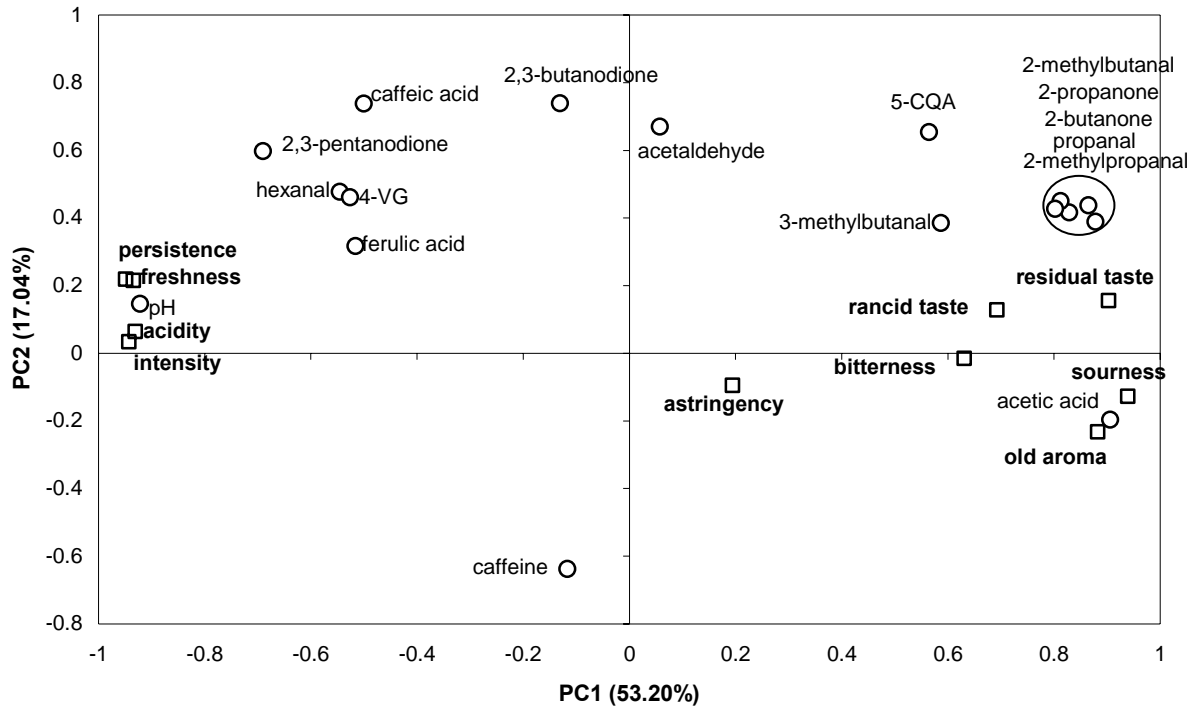
Figure 4.-Principal Component Analysis (PCA) of the coffee brews throughout storage at 4ºC, a) parameter loadings, b) sample scores.
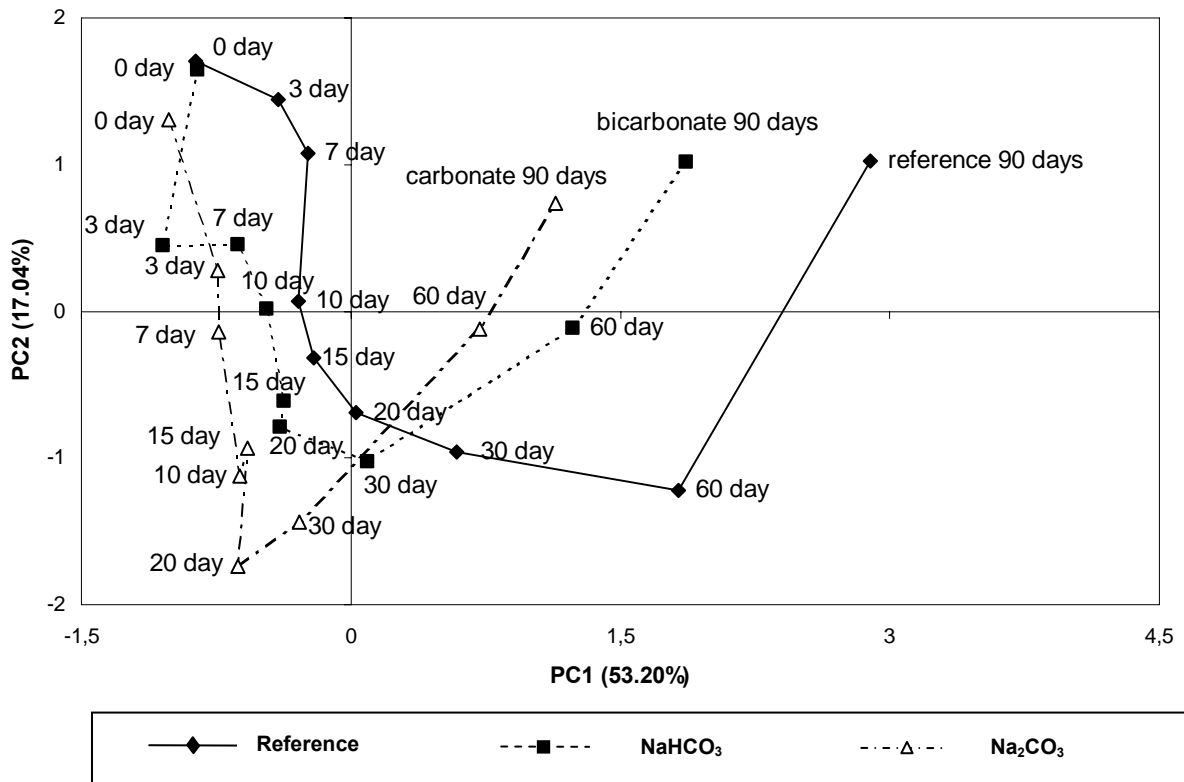
a) parameter loadings

b) sample scores

Figure 5. Discriminant scores and centroid values of the coffee brew samples.