DETERMINATION OF THE RELATIONSHIP BETWEEN PHOSPHATE CONCENTRATION AND PERCEIVED ACIDITY IN COFFEE

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INTRODUCTION

In 1997 Kenya Kagumo coffee was purchased in Nairobi at a record high price due to its pronounced acidity.[1] After initial analysis by Michelson Laboratories using ion exclusion chromatography, it was concluded that the phosphoric acid concentration was significantly higher in the Kenya Kagumo sample than in other Kenyan coffees.[2] It was hypothesized that the increase in hydrogen ion concentration from the dissociation of phosphoric acid governs the perceived acidity of coffee.[3]

Currently, two positions are held on the role of phosphoric acid in coffee. One explanation is that phosphoric acid is present in coffee, but is neutralized by excess potassium in solution and therefore makes no direct contribution to the perceived beverage acidity.[4] Another explanation is that phosphoric acid is present in coffee and strongly influences flavor as a result of the low pK_a of phosphoric acid.[3,5] No studies have reported a precise comparison of the phosphate content of coffees of different origins.

Given the uncertainty regarding phosphate levels in coffee and the influence of phosphate on the taste of coffee, we have measured phosphate concentrations in Kenyan Kagumo (1997), Kenyan Mweiga (1998), Aged Java Old Brown (1996/1997), Sumatran Mandheling Golden Pwani (1998/1999), Costa Rican Tarrazu Papagayo (1998/1999), and Indian Cherry Robusta (1998/1999) coffees to determine if there is a correlation between perceived acidity and phosphate concentration. The perceived acidity is also strongly affected by the degree of roasting of the coffee. The possible connection between roasting, acidity, and phosphate concentration was studied by determining the phosphate concentration in Colombian La Vareda (1999) coffee of different roast degrees.

Upon extraction of a coffee, acid-base reactions involving phosphate and numerous other species occur, ultimately leading to a coffee solution of approximately pH 5. Phosphoric acid has dissociation constants[6] of 7.1×10^{-3}, 6.3×10^{-8}, and 4.2×10^{-13}; therefore, in a coffee solution one will find almost exclusively the

dihydrogen phosphate ion, irrespective of whether the phosphate originally existed as phosphoric acid or as a phosphate salt. For this reason, we have focused our investigation on the total concentration of phosphate in coffee and have not attempted to identify the original form in which the phosphate existed.

EXPERIMENTAL METHODS

Materials. ACS reagent grade standards of acetic acid, citric acid monohydrate, formic acid, lactic acid, malic acid, oxalic acid, potassium nitrate, pyruvic acid, quinic acid, sodium dihydrogen phosphate monohydrate, sodium chloride, sodium sulfate, sodium sulfite, and succinic acid were used as received from the supplier. Acid-base buffers for pH calibrations were obtained from Fisher.

Coffee Samples. Coffee samples were roasted by the Specialty Coffee Institute in a STA Impianti (Bologna, Italy) laboratory roaster and used within 36 hours of roasting. Table 1 shows the Agtron number, roasting time, the initial and final temperatures, and percent weight loss for the Colombian La Vareda coffee. All other coffees were roasted to a medium-brown roast (Agtron # 55 ± 2). Coffee samples were ground on a Ditting grinder setting 8.

Table 1. Roasting specifications for the Colombian La Vareda coffee.

Agtron Number	Roast Time (min)	Initial Temperature (°C)	Final Temperature (°C)	Percent Weight Loss
74.0	9.3	176.6	179.4	8.77
65.1	10.1	176.6	185.0	11.13
55.0	10.8	176.6	196.1	13.71
43.7	11.5	176.6	198.3	15.90
36.7	12.2	176.6	205.0	18.09
26.5	13.0	176.6	208.9	23.27

Coffee Extraction. Ground coffee (5.500 g) was submitted to extraction by pouring 100.0 mL of boiling de-ionized water over the ground coffee in an insulated beaker. The coffee was extracted for 4.5 minutes with constant stirring and isolated by suction filtration. The coffee sample was cooled to 25°C, and the pH was determined using an Accumet AR10 pH meter equipped with a standard single-junction glass-Ag/AgCl combination electrode, which was calibrated at pH 4 (0.5 M potassium hydrogen phthalate buffer) and pH 7 (0.5 M potassium dihydrogen phosphate/sodium hydroxide buffer). The percent extracted solids was determined by drying 20.0 mL of coffee extract in a pre-weighed beaker to a constant weight. Each coffee was brewed twice, and two trials were performed with each extract. Coffee extraction was performed five minutes prior to analysis.

Qualitative Analysis of Phosphate. Activated carbon (5.0 g) was rinsed (5x) with boiling de-ionized water to remove residual phosphate. The coffee was de-colorized by mixing 10 mL of coffee extract with 2.5 g of the phosphate-free activated carbon (2x) for 5 minutes. Nitric acid (3 mL, 6 M) and ammonium molybdate (3 mL, 2 M) reagents were added to the decolorized filtered coffee extract.

Anion Exchange Chromatography. Anion analysis was performed using a Dionex DX-100 Chromatograph equipped with a conductivity detector. A Dionex IonPac AS14 4-mm (10-32) anion exchange column, a Dionex AG14 4-mm (10-32) guard column and a Dionex ASRS-I 4-mm self-regenerating suppression system were used. All samples were filtered through a 0.2 μm Nylon membrane filter (Millipore) and 25.0 μL of the each sample was injected onto the column. The aromatic anions present in coffee did not elute under normal chromatographic conditions. In order to maintain good column performance, the columns were cleaned every 25 trials with de-ionized water for 15 minutes, a solution of 5% acetonitrile and 20% sodium chloride (1 M,

adjusted to pH 2 with HCl) for 10 min, a solution of 80% acetonitrile and 20% sodium chloride (1 M, adjusted to pH 2 with HCl) for 60 minutes, and de-ionized water for 180 minutes. Calibration standards were re-run after each cleaning.

Anion standards were run individually by diluting 5 mL of coffee extract to 10 mL using 150 mg L^{-1} solutions of either acetic acid, citric acid, formic acid, lactic acid, malic acid, oxalic acid, potassium nitrate, pyruvic acid, sodium chloride, sodium dihydrogen phosphate, sodium sulfate, sodium sulfite, succinic acid, or quinic acid. Chromatographic analysis for standard additions of mono-anions was performed using a sodium hydroxide (1 mM) mobile phase, and analysis for standard additions of di-anions and tri-anions was performed using a mobile phase of sodium carbonate (1.75 mM) and sodium hydrogen carbonate (0.50 mM) at a flow rate of 2.0 mL min^{-1}.

Quantitative analysis of phosphate was performed using a 6.0 mM sodium hydrogen carbonate and 0.6 mM sodium carbonate mobile phase at a flow rate of 1.5 mL min^{-1}. Phosphate standards ranging from 40-130 mg L^{-1} phosphate in increments of 30 mg L^{-1} were prepared from successive dilutions of a standard 200 mg L^{-1} phosphate solution prepared from sodium dihydrogen phosphate monohydrate. Two runs were made with each phosphate standard.

RESULTS AND DISCUSSION

Qualitative Analysis of Phosphate. The presence of phosphate can be confirmed by treating a solution with nitric acid and ammonium molybdate. Upon addition of the ammonium molybdate, a bright yellow complex of triammonium dodecamolybdophosphate ($(NH_4)_3[PO_4{}^{\$}12\ MoO_3]^{\$}12\ H_2O$) is formed if phosphate is present.[7]

$$PO_4^{3-} + 12\ H_2MoO_4 + 3\ NH_4^+ \rightarrow (NH_4)_3[PO_4{}^{\$}12\ MoO_3]^{\$}12\ H_2O$$

Prior to subjecting a coffee solution to this test, it proved necessary to decolorize the coffee with activated carbon in order to permit careful observation of the yellow $(NH_4)_3[PO_4{}^{\$}12\ MoO_3]^{\$}12\ H_2O$ solid. To ensure that the activated carbon did not contain phosphate, 5 mL of boiling water was added to 5.0 g of the rinsed activated carbon, and allowed to sit for 5 minutes while boiling. The extract was isolated by suction filtration and analyzed both by ion chromatography and by adding nitric acid and ammonium molybdate reagents. No peak corresponding to phosphate ion was observed in the ion chromatogram, and the qualitative test did not

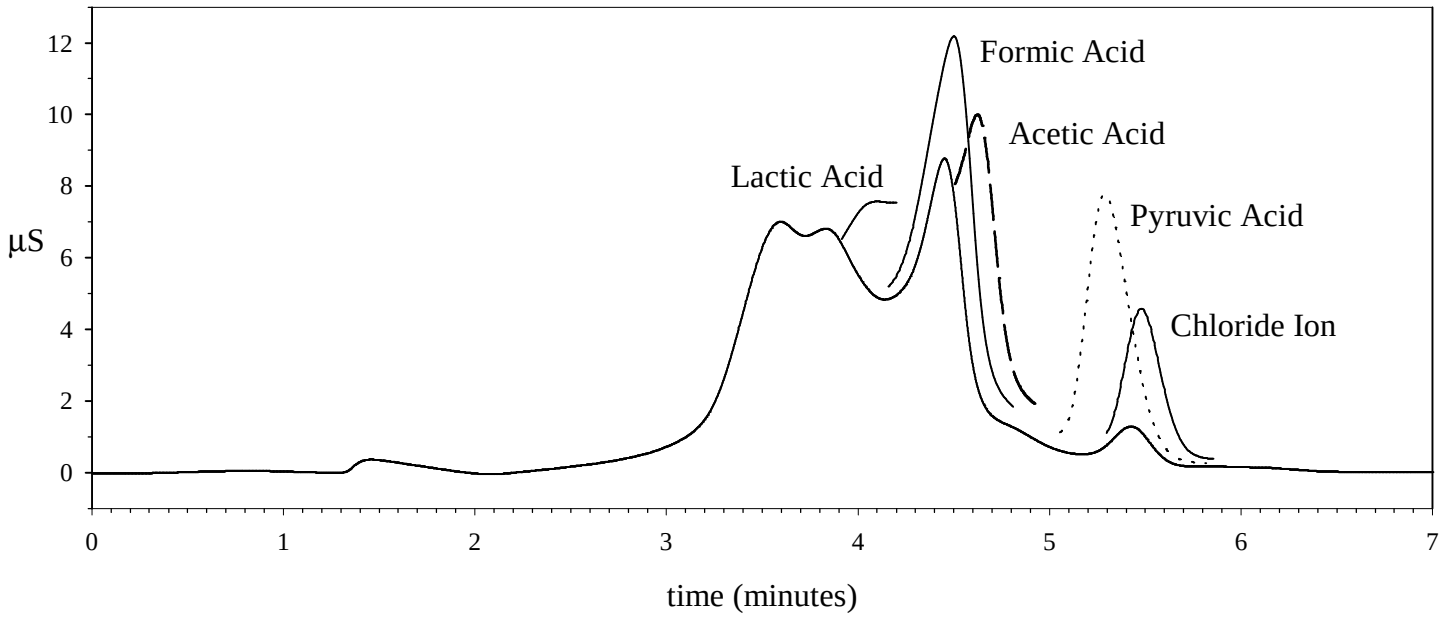

Figure 1. Chromatogram of Kenyan Kagumo coffee with standard additions of lactic acid, formic acid, acetic acid, pyruvic acid, and sodium chloride using a sodium hydroxide (1 mM) mobile phase at a flow rate of 2.0 mL min^{-1}.

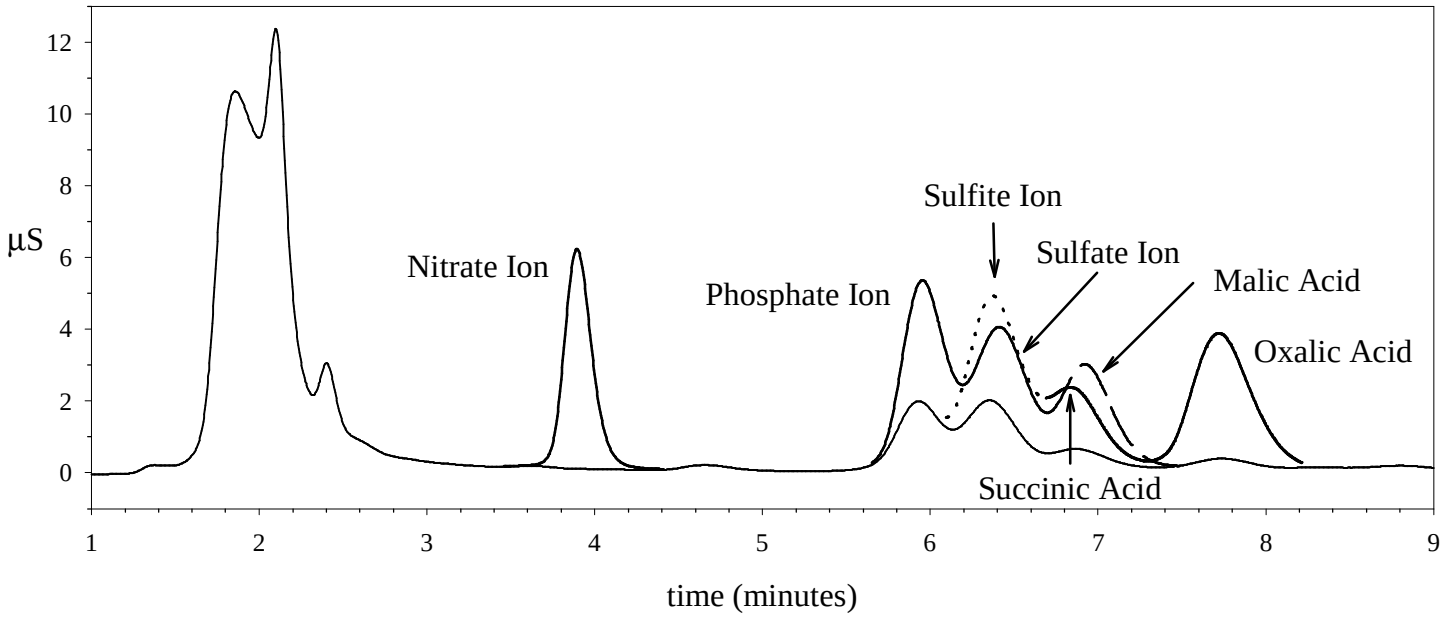

Figure 2. Chromatogram of Kenyan Kagumo coffee with standard additions of potassium nitrate, sodium dihydrogen phosphate, sodium sulfite, sodium sulfate, succinic acid, malic acid, and oxalic acid using a sodium carbonate (1.75 mM) and sodium hydrogen carbonate (0.50 mM) mobile phase at a flow rate of 2.0 mL min^{-1}.

result in the formation of a yellow solid, verifying that no phosphate was released from the activated carbon. When nitric acid and ammonium molybdate were added to a decolorized solution of Kenya Kagumo coffee a bright yellow solid precipitated verifying the presence of phosphate in the coffee.

Ion Chromatography. Optimal chromatographic conditions were determined using Kenyan Kagumo coffee. Coffee contains a complex mixture of anions, and it proved impossible to clearly resolve all detectable components. Figures 1 and 2 show representative chromatograms of the mono-anion and di-anion regions, respectively. Tri-anions and aromatic anions did not elute under the chromatographic conditions that were employed. These analytes were removed by frequent washing of the columns as described in the Experimental section.

Attempts were made to determine whether any common anions co-eluted with phosphate ion by co-injecting known anions with the coffee sample. Figure 1 shows the chromatogram of the Kenyan Kagumo coffee with standard additions of lactic acid, formic acid, acetic acid, pyruvic acid, and sodium chloride overlaid on a chromatogram of pure coffee to facilitate comparison. Figure 2 shows the chromatogram of the Kagumo coffee with standard additions of potassium nitrate, sodium dihydrogen phosphate, sodium sulfite, sodium sulfate, succinic acid, malic acid, and oxalic acid. No standard co-eluted with the phosphate standard.

The composition of the mobile phase and the flow rate were varied to shift the phosphate peak relative to other di-anionic components in order to check for unidentified co-eluting components. The integration of the phosphate peak remained constant under all chromatographic conditions, indicating that no significant component co-eluted with phosphate and that phosphate concentrations could be accurately determined. No peaks were observed in the mono- or di-anion region when a blank sample of de-ionized water, treated using the same extraction procedure but without the addition of coffee grounds, was injected onto the column. Thus, no extraneous phosphate was introduced during the handling of the samples.

Quantitative analysis of phosphate concentrations were performed using 6.0 mM sodium hydrogen carbonate and 0.6 mM sodium carbonate mobile phase and a flow rate of 1.5 mL min^{-1}. A calibration curve was created from peak area and concentration data, and a second-order polynomial was fit to the data as shown in Figure 3. A representative chromatogram of the di-anion region of coffee under these chromatographic conditions is shown in Figure 4.

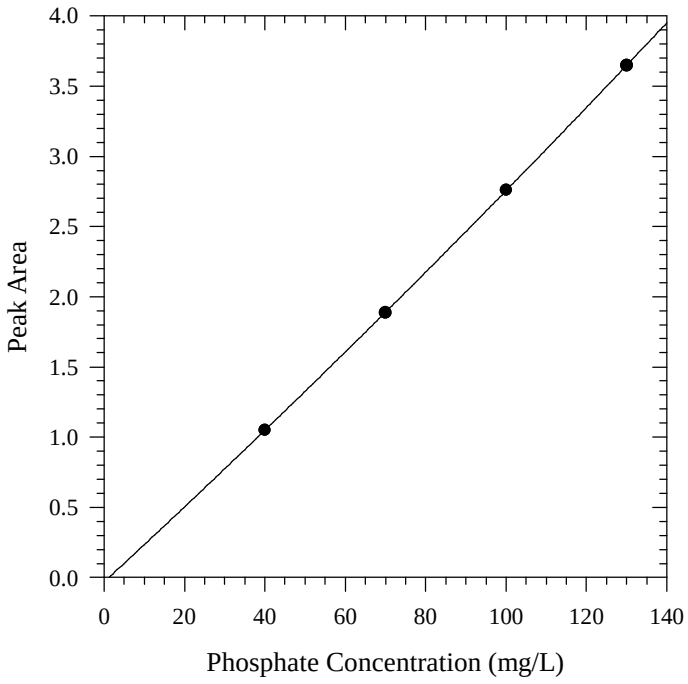

Figure 3. Calibration curve prepared from peak areas and concentration of phosphate standards. The solid line is a second-order polynomial fit to the experimental points. Two independent trials are plotted at each concentration.

Concentration of Phosphate in Coffees. The phosphate concentration of each coffee was determined to examine the relationship between phosphate concentration and perceived acidity. Phosphate concentrations were found to vary nearly linearly with the amount of extracted solids within the small range of concentrations employed in this study. In order to account for variations in phosphate concentration with the effectiveness of the extraction, the phosphate concentrations were normalized to 20 % extracted solids. The experimental results are given in Table 2. The average phosphate concentrations (at 20% extracted solids) were found to be: Kenya Kagumo 81.7 ± 2.7 mg L^{-1}, Costa Rica Tarrazu Papagayo 89.5 ± 0.8 mg L^{-1}, Kenya Mweiga 94.0 ± 1.2 mg L^{-1}, Sumatra Mandheling Golden Pwani 104.3 ± 2.3 mg L^{-1}, Aged Java Old Brown 128.0 ± 0.5 mg L^{-1}, and Indian Cherry Robusta 136.8 ± 0.8 mg L^{-1}.

The Kenya Kagumo sample, which was prized for its high acidity, had the lowest phosphate concentration. The Sumatra Golden Pwani, which was valued for its lack of acidity, had a relatively high phosphate concentration. Indeed, the coffee with the lowest perceived acidity, the Aged Java Old Brown, had the second largest phosphate concentration. In terms of the taste of the coffee, acidity was inversely correlated with the phosphate concentration. The coffees regarded as highly acidic had lower phosphate levels than those coffees known for being less acidic. On the other hand, the concentration of phosphate in the coffee was uncorrelated with the pH of the coffee, suggesting that phosphate species play a relatively minor role in establishing the pH of the coffee. The observed pH of coffee (4.9-5.5) lies outside the effective buffering regions of phosphate species (1.1-3.1 for

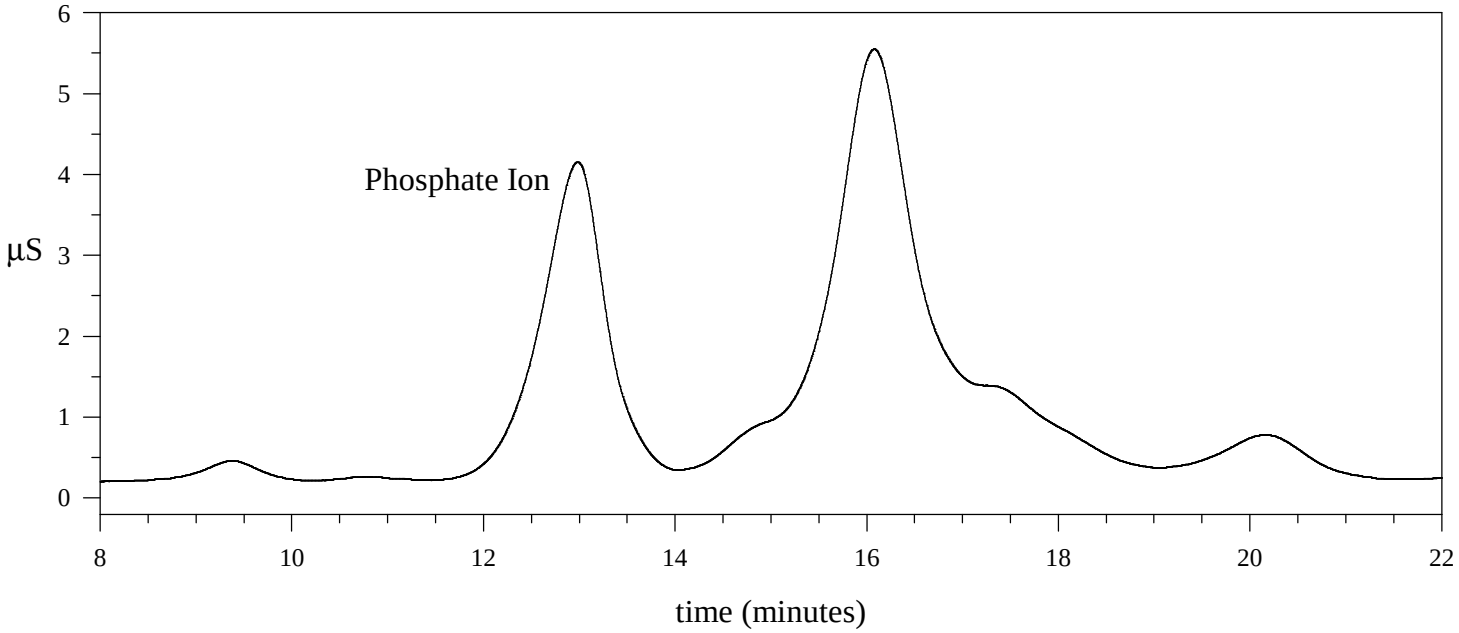

Figure 4. Di-anion region of Kenyan Kagumo coffee using 6.0 mM sodium hydrogen carbonate and 0.6 mM sodium carbonate mobile phase at a flow rate of 1.5 mL min^{-1}.

Table 2. Experimental phosphate concentration, pH, percent of solids extracted, and normalized phosphate concentration for coffees roasted to an Agtron value of 55 ± 2. Each coffee was extracted twice (indicated by the 1 or 2) and two trials (a or b) were performed with each extract.

Coffee	Extract (Trial)	Phosphate Concentration (mg L^{-1})	pH	Percent Solids Extracted	Normalized Phosphate Concentration (mg L^{-1})
Kenya Kagumo	1(a)	81.1	4.97	20.46	79.3
Kenya Kagumo	1(b)	81.1	4.97	20.46	79.3
Kenya Kagumo	2(a)	82.4	4.95	19.57	84.2
Kenya Kagumo	2(b)	82.1	4.95	19.57	83.8
Costa Rica	1(a)	78.6	5.13	17.65	89.0
Costa Rica	1(b)	78.7	5.13	17.65	89.1
Costa Rica	2(a)	78.7	5.13	17.62	89.3
Costa Rica	2(b)	79.9	5.13	17.62	90.7
Kenya Mweiga	1(a)	88.1	4.90	18.89	93.3
Kenya Mweiga	1(b)	87.7	4.90	18.89	92.8
Kenya Mweiga	2(a)	88.2	4.91	18.45	95.6
Kenya Mweiga	2(b)	87.2	4.91	18.45	94.5
Sumatra Golden	1(a)	88.1	5.07	17.10	103.1
Sumatra Golden	1(b)	86.9	5.07	17.10	101.7
Sumatra Golden	2(a)	91.4	5.08	17.20	106.3
Sumatra Golden	2(b)	91.4	5.08	17.20	106.2
Aged Java	1(a)	119.5	5.05	18.72	127.7
Aged Java	1(b)	120.2	5.05	18.72	128.4
Aged Java	2(a)	118.7	5.05	18.63	127.5
Aged Java	2(b)	119.6	5.05	18.63	128.4
Indian Robusta	1(a)	131.1	5.48	19.28	135.9
Indian Robusta	1(b)	132.9	5.48	19.28	137.8
Indian Robusta	2(a)	127.4	5.49	18.66	136.6
Indian Robusta	2(b)	127.6	5.49	18.66	136.8

$H_3PO_4/H_2PO_4^-$, 6.2-8.2 for $H_2PO_4^-/HPO_4^{2-}$, and 11.4-13.4 for HPO_4^{2-}/PO_4^{3-}), indicating that phosphate species do not provide any significant buffering action in coffee.

Effect of Roast Degree on Phosphate Concentration. A study of the variation in extractable phosphate concentration with roast degree was performed using Colombian La Vareda coffee. During the course of our study, it was observed that larger volumes of coffee grounds were brewed at the darker roasts, because each coffee was weighed out to 5.500 g, and the density of the grounds decreased with darker roasts. In order to account for this effect, phosphate concentrations were normalized to a constant mass of coffee prior to roasting. Each extraction was performed using 5.500 g of roasted coffee. If *w* represents the fraction of weight lost during roasting, then the original mass of the 5.500 g roasted sample prior to roasting would have been 5.500 g/(1-*w*). Over the relatively narrow range of conditions employed in this study, the phosphate concentration was found to vary approximately linearly with the mass of coffee brewed. The phosphate concentration was therefore multiplied by (1-*w*) in order to normalize each concentration to the value expected had 5.500 g of unroasted coffee been roasted and then extracted. The variations of phosphate concentration and pH with roast degree for the Columbian La Vareda coffee are given in Table 3.

The extractable phosphate concentration increased significantly early in the roast when other chemical changes such as fat hydrolysis, oxidation of acids, thermal degradation of esters and autoxidation of aldehydes and ketones are known to occur.[8] The normalized phosphate concentration at the lightest roast (Agtron #

Table 3. Agtron number, percent weight loss during roasting, percent solids extracted in each brew, phosphate concentration, pH, and normalized phosphate concentration for Colombian La Vareda coffee at various roast degrees. Each coffee was extracted twice (indicated by the 1 or 2) and two trials (a or b) were performed with each extract.

Agtron Number	Extract (Trial)	Percent Weight Loss	Percent Extracted Solids	Phosphate Concentration (mg L^{-1})	pH	Normalized Phosphate Concentration (mg L^{-1})
74.0	1(a)	8.77	18.30	64.1	4.90	58.5
74.0	1(b)	8.77	18.30	64.9	4.90	59.2
74.0	2(a)	8.77	19.47	65.0	4.85	59.3
74.0	2(b)	8.77	19.47	64.8	4.85	59.1
65.1	1(a)	11.13	19.01	77.5	4.89	68.9
65.1	1(b)	11.13	19.01	77.9	4.89	69.2
65.1	2(a)	11.13	18.43	77.7	4.89	69.1
65.1	2(b)	11.13	18.43	77.7	4.89	69.1
55.0	1(a)	13.71	20.55	89.3	5.08	77.1
55.0	1(b)	13.71	20.55	89.5	5.08	77.2
55.0	2(a)	13.71	20.57	89.8	5.06	77.5
55.0	2(b)	13.71	20.57	89.3	5.06	77.1
43.7	1(a)	15.90	20.05	92.1	5.37	77.5
43.7	1(b)	15.90	20.05	92.4	5.37	77.7
43.7	2(a)	15.90	20.29	92.8	5.36	78.0
43.7	2(b)	15.90	20.29	92.9	5.36	78.1
36.7	1(a)	18.09	20.81	93.8	5.71	76.9
36.7	1(b)	18.09	20.81	93.3	5.71	76.4
36.7	2(a)	18.09	20.39	95.6	5.72	78.3
36.7	2(b)	18.09	20.39	95.4	5.72	78.1
26.5	1(a)	23.27	21.25	99.2	6.13	76.1
26.5	1(b)	23.27	21.25	99.2	6.13	76.1
26.5	2(a)	23.27	21.28	99.9	6.13	76.6
26.5	2(b)	23.27	21.28	100.7	6.13	77.3

74.0) was determined to be 59.0 ± 0.4 mg L^{-1}. At the next degree of roast analyzed (Agtron # 65.1), the extractable phosphate concentration increased to 69.1 ± 0.1 mg L^{-1}. At roasts darker than Agtron number 55.0, the concentration of extractable phosphate stabilized to approximately 77 mg L^{-1}.

It is probable that the increase in phosphate concentration is the result of the decomposition of inositol-hexaphosphoric acid or other phosphate containing organic compounds.[9,10,11] A recent study[10] reported that the concentration of phosphoric acid concentration increased as the concentration of inositol-hexaphosphoric acid decreased during extended storage of brewed coffee at 60 °C, while another study[9] indicated that inositol-hexaphosphoric acid is decomposed at darker roasts. In both studies, higher temperatures resulted in increased phosphate concentrations in coffee.

This trend in phosphate concentrations, however, runs opposite to the observed decrease in perceived acidity at darker roasts. The relatively acidic lightly roasted coffees displayed the lowest phosphate levels, while the less acidic darker roasted coffees were found to possess the highest phosphate concentrations, consistent with the trend observed in the preceding section. Interestingly, the light roast coffees (with the highest perceived acidities) also displayed the lowest pH (highest chemical acidity), and the chemical acidity was observed to decrease (as evidenced by an increase in pH) as the roast degree increased. In this case, the pH trend

correlates well with the trend in perceived acidity. Phosphate concentrations, however, were observed to increase as the coffee pH increased.

CONCLUSIONS

The experimental data indicates that phosphate concentrations are relatively low in coffees of high perceived acidity, and phosphate concentration increases with an increase in pH as detailed in the roast study. While our data clearly indicates an inverse correlation between perceived acidity and phosphate concentration, this evidence is not sufficient to conclude that phosphate directly lowers the perceived acidity. When examining several different coffees of the same roast, no correlation between coffee pH and phosphate concentration was observed, despite relatively large variations in phosphate concentration. Conversely, the series of measurements made on Columbian La Vareda coffee at different roast degrees showed a strong inverse relationship between pH and phosphate concentration, with the phosphate concentration increasing and the chemical acidity of the coffee decreasing as the roast degree increased. This behavior is likely attributable to thermal degradation of organophosphorus compounds. If this degradation releases PO_4^{3-}, the basic PO_4^{3-} would quickly become protonated to form $H_2PO_4^-$, thereby increasing the pH of the coffee. It is also possible that the pyrolysis of the coffee releases other bases, which might also contribute to the change in pH.

Although we did not undertake a detailed study of the other anions, the chromatographic data clearly shows coffee to contain significant concentrations of numerous carboxylic acids. It should be noted that conductivity detection under the conditions employed in this study displays a higher sensitivity for the phosphate ion than for carboxylic acids. One can conclude, consistent with other findings[4], that carboxylic acids such as citric acid, malic acid, and the chlorogenic acids are much more important sources of hydrogen ions in coffee than is the phosphate ion. Since phosphoric acid is only a minor source of hydrogen ions it is reasonable to expect that it will not govern the acidity of coffee. The measured pH of coffee is very close to the pK_a values of carboxylic acids, indicating that these carboxylic acids are only partially deprotonated in coffee solution. Carboxylic acids undoubtedly play the dominant role in establishing the pH of coffee and in buffering the coffee.

SUMMARY

The phosphate concentration of Kenyan Kagumo, Kenyan Mweiga, Aged Java Old Brown, Sumatran Mandheling Golden Pwani, Costa Rican Tarrazu Papagayo, and Indian Cherry Robusta coffee was determined using anion exchange chromatography. The change in extractable phosphate with roast degree was determined using Colombian La Vareda coffee at roasts ranging from very light to very dark (Agtron # 75-25). Using a medium-dark roast (Agtron # 55 ± 2), the average phosphate concentrations (normalized to 20 % extractable solids) were: Kenyan Kagumo 81.7 ± 2.7 mg L^{-1}, the Costa Rican Tarrazu Papagayo 89.5 ± 0.8 mg L^{-1}, the Kenyan Mweiga 94.0 ± 1.2 mg L^{-1}, the Sumatran Mandheling Golden Pwani 104.3 ± 2.3 mg L^{-1}, the Aged Java Old Brown 128.0 ± 0.5 mg L^{-1}, and the Indian Cherry Robusta 136.8 ± 0.8 mg L^{-1}. It was observed that the concentration of extractable phosphate increased significantly during the beginning of the roasting process. The normalized phosphate concentration at the lightest roast (Agtron # 74.0) was determined to be 59.0 ± 0.4 mg L^{-1}, while the concentration at the darkest roast (Agtron # 26.5) was determined to be 76.5 ± 0.6 mg L^{-1}.

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