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Cations and anions in fresh fruit juice by fast ion chromatography

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ABSTRACT

Food products are analyzed to meet compliance with labeling and legal requirements, establish nutritive value, determine product quality, identify defects, research and development. This information is vital for economically producing safe and nutritious food. Ion chromatography is known to be an effective tool in identifying many types of cations and anions, with this work focusing on water soluble ions that are readily available in common fresh fruit products. Extraction into an aqueous partition layer revealed numerous anions and cations that were effectively separated by fast ion chromatography. Cation analysis utilized a Metrosep Cation 1-2 column and a ICSep AN1 column for anion analysis. Ions identified and measured included sodium, ammonium, potassium, calcium, magnesium, fluoride, chloride, nitrate, phosphate, and sulfate. Chromatograms identified the well resolved anion and cation species associated with consumption of fruits. Their concentrations were determined for dietary and nutritive considerations. Fast ion chromatography under conditions specified in this study coupled with non-suppressed conductivity detection is an efficacious approach to measure ion species associated with fresh fruits. Nutritive considerations, manufacturing regulations, and response to consumer demands make important the monitoring and determination of minerals and vitamins in fresh produce. Ion chromatography effectively measures various ions found in fresh fruits that are necessary nutriments for dietary sustainment. A broad spectrum of monovalent and multivalent ions were identified and measured utilizing these ion chromatography parameters.

KEYWORDS

Ion chromatography;
Cations;
Anions;
Fruits;
Minerals.

INTRODUCTION

Ion chromatography (IC) has been found to be an indispensable tool of analytical chemistry[1]. Various methodology has been developed to study complex mixtures of anions and cations and their separation for identification and measurement[1]. Polyatomic inorganic ions and even larger organic ions can be identified by IC when utilizing columns packed with the appropriate solid ion-exchange material. These types of analysis reach into many branches of physical sciences[1]. The capability to identify multiple ion species and polyvalent ions with a single sample injection is powerful characteristic of ion chromatography.

Whereas the types and amount of ions are a issue for dietary nutritionists the application of IC enables better understanding of the nutritional value of food preparations. With the application of judicious approaches to sample preparation it is possible to determine inorganic ions in a wide variety of food products[2]. Manufacturers have specific parameters to comply in order to adhere to governmental requirements and product quality. Accordingly the measurement of the amount of chloride anion in mustard sauces can be effectively monitored utilizing a suitable eluent system with ion chromatography[3]. Previous studies have determined bromide ions by microwave digestion followed by ion chromatography with non-suppressed conductivity detection[4]. Single column IC has been utilized to separate and measure nitrate and chloride anions derived from the same vegetable samples of lettuce, potato, carrot, and cucumber[5].

A method to detect free and total sulfites in several dehydrated foods accomplished by IC that utilizes electrochemical detection[6]. IC can be applied to detect phytic acid in soy flour, soy isolate, wheat bran, wheat bread, and infant formula powder[7]. The EDTA concentration in synthetic drug injection drug solutions, contact lens solutions, canned mayonnaise, and canned mushrooms is measured in the presence of interfering analogous compounds, such as EGTA, by utilizing ion chromatography coupled with suppressed conductivity detection[8]. Pretreatment of food samples can enhance analysis of organic as well as inorganic ions[9]. Carboxylic acids and metallic ions are determined from food samples[10]. IC coupled with ultraviolet detection has been applied in determination of hypophosphite, phosphate, and orthophosphate ion[11].

The total amount of nitrogen in environmental, food, and other samples is detectable by IC[12]. Mass spectrometry coupled with IC has been shown to allow measurement of perchlorate in beverages and various foods[13]. Sodium azide is utilized as a preservative for laboratory reagents and has a high acute toxicity[14]. Sodium azide is applied as a preservative in some wines[14]. Determination of sodium azide is done in beverages using ion chromatography through combination of sample preparation with a bubble and trap apparatus (removing any interferences) and sample acidification, converting azide into volatile hydrazoic acid[14].

This work presents a fast ion chromatography analysis of various fresh fruits for ion species considered of nutritive importance, such as potassium, calcium, chloride, magnesium, sulphate (sulfur), and phosphate (phosphorous). The identity and relative amounts of ion species present can be determined, which provides greater knowledge of the actual nutrient capability of these commercial products.

EXPERIMENTAL

Reagents and instrumentation

All reagents were acquired from Sigma-Aldrich Company (Sigma-Aldrich, P.O. Box 2060, Milwaukee, WI 53201 USA). Where further treatment of commercial products was necessary (ie. dilution, extraction, mixing) then distilled water was utilized. For the reagents necessary to operate the single column Metrohm 792 Basic IC, then 18 mOHM water was utilized as the solvent. For cation analysis a Metrosep Cation 1-2 column was used (size 7.0 μm, pressure 6.5 MPa, cond 829.2 μS/cm), with eluent 0.1 M H_2SO_4, 0.002 M HNO_3, and 0.00075 M 2,6-pyridinedicarboxylic acid in 18 mOHM water. For anion analysis a ICSep AN1 column (size 10 μm, pressure 6.8 MPa, cond 12.9 μS/cm) was used with eluent 0.1 M H_2SO_4, 0.0018 M Na_2CO_3, and 0.0017 M $NaHCO_3$ in 18 mOHM water. Detection of eluted ions was accomplished by non- suppressed conductivity. Units of conductivity detection is microSiemens per centimeter, where Siemens is unit of the conductance (one Siemens is equal to the reciprocal of one ohm). Complete elution required less than nine minutes for both cations and anions.

Preparation of samples

All raw juice/pulp derived from fruits were filtered through Whatman #1 filter paper prior to dilution and injection into instrument. For preparation of strawberry, orange, and tomato samples a mass of 61.80 g, 33.50 g, and 39.40 gram portions, respectively, were ground in mortor and pestle. Then 1.00 milliliter aliquot of obtained liquid was diluted into 10.00 mL volumetric flask utilizing distilled water, then injected into anion and cation instrument. All strawberry samples originated from California, USA.

Similarly, for peach, pear, nectarine, and Chilean red plum, a mass of 35.10 g, 35.10 g, 33.50 g, 25.60 gram portions, respectively, were ground in mortor and pestle with extraction into 13.0 mL, 3.0 mL, 12.0 mL, and 15.0 mL, respectively. Then a 1.00 mL aliquot taken of extract to dilute (distilled water) to 10.00 mL volumetric flask followed by injection for cation/anion analysis.

For grapefruit, cantelope, lemon, and lime, an amount of 62.70 g, 62.20 g, 112.6 g, and 57.30 grams of pulp is ground in mortor and pestle. Then 4.00 mL of filtered juice is diluted to 100.00 mL volumetric flask using distilled water, prior to injection into cation/anion instrumentation. In the case of Chilean green seedless grapes and Chilean red seedless grapes an amount of fruit pulp weighing 67.30 g, 63.40 g, respectively, were ground in mortor/pestle, juice filtered through Whatman #1, then 4.00 mL aliquots of filtered juice diluted to 50.00 mL with distilled water in volumetric flask. Diluted juice was injected into IC instruments.

For raspberries, a mass of 31.00 grams of fruit was ground by mortor/pestle, the juice filtered through Whatman #1, then a 2.00 mL aliquot of filtered juice was diluted to 10.00 mL volumetric flask utilizing distilled water. Then injected into cation and anion IC instrumentation.

RESULTS AND DISCUSSION

Food products are analyzed in order to meet compliance with labeling and legal requirements. In addition, analysis establishes nutritive value, determines product quality, identifies defects, enhances research, and product development. This information is vital for the economical production of safe and nutritious food products. Medical studies have shown previously that judicious choices of food consumption can help deter adverse reactions to food allergies and food sensitivities. It is clear that nutrition can play a role in inhibiting carcinogenesis as well as deter aggravating conditions of diabetes, digestive disease, eating disorders, heart health, osteoporosis, weight, and obesity.

Knowledge of the type and concentration of specific minerals found in food products is important to the food industry. While ion chromatography is known to be an effective tool in identifying many types of cations and anions[15, 16], this study focuses on water soluble ions that are readily available in fresh fruit products. This being information being useful for deciding the nutritive potential for dietary goals.

Potassium is utilized clinically as a diuretic[17] and is a major cation within cells. Deficiency in potassium can be manifested by impaired neuromuscular function, changes in gastric secretion, myocardium abnormalities such as conduction defects and disturbed ECG patterns, and abnormal functioning of the kidney[17]. It is known that excessive potassium consumption runs the risk of cardiotoxicity[17].

Calcium is present in small quantities within extracellular fluid and is among the most abundant elements in the body[17]. Calcium is vital for various important physiological activities, it being essential for function of the nerve transmission, intracellular signaling and hormonal secretion, muscle function, for normal cardiac function, and vascular contraction and vasodilation[17]. Over 90% of calcium within the body is found in the skeleton, as phosphates and carbonates, although calcium is in constant exchange with the calcium of the interstitial fluids[17]. Deficiency or insufficient uptake of calcium may lead to: osteomalacia; osteoporosis; Rickets; or Tetany.

For aqueous extracts of orange, raspberry, and tomato; the instrument parameters and solvent system quickly (less than nine minutes) resolved sodium, potassium, calcium, ammonium, and magnesium (see Figure 1). Potassium is a dominant species through-

TABLE 1 : Molarity of ions in raw juice samples

Sample	Na^+	NH_4^+	K^+	Ca^{2+}	Mg^{2+}	F^-	Cl^-	NO_3^-	PO_4^{3-}	SO_4^{2-}
Orange	9.848E-04	ND	4.163E-02	1.999E-03	4.201E-03	ND	1.061E-03	4.241E-05	ND	3.325E-03
Raspberry	7.965E-04	3.19E-05	3.158E-02	1.647E-03	5.585E-03	ND	1.771E-03	8.42E-05	2.724E-04	6.435E-04
Tomato	1.393E-03	ND	4.432E-02	ND	1.224E-03	4.566E-04	3.386E-03	4.725E-05	9.916E-04	1.263E-03
Strawberry	1.63E-03	3.55E-05	2.350E-02	3.617E-03	2.747E-03	ND	1.013E-03	1.621E-04	1.073E-03	1.855E-03

ND= not detected

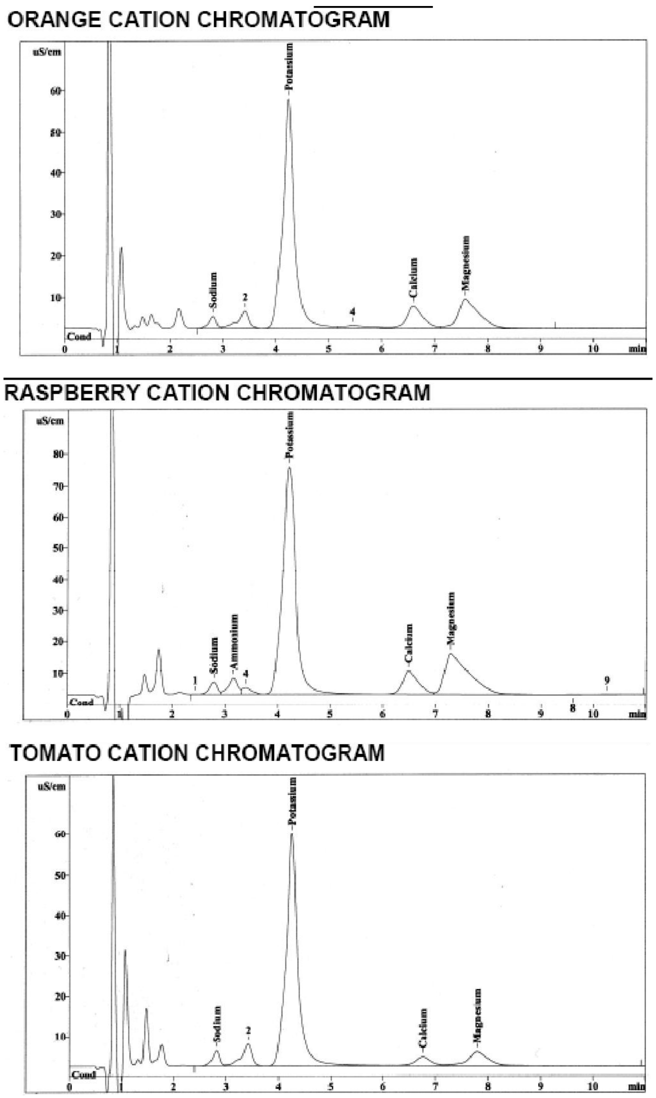


Figure 1 : Orange cations separation: The primary ion is potassium determined to be 0.04163 molar in raw fruit. Other cations identified include sodium, calcium, and magnesium. Raspberry cations separation: The major ion is potassium assayed to be 0.001263 molar in raw fruit. Other cations identified include sodium, ammonium, calcium, and magnesium. Tomato cations separation: The leading ion is potassium measured to be 0.04432 molar in raw fruit. Other cations determined include sodium, calcium, and magnesium. Units of conductivity detection is microSiemens per centimeter

out, followed by calcium and magnesium. Smaller amounts of ammonium (NH_4^+) are found in strawberry and raspberry (see TABLE 1). By molar concentration, there is 990 times more potassium than ammonium in raspberries. Likewise by molar concentration, for strawberries, there is 662 times more potassium than ammonium. The sodium molar concentration in raw juice from fruit orange, raspberry, tomato, and strawberry ranged from the low value of 7.965E-04 molar (raspberry) to the high of 1.63E-03 molar (strawberry). For the same four fruits the range for potassium was a lower 2.350E-02 molar (strawberry) to the higher 4.432E-02 molar (tomato). For the same four fruit the ranges for calcium and magnesium, ranged from lower value 1.647E-03 molar (raspberry) to 3.617E-03 molar (strawberry), and the lower 1.224E-03 molar (tomato) to the higher 5.585E-03 molar (raspberry), respectively. A very small amount of fluoride was found in tomato (4.566E-04 molar), but none in the remaining three fruits above. Nitrates were very low in concentration, with lowest in orange (4.241E-05 molar) to highest in strawberry (1.621E-04 molar). Phosphate was not detected in orange, however ranged from low in raspberry (2.724E-04 molar) to high in strawberry (1.073E-03 molar). Chloride (Cl^{1-}), was identified in all four fruits above, ranging from low in strawberry (1.013E-03 molar) to highest in tomato (3.386E-03 molar). Sulfate (SO_4^{2-}), was found in all four fruits above from the lower 6.435E-04 molar (raspberry) to the highest in orange (3.325E-03 molar).

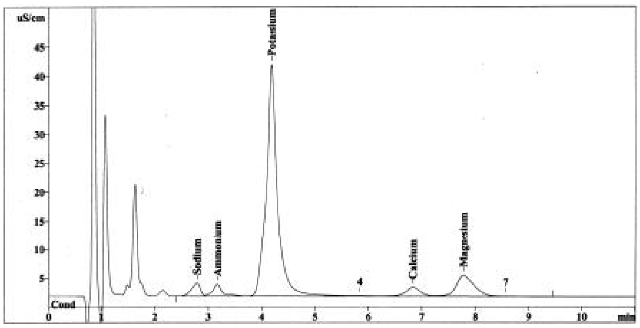
The bulk of phosphate found within the body is located in the bones and it plays an important role in virtually all the organs and tissues of the body, with even notable measured activity in acidifying the urine[17]. Insufficiency in phosphorous can lead to: anaemia; demineralization of bones; nerve disorders; respiratory problems; weakness; or weight Loss. Magnesium is the second most plentiful cation within cellular fluids and is an important activator of many enzyme systems found in the body[17]. Deficiency can occur gradually and leads to: anxiety; fatigue; insomnia; muscular problems; nausea; premenstrual problems, with more extreme cases of deficiency being associated with arrhythmia. Studies have strengthen evidence for the importance of potassium for blood pressure regulation in the general population[18]. Potassium in particular has been shown to have a modest blood pressure–lowering effect in normotensive persons with low dietary intake[18]. An important role for magnesium is the regulation of blood pressure. It appears that an inverse relationship between magnesium intake and blood pressure is strongest for magnesium that is obtained from food rather than that obtained via supplements[19]. Some studies suggest that lifestyle changes that include an adequate magnesium

TABLE 2 : Molarity of ions in raw juice samples

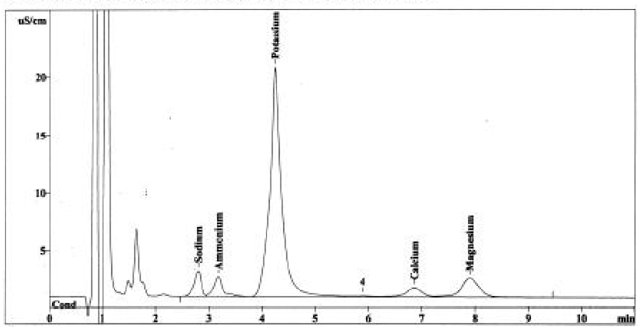
Sample	Na^+	NH_4^+	K^+	Ca^{2+}	Mg^{2+}	F^-	Cl^-	NO_3^-	PO_4^{3-}	SO_4^{2-}
Red Plum	8.770E-04	2.605E-05	2.860E-02	ND	9.214E-04	1.281E-02	ND	4.064E-05	ND	1.295E-02
Peach	8.173E-04	1.607E-05	2.319E-02	ND	2.353E-04	3.487E-02	ND	3.757E-05	ND	5.214E-03
Pear	1.117E-03	7.205E-06	2.297E-02	6.113E-05	7.219E-04	9.326E-04	8.57E-04	3.903E-05	ND	4.502E-03
Nectarine	6.686E-04	2.106E-05	1.496E-02	ND	ND	2.522E-03	1.02E-03	6.064E-05	ND	2.027E-03

ND= not detected

CHILEAN RED PLUM CATION CHROMATOGRAM



NECTARINE CATION CHROMATOGRAM



PEACH CATION CHROMATOGRAM

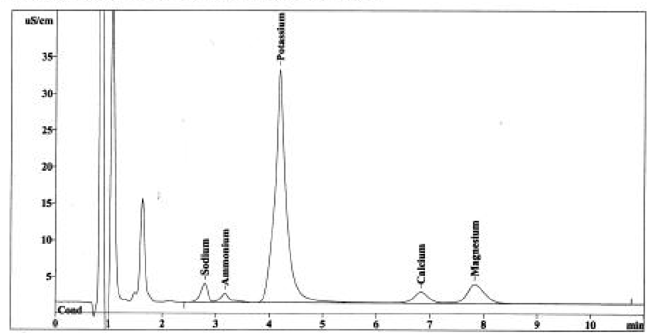


Figure 2 : Chilean red plum cations separation: The primary ion is potassium assayed to be 0.02860 molar in raw fruit. Other cations identified include sodium, ammonium, calcium, and magnesium. Nectarine cations separation: The primary ion is potassium measured to be 0.01496 molar in raw fruit. Other cations identified include sodium, ammonium, calcium, and magnesium. Peach cations separation: The leading ion is potassium appraised to be 0.02319 molar in raw fruit. Some other cations evaluated include sodium, ammonium, calcium, and magnesium.

intake can benefit blood pressure control, promote weight loss, and improve chronic disease risk[19]. For type 2 diabetic patients, a combination of vitamins and minerals (rather than vitamin C and E or an Mg and Zn combination), can decrease blood pressure[20].

IC cation chromatograms are presented in Figure 2 for Chilean red plum, nectarine, and peach fruit extracted juice. Peaks for sodium, ammonium, potassium, calcium, and magnesium are resolved and well defined. All ion species are eluted in less than nine minutes. Concentrations of cations sodium, ammonium, and potassium, found in raw juice of red plum, peach, pear, nectarine, ranges are (see TABLE 2): 6.686E-04 molar (nectarine) to 1.117E-03 molar (pear), 7.205E-06 molar (pear) to 2.605E-05 molar (red plum), 1.496E-02 molar to 2.860E-02 molar, respectively. Calcium was not detected in red plum, peach, and nectarine (pear having 6.113E-05 molar). Magnesium was not detected in nectarine, however concentration in raw juice for red plum, peach, and pear, was 9.214E-04 molar, 2.353E-04 molar, and 7.219E-04 molar, respectively. The anion detection for these four fruits (above) did not reveal any phosphates and no chloride was detected in red plum and peach. Concentration of fluoride in raw juice ranged from low of 9.326E-04 molar (pear) to high 3.487E-02 molar (peach) (see TABLE 2). Concentration of nitrate (NO_3^{1-}) ranged from low 3.757E-05 molar (peach) to high 6.064E-05 molar (nectarine). Concentration of sulfate (SO_4^{2-}), found in all four of these fruit, ranged from 2.027E-03 molar (nectarine) to high of 1.295E-02 molar (red plum).

IC cation chromatograms are presented in Figure 3 for fruit Chilean cantelope, Chilean green grapes, and Chilean red grapes extracted juice. Peaks for sodium, ammonium, potassium, calcium, and magnesium are resolved and well defined. All ion species are eluted in less than nine minutes. Calcium cation was not detected in lemon, red grape, lime, grapefruit, and cantaloupe

TABLE 3 : Molarity of ions in raw juice samples

Sample	Na^+	NH_4^+	K^+	Ca^{2+}	Mg^{2+}	F^-	Cl^-	NO_3^-	PO_4^{3-}	SO_4^{2-}
Green Grape	7.582E-04	9.54E-03	2.459E-02	7.230E-04	1.813E-03	ND	ND	3.38E-05	ND	2.382E-02
Lemon	5.29E-03	1.574E-05	5.10E-01	ND	1.779E-04	ND	ND	9.27E-03	ND	1.053E-03
Red Grape	7.384E-04	3.547E-05	1.97E-02	ND	3.485E-04	9.078E-03	ND	ND	ND	2.088E-02
Lime	3.462E-05	2.416E-05	2.36E-03	ND	4.020E-03	ND	ND	2.01E-04	ND	2.992E-03
Grapefruit	6.720E-03	6.650E-04	8.17E-01	ND	2.546E-04	2.275E-05	3.90E-04	ND	ND	5.740E-02
Cantaloupe	1.114E-03	ND	1.04E-02	ND	4.550E-02	ND	2.30E-03	8.77E-03	1.09E-04	5.840E-02

ND= not detected

(see TABLE 3), but found in green grapes at 7.230E-04 molar. For sodium and ammonium cation, the levels for green grape, lemon, red grape, lime, grapefruit, and cantaloupe ranges for sodium low of 3.462E-05 molar (lime) to high of 6.720E-03 molar (grapefruit), and ammonium low of 1.574E-05 molar (lemon) to high of 9.54E-03 molar (green grape), respectively. For potassium and magnesium cations the concentration ranges for these six fruits (above), are potassium low of 2.36E-03 molar (lime) to high of 8.17E-01 molar (grapefruit), and magnesium low of 1.779E-04 molar (lemon) to high 4.550E-02 molar (cantaloupe). Chloride anion was not detected in green grape, lemon, red grape, and lime, but found at 3.90E-04 molar and 2.30E-03 molar in grapefruit and cantaloupe, respectively. Phosphate anion was not detected green grape, lemon, red grape, lime, and grapefruit but found at 1.09E-04 molar in cantaloupe. Fluoride anion was not detected in green grape, lemon, lime, and cantaloupe, but found at 9.078E-03 molar and 2.275E-05 molar in red grape and grapefruit, respectively. Sulfate anion was detected in all these six fruits (above) with range of low 1.053E-03 molar (lemon) to high of 2.088E-02 molar (red grape).

Detection of anions such as fluoride, chloride, nitrate, phosphate, and sulfate was effective under instrument parameters and solvent conditions. Several chromatogram results of anion elution are presented in Figure 4 for nectarine, Chilean red plum, and pear; showing distinct peaks. Elution of all anions was complete in less than nine minutes. The use of non-suppressed conductivity as detection mode will utilize a low capacity column coupled with dilute acidic eluents to achieve the low background signal. Applying non-suppressed conductivity detection does produce linear calibration curves for ammonium and weakly basic amines[21].

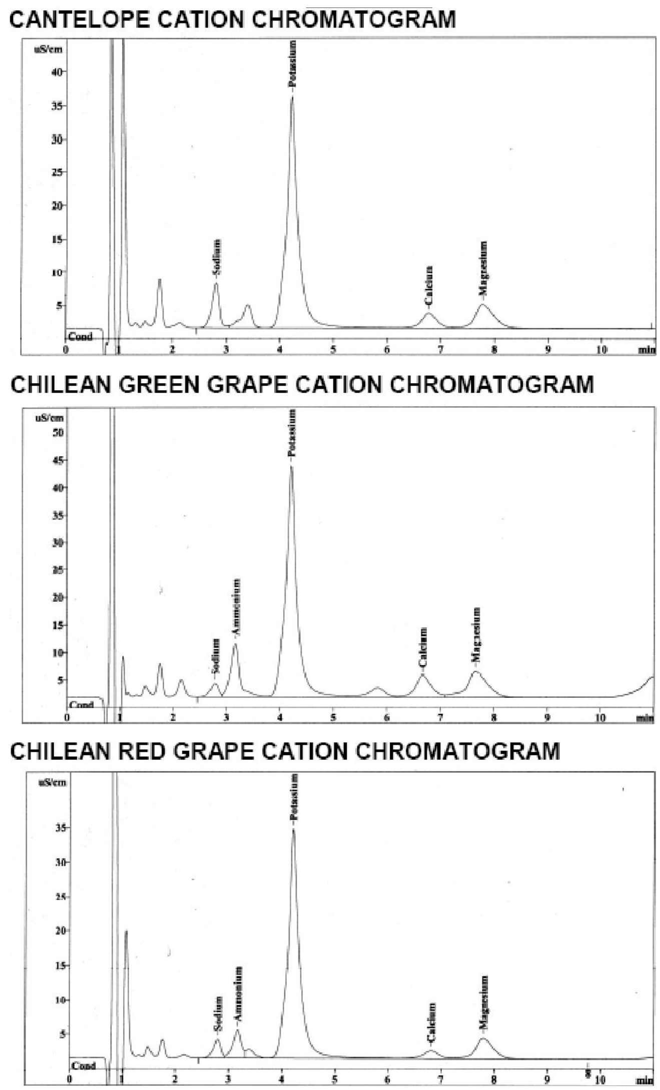


Figure 3 : Cantelope cations separation: The major ion is potassium valuated to be 0.0104 molar in raw fruit. Some other cations identified include sodium, calcium, and magnesium. Chilean green grape cations separation: The leading ion is potassium measured to be 0.02459 molar in raw fruit. Other cations identified include sodium, ammonium, calcium, and magnesium. Chilean red grape cations separation: The leading ion is potassium appraised to be 0.0197 molar in raw fruit. Some other cations evaluated include sodium, ammonium, calcium, and magnesium.

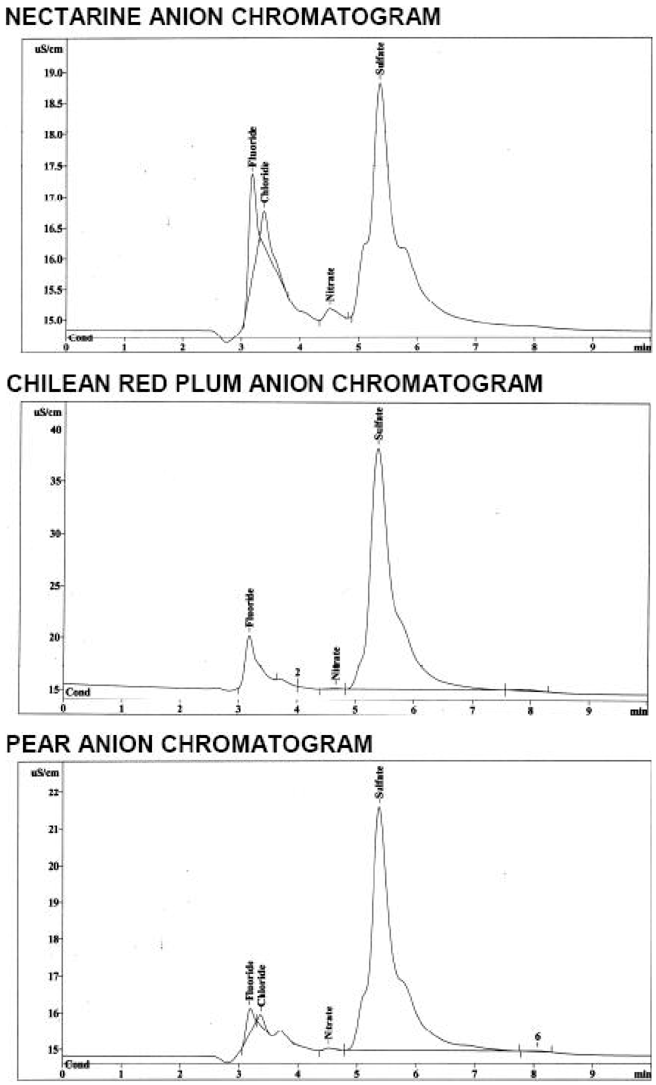


Figure 4 : Nectarine anions separation: The primary anion is fluoride at 0.002522 molar in raw fruit. Other anions identified include chloride, nitrate, and sulfate. Chilean red plum anions separation: Anions such sulfate measured at 0.01295 molar and fluoride at 0.01281 molar are major components. Other anion identified is nitrate. Pear anions separation: Leading anion is sulfate determined at 0.004502 molar in raw fruit. Some other anions recognized are fluoride, chloride, and nitrate. Units of conductivity detection is microSiemens per centimeter.

Information regarding the amounts and types of specific minerals present in food products is important in the food industry. Various physicochemical characteristics of minerals useful for determination are: 1) Their low volatility; 2) Reactivity with specific chemical reagents; 3) Unique electromagnetic spectra. Minerals are of nutritional and functional importance and calcium, phosphorus, sodium, potassium, magnesium, chlorine, and sulfur constitute members of dietary macro minerals. For reasons of food quality and relevance to dietary significance, the determination of minerals in food products is useful. The intake of sufficient minerals is necessary for proper bodily functions, in particular with regard to the building of bones and soft tissues, the regulation of processes such as heartbeat, blood clotting, nerve response, and oxygen transport.

CONCLUSIONS

Utilizing a single column IC instrument with cation analysis by Metrosep Cation 1-2 column and anion analysis with a ICSep AN1 column the minerals of various fresh fruits were detected and measured. Cations and ions were identified in chromatograms, made resolved and distinct under these instrumental conditions. Important dietary minerals such as sodium, calcium, potassium, magnesium, and phosphorous (as phosphate) were identified and assayed at levels of concentration advantageous for nutritive considerations. Fast ion chromatography is shown to be an effective approach to investigate raw juice extract of various fruit types and derived from diverse geographical regions. The approach presented in this study utilizes extraction into aqueous phase, followed by expedient pre-conditioning (ie. Filtration, dilution) prior to injection into the single column Metrohm 792 Basic IC. Elements detected include fluorine and sulfur (as sulfate), are considered useful for nutritive considerations. IC is an effective methodology to analyze food products and for fresh fruits the efficacy of establishing nutritive benefits for macro minerals is presented here. Any variation of mineral concentration from fruit type to type is detectable under conditions presented.

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