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Microwave- assisted digestion of organic materials with $KClO_3$/ HNO_3 for the analysis of trace metals and non-metals

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ABSTRACT

A new microwave assisted pressure bomb digestion method employing an almost saturated potassium chlorate solution acidified with nitric acid, has been introduced for the digestion of plant and food samples, which permits to use up to 4 times the usual sample weight within some shorter time. The digest is especially suitable for the analysis of non- metals (boron, silicon, germanium, total sulphur, iodine), but also for other trace elements like Al-Ba-Be-Cd-Co-Cr-Cu-Fe-Li-Mn-Mo-Ni-Pb-Sb-Sn-Tl-V-Zn, as well as for main elements Ca-Mg-Na-P after dilution. For ICP-OES measurements, calibrants have to be matched with the same amount of digestion solution. ICP-MS measurements were done after dilution and addition of internal standard indium. ICP-OES determination of iodine determination is interfered by phosphorus, and could be done in the ICP-MS by standard addition as the iodate. Due to salt matrix and blanks, K and Cl, as well as Rb and Br, cannot be determined, whereas blanks for B and Si are lowered. In the ICP-MS, the isotopes V-51, Cr-52, As-75, Se-77 and Mo-95 are positively interfered. Arsenic can be determined by hydride AAS. The digestion efficiency versus some aromatic compounds may be lower than from concentrated HNO_3, therefore intermediate degradation products of phenylalanine, tyrosine, and salicylic acid were studied in detail. The proposed method was checked in ring tests of various plant and food samples.

KEYWORDS

Pressure digestion;
Potassium chlorate;
Non-metals;
Trace elements;
Food;
Green plants.

INTRODUCTION

The need of multi-element methods

In a modern trace analytical laboratory, multi-element techniques like ICP-OES (optical emission spectroscopy) or ICP-MS (mass spectrometry) can be used to get most elements of interest in one run, or at least within a few suitable dilutions. The number of available elements, however, is limited by insufficient dissolution, volatilization of analytes, or blanks in the preceding digestion. In particular, non-metals get frequently lost by volatilization. Sulphur may be volatile as SO_2 or H_2S, or remain as insoluble elemental sulphur. Silicon is prone to the introduction of blanks from glass and dust, as well as from incomplete dissolution. Germanium is volatile as the anhydrous chloride, even from hydrochloric acid, and recovery, particularly from sulfides, is pure. Iodine is volatile as the element or as alkylated compounds.

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Microwave-assisted pressure digestion

Microwaves interact with dipole molecules or with ions in solution to get them moving, which means formation of heat. Microwave assisted pressure bomb digestions are very clean with respect to trace elements, because there is limited amount of reagents, handling, and input of dust. At elevated pressure, higher temperatures than the normal boiling points can be reached, leading to increased reaction rates and sample decomposition. Teflon as an inert material is transparent to microwave radiation. After initial power input, a break in the program should be maintained, because some organic matrices (e.g. oyster tissue, rice flour) might cause additional temperature and pressure attributed to an exothermic reaction[5]. The absorption of microwave energy of conc. HNO_3 was measured to be 80%, of HF 57%, and of H_2SO_4 45 % with respect to pure water, but salt solutions absorb much more energy[5].

Using nitric acid or aqua regia, however, sample weight is limited to about 200 mg of dry substance[4], because there is limitation from evolved pressure.

Merits and limitations of pressure digestions with HNO_3

In case 65% HNO_3 is heated by microwaves in tightly closed vessels, the inside pressure is less than heating pure water, but additional CO_2 and possibly NO from the degradation of organic compounds have to be considered[3,5].

In pressure composition with HNO_3 (2 ml HNO_3 + 90 mg organic carbon) at 180°C, some components of biological matrices resisted complete mineralization, like phenylalanine, histidine, tryptophane or methionine, due to the determination of residual carbon in the resulting digest[10]. Voltammetric sweeps as well as mass spectra indicated the presence of nitro- benzoic acids[2,11].

Digestions with HNO_3 or the like are suitable to produce clean digests for the analysis of metals and semi- metals, but volatilization of some non-metals (boron, sulphur, germanium, iodine) may occur. In particular, iodine is easily lost in the digestion step, if the element can be intermediately formed. In sample digests containing nitric acid or nitrite, iodide is partially oxidized to yield just J_2. Elementary iodine is easily volatile and also strongly adheres to plastic surfaces.

Boric acid is volatile with hot vapour from acid solution. When sulphur goes to SO_2, it leaves the acid digest as a gas immediately. Silicon may be precipitated as silicious acid from acid solution, and germanium is volatile as the $GeCl_4$ (boiling point 84°C).

Selection of $KClO_3$, acidified with HNO_3

A method had to be developed to enable the determination of non-metals, main and trace elements in one run, or at least from the same digest. Organic compounds frequently should be oxidized as much as possible, because they produce carbon soot in or after the plasma torch. To achieve suitable sample digests for cations, they should be acid enough to avoid hydroxide precipitation or coprecipitation. This excludes alkaline reagents like tetraammonium-hydroxide, which has been used for the extraction of iodide[1]. Among the non- metals, sulphur should rapidly go to sulphate, and iodine to iodate.

A mixture of chloric/nitric acid in open vessels[1], and later with $HClO_4/HClO_3$ in microwave-assisted pressure bombs[1] readily destructed organic materials except fatty samples, yielding complete recovery of any iodine as the non- volatile iodate. Toxic and thermally labile fumes of ClO_2, as well as residues of immiscible fat were, however, disadvantageous. Currently, the chloric acid is not commercially available any more because of safety reasons. This led to the use of $KClO_3$ instead. Solid potassium chlorate has been known for long to oxidize elemental sulphur to sulphate, and iodine to iodate.

For the first experiments, bread crumbs were selected as a test material. Heating with neutral 10% $KClO_3$-solution (which is almost at saturation) yielded black tar. Within a series of experiments, more and more nitric acid was added, until complete dissolution was achieved.

This led to the final procedure, to mix 200 ml of 10% $KClO_3$-solution with 80 ml concentrated nitric acid.

Microwave energy absorption in salt solutions is known to be significantly higher than in water, e.g. in 0,5M (molar) NaCl solution it is 4-times more[2]. The $KClO_3$- solution proposed in this method is 0,58 M, which means a much more efficient absorption of

microwave energy than in conventional HNO_3/H_2O_2 digests. Therefore, the power-time program was set lower than usual.

The amount of digestion solution was increased from the usual 4 ml HNO_3 to a value as much as possible to ensure the tightness of the vessels. Water in the system presumably dissolves the emerging CO_2, NO_2 etc to yield much less pressure, so that 8 ml of digestion solution were possible.

MATERIAL AND METHODS

Reagents

Potassium chlorate $KClO_3$, Merck p.a. Art 4944
Potassium iodate KJO_3, Merck p.a. Art 5053
Boron - calibrant solution 5,000g B/L, Merck Titrisol Art 9923
Germanium Atomic Spectroscopy Standard Solution, Fluka Nr. 48843
Sulfate standard solution 1000 mg/l SO_4^{2-}, Merck Certipur Nr. 1.19813
Silicon standard 1000 mg, Merck Nr. 9947
Phosphate standard 1000 mg, Merck Nr. 9870
Various other standard solutions for main and trace cationic elements
Nitric acid, 65% suprapure, Merck 1.00441.1000
Test substances: L-phenylalanine, FLUKA Nr. 78019
L-tyrosine,FLUKA Nr. 93830
Salicylic acid: Merck Art. 635, extra pure
L-Arginine, FLUKA 11010
L-Iso-leucine, FLUKA Nr. 58880
Saccharose Merck Art 7651, for biochemistry and microbiology
Digestion reagent solution: dissolve 20 g of $KClO_3$ in 200 ml ultrapure water, add 80 ml 65% HNO_3, and store in a plastic bottle.

Equipment

Microwave digestion unit: mls 1200 mega high performance microwave digestion unit, MLS GmbH, D-88299 Leutkirch
ICP-OES: Perkin Elmer Optima 3000XL with axial plasma
ICP-MS: Perkin Elmer Sciex ICP mass spectrometer ELAN DRC II
Hydride AAS: Perkin Elmer MHS 10 at Perkin Elmer 3030
NMR: Bruker, 600 MHz
Milli-Q plus ultra pure water purification unit, Millipore

Procedure of digestion

1 g of solid dry plant material is weighed into digestion vessels, and mixed with 8 ml digestion solution. 2 blanks are run with each batch.

For samples containing fat, 0,5 - 1,0 g sample weight due to fat or aromatic carbon content is taken. For milk or urine, 3 ml of milk + 5 ml of digestion solution were optimum; for serum, 2ml need 6 ml of digestion solution.

After closing, the subsequent program was run:

1 min 250 W / 2 min 0 / 5 min 250 W / 5 min 400 W / 5 min 500 W / 15 min ventilation

Samples containing appreciable fat content were run 2 times without intermediate opening

The power-time program was shortened with respect to the routinely used program for the HNO_3 resp. HNO_3/H_2O_2 digestions, due to an estimated increased uptake of microwave energy by the salt solution; it was not varied throughout.

After opening the pressure vessel, 1ml of digestion solution is added, and the contents transferred to a 25 ml volumetric plastic flask through a plastic funnel. This prevents formation of gas bubbles, which might extinguish the ICP-torch.

After every sample digest, a cleaning procedure was run using 2ml of digestion solution, and the same energy-time microwave program, to be sure to clean the pressure vessels. The vessels have high memory for iodine.

Procedure of the characterization of intermediate products from test substances

Residual carbon in the obtained digest was determined by ICP-MS reading the counts of C-13, after 1+9 dilution with 2M-HCl to expel CO_2. For calibration, 1g/L carbon equals 250 mg saccharose in 100 ml 0,1% HNO_3.

0,25 – 1 g of the test substances were digested according to the proposed procedure, but finally not made up to the mark just with water, but the vessel was cleaned with 5ml of di-isopropylether plus water, and made up to the mark in glass volumetric flasks. The organic solvent dissolved most of the precipitates

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formed, of any. The remaining aqueous phase was extracted with ethyl acetate several times, the organic phases combined, and the solvent evaporated. The residue was characterized by NMR (nuclear magnetic resonance) spectra in CH_3OH-d_4 and compared with known substances.

Reaction conditions in the $KClO_3$- HNO_3 mixture might be complicated, therefore experiments were run in which nitric acid was substituted with equivalent sulphuric acid, and vice versa, $KClO_3$ was omitted.

RESULTS

Organic carbon degradation and recovery studies

There might be complaints about incomplete destruction of organic materials. Digests of green plants (ALVA ring test 2009 and animal feeds) had still 1-5% residual carbon with respect to sample weight. 1g of cellulose, glycine, arginine and iso-leucine easily yielded colourless digests; the latter contained just some iso-butyric acid. Aromatic compounds are more resistant towards degradation (TABLE 1). 1 g of salycilic acid turned into an orange precipitate plus yellow solution, and 0,2 g of 3,4 dimethoxy benzoic acid even yielded a black precipitate, whereas from pyrocatechol-disulfonic acid ("tiron"), colourless digests were obtained.

TABLE 1 : Residual dissolved carbon

Test substance	´meq needed for oxidation to CO_2+N_2	Carbon content	Carbon found after digestion
755 mg phenylalanine	197	494 mg C	20,2 mg residual C
595	155	389	17,5
224	56	147	48
117	29	76	47
758 mg tyrosine	172	452	170
482	109	288	99
169	38	101	38
516 mg salicylic acid	90	314	26

8 ml of the digestion solution contain 181 meq (milli-oxidational equivalents) from HNO_3 and 23 meq from the $KClO_3$, making 204 meq together, which should be sufficient to go to CO_2 + N_2. Thus, the mechanism is more complicated, and intermediate oxidational products have been isolated and characterized (see section 4). If there is no complete degradation of the sample, less sample weight and double time is helpful; but high levels of aromatic amino acids like tested in TABLE 1, are hardly encountered in food or environmental samples.

There was quantitative recovery of 10 µg B/J/Ge + 25 µg Sn + 100 µg SO_4, and of 20 µg B/J/Ge + 50 µg Sn + 200 µg SO_4, added to 1g of bread crumbs. Also, complete recovery of 5/10 µg of B and Ge was obtained from mixed feed. Sulfur was completely recovered from self-prepared mixtures of 1% elemental sulfur in cellulose. Recovery of 4-20 µg Fe-Mn-Mo-Sn-Sb-V-Zn added to 0,4 g of pumpkin seed oil was within 100-110% (sample 08020430). Recovery of 100 ng As added to milk (3,5% fat content) was 89% after double time of digestion.

Blanks

In trace element analysis, salt solution reagents should be generally omitted because of blanks. Surprisingly, the $KClO_3$ was sufficiently pure to permit determinations of all trace elements requested. Two blanks were run with any series, and they were clean even for ICP-MS. Just K and Cl (matrix), as well as Rb and Br cannot be analyzed. The suprapure HNO_3 is supplied in glass bottles, thus, in case of B and Si, the blanks are lowered, because just 1/3 of the suprapure HNO_3 is used for 4 times the sample weight. The detection limit of B is estimated at 0 – 0,3 mg/kg, but it is not limited by the blank but by the memory of the ICP-torch; therefore, after calibration, H_2O and 2 blanks are run twice before the samples. For meat and egg samples, however, samples were below detection limit for B and Si. Milk samples might dissolve some boron from glass and should be kept in plastic bottles prior to analysis of boron throughout.

Application to various matrices

1g of high- carbohydrate samples, like green plants, cereals, bread and animal feeds, can be digested with 8 ml digestion solution. For milk and urine samples, 3 ml sample + 5ml digestion solution is still possible, or 2 ml serum sample + 6 ml digestion solution. Fat needs more oxidants and more vigorous conditions. Therefore, sample weight has to be reduced to ½ g, and the time-power-program in the microwave oven has to run twice without intermediate opening. In case of chocolate, freeze dried liver, egg white and egg yolk samples, the

sample weight was reduced to 0,5 g in order to achieve complete dissolution. Samples of high fat content (e.g. liver samples) were run twice with the same program, without intermediate opening the vessels. For ready – made commercial meals, about 1,5 – 2,5 g wet sample was taken.

For the analysis of biowaste samples (water content 72,3 ± 6,5 %), 2-3 g of wet sample were digested the same way. This turns all sulphur into sulphate, all iodine into iodate, and hydrolyzes silica up to about 0,2% in the sample. Rock silicates, however, precipitate from the final solution, and total silicon had to be determined gravimetrically as SiF_4 – loss. Sometimes, a black film appeared inside the pressure vessel, which can be finally removed with acetone.

Use of the digests for ICP- and hydride-AAS measurements

Calibrant solution for ICP-OES multi-element measurements should contain the same amount of digestion solution as the sample. This copes with signal depression (till 30% of the signal) and yields exactly the same spectral background. For the main elements, dilutions 1+19 or more can be calibrated versus aqueous calibrants. For determination of boron, silicon and germanium, contact with glass has to be avoided, and dilutions to be done by hand. Compared with the conventional procedure, just 1/3 of nitric acid is used which is supplied in borosilicate bottles. This lowers the blank to about 0-0,3 mg/kg for B and 0 – 1,4 mg/kg for Si, if 1g sample ends up in 25 ml, without the need to use a clean room. The iodine line at 178 nm is severly overlapped by a neighbouring P-line and can only be used in low-P samples (e.g. table salt).

For ICP-MS measurement, 1+9 or 1+19 dilution of the digest and addition of indium as an internal standard has led to correct results for Pb, Cd, Tl, Bi, Co and Mo (Mo-98 only). The isotopes Cr-52, V-51, As-75 and Se-77 yield far too high results because of chloride or chlorate interference (equimolar amounts of chloride and chlorate result in the same interferences).

In flame-AAS, matrix effects of the $KClO_3$ were less pronounced (Cu, Fe, Na were tested), but matrix matched calibrants are recommended.

For hydride-AAS (in the batch mode), up to 2 ml of the digest could be used directly for the determination of arsenic. Results were correct, but there are limitation due to foaming and signal depression from residual oxidants in the sample solution. Recovery of selenium in the hydride AAS was incomplete (about 70%), it might be lost during conversion to Se(IV).

Quality checks

The proposed method has been used to analyze samples within the frame of International Plant Exchange (IPE), organized by Wageningen Agricultural University (The Netherlands) since 2007.1. Within this program, 4 times 4 samples are sent each year, the results are collected and evaluated. The results are published in a booklet open to all participants; my code is WELE-136. From the digests obtained with $KClO_3/HNO_3$, data for (in alphabetical order) Al-Ba-Be-Cr-Cu-Fe-Mn-Ni-V-Sr-Zn, as well as B-S-Si (from a second run) were successfully taken from ICP-OES measurement versus calibrants prepared in $KClO_3/HNO_3$ matrix. Ca-Mg-Na-P were obtained from ICP-OES after dilution 1+19 versus aqueous calibrants. For the elements Bi-Cd-Co-Mo-Pb, the ICP-OES was not sensitive enough to reach the level of non-contaminated green plants, thus ICP-MS was used utilizing In-115 as internal standard. Iodine was determined by ICP-MS and standard addition. Arsenic was obtained correctly by hydride AAS (IPE 2008.3), but not submitted to the ring test.

For Ge and Tl, complete recovery in the digestion procedure was obtained, but they are not included in the IPE ring test yet, and all results were at or below detection limits (0,2 mg/kg for Ge in ICP-OES and 0,001 mg/kg for Tl in the ICP-MS). Mercury was never tried.

Iodine in animal feeds were analyzed within the frame of ring tests organized by the ALVA (Austria) and VDLUFA (Germany). Contrary to the conventional extraction of iodide with tetramethylammonium hydroxide (TMAH), total iodine is obtained, and the results are higher, except for mineral mixtures. Comparison of data obtained for human urine by traditional Sandell-Kolthoff method, however, gave the same results (paper in preparation).

Before 2007, a wet digestion of green plant samples with $HNO_3/HClO_4$ in open glass Erlenmeyer flasks was used in this lab, which yielded many good results.

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Improvements of the proposed method were noted for Al-Ba-Cr-Fe, which were sometimes too low because of either incomplete dissolution or interaction with the glass. From the open digests, As-B-Ge-I-Si-Se as well as low Na data are not available because of volatilization or blanks. The proposed method, however, is not suitable for determinations of K and Cl (matrix), as well as Rb and Br (blanks from the reagent).

In the IPE program, the silica data were very scattering, therefore exchange of samples with a German lab to achieve compatible data for (biogenic) silica is still in progress.

In addition, the proposed method was checked by the international reference materials NBS-1566a Oyster tissue and BCR-CRM 129 hay powder 1, which are certified for P-S-I as well as an appreciable number of cations. To check for the results of non-metals, IAG-feed samples were taken which contain known amounts of P-S-I-B.

DISCUSSION

Materials mainly consisting of cellulose and other carbohydrates, yield clear solutions and less pressure than the HNO_3- digests, even starting from 1 g sample weight, but some more residual carbon remains in the sample digest as well. When e.g. liver samples, chocolate or egg yolk were tried, sometimes white precipitates appeared, which hardly dissolved in the resulting aqueous acid solution. As these precipitates might occlude parts of the analytes, this has to be avoided, which could be achieved by reduction of sample weight to 0,5 g, and in case of high fat content, by running time-power program two times without intermediate opening.

Nevertheless, experiments were made to check which kind of substances were formed which resisted the degradation to CO_2 or at least to water soluble compounds.

Whereas chloric acid resp. ClO_2 are known to degrade or at least solubilise carbohydrates (including cellulose) and low molecular aliphatic organic substances easily, aromatic compounds might be more stable. Thus, the aromatic amino acids phenylalanine and tyrosine, as well as salicylic acid, were chosen as chemically pure test substances. Egg yolk may contain phenylalanine at high levels as an essential amino acid. 200 – 1000 mg of test substance were reacted according to the proposed procedure, which is much more than expected to be present in an environmental sample.

In order to elucidate the mechanism of degradation and the limits of the method, a short literature survey was made about the reactions of chloric acid, nitric acid, and their mixture towards organic materials.

Reactions with dilute chloric acid

When the vessels containing the blanks are opened, a faintly green solution and the characteristic smell indicate the formation of ClO_2.

Reactions with chloric acid have been investigated mainly about 170 years ago, but at this time, no exact characterization methods for the resulting organic products were available. Dilute chloric acid oxidizes e.g. ethanol to yield acetic acid and chlorine gas. Sugars get easily oxidized, they yield CO_2 at 130°/24h[12]. In dilute sulphuric acid solution, oxalic acid reacts to HClO + CO_2, in presence of manganese as a catalyst till CO_2 + HCl. In neutral aqueous solutions chlorate the addition of OH to C-double bonds without C-C- cleavage. Thus, ethylene gets glycol, allyl alcohol gets glycerol, malein acids gets tartaric acid, and acetylene gets acetic acid. Chloric acids also directly reacts with NH_3 and NO to yield N_2 or nitrate. ClO_2 directly reacts with amines under cleavage of C-N bonds, yielding aldehydes and subsequently the respective carbonic acids. Identical ratio of benzyl to methyl cleavage by ClO_2 was observed[6].

In water potabilization, the use of ClO_2 as a pre-oxidant instead of Cl_2 is known to have a beneficial influence of minimizing the trihalomethane formation following post-chlorination. During the reaction of amino acids with ClO_2, in dilute aqueous solutions, acid gets formed. Tryptophan reacts with excess ClO_2 to unknown brown compounds besides oxalic acid, fumaric acid, and lesser 2- aminobenzoic acid. The indole ring undergoes oxidative ring opening via initial hydroxylation and formation of a carbonyl group. The amino acid nitrogen forms hydroxylamines, oximes and imines and final oxidative C-C bond breaking in the alpha positions to hetero-atoms. The benzene structure remains more stable. Histidine gets preferably attacked by ClO_2 at the amino N- atom, leading to the corresponding 4-

imidazolyl acetic acid, and further on, to hydroxylated heterocyclic compounds besides opening of the ring. Tyrosine reacts with excess ClO_2 in presence of O_2 to short –chain, highly functionalized carboxylic acids. Hydroxylation of the aromatic ring ruptures the ring to yield carboxylic acids. Tyrosine finally loses the lateral chain by decarboxylation, and forms phenolic carboxylic acids[9].

Chlorination of dissolved amino acids in aqueous solutions leads to the formation of mono- and dichloramines, further on to specific aldehydes and nitriles, as well as halogenated byproducts, some of them mutagenic. Chlorine finally oxidizes alcohol functions to ketone and acid groups, and thiols to sulfons and sulfoxides, whereas amide linkages remain largely untouched. The chlorine demand of the aromatic amino acids tyrosine and tryptophan was 5 times higher than of phenylalanine[7].

Unlike the reaction mixtures of aqueous chlorine with amino acids and humic acids, in which trihalomethanes are formed, the reactions of aqueous ClO_2 with organic compounds are primarily oxidative and do not produce trihalomethanes. In general, amino acids in aqueous medium undergo oxidation with ClO_2 to form imine type interdediates, which are hydrolyzed followed by decarboxylation to produce aldehydes[8]

Reactions with dilute nitric acid

Amino groups get lost by reaction with nitrite. Salicylic acid reacts with nitrite towards 2-nitrophenol. Tyrosine gets nitrated to yield p-nitro-tyrosine in dilute nitric acid, and picric acid + 3,5, dinitro-hydroxy benzoic acid in 1+1 nitric acid. Phenylalanine gets nitrated in 1+1 nitric acid to p-nitro-phenylalanine[13].

Reactions with chloric acid + nitric acid

In the sample digestion mixture, chloric acid is intermediately formed. Either the nitric acid acts as an oxidant, and resulting nitrous acid or NO are re-oxidized by the chloric acid, or the chloric acid reacts itself with the various organic molecules of the sample. Chloric acid rapidly oxidizes elemental sulphur and selenium to sulphate and selenate.

Results of the digestion of test substances by the proposed procedure

In case of phenylalanine, a white precipitate appeared in the digest, which was soluble in di-isopropylether and ethyl acetate. NMR (nuclear magnetic resonance) spectroscopy revealed that this was a mixture of p-chlorobenzoic acid (about 56 mole%), benzoic acid (about 37 mole%, and o-chlorobenzoic acid (about 6 mole%). Thus, the aliphatic side chain got completely lost. After an experiment starting with more than double the phenyl alanine, p-chloro-benzoic acid and p-nitro-benzoic acid could be identified. Within a further experiment, nitric acid in the reaction mixture was substituted by an equivalent amount of sulphuric acid. This yielded mainly a brownish tar besides 34 mole% benzoic acid and 17% phenyl-acetic acid. If, on the other hand, the $KClO_3$ is omitted, and the digest is done with 1+2 HNO_3, this results in a greenish tar and maybe water soluble compounds.

Tyrosine was nitrated in parts till picric acid, besides other water-soluble compounds of orange coloration. Without HNO_3, the $KClO_3$ in equivalent H_2SO_4 yielded mainly brownish tars, and minor amounts of p-hydroxy-phenylacetic acid, plus o-chloro-p-hydroxy-benzoic acid. There was definitely no second chlorine added to the aromatic ring.

Salicylic acid immediately reacted to an orange product with the digestion mixture, from which o-chloro-p-nitro-benzoic acid (11 mole%), and picric acid (3,5 mole%) could be isolated and identified. $KClO_3$ in dilute H_2SO_4 formed p-and o- chloro-salicylic acid (10 mole%) resp. 4 mole%), and major amounts of brownish tar. 1+2 diluted HNO_3 yielded orange products as well, from which 16 mole% were identified as picric acid and 10% as dinitro- salicylic acid.

Dimethylamino- benzaldehyde and 3,4 dimethylamino benzoic acid yielded just blackish tar, whereas pyrocatechol-3,5-disulfonic acid (commonly known as "tiron") degraded completely.

All the produced tars were soluble in acetone and aqueous ammonia, which was important for cleaning purposes.

CONCLUSIONS

Microwave assisted pressure digestion with $KClO_3/HNO_3$ solution can be almost universally applied to digest up to 1g of green plants, feed and food samplesin order to screen for various metals and non-

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metals by e.g. ICP- multi-element techniques.

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