# Degradation of Some Resistant Organic Compounds during Pressure Decomposition with Nitric Acid – Potassium Chlorate Reagent Solution

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***Authors' contributions***

*This work was carried out in collaboration between both authors. Author MS designed the study, performed the digests managed the literature search and wrote the protocol. Author FH prepared, performed, and interpreted the NMR-spectra. Both authors read and approved the final manuscript.*

***Article Information***

**Short Communication**

## ABSTRACT

In a mixture of nitric acid and chloric acid, nitrate has been proven to be the oxidizing agent for the organic matrix to be digested. White precipitates obtained occasionally after microwave-assisted pressure digestion with potassium chlorate in dilute nitric acid solution, have been identified as nitrification products of phenyl alanine and tyrosine by ^{1}H- NMR methods. Lowering sample weight leads to clear digests.

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## 1. INTRODUCTION

In trace element analysis, pressure digestion methods of environmental and nutritional materials can be done with a minimum of reagents and dust contaminations. In order to overcome inhomogeneity problems, the objective should be to use as large sample weights as feasible. Microwave-assisted pressure digestion with almost saturated potassium chlorate solution, acidified with nitric acid, permits to obtain digests suitable for both cation and anion analysis; for safety reasons, chloric acid is not commercially available. Besides low blanks, particularly for boron and silicon, the sample weight can be increased four times with respect to nitric acid only (one gram dry substance in 25 ml), thus permitting more representative subsamples. Water in the system reduces the pressure obtained, and improves the recovery of boron and germanium. All iodine is transferred to iodate, and all sulfur is transferred to sulfate, which are not volatile. Just for rubidium, some blanks have been noted. The author uses this method in his routine for main and trace elements, and valid results have been obtained in ring tests for a range of green plants and food items [1-6]. In soil analysis, the procedure may substitute aqua regia, the accesibility of total sulfur and iodine are advantageous.

Salt solutions significantly increase the absorption of microwaves, thus full power of the device is not needed. According to Kingston and Jassie [7,8], concentrated $HNO_3$ absorbs 80% of the energy with respect to $H_2O$, but 0,5M NaCl (29 g/L) absorbs 4 times more than water.

Originally, the procedure aimed at complete digestion was optimized for bread crumbs, yielding a clear solution without visible residues, but some problems arose with samples of high fat content, as well as with eggs, and liver samples. Contrary to nitric acid only, the level of oxidation equivalents in the $KClO_3$-$HNO_3$-reagent is lower; it is sufficient to cope with 4-fold sample weights of carbohydrates, but not for fats. Therefore, sample weights of cream or chocolate have to be reduced to 0.7 g [4,5], egg yolks to 0.6 g [3]. The 8 ml reagent solution applied (see below), contain 181 meq for oxidation from $HNO_3$ and 23 meq from $KClO_3$, together 204 meq. This is sufficient to oxidize 1 g of glucose to $CO_2$ (needs 133 meq), but not for fat (1 g stearic acid needs 359 meq).

Egg samples yielded a white precipitate, which proved to be soluble in acetone. This short communication, is about to document the attempts to identify these compounds, whether this has been just fat, or something else.

Aromatic compounds are much more resistant to oxidative digestion than aliphatics. It is known that eggs contain a lot of phenylalanine. Pressure digestion at 180ºC with pure $HNO_3$ within 3 hours yielded incomplete oxidation of phenylalanine, histidine, tryptophane, nicotine, betaine and benzoic acid, which interfered in subsequent voltammetric determination of trace elements [9]. 2-nitro-benzoic acid and 2,4-dinitro-benzoic acid were identified as main reaction products by IR and NMR-spectroscopy [10]. Modern multi-element determination methods, like ICP-OES or ICP-MS, are tolerant towards these items, however.

High pressure ashing can be more efficient, but requires more manpower and special equipment. Therefore, within this work, the digestion of some aromatic compounds by the $KClO_3$-$HNO_3$-reagent was tested.

## 2. PROCEDURE (AFTER SAGER 2011)

$KClO_3$-$HNO_3$-reagent: 20g $KClO_3$ p.a. + 200 ml $H_2O$ + 80 ml $HNO_3$ suprapure (the reagent contains 7% $KClO_3$ in 4,5 M $HNO_3$). Equipment: mls 1200 mega, high performance microwave digestion unit, 50 ml pressure bombs Weigh 1,00 g sample into a PTFE pressure bomb vessel, add 8 ml reagent.

Sufficient digestions have been obtained with the following program for the microwave unit: 3 min 250 W / 2 min – / 5 min 400 W / 5 min 500 W. After coooling, the digest is made up to 25 ml in plastic volumetric flasks. Unfortunately, our equipment does not provide internal temperature and pressure.

Residual carbon was determined by ICP-MS via the counts of $^{13}C$, calibrated with glucose. Prior to this, all solutions must be acidified and purged with nitrogen, to expell $CO_2$. As test substances, phenylalanine, tyrosine, salicylic acid, arginine and tiron (1,2-dihydroxy-3,5-di sulfonic acid, disodium salt), were used.

Table 1 shows the experiments performed, and its results. For each digestion, 204 meq were added, which were considered sufficient for complete transformation into $CO_2$ and $N_2$, but aromatics are more resistant. In order to look for the composition of the precipitates obtained, in a

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second run, 5 ml of diisopropyl-ether was added to the respective digests to dissolve all precipitated in the pressure vessel, made up with water to 25 ml in the volumetric flask, and brought to the organic chemistry laboratory.

There, the aqueous phase was additionally extracted with ethyl acetate, added to the di-isopropylether extract, evaporated at a rotavapor, taken into acetone-d6 and submitted to 1H-NMR spectroscopy.

**Table 1. Experiments performed**

|  | meq | mg C | Residual C in solution | precipitate | Solution |
|---|---|---|---|---|---|
| 755 mg phenyl alanine | 197 | 494 | 4,5% | White | Yellow |
| 653 mg phenyl alanine | 170 | - | - | White | Yellow |
| 595 mg phenyl alanine | 155 | 389 | 5,0% | White | Clear |
| 224 mg phenyl alanine | 56 | 147 | - | White | Clear |
| 117 mg phenyl alanine | 29 | 77 | - | White | Clear |
| 758 mg tyrosine | 172 | 452 | 37,5% | Orange oil | Yellow |
| 482 mg tyrosine | 109 | 288 | 34,3% | Orange oil | Yellow |
| 169 mg tyrosine | 38 | 101 | 29,7% | - | Clear |
| 1038 mg salicylic acid | 181 | 632 | 16% | Orange | Yellow |
| 516 mg salicylic acid | 90 | 314 | 5,8% | - | Yellow |
| 879 mg tiron | 73 | 201 | 5,1% | - | Clear |
| 531 mg arginine | 113 | 239 | 3,3% | - | Clear |

## 3. RESULTS

The white precipitate obtained from phenyl alanine proved to consist of p-chloro-benzoic acid and p-nitro-benzoic acid = 2:1, and o-chloro-benzoic acid as a by-product (Fig.1).
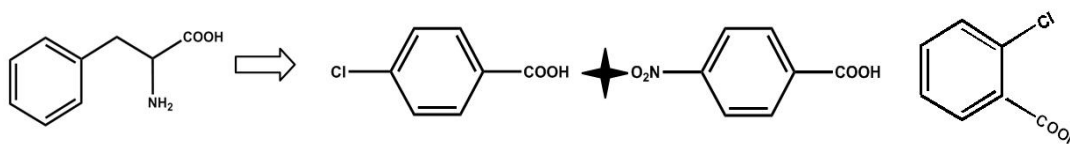


**Fig. 1. Reaction of phenylalanine**

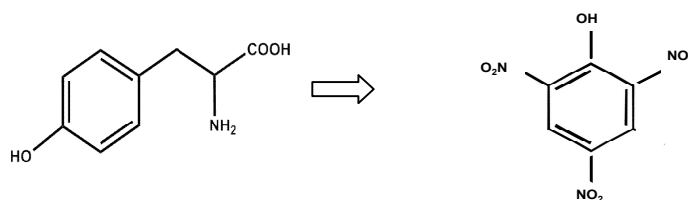More than 80% of the tyrosine was transformed to picric acid (Fig. 2).



**Fig. 2. Reaction of tyrosine**

The salicylic acid yielded picric acid and 2-hydroxy-3, 5 dinitrobenzoic acid = 3:2 (Fig. 3).
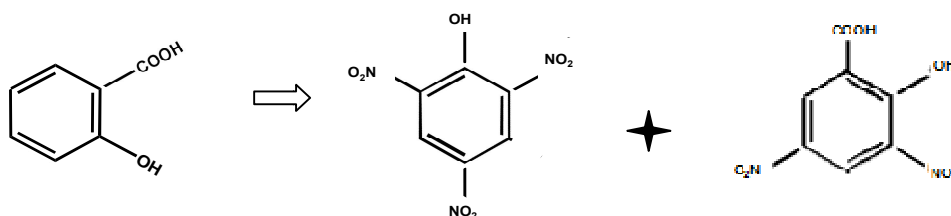


**Fig. 3. Reaction of salicylic acid**

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To prove the identity and quantity of products, NMR-spectra were taken of the product in question, as well as from a mixture of pure substances in hexa-deutero-acetone (Fig. 4).
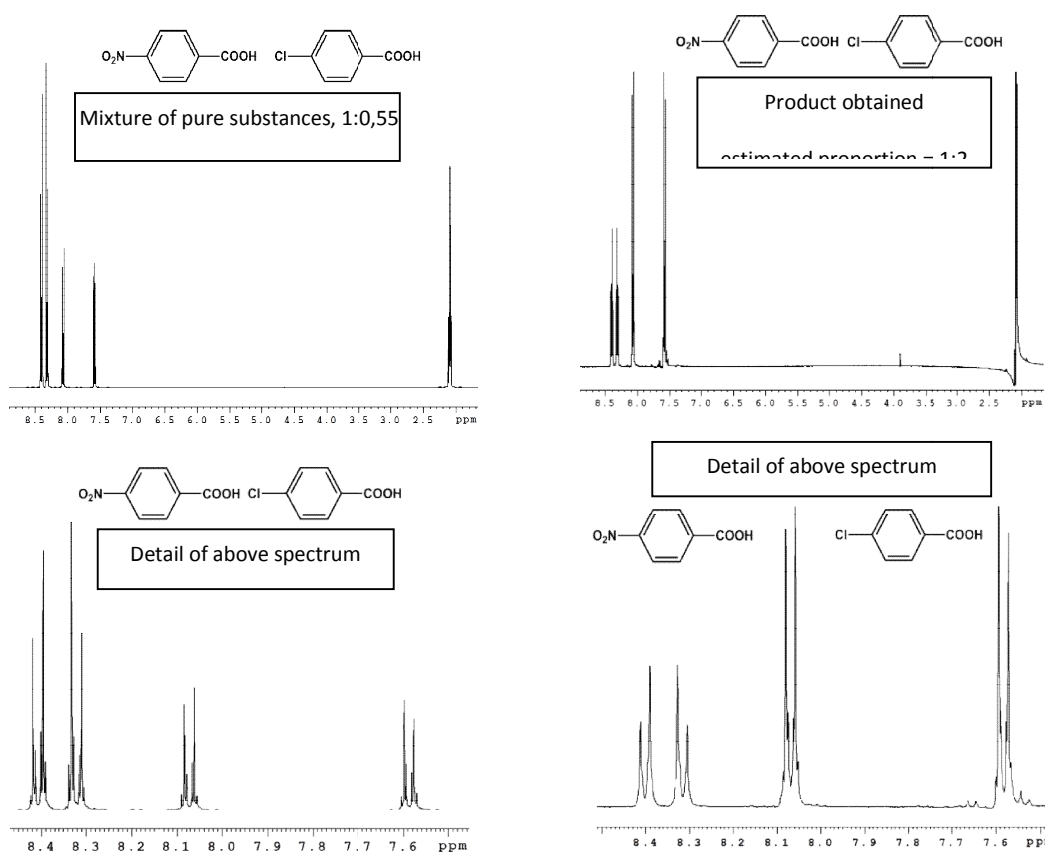


**Fig. 4. ^{1}H-NMR spectra of presumably obtained pure substances, compared with reaction products pf phenylalanine after incomplete digestion**

## 4. DISCUSSION

Residues obtained after acid oxidative digests for elemental analysis, should be of concern. Digestions with chlorate in 4,5M $HNO_3$ permit to use up to 4 times larger sample weights in closed digestion vessels, but the amount of added oxidation equivalents is lower than with $HNO_3$ only. About 100 mg phenyl alanine resp. 200 mg tyrosine have been digested fairly well by the proposed procedure, like 1 g of green plants, fruits or milk samples [1-6], which was controlled by digesting 0.25 g of the same samples with $HNO_3$ only (to get K and Rb). If the sample contains substantial aromatic amino acids, white precipitates might appear which were identified as p-nitro-benzoic acid, p-chloro-benzoic acid and the like. These resultant reaction products do not yield precipitates with inorganic ions in the digest (except occlusions), and will not interfere with subsequent trace element analysis. This permits the utilization of the $KClO_3$-$HNO_3$-digestion method to digest samples high in phenylalanine and/or tyrosine for subsequent trace element analysis, without further concerns.

When phenylalanine or tyrosine gets digested, the aliphatic chain gets completely disrupted. At the aromatic ring, the $HNO_3$ is the primary reactant. The $HClO_3$ present in the sample digest can add just one Cl to the aromatic ring at maximum, the Cl and $NO_2$ substituents stabilize the ring. More Cl can only be added from $HClO_3$-HCl solutions. This agrees with the known fact that $ClO_2$ in neutral solution does not oxidize amines to imines or aldehydes, and does not introduce Cl to the aromatic ring [11,12].

Even as early as 1843, the reduction of chlorate by various organic compounds (tartaric, citric and

fatty acids, meat, protein etc.) in presence of nitric acid, was described to yield hypochlorite, and intermediately, nitrous acid was formed, which was re-oxidized by the chlorate, whereas chlorate hardly reacted directly. At that time, however, the organic products could not be identified [13].

## 5. CONCLUSION

During digestion of organic comounds with potassium chlorate in dilute nitric acid, chlorate hardly oxidizes the organics directly, but re-oxidizes nitrite. Samples containing high amounts of aromatic compounds like phenyl-alanine yield chloro- and nitro-benzoic acids, which hardly interfere with subsequent determinations of total element contents in the resulting digests.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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