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RESEARCH ARTICLE

COMPARISON OF TWO METHODS OF TETRAMETHYLAMMONIUM DOSING IN FRUIT MAERUAPSEUDOPETALOSA (GILG AND BENEDICT) OF WOLF

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ABSTRACT

The population of poor agricultural yields areas have potentially very nutritional substitution food at their disposal. Among these sources, *Maeruapseudopetalosas* fruit (syn.*Courboniavirgata*) whose iron and potassium content highlighted allow the compensation of various nutritional deficiency. However, the fruit presents a toxicity that can be inhibited by lixiviation. The dosage method of this toxin, the tetramethylammonium (TMAH), is old and has been used earlier**.** This current scientific work suggests a new volumetric dosagemethod of the TMAH in *Maeruapseudopetalosa* fruit. Ripe *Mareuapseudopetalosa* fruit have been collected in two distinct vegetable areas, located in Kieneba and Koussane in the region of Tambacounda in Senegal. The dosage carried out on this fruit is essentially based on the volatility of ammonia derived from TMAH. Indeed, in highly alkaline environment, ammonia is distilled, then being dosed volumetrically. The results derived from operating conditions (aqueous extraction with a ratio of 1g/ml shaken for two hours at 25 C) have allowed in getting an average quantity of 5.69 ± 0.3 g of TMAH in 100 grams of seeds.

INTRODUCTION

Maeruapseudopetalosa of Wolf is a plant belonging to the family of Capparidacées which is found mainly in eastern Senegal around Tambacounda. It usually grows in the gold zones but also in aridzones (Kheraro, 1974) such as Sudan (southern), northern Uganda and north-eastern Kenya. (Becker, 1986) The fruit of Maeruapseudopetalosa is consumed during the lean period and ensures energy intake of 375.75 kcal • 100 g-1 on average (Ayessou *et al*., 2009). However, it is known for its toxicity (Kheraro, 1974; Henry, 1948) due mainly tetramethylammonium hydroxide (TMAH). (Henry, 1948). This toxin is removed before consumption by a technique called leaching fruit (Henry, 1948; Henry and Grindley, 1954). In fact, after picking, they are soaked in successive baths of water, then rubbed with ash, rinsed and dried before being cooked on fire. The dosage method used in these tests is very old and is based on a series of unit operations. (Henry and Grindley, 1949) This work suggestsa new volumetric method based on the physicochemical characteristics of TMAH.

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MATERIALS AND METHODS

Sampling of fruits

The pseudopetalosafruits were harvested at maturity on two distinct plant communities located in Kiénéba (latitude 14 ° 05 ' 54.4 ' 'N , longitude 12 ° 03' 31.8 ' ' W) and Koussané (latitude 14 ° 07 ' 53.3 ' 'N , longitude 12 ° 26' 37.1 " W) in the region of Tambacounda in Senegal. Sampling allowedto form a batch on the site of Kiénéba and two lots at Koussané. The fruit is a berry Maeruapseudopetalosa splenic the top corner which is stripped of its envelope and edible (Figure 1).

Characteristics of tetramethylammonium hydroxide (TMAH)

The TMAH salts are formed from Tetramethylammonium hydroxide (TMAH) whichis a strong base of crude molecular formula C4H13NO or (CH 3) 4 NOH. TMAH is soluble but unstable in aqueous solution and emits ammonia and methanol when heated. Its semi-developed formula is shown in Figure 2. The dosage is essentially based on the volatility of ammonia. Indeed, in a highly alkaline medium, ammonia is distilled and then driven by the steam.

Figure 1. Maeruapseudopetalosafruits before harvest and seeds without their shell

The dosageis then carried out on the distillate by titrimetric referring to the standard method of thedosageof ammonium in water (Rodier, 1996). The equations that take place during the dosage are as follow:

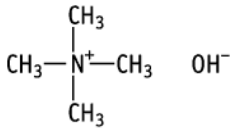

Figure 2. Semi-developed Formula of Tetramethylammonium [http://dra4.nihs.go.jp/mhlw_data/home/file/file75-59-2.html]

$(CH_3)_4NOH + 4H_2O \rightarrow NH_4OH + 4CH_3OH$ (Aqueous extraction of TMAH) $NH_4OH \rightarrow NH_3$ (gazeux) + H_2O (Distillation)

The reactants

The various reactants that were used are:

- Distilled water
- Boric acid solution mass concentration of 10g / L
- Sodium hydroxide solution of normality concentrated 40N
- Solution sulfuric acid normality of 0.05N
- Mixed colored indicator compound methylene blue and bromocresol green

Procedure

In the flask of the distillation apparatus, introduce the test sample containing 0.2 to 20mg of ammonium. Add 20 mL of sodium hydroxide solution (40N) and optionally an anti-foaming agent. Start distillation for 20 minutes at least. Collect the distillate in 5 ml of boric acid solution. Check that no ammonium in the last fractions of the distillate.

Sample Preparation

- In a 500 ml flask, collect 1g of fruit. Adjust to the mark with distilled water and extract with magnetic stirring for 2 hours then strain
- Take an aliquot of filtrate of 100 ml and 100 ml of distilled water
- Add 20 ml of concentrated solution of sodium hydroxide.
- Collect 150 ml of distillate in 10ml boric acid containing the mixed indicator (NB : ensure that all of the ammonia present in the balloon was moved)
- Perform assay using a sulfuric acid solution 0.05 N.

Expression of the nitrogen concentration

$$\%N = \frac{N1 \times V1 \times 14}{V2} \times \frac{F \times 100}{PE \times 1000}$$

N1 and V1: normality and volume of sulfuric acid 0.05 N

V2: volume of filtrate distilled

PE: mass of the test sample

F : Volume of solution- Vial

That is to say: $\%N = 0,35V1$

Expression of the concentration of tetramethylammonium

$$\%TMAH = \%N \times 6,5$$

RESULTS AND DISCUSSION

The tests performed in duplicate on three batches of samples have given the respective following percentage contents of Tetramethylammonium (TMAH): 5.68 ± 0.3 on the lot Kéniéba; 5.65 ± 0.29 and 5.68 ± 0.3 on the lot Koussané an overall average of 5.67 ± 0.3 g / 100g of seeds. These values are very different from those found by Henry and Grindley (Henry and Grindley, 1949) who evaluate to 15g / 100g. The gap is explained by a difference in methods at the point of extraction and the principle of the proper dosage. Indeed, the method used by Henry and Grindley (Henry and Grindley, 1949) is done in several steps declined as follows:

- Cold extraction in alcohol
- Alcohol evaporation and taking up the residue with water and filtration.
- Addition of filtrate sodium acetate
- Neutralization of the excess sodium hydroxide with sulfuric acid.
- Evaporation of the supernatant
- The residue is again recovered with water and filtered. The filtrate was then heated to 50 - 60 ° C and potassium iodide is added drop by drop while stirring.
- The solution is left to cool in a refrigerator overnight. This forms a precipitate. We proceed againwith a filtration and washing with a small amount of iodine water. The precipitate obtained is green in color and crystal form. This precipitate is decomposed by prolonged treatment with hot water until free iodine is expelled.
- The gross TMAH iodine solution obtained is evaporated at 110 ° C overnight followed by addition of water. A certain amount of insoluble impurities is formed and removed by filtration.

- The filtrate is colorful at this stage of the method is again evaporated and the residue is washed with alcohol until the color disappears. At this stage, iodide TMAH is almost pure. It is then dissolved in hot water and filtered. The filtrate is boiled until crystallization starts. The solution is cooled and iodide TMAH in crystalline form is filtered and washed with absolute alcohol.
- The product obtained is then white, crystalline form and corresponds to the TMAH content in the starting sample.

This method is still very questionable for the following reasons

- Too long extraction to give repeatable results
- Solubility TMAH remains unproven in semi -polar solvents such as alcohol
- The evaporation and heating temperatures are too high since the action of heat on the molecule TMAH
- Releases filtrates are potentially possible losses TMAH

Therefore, the proposed method is based on physico-chemical properties including solubility of the hydroxide TMAH in water and the volatile properties of ammonium. Indeed, the TMAH is soluble in aqueous medium. It emits ammonia and methanol when heated (http://dra4.nihs.go.jp/ mhlw_data/ home/file/file75-59-2.html). The ammonia was then distilled and then assayed by an acid. This dosage approach is more objective because despite the announced toxicity, the fruit and leaves of Maeruapseudopetalosa are highly palatable to cattle (Gueye, personal communication).

These observations courses seem to militate for an over-estimation of the load TMAH announced by henry and Grindley (Henry, 1949) 15%. In addition that LD50 in rats is estimated between 34 and 50mg / kg.

Conclusion

The extraction principles and tetramethylammonium dosage used are based on the physicochemical properties of the molecule and allow to obtain objective results and consistent with the toxic power of the fruit. However, a more sophisticated technique, including chromatographic would refine the quantitative analysis of tetramethylammonium. This improvement will also facilitate a better assessment of risks related to the consumption of fruit Maeruapseudopetalosa.

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