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Short communication

Effects of moisture content in cigar tobacco on nicotine extraction Similarity between Soxhlet and focused open-vessel microwave-assisted techniques ☆

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Abstract

The effects of tobacco moisture on nicotine yield were investigated in this study. Soxhlet and microwave-assisted techniques were used to extract nicotine from cigar fillers of varying moisture contents (5–20%), using a polar (methanol) and a non-polar (isooctane) solvent. The extracts were analyzed by a gas chromatograph equipped with a flame-ionization detector. For both extraction techniques, higher nicotine yields were consistently obtained with methanol than with isooctane from the same samples. Solubility of nicotine salts in methanol but not in isooctane is the major cause of this observation. Moreover, pronounced effects of the tobacco moisture content on extraction efficiency were observed with isooctane but not with methanol. For microwave assisted extraction (MAE) with isooctane, nicotine yield increased from 3 to 70% as the moisture level in tobacco was raised from 3 to 13%, and leveled off thereafter. Similar observations were made with Soxhlet extraction. While MAE results were rationalized by the known cell-rupture process, a mechanism based on the interaction between the solvents and the structural components of the plant cells has been proposed to account for the observations made with Soxhlet extraction.

Keywords: Tobacco; Microwave-assisted extraction; Soxhlet extraction; Extraction methods; Cellular mechanism; Moisture effects; Nicotine; Alkaloids

1. Introduction

In recent years, microwave-assisted technique has been used to extract various analytes from plant materials [1–9]. The use of microwaves as a heat source is based on the principle that microwave energy, when absorbed, induces rotation of polar molecules, and the resulting molecular friction produces heat. Generally, absorption of the energy increases with the dielectric constant of the molecule, resulting in more effective heating. In the microwave-assisted process (MAP) originated by Paré et al. [1], solvents of low dielectric constants were used to extract plant chemicals. Free water in the plant cells, glands, and other microstructures was selectively heated over the solvent, which eventually leads to rupture of the walls when the internal pressure caused by heating reaches a certain threshold, and the soluble chemical species within the

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cells are extracted into the surrounding solvent. This mechanism of extraction, based on exposing the analytes to the solvent through cell rupture, is different from Soxhlet extraction that depends on a series of permeation and solubilization processes to bring the analytes out of the matrix. Based on the mechanism of MAP [1,2], it is conceivable that the water content in a plant sample will have great influence on the extraction efficiency of the target analyte(s) contained within plant cells or the likes. For example, in the extractions of ginger by hexane [4], withanolides from dried leaves of *Iochroma gesnerioides* by dichloromethane [7], and piperine from *Piper nigrum* [8] by light petroleum, significant increase in extraction efficiency was observed when a small amount of water was added to the plant materials prior to microwave extraction. Review of current literature indicates that comparisons have been made to demonstrate the various advantages of microwave-assisted extraction (MAE) over Soxhlet extraction of plant chemicals [2–9], but comparative studies on relating moisture level in plant samples with extraction efficiency between these two techniques have never been reported. This is probably a result of the assumption that in Soxhlet extraction, solubility of the analyte in the solvent is the limiting factor, and the yield of analyte is independent of the water content in botanical tissues. To clarify this notion, we compared the extraction behaviours of Soxhlet technique with MAE, using both polar (methanol) and non-polar (isooctane) solvents to recover nicotine from cigar tobacco leaves containing different amounts of water. Tobacco nicotine was chosen because it is an analyte known to be present within the cell cavity [10]. Hence, it allows the study of extraction by MAE via cell rupture, as well as by Soxhlet via permeation/solubilization through cell walls. In this investigation, open-vessel MAE was used for comparison with Soxhlet extraction. The amount of nicotine extracted was determined by gas chromatography–flame ionization detection (GC–FID). Attempts were made to rationalize the observations at cellular level.

2. Experimental

2.1. Chemicals and materials

Methanol and acetonitrile, HPLC grade, were purchased from Fisher Scientific (Ottawa, Canada). Isooctane and *N*-methyl-*N*-trimethylsilyl-trifluoroacetamide (MSTFA) were supplied by Caledon (Georgetown, Canada) and Supelco (Oakville, Canada), respectively. Cigars were obtained from a local store.

2.2. Sample preparation

Cigar is composed of three components: wrapper, binder and filler. Filler constitutes the major part of cigar. The fillers from several cigars of the same brand were mixed and ground in a Wiley mill (Arthur H. Thomas, Philadelphia, PA, USA), passed through a 20-mesh sieve, and used without further treatment. The moisture content of the ground tobacco was determined by using an HR73 Moisture Analyzer from Mettler Toledo (VWR Canlab, Montreal, Canada). Samples of lower moisture levels were obtained by leaving 5 g of the ground tobacco in a desiccator with a bed of Drierite at the bottom for different period of time (for example, a 9% moisture sample was obtained after 4 h). To obtain samples of higher moisture contents, a glass dish containing 5 g of the tobacco sample was placed in a sealed desiccator-like container, and the tobacco was exposed for different lengths of time to the vapour of a pool of deionised water kept at 45 °C at the bottom of the container (for example, a 19% moisture sample was obtained after 6 h).

2.3. Trimethylsilylation

Methanol MAE extract, 500 μl, was dried by a stream of nitrogen gas over the solution. The residue was heated with equal volumes (500 μl) of acetonitrile and MSTFA at 70 °C for 30 min. The solution (1 μl) was analyzed for the presence of organic acids on a GC–mass spectrometric (GC–MS) system (Agilent Technologies, Palo Atlo, CA, USA) composed of a HP GC 6890 series II GC instrument and a HP 5973 MS detector, using the same GC column and conditions as described in Section 2.5.

2.4. Extraction procedure

2.4.1. Soxhlet extraction

One g of ground cigar filler was extracted with 75 ml isooctane or methanol for 2 h in a Soxhlet

system at refluxing temperatures 99.2 and 64.5 °C, respectively, according to a method described by Burns and Collins [11]. The liquid was allowed to cool and transferred into a 100-ml volumetric flask, and the same solvent was added to make up to the mark. The diluted solution was used for GC–FID analysis.

2.4.2. Microwave-assisted extraction

One g of ground cigar filler was mixed with 25 ml isooctane or methanol in a quartz extraction vessel. The mixture was irradiated with microwave at 200 W for 6 min in a Prolabo Soxwave 3.6 (Fontenay-Sous-Bois, France), a focused microwave system operated at 2.45 GHz with a full power of 200 W at atmospheric pressure. Milder conditions were used for methanol extraction: 100 W for 30 s followed by 20 W for 5.5 min. After extraction, the mixture was allowed to stand for 10 min to let the co-extracted hydrocarbon waxes precipitate. The supernatant liquid was decanted into a 100-ml volumetric flask and additional solvent was used to rinse the tobacco residues. The rinses were added to the solution to make up to the mark. The volume-standardized extract was used for GC–FID analysis.

2.5. Quantification of nicotine by GC–FID

The extracts were analyzed on a HP5890 series II gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a flame ionization detector, a HP 6890 autosampler, and a DB5MS column, 30 m×0.25 mm I.D.×0.25 μm film (J&W Scientific, Folsom, CA, USA). One μl of each volume-standardized extract or the calibration solution was injected splitless into the GC with the injector and the detector set at 250 and 300 °C, respectively. Helium flow was maintained constant at 1.0 ml/min, while the column was heated at 80 °C for 1 min, then ramped at 15 °C/min to 300 °C at which it was held for 1.5 min. Each solution was analyzed in triplicate and the average response was used for calculating nicotine yield. The percent yields were calculated on a dry basis by considering the yield obtained through 2 h Soxhlet extraction of the sample with methanol to be the maximum [11].

3. Results and discussion

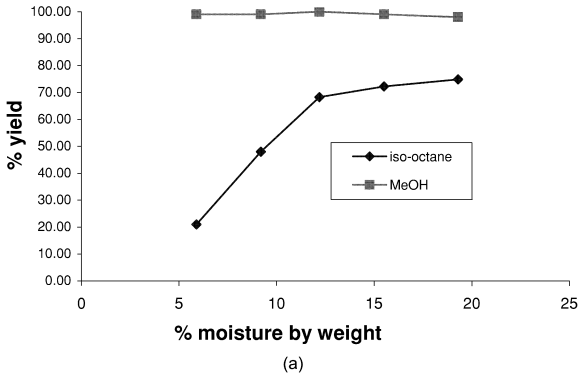
Previously, rehydration of dried plant materials was carried out by soaking [8] or adding water to the plant sample prior to extraction [4,7]. We have found that when enough water was added to ensure that the tobacco was homogeneously moisturized, it was inevitable that some excess water wetted the sample surfaces and eventually mixed with methanol, or formed a barrier between the sample and isooctane. It also complicated the determination of moisture content in the matrix. To avoid these complications, samples of high moisture contents were prepared by exposing a filler tobacco (~13%) to water vapour in a closed container. Drier samples were obtained by dehydration in a desiccator.

Nicotine is present in tobacco as a free base and salts. The salts are converted to free nicotine at the heated injection port of the gas chromatograph [12,13]. The free base is soluble in both isooctane and methanol, but the ionic salts are soluble only in methanol. This accounts partly for the higher nicotine yields consistently obtained with methanol than with isooctane at all moisture levels (Fig. 1a and b, respectively). The acid components of nicotine salts, such as malic, citric, succinic and pyroglutamic acids, are highly polar and cannot be detected unless they are derivatized [14]. The presence of nicotine salts in the methanol extract was confirmed by the detection of these acids as trimethylsilylated (TMS) derivatives along with nicotine by GC–MS.

Both methanol and isooctane extracts gave similar chromatograms (Fig. 2), in which nicotine displayed good peak shape and was well separated from the co-extracted plant hydrocarbons as confirmed by GC–MS using the same GC column and conditions. Nicotine was reproducibly integrated with <0.5% RSD (n=3). All extractions were carried out in duplicates with the nicotine yields of the two extractions agreeing within 1%.

3.1. Soxhlet extraction

Our results show that generally, methanol has higher extraction efficiency than isooctane in Soxhlet extraction of nicotine. For example, for a filler



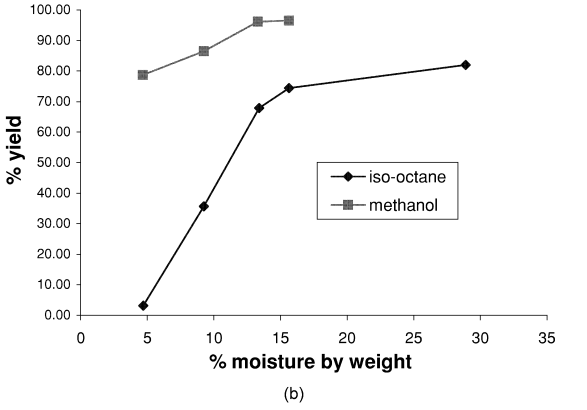


Fig. 1. (a) Nicotine yields obtained from 2 h Soxhlet extraction of tobacco samples of varying moisture contents. (b) Nicotine yields obtained from MAE of tobacco samples with varying moisture contents (sample–solvent=1 g:25 ml; isooctane MAE: 200 W for 6 min, methanol MAE: 100 W for 30 s, then 20 W for 5.5 min).

tobacco with ~13% moisture, 100% yield was obtained after 30 min extraction with methanol, but it took 70 min to reach an optimal yield of 70% with isooctane. For the study of effects of tobacco moisture on extraction efficiency, Soxhlet extractions with methanol and isooctane were carried out for 2 h for tobacco samples containing 5–20% moisture. The results are summarized in Fig. 1a. Nicotine yield obtained from the extraction with isooctane was dramatically reduced at moisture levels below ~13%, but only increased slightly beyond that point. On the contrary, the extraction efficiency was not significantly affected over the entire moisture range of 5–20% when methanol was used as the solvent. An understanding of the anatomy of plant cells (Fig. 3a) helps explain the differences observed between methanol and isooctane. A plant cell is composed of a large vacuole and other organelles in a sea of cytoplasm contained within a cell membrane. The membrane-bound cell is enclosed by a rigid cell wall, which is a composite material consisting of a framework of cellulose microfibrils embedded in a hydrophilic gel-like matrix of pectins and hemicelluloses. An amorphous pectin layer termed middle lamella connects cell walls of adjacent cells to each other. Nicotine, together with other solutes and metabolites, accumulates in the vacuole [10] that is separated from the cytoplasm by a membrane called tonoplast. For tobacco containing high % moisture, hot isooctane permeates through the porous middle lamella and cell wall [15]. Once inside the wall, it collapses both the hydrophobic phospholipid cell membrane and tonoplast successively as it comes into contact with them, thereby releasing nicotine from the vacuole. For dry tobacco, the aqueous volume in the cell is low, thus the membrane-bound cytoplasm shrinks away from the rigid cell wall, creating a space between the cell membrane and the cell wall (Fig. 3b). The dehydrated gel matrix in the cell wall also shrinks considerably such that the pores on the wall are not well exposed for free passage of isooctane [15,16]. As a result, less solvent comes into contact with the cell membrane. The extraction mechanism described above becomes less efficient, resulting in lower nicotine yield. Methanol, because of its compatibility with the hydrophilic cell wall matrix, is able to swell the dehydrated gel and thus retains the ability to permeate through the cell wall. It is also highly permeable to the cell membrane and the tonoplast [17]; and at the refluxing temperature, it can destabilize them [18] to facilitate the release of nicotine. All these factors contribute to the high extraction efficiency of methanol even at low moisture levels. The trend for isooctane continued until the moisture content reached ~13%, where the yield started to level off. Although fresh tobacco leaves could consist of as high as 90% water [19], the moisture content of cigar tobaccos kept in a humidor usually ranges from 10 to 15%. Cigar tobaccos are usually air-cured, fermented, and aged before being manufactured into cigars. During these processes, the leaves become dehydrated as some of the plant cells and other microstructures are broken

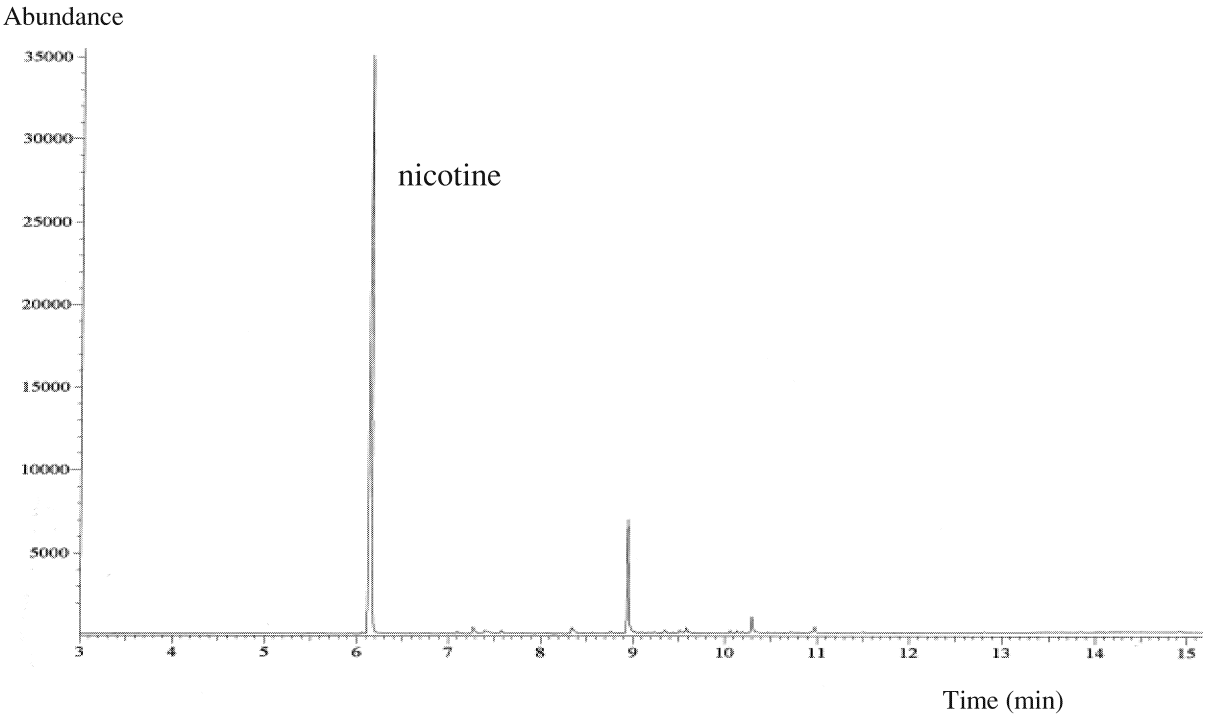


Fig. 2. A typical GC–FID chromatogram of underivatized MeOH extract of cigar filler. Unlabelled peaks are tobacco hydrocarbons.

and can no longer hold water. Grinding the sample before extraction also causes further damage to the plant cells. It could be that at ~13% moisture level, all the remaining intact cells and their cell walls have been saturated with water. Any additional moisture would remain on the exposed surface of the tissues, without contributing to a significant increase in nicotine yield.

3.2. MAE with isooctane and methanol

With isooctane as the extracting solvent, optimal yield of nicotine was achieved by treating 1 g of ground cigar filler in 25 ml of the solvent with microwave irradiation at 200 W for 6 min. For all samples containing different amounts of moisture, extending the extraction time to 10 min or re-extracting the samples with fresh isooctane did not increase the yield significantly. At the end of the extraction, the mixture was slightly warm to touch with a temperature ranging from ~30 to 45 °C, which was caused by heat transferred from the water-containing tobacco matrix. In addition, condensation immiscible with isooctane was observed on the wall of the extraction vessel just above the solvent level, indicating that water in the tobacco sample had been selectively heated and rapidly distilled off from the mixture. This also explains why condensation was not observed, and the temperature of the tobacco–solvent mixture remained close to ambient when a second extraction was applied to the same sample. Fig. 1b illustrates the dependence of nicotine yield on the water content in the tobacco sample. As the moisture level increases, the number of water-containing plant cells increases. Consequently, more cells are ruptured and higher nicotine yield is obtained. It could also be rationalized on the basis that as the aqueous volumes of the cells become larger, the internal pressure created upon microwave irradiation is higher; hence it is more likely for the cell to rupture and release nicotine into the solvent.

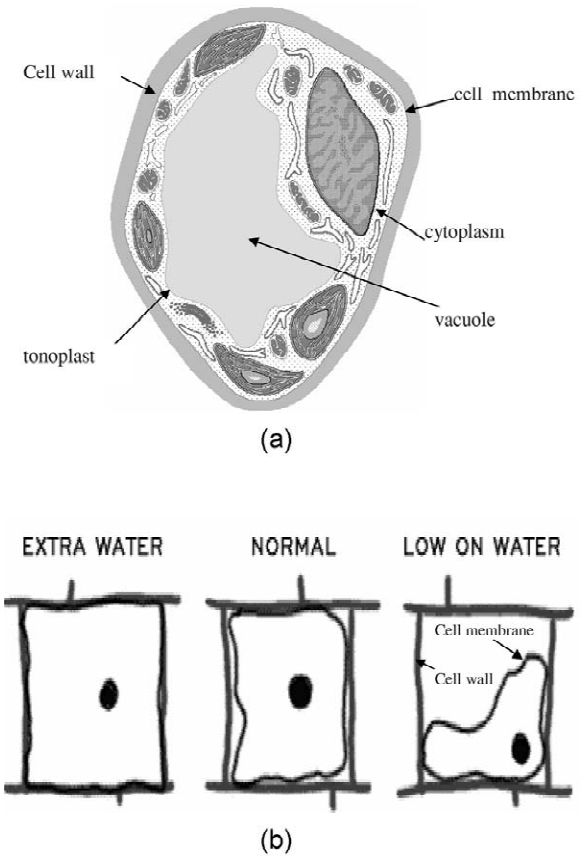


Fig. 3. (a) Anatomy of plant cell. Unlabelled structures are other organelles. (b) Contact between cell wall and cell membrane at different moisture levels.

For the same reason described above for Soxhlet extraction, the yield was not as much affected at moisture level >13%. Methanol, because of its high dielectric constant, is readily heated upon microwave irradiation. Therefore, milder conditions were used for MAE with methanol to avoid uncontrolled refluxing. For comparison purposes, the same extraction time (6 min) for isooctane MAE was used for methanol extraction. Unlike isooctane, the boiling methanol also allows Soxhlet-like extraction of nicotine through permeation–solubilization process, which occurs efficiently in conjunction with MAE via cell rupture. This conventional process becomes the major mode of extraction at low moisture levels where cell rupture is not favoured. The net result is that nicotine yield obtained from methanol MAE is less sensitive to the change of sample moisture content in comparison with isooctane MAE (Fig. 1b).

4. Conclusions

This paper reports a systematic study of the effects of tobacco moisture on the extraction of nicotine, a chemical existing within tobacco cells, by Soxhlet and microwave-assisted techniques using isooctane and methanol as solvents. For the first time, it has been shown that Soxhlet exhibits extraction behaviours similar to MAE. The effects of sample moisture observed with MAE were consistent with the cell-rupture mechanism [1]. To account for the results obtained from Soxhlet technique, a cellular mechanism has been proposed in this paper. The pores on the gel-like matrix of pectins and hemicelluloses in the cell wall play an important role in the extraction of nicotine. At low moisture levels, the pores shrink, resulting in reduced permeability and consequently lower nicotine yield with isooctane. Such effect was not observed with methanol because the solvent is able to swell the polar cell–wall matrix and restore the porosity. This study demonstrates that an understanding of the interaction between the solvent and the structural components of plant cells is essential for rationalizing the Soxhlet extraction behaviors of cellular chemicals. Further research aimed at comparing extractions of plant chemicals that exist within and without enclosed microstructures may provide further insight into the underlying physical phenomenon of extraction of plant chemicals.

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